EVALUATION OF THE ATTENUATING PROPERTIES OF VITAMIN C ON SOME ISONIAZID INDUCED NEUROPATHIES IN RATS

BY

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DOCTOR OF PHARMACY (PHARM. D) DEGREE OF THE FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

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CERTIFICATION

We certify that this project work was carried out by Miss **Hajarah-Ummih Bello** in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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DEDICATION

I dedicate this work to Almighty Allah, the Source of Knowledge and Wisdom. I offer gratitude for the blessings bestowed upon me in my pursuit of understanding.

"Read, and your Lord is the most Generous - Who taught by the pen - Taught man that which he knew not." (Quran, Surah Al-'Alaq, 3-5).

Also, in loving memory of my mother, Pharm. Zainab Yahaya who left too soon, her kindness, curiosity and passion for knowledge continue to guide my efforts.

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LIST OF ABBREVIATIONS

DS-TB: Drug Sensitive Tuberculosis

DR-TB: Drug Resistant Tuberculosis

ART: Anti Retroviral Therapy

HAART: Highly Active Antiretroviral Therapy

INH: Isoniazid

NRS: Numeric Rating Scale

PN: Peripheral Neuropathy

SOD: Superoxide Dismutase

GPx: glutathione peroxidase

GR: Glutathione Reductase

CAT: Catalase

ABSTRACT

Isoniazid is a widely used drug in tuberculosis treatment regimens. Its application in ❸direct observed therapy short course ❾(DOTS) along with other medications has been well documented to be efficacious and effective.

However, since its introduction over 70 years ago, it has been found to possess adverse effects such as the induction of neuropathies. There are estimates that as many as 10 % of patients receiving isoniazid will develop some form of neuropathy. Introduction of new medications to stop these neuropathies still pose a challenge. Pyridoxine (vitamin B6) is currently recommended with isoniazid therapy to avert induction of neuropathy. Although ,the potential of vitamin C as an antioxidant to prevent induced neuropathies has been suggested based on previous studies, the findings from this study were intended to contribute valuable insights into the potential therapeutic role of vitamin C as an adjuvant to mitigate neuropathic complications in isoniazid-based therapies.

Using well-established animal models, we assessed the effects of vitamin C supplementation on the development and progression of some neuropathic symptoms induced by isoniazid administration. Male Wistar rats were divided into six groups: control, isoniazid-treated (800 mg/kg), and combination-treated; Isoniazid with vitamin C in low (7.5 mg/kg), medium (15 mg/kg), high (30 mg/kg) daily doses and isoniazid with pyridoxine (50 mg/kg). Behavioural assessments, including sensory and motor function tests, were conducted at the end of a seven day period to monitor the onset and severity of neuropathy.

In conclusion, our findings revealed that isoniazid administration led to a significant decline in sensory and motor functions indicative of peripheral nerve damage. Vitamin C supplementation did not demonstrate a remarkable attenuation of these neuropathic manifestations. Rats co-administered with isoniazid and vitamin C did not exhibit any improvement in sensory and motor functions when compared with the control and standard therapy of pyridoxine.

These results negate the potential neuroprotective effects of vitamin C against isoniazid-induced peripheral neuropathy.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction to Peripheral Neuropathies

Peripheral neuropathy, also known as peripheral polyneuropathy, is a general term for a broad range of disorders that cause damage and dysfunction of the nerves of the peripheral nervous system in several different patterns (Novello and Pobre, 2022). Peripheral neuropathy (PN) is a serious condition affecting the nerves responsible for transmitting information from all parts of the body to the spinal cord and brain (Torpy e*t al*., 2010).

Peripheral neuropathy is a complex and debilitating neurological disorder characterized by damage to the peripheral nerves, leading to a wide range of sensory, motor, and autonomic disturbances (Offermanns and Rosenthal, 2008). It refers to symmetrical, widespread damage to nearby nerves. The damage and clinical symptoms are typically proximally progressing and distal in location. Peripheral neuropathy can come in more than 100 different forms, each with its unique symptoms and prognosis. It is estimated that as many as 500 million people in the world suffer from peripheral neuropathy (Smith and Torrance, 2012). It has been known to affect sensory nerves, motor nerves, or autonomic nerves.

1.2 Pathophysiology of Peripheral Neuropathies

The pathophysiology of PN depends significantly on the underlying illness. However, it results from an insult to the body of the nerve or to the myelin sheath, leading to loss of normal function and development of deficits in the affected individual (Azhary *et al.*,2010).

The pathophysiology of neuropathic pain is complex and involves the, sensitization of nociceptors, spontaneous activation of afferent fibers and nociceptors, ascending regulation of sodium channels, sensitization of primary afferent fibers and catecholamines, ectopic discharges of the dorsal root ganglion,activation of the immune system and glial cells with release of proinflammatory cytokines, chemokines, and other neuroexcitatory compounds (Kraychete *et al.*, 2008).

In diabetic neuropathy, changes due to excess glucose out of the cells are observed which causes; increased glucose flow to the polyol pathway or hexosamine pathway, excessive or inappropriate activation of the c-protein phosphokinase, accumulation of glycosylated end products, imbalance of the reduced state in the mitochondrial pathway, and increased formation of superoxide radicals (Cornell and Ducic, 2008).

Peripheral neuropathies can ultimately result from a wide range of unique disorders, yet the methods by which peripheral nerves are injured have patterns in common. Upon electrophysiological findings, axonal, demyelinating (with or without conduction block), or mixed neuropathies are differentiated (Kraychete and Sakata, 2011).

These patterns of injury include:

(a) Segmental demyelination: This process refers to the process of degeneration of the myelin sheath, with sparing of the nerve axon (Hammi and Yeung, 2022).Myelinating Schwann cells (SC) ensheath the peripheral nervous system axons in a symbiotic developmental relationship.(Moss and Bopp, 2021).Schwann cell precursors (SCPs)

provide trophic support for growing axons and axons in turn support SCP survival and migration. Immature Schwann cells (iSCs) initiate myelination in a multi-step process called radial sorting. Three to eight iSCs surround bundles of axons to form units sharing a common basal lamina and then iSC lamellipodia-like processes invade the axons to categorize them by caliber (Bhagavati *et al*., 2009). Large caliber axons are surrounded by promyelinating Schwann cells in a 1:1 relationship and small caliber axons remain in Remak bundles. Myelinating SC then continue to polarize radially and longitudinally to ensheath axons; a process that requires specific localization of myelin proteins and lipids (Moss and Bopp, 2021).

In classic demyelination, the loss of myelin sheaths following proper development, is commonly observed in the acquired demyelinating neuropathies such as Acute inflammatory demyelinating polyneuropathies (AIDP) and Chronic inflammatory demyelinating polyneuropathies (CIDP). (Chanson and Echaniz-Laguna, 2014). However, with dysmyelination, the myelin sheaths likely never develop properly and may undergo a process very similar to classic demyelination later in disease progression, is usually observed in the inherited demyelinating neuropathies Charcot-Marie-Tooth disease type 1a (CMT1A-F and -X) (Brennan *et al.,* 2015).

Secondary axon degeneration is a common feature of demyelinating neuropathies, and this process is often correlated with clinical deficits and long-lasting disability in patients. These are often inflammatory and sometimes immune-mediated. About 20% of symmetrical peripheral neuropathies result from damage to the myelin (Hammi and Yeung, 2022).

(b) Wallerian degeneration: This tends to occur due to lack of necessary nutrients in the body. The nerve axon degenerates as a result of a lesion or physical compression, leading to the area distal to the axon slowly wasting away. This reaction results in focal mononeuropathy that is secondary to trauma or infarction of the nerve (Freeman, 2014).

(c) Axonal degeneration: Here, the axon degenerates in a pattern that starts distal and progresses proximally; this is thought to be because the most distal portion of the axon is particularly vulnerable due to its distance from the cell body, which provides metabolic support (Sumner and Asbury, 1975). This degeneration also known as "dying back phenomenon" usually manifests as trophic alterations to muscle, alongside accompanying symmetrical polyneuropathy. It is associated with weakness, most notably weakness in dorsiflexion of the feet and ankles. Examples of diseases causing axonal degeneration include diabetes, HIV, HCV, and Guillain-Barre syndrome (Cashman and Höke, 2015).

These among other factors cause inflammatory reactions, changes in angiogenesis, capillary basement membrane thickening, increased proliferation of the vascular smooth muscle, altered capillary permeability, reduced neurovascular flow and metabolism, and activation of transition factors (NF-κB, TGFβ) alongside neural dysfunction, mitochondrial and cellular death that results in excitation of the nerve excitation and pain. (Rasmussen *et al*., 2004, Bennett *et al.,*2007).

1.3 Classification of Neuropathies

Neuropathies of peripheral nerves may be classified according to various criteria (Figure. 1.1). The most important classification is the one according to its origins, which differentiates between acquired or inherited forms (Finsterer *et al.,*2021).

Peripheral polyneuropathies can be acquired, such as in diabetes mellitus, amyloidosis, HIV, or cis-platinum chemotherapy, while some may be inherited, as in Charcot-Marie-Tooth (Novello and Pobre, 2022). Both hereditary and acquired neuropathies may or may not be accompanied by involvement of other systems, organs, tissues. The latter are further classified according to the degree of co-affection of other organs or tissues as dominant or non-dominant. In non-dominant cases, neuropathy is overshadowed by manifestations of other organs (Finsterer e*t al.,*2021).

Acquired and hereditary neuropathies are also classified according to the course as either acute (e.g., Charcot-Marie-Tooth disease) which is a medical emergency or chronic (e.g., Guillain-Barre syndrome (Offermanns and Rosenthal, 2008).

According to the types of nerve fibers affected they may be classified as motor, sensory, autonomic, or mixed neuropathies.

- **Sensory nerves** help you feel pain, touch, temperature, position and vibration. Negative sensorial manifestations include hypoalgesia and hypoesthesia; and positive manifestations include paresthesia, dysesthesia, hyperpathia, and allodynia in addition to sensations of stinging, tingling, or tinnitus. Positive or negative sensorial manifestations usually indicate involvement of small type Aδ and C fibers. However, positive sensorial manifestations can suggest acquired neuropathy, since hereditary neuropathies have a tendency to present more physical changes than symptoms (Kraychete and Sakata, 2011)
- **Motor nerves** help you move and maintain muscle tone. The most common motor manifestations include muscle spasms, clonus, fasciculations, amyotrophies, and loss of dexterity and muscle strength (Kraychete and Sakata, 2011).
- **Autonomic nerves** help control the function of some of your body❼ organs, such as your bladder or bowel.

Motor and sensory axons essentially run in the same nerves, both motor and sensory functions are usually affected in this condition. Neuropathies with large fiber dysfunction (motor or sensorial) with loss of proprioception, vibration, or light touch related to demyelination cause muscle weakness with or without ataxia and positive sensorial manifestations, such as tingling (Kraychete and Sakata, 2011).

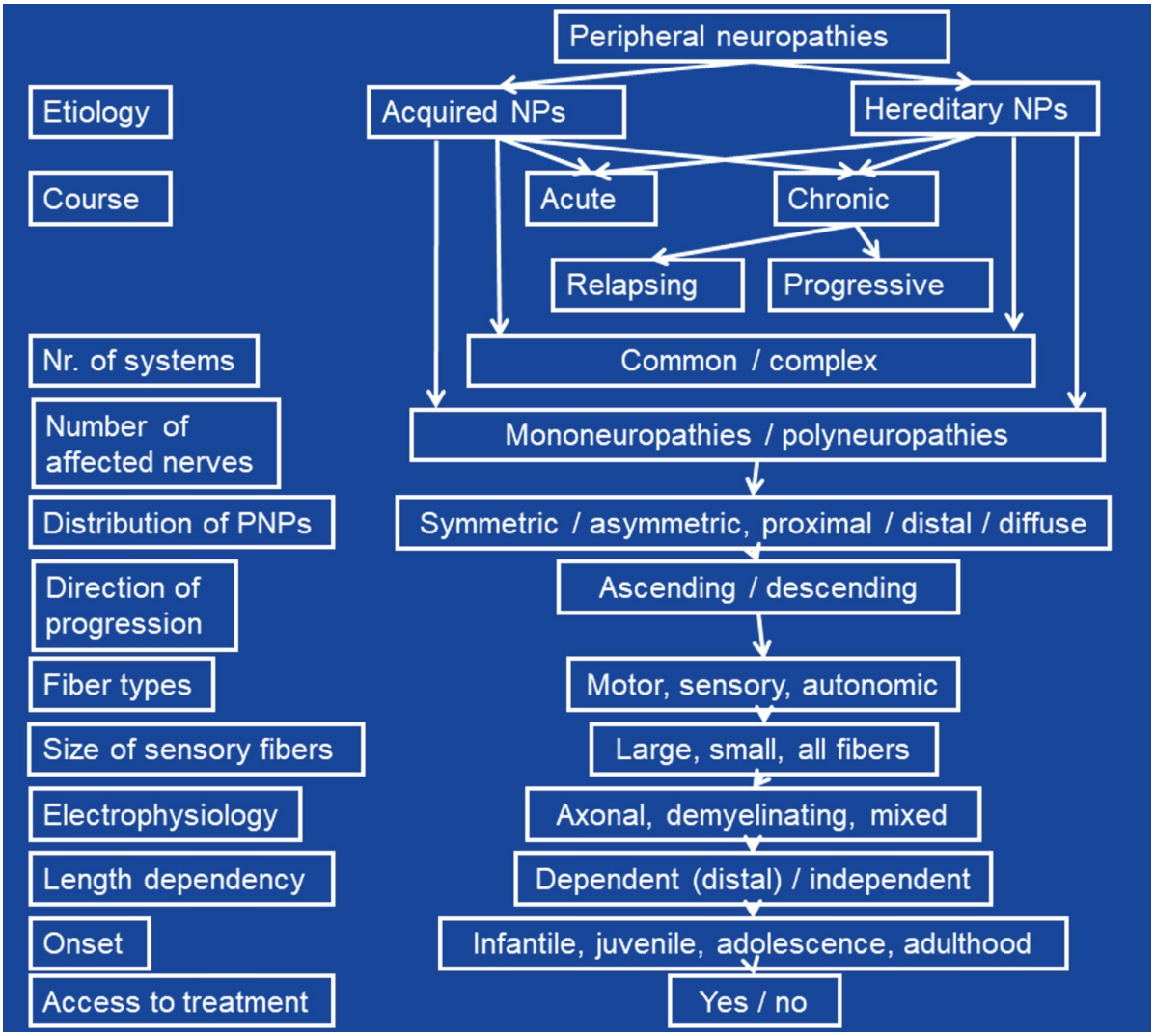

Figure 1.1 Classification of peripheral neuropathies (Finsterer *et al.,*2021).

1.4 Symptoms and Epidemiology of Peripheral Neuropathies

1.4.1 Symptoms

Peripheral neuropathy (PN) can manifest with a variety of symptoms, and a diagnosis can usually be made on a clinical basis (Mafukidze *et al*., 2016). Treatment and prognosis of PN vary depending on the underlying cause, but often the condition can lead to permanent disability especially in individuals with TB. For this reason, primary prevention is key as is early identification and management of symptoms (Mafukidze *et al*.,2016).

While many begin more slowly, some can manifest suddenly. When patients first experience symptoms, they are frequently bilateral and typically limited to the distal extremities such as the feet or hands in what is known as the ❽**tocking and glove**❾distribution and often manifests as paresthesias, numbness, or pain as common symptoms of peripheral polyneuropathies. Inherited polyneuropathies may present with ataxia and muscle cramping while acquired polyneuropathies typically present as burning or paraesthesias (Novello and Pobre, 2022).

Typically, the development of these sensory symptoms precedes the development of any muscular or proprioceptive symptoms, but not always, and patients may complain of losing their balance or increased frequency of falls.

1.4.2 Epidemiology

It is estimated that the prevalence of peripheral neuropathies is 2–3% in the general population. However, in patients >55 years of age the prevalence increases to 8%. (Burns and Mauermann, 2011).

Although most of this data come from western countries and there is a need for more general baseline data especially in high-burden TB settings (Hoffman *et al*.,2015). Rates between 0 and 10% among those with drug susceptible tuberculosis have been reported in the literature (Kass and Shandera, 2010).

1.5 Etiology of Peripheral Neuropathies

Although peripheral polyneuropathies are commonly found in patients with diabetes mellitus or excessive alcohol use, many medical conditions have associations with peripheral neuropathies.

Causes of PN in patients are multiple, and can include tuberculosis, other co-morbid conditions, such as Human Immune-deficiency virus (HIV) disease, malnutrition, or diabetes mellitus (DM), and several anti-tuberculous medications (Mafukidze *et al*., 2016). Generally, acquired neuropathies are more frequent than hereditary neuropathies. Acquired neuropathies categorized into **metabolic, toxic, neoplastic/paraneoplastic and drug-induced neuropathies** (Burns and Mauermann, 2011). It may also be the exclusive manifestation of a disease or may occur together with affection of other organs (Finsterer e*t al*., 2021).

1.5.1 Metabolic neuropathies

Generally, metabolic neuropathies include diabetic, uremic, endocrine, and nutritive neuropathies. (Finsterer *et al*., 2021).

(a) Diabetic neuropathy: is a slowly progressive painful sensory neuropathy evolving with a length dependent pattern. Slight distal weakness of toes extensor can be observed (Lozeron and Adams, 2008). It is the most frequently observed form of neuropathy in industrialized countries, and it possesses a vast array of clinical manifestations. Most clinical patients presenting with diabetic neuropathy have a distal symmetrical form of the condition that progresses following a fiber-length-dependent pattern, with predominant sensory and autonomic observations. This pattern of neuropathy is associated with a progressive distal axonopathy. Patients experience pain in the feet, and autonomic disturbances (Said, 2007).

(b) Nutritive neuropathies: are most frequently length-dependent, sensory neuropathies with the exception of vitamin-B12 deficiency neuropathy (Gwathmey and Grogan, 2019). Neuropathy due to vitamin deficiency, with/without involvement of other organ systems, has been reported much more rarely. Reduction in vitamin levels may be due to diet (malnutrition, emesis, malabsorption, diarrhea), autoimmune conditions, certain medications, chronic colitis, excessive alcohol consumption, bariatric surgery, or because of some form of gastrointestinal compromise (Finsterer *et al*., 2021). Vitamins deficiencies that have been associated with neuropathy include vitamin-B12, folic acid, thiamine (vitamin-B1), vitamin-B6, and vitamin-E.

Vitamin B12 (Cobalamin): About 4% of the distal symmetric PNs are caused by vitamin-B12-deficiency (England *et al*., 2009). Unlike other nutritive neuropathies, vitamin-B12-deficiency manifests as a length-independent, sensory neuropathy and affected patients usually present with concomitant myelopathy (Gwathmey and Grogan,

2019). Cobalamin plays an important role in the folate dependent intracellular methylation process of converting homocysteine to methionine . Therefore, any deficiency in cobalamin would result in hyperhomocysteinemia, which has toxic effects on neurons (especially for myelin sheaths) and vascular endothelium (Pratama e*t al*., 2022). Another possible mechanism arises from the disruption of Methylmalonyl-CoA Mutase (MCM) function due to Cobalamin insufficiency. This essential enzyme is responsible for the conversion of methymalonyl-CoA to succinyl-CoA (a known substrate in the Citric acid cycle) whilst using adenosyl cobalamin as a cofactor (Takahashi-Iñiguez *et al*., 2012). The dysfunction of MCM causes the methylmalonyl-CoA to accumulate, which results in the formation and insertion of non-physiologic fatty acids into neuronal lipids. This eventually leads to the degeneration of myelin sheath and interferes with myelin formation, causing neuropathy (Kalarn and Watson, 2017). Methylmalonyl-CoA on accumulation can be also converted to methylmalonic acid (MMA), which has been shown to contribute to myelin damage (Infante *et al*., 2021). Patients with cobalamin neuropathy characteristically have concomitant myelopathy (Gwathmey and Grogan, 2019).

Folic acid: PN due to folate deficiency is rare and manifests with slowly progressive distal symmetric, sensory neuropathy (Koike *et al.,* 2015). Its development is related to vitamin B12 deficiency neuropathy discussed previously which predominantly affects the lower limbs. Cognitive and affective symptoms of folate-deficiency are far more common than PN.

Vitamin E: deficiency is uniquely associated with a spinocerebellar syndrome. (Gwathmey and Grogan 2019).

Vitamin B6 (Pyridoxine): Vitamin-B6 deficiency almost exclusively develops in the context of drugs that reduce vitamin-B6 re-absorption and cause axonal distal sensory-motor PN. (e.g. isoniazid) (Kulkantrakorn, 2014). A further cause of vitamin-B6 deficiency can be chronic dialysis (Moriwaki *et al.,* 2000) Pyridoxine, like cyanocobalamin and folate, is required in the metabolism of methionine to cysteine and hence necessary for neuronal survival (Ghavanini and Kimpinski, 2014). Pyridoxine deficiency is known to impair trans cellular signaling between neurons and usually presents itself as a culmination of various nonspecific symptoms sometimes difficult to put together (Brown *et al.,* 2022). Pyridoxine in high doses interferes with the efficacy of chemotherapeutic drugs and can also precipitate neuropathy (Galluzzi *et al*., 2012). Daily dosing greater than 50 mg/day if used for longer than six months has been proven harmful (Ghavanini and Kimpinski, 2014).

A Hypothesis for why a small portion of patients with vitamin deficiency develops neuropathy is that pre-existing nerve pathology may be necessary for the development of a vitamin-deficiency-related neuropathy (Finsterer *et al*., 2021).

(c) Endocrine neuropathy: Neuropathy attributable to hypothyroidism or hyperthyroidism is extremely rare. More frequent than neuropathy is myopathy due to hypothyroidism. In the majority of the cases, thyroid disorders are well controlled hence the prevalence of a resulting neuropathy has considerably declined. Onset of hypothyroid neuropathy is usually in early adulthood. Neuropathy due to hypothyroidism typically starts as Small

fiber neuropathy (SFN) and may gradually affect larger fibers with disease progression (Sharma *et al*., 2018). For hypothyroidism neuropathy, nerve conduction study (NCS) reveals a length-dependent, sensory-motor axonopathy (Jin and Shin, 2019). The clinical presentation of neuropathy in hyperthyroidism can be similar to that of hypothyroidism but may also show up as a Guillain-Barre syndrome (GBS)-like presentation. NCSs in this case often reveals a symmetric, sensori-motor, mixed, neuropathy with active denervation in lower extremity muscles (Al-Wahaibi *et al*., 2017).

1.5.2 Toxic Neuropathies

Industrial and environmental toxins can damage the peripheral nervous system. Metal intoxications most frequently not only cause isolated neuropathy but also systemic manifestations. (Finsterer and Löscher, 2021). Acquiring a detailed history of possible occupational and home exposures is essential in establishing toxic neuropathy diagnosis. However, exposure does not always equal causation and an alternative cause may better explain what is initially suspected to arise from toxic exposure (Toledano, 2020).

Neuropathy due to hypocupremia is rare and predominantly occurs after bariatric surgery, gastrectomy, or due to alcoholism. Here, neuropathy is progressive, ascending and associated with gait ataxia, cerebellar ataxia, fatigue, dyspnea, macrocytic anemia, neutropenia, and unintentional weight loss. It is usually sensory and symmetric (Rapoport and Lavin, 2016).

(a) Lithium: Lithium has long been used in psychiatry as an adjuvant therapy for bipolar disorders. Frequently, long-term lithium treatment leads to chronic intoxication, clinically presenting as nystagmus, ataxia, tremor, memory impairment, myoclonus with generalized triphasic epileptiform discharges, and PN (Stetkarova *et al.,* 2017). Lithium

neuropathy shows a rapidly progressive course and its discontinuation is often followed by improvement or resolution of the PN (Jin and Shin, 2019).

(b) Lead: Lead toxicity results from industrial exposure and can cause a progressive, mostly asymmetric motor PN that affects the upper extremities more than the distal extremities. Classical lead neuropathy manifests with weakness of the wrist and finger extensors and the prognosis of recovery is favorable as long as exposure is terminated promptly (Thomson and Parry, 2006). Lead intoxication also causes anemia, dental, and cerebral changes (lead encephalopathy) (Helmich and Lock, 2018).

(c) Mercury: Mercury intoxication causes an axonal, sensory PN with autonomic dysfunction. In addition to neuropathy, patients with mercury intoxication may present with diffuse full-body rash, fever, myalgias, headache, oral paresthesias, and tender cervical posterior lymphadenopathy (Huan, 2010).

1.5.3 Drug induced neuropathies

Among the diverse etiologies of neuropathy, drug-induced neuropathy stands as a significant concern, particularly in patients undergoing prolonged medication regimens for the treatment of various diseases. One such drug associated with neuropathy is isoniazid (INH), an essential component of the first-line treatment for tuberculosis (TB). Latent *M. tuberculosis* infection is defined by WHO (World Health Organization) as a state of persistent immune response to *M. tuberculosis* antigens with no clinical evidence of active disease. During their lifetime, up to 10% of people with latent *M. tuberculosis* infection are at risk of progressing to active tuberculosis disease (WHO Global Tuberculosis Report, 2021).

Giving daily isoniazid preventive treatment (IPT) to immunocompromised people; pregnant or lactating women; and people living with HIV, malnutrition, diabetes, chronic liver disease, or renal failure with latent *M. tuberculosis* infection can prevent progression to active tuberculosis disease and save lives. In countries with a high burden of tuberculosis and HIV (Human Immunovirus), the tuberculosis drug isoniazid is part of the management of people living with HIV taking highly active antiretroviral therapy (HAART) (WHO Consolidated Guidelines on Tuberculosis, 2020).

However, so many of the medications used for DR-TB (Drug Resistant TB) treatment can cause peripheral neuropathy as shown in table 1.1 below. It is important that, whenever possible, treatment for DR-TB be based on drug-susceptibility testing with a goal of maximizing effectiveness while limiting toxicity.

Table 1.1: Medications associated with the development of PN in patients with TB

Medication Class	Medication	Comment
DS-TB		
	Isoniazid	The combination of INH and pyridoxine to form a hydrazone which is excreted in the urine, results in a relative deficiency of biologically active pyridoxine.
	Ethambutol	Optic nerve toxicity resulting from the administration of ETH is a well-recognized complication of therapy
DR-TB		
	Cycloserine	A structural analogue of alanine, a central neurotransmitter. Interestingly d-cycloserine may help lessen pain and other symptoms of PN caused by chemotherapy
	Ethionamide	A member of the thioamide family and structurally related to INH. Causes pyridoxine deficiency
	High dose-INH	
	Linezolid	May be a result of disrupted mitochondrial function in neurons.
ART		
	Stavudine (D4T)	NRTI-associated mitochondrial dysfunction, inflammation and nutritional factors are implicated in the pathogenesis PN among ART patients.

(Mafukidze *et al.,* 2016).

(a) Antineoplastics: Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent complication of various antineoplastic agents especially with taxanes, vinca-alkaloids, platins, and bortezomib classes. Neuropathy can be severe but resolves upon discontinuation of the drugs, and treatment with steroids, IVIG, or plasmapheresis (Jin and Shin, 2019). However, a new class of drugs known as ‘‘**Immune checkpoint inhibitors’’** (e.g., pembrolizumap, ipilimumab) effective with refractory cancers (Jin and Shin, 2019). Peripheral nervous system (PNS) side effects are rare and occur in <1% of the patients. Eribulin is also a chemotherapeutic agent used to treat acute lymphatic leukaemia, myeloma, and metastatic breast cancer. The most frequent non-hematological side effect of eribulin is a sensory PN of mild to moderate severity. Vinca-alkaloids, platins, taxanes, and thalidomide are more neurotoxic than eribulin (Islam *et al.,* 2019).

Other drugs neuropathy is most frequently caused by include: phenytoin, disulfiram, fluoroquinolones, metronidazole, chloroquine, nitrofurantoin, nucleoside-analogs (thalidomide, tacrolimus, leflunomide, amiodarone, or colchicine) (Al-Kuraishy *et al.,* 2019).

(b) Isoniazid induced neuropathy: An estimated 1.7 billion people globally are latently infected with *Mycobacterium tuberculosis* (WHO. Global tuberculosis report 2021).

Isoniazid (INH) is a widely used drug in tuberculosis treatment, but its administration is associated with dose-dependent peripheral neuropathy. The recommended dose of INH in adults is 300 mg (5 mg/kg) daily to 900 mg (15 mg/kg) once or twice weekly. Isoniazid toxicity is more frequently associated with adverse effects on the nervous

system, most prominently peripheral neuropathy, psychosis, and seizures (Kass and Shandera, 2010).

A factor that is responsible for the association of Tuberculosis and Peripheral Neuropathy are the medications used to treat TB. Drug sensitive tuberculosis (DS-TB) is usually treated with a combination of four drugs given for a six-month period. These drugs are isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (EMB), and of these medications, both INH and EMB have been associated with neuropathy (Mafukidze *et al.*, 2016).

In Swaziland, a cohort of 250 patients from one hospital with drug sensitive tuberculosis(DS-TB) revealed a rate of 12%; however male patients had a higher rate 18% compared to females 7% ; the older the patient, the higher the rate of neuropathy (20% in those older than 45 years old compared to 9.6% in those less than 45 years old (Piggs Peak Hospital Annual TB Report, 2013).

INH is one of the medications most commonly associated with PN, and there are estimates that as many as 10 % of patients receiving INH will develop some form of PN (Centre, 1963). It typically causes a sensory peripheral neuropathy which presents with burning and numbness of the extremities. In a few cases, sensory symptoms may progress rapidly with the development of ataxia and motor weakness (Arsalan and Sabzwari, 2015). Isoniazid acts as a competitive inhibitor of pyridoxine (B6), making its biologically active form less available for proper functioning of nerve cells (Fekih *et al*., 2011).

While it is highly effective against TB, its link to adverse effects on the peripheral nervous system, affects treatment adherence. The mechanisms underlying INH-induced neuropathy involves oxidative stress, mitochondrial dysfunction, and interference with nerve conduction, ultimately resulting in nerve injury (Weber and Hein, 1979). INH-induced neuropathy can be caused by two different mechanisms. First, metabolites of INH directly inactivate pyridoxine species. It also acts by inhibiting the enzyme pyridoxine phosphokinase which is a necessary enzyme to convert pyridoxine to its active form of pyridoxal 5' phosphate which is a very important cofactor in many reactions (Badrinath and John, 2023).

Since the discovery of isoniazid 70 years ago, peripheral neuropathy is a well-documented side-effect that can be prevented by co-administration of vitamin B6 (WHO Consolidated Guidelines on Tuberculosis, 2020). Neurotoxicity of INH can be ameliorated by giving pyridoxine supplementation, although high doses (>50 mg per day) of pyridoxine have themselves been shown to be toxic to the nerves (Nisar *et al*., 1990). Pyridoxine-associated neuropathy due to excessively high doses of pyridoxine tends to present with loss of vibration and proprioception, and adherence to recommended doses of pyridoxine should be followed strictly to avoid this iatrogenic complication. Pyridoxine is only effective when given alone and not as part of B complex vitamin therapy and should be discontinued after TB therapy is complete (Mafukidze *et al*., 2016).

Ever since WHO recommended fortification of food with vitamin supplements, only sporadic cases of pellagra have been reported, which are associated with tuberculosis therapy in people living with HIV or malabsorption disorders, and in populations in sub-

Saharan Africa that rely on unfortified maize as their primary food source (Prabhu and Dawe, 2021).

Secondly, acute INH toxicity presents as seizures, and the important mechanism underlying this is a depletion of gamma-aminobutyric acid (GABA) an essential inhibitory neurotransmitter. INH induces pyridoxine deficiency which leads to reduced production of GABA, as it is usually a product of pyridoxine-dependent decarboxylation reaction. Therefore, GABA deficiency may manifest as seizures especially in the acute setting of toxicity (Badrinath and John, 2023).

This complication is normally rare when small dosages of the drug are used, but a high incidence of the neuropathy has been observed in East Africa in a group of malnourished tuberculous patients receiving isoniazid in comparatively low dosage (4-6 mg/kg body weight daily) (Money, 1959).

The management of PN can be complicated once symptoms have developed and it depends on the type of problems the patient is having as well as the severity of the symptoms (Brannagan, 2012). Cessation of ongoing nerve damage is a primary concern since PN is frequently irreversible and can cause considerable morbidity and lifelong disability.

Withdrawal from potentially harmful substances, vitamin supplementation, physical therapy, analgesics, and targeted medications such tricyclic antidepressants, selective serotonin reuptake inhibitors, and gabapentin are all feasible forms of treatment.

1.6 The Role of Oxidation and Antioxidants in Peripheral Neuropathies

Drug-induced peripheral neuropathy is an adverse side effect of many chemotherapeutic treatments. CIPN (Chemotherapy Induced Peripheral Neuropathy) often causes neuropathic pain in extremities, and oxidative stress is known as a contributing factor to this pain (Shim *et al*., 2019).

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defense systems, has been implicated in the pathophysiology of various pain conditions, including neuropathic pain and thus neurodegeneration. Ever-increasing evidence indicates that reactive oxygen species (ROS) sustain pain hypersensitivity in a variety of neuropathic pain models, including diabetic neuropathy (Cameron *et al.,* 2001), peripheral nerve injury (De Logu *et al*.,2017), and chemotherapeutic-induced peripheral neuropathy (CIPN) (Shim *et al*., 2019).

The number of free radicals produced in the brain is inversely correlated with brain activity. The brain is particularly vulnerable to free radical damage because of its limited antioxidant capacity which may affect lipids, nucleic acids, and proteins. (Łukawski and Czuczwar, 2023).

1.6.1 Vitamin C (Ascorbic acid) In Peripheral Neuropathies

Antioxidants, such as vitamins (e.g., vitamin C, vitamin E), enzymes (e.g., superoxide dismutase), and phytochemicals (e.g., polyphenols), exert their pain-relieving effects through multiple mechanisms. Possible neuroprotective activities of antioxidants could lead to less structural damages, reduced epileptogenesis and milder cognitive deterioration. Numerous beneficial effects of antioxidants on oxidative stress markers and in some cases also

neuroprotective effects were observed in animal seizure models (Martinc *et al*., 2014). Possible protective mechanisms include the scavenging of ROS, modulation of pro-inflammatory cytokines and immune responses, restoration of mitochondrial function, and preservation of neuronal integrity.

Vitamin C, with its antioxidant and neuroprotective properties, has been proposed as a potential adjunct therapy to ameliorate these neurological complications. It is a powerful antioxidant that plays a crucial role in protecting cells from oxidative stress and scavenging free radicals. Its neuroprotective properties have garnered increasing interest in the context of neuropathies. Not unlike inflammation, elevated ROS is thought to be both a cause and consequence of seizures (Patel, 2004). Inflammation and ROS production are closely related as ROS production is modulated by inflammatory pathways, while inflammatory processes are modulated by ROS (Eastman *et al.,* 2020). Excessive ROS generation increases the concentration of calcium ions (Ca^{2+}) via mechanisms outlined in **Figure 1.2** There is a balance between beneficial and detrimental effects of Ca^{2+} and ROS on mitochondria,however, Ca^{2+} overload may be associated with neuronal death, as well as in necrosis and apoptosis (Martinc *et al.*, 2012).

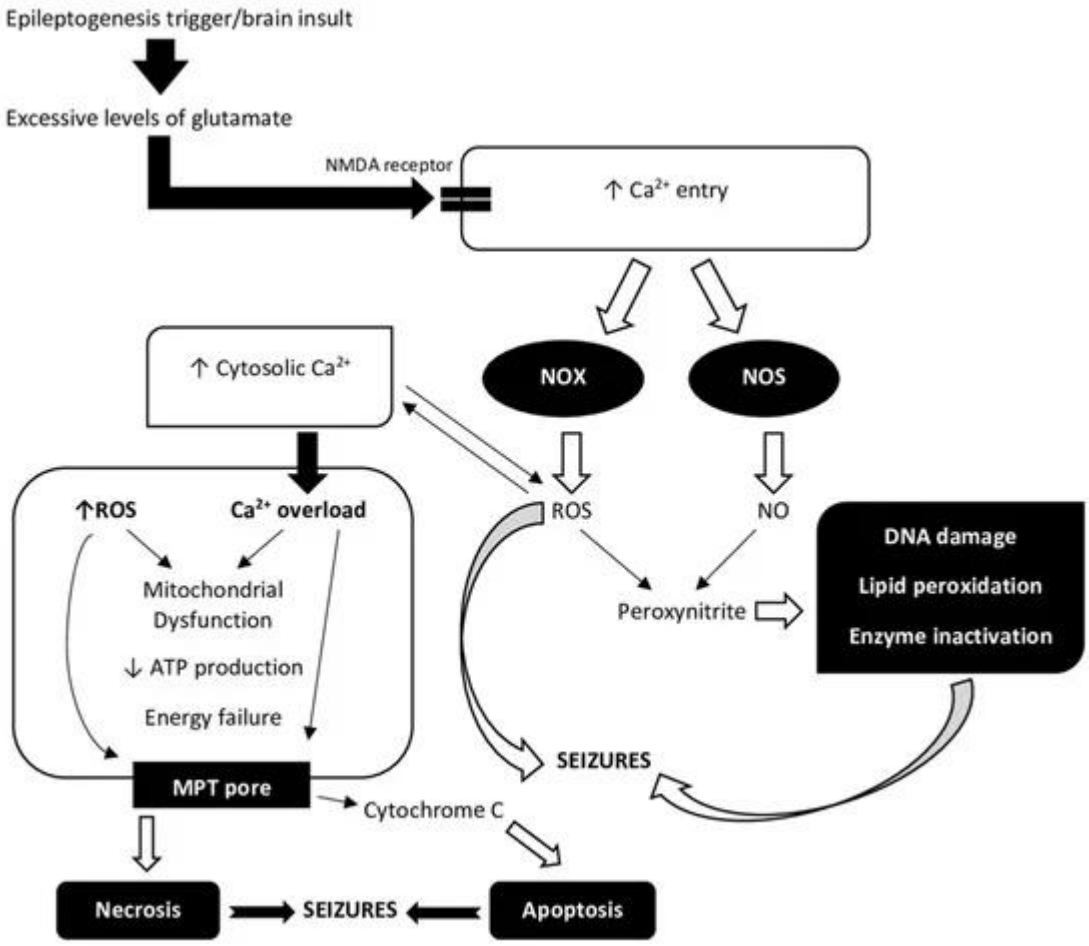

Figure 1.2: A relationship between calcium ions and reactive oxygen species (ROS) in the process of epileptogenesis (Łukawski and Czuczwar, 2023).

Following in vitro and in vivo studies, oxidative stress is linked to the generation of seizure-induced cell death (Frantseva *et al.,* 2000). The activation of glutamate receptor and the ensuing calcium-dependent depolarization of the mitochondrial membrane potential cause inadequate oxygen consumption, decreased adenosine triphosphate (ATP) synthesis, excessive ROS, nitric oxide, and peroxynitrite generation, and consequent damage to cell components including lipids, proteins. As a result, in susceptible brain areas, impaired mitochondrial respiratory chain function and the resulting lipid peroxidation linked to seizure activity may precede neuronal injury and death in vulnerable brain areas (Shin *et al.,* 2011). Neuronal death is often involved in neurodegeneration and neuropathic pain.

Vitamin C may serve as a potential therapeutic intervention to ameliorate or prevent nerve damage, modulate oxidative stress, and enhance endogenous antioxidant systems. Additionally, the role of vitamin C in supporting mitochondrial function and promoting nerve regeneration is well known.

1.7 Animal Models of Neuropathies

1.7.1 For sensory impairment and analgesia

Lesions to the somatosensory nerve system, which change its structure and function and cause pain to occur spontaneously and responses to unpleasant and harmless stimuli to be pathologically magnified, this results in neuropathic pain which becomes a manifestation of maladaptive behaviour. Ectopic action potential production, facilitation and disinhibition of synaptic transmission, loss of synaptic connection and the development of new synaptic circuits are a few of the changes that occur (Costigan *et al.*, 2009).

Allodynia (pain due to a stimulus that does not usually provoke pain) and hyperalgesia (increased pain from a stimulus that usually provokes pain) are both present in peripheral neuropathy and impact 15–50% of neuropathic pain sufferers. Based on the sensory modality employed (pressure, pinprick, cold, and heat), allodynia and hyperalgesia can be categorized (Jensen and Finnerup, 2014).

It is, however, important to note that neuroplastic processes may result in gain (sensitization) or loss (desensitization) of function in relation to the incoming nociceptive signals (Arendt-Nielsen *et al.,* 2018).

a) **Skin sensation test:** Loss of tactile sensation is a common occurrence in patients with peripheral nerve damage which impairs sensory systems causing allodynia (Shlomy *et al.,* 2021). The skin sensitivity test in rodents is a behavioral assay used to assess the animals' sensitivity to tactile or thermal stimuli on their skin. As a result of the significant deficits in sensory nerve terminal function that are associated with distal fiber loss, morphological damage, and behavioral hyposensitivity in neuropathy. Thus, leading to the loss of tactile acuity and pain sensation associated with insensate peripheral neuropathy (Lennertz *et al*., 2011).

   PN damages the distal ends of sensory axons and suppresses axon regeneration, ultimately leading to chronic denervation of cutaneous tissues. Multiple studies have established that experiencing decreased tactile sensation concomitantly have significantly reduced dermal and epidermal innervation (McCarthy *et al.,* 1995; Kennedy *et al.,* 1996; Lauria *et al*., 2005). This test helps researchers understand the

animals' sensory perception and can be used to study various conditions, including neuropathic pain, analgesic effects of drugs, and sensory deficits.

b) **Tail immersion test:** The tail immersion/flick test is a commonly used test first describeded by D;Amour and Smith in 1941 as an objective measure to quantify nociception and analgesic response. In this sensory assessment, the rodent is restrained in a loose manner and radiant heat is focused on its tail. The latency to flick the tail away from the noxious thermal stimuli is recorded. This test is easy to learn, simple to conduct, produces only transient thermal pain and can be repeatedly applied to individual animals (Otto *et al.,* 2011). The response was predominantly considered to be a spinal reflex, according to many groups in the decades after its discovery (Irwin *et al.,* 1951; Grossman *et al.,* 1982). More recent studies have noted that learning (King *et al*., 1997) and supraspinal systems (Jensen and Yaksh, 1986) may modify the response based on the intensity of the thermal input. It is well suited to modern animal ethics concerns and for practical use by undergraduates. Care is taken so as not to misinterpret a reduced skin temperature as analgesia, and an increased skin temperature as hyperalgesia (Hole and Tjølsen, 1993). The animals should be kept at room temperature and not overstimulated by repeated testing.

1.7.2 Motor coordination

(a) Static rod test: Brain injury, genetic manipulations, and pharmacological treatments can result in alterations of motor skills in mammals (Luong *et al.,* 2011). The behavioral characterization of animal models is crucial in order to properly utilize the models of this

disease states and to comprehend the underlying pathophysiology of neurodegenerative illnesses. In acute trials such as this, it becomes necessary to separate motor from behavioral and cognitive function in determining the functional specificity of the drug.

Deterioration in the performance of motor tasks may also indicate the point when motor deficits begin to be compromised (Brooks *et al*., 2012). The motor coordination test using a static rod is a behavioral assay designed to assess an animal's ability to balance and maintain its grip on a stationary rod. his technique eliminates the need for training (as in other test variants) as the innate reaction of a mouse to being placed near the end of an elevated rod is to hold on and attempt to reach the supported end (Deacon, 2013).

The animals are allowed to acclimate to the testing environment for a period of time before the actual testing begins. This helps reduce stress and anxiety that might affect their performance. This test requires both strength and coordination for successful performance but only the minimal degree of muscle tone necessary simply for the mouse to use its limbs. Animals with better motor coordination and balance skills will exhibit longer durations on the rod (Deacon, 2013).

1.7.3 For Anxiety

(a) Hole board test: Hole board test was first described by Boissier and Simon in 1962 and remains one of the standard procedures applied in psychopharmacology and behavioral studies. The hole-board is generally used as a test for anxiety and spatial memory (Lalonde and Strazielle, 2022). The hole-board apparatus has small cylindrical holes at the bottom of the experimental arena that allows experimenters to conduct more complex

behavioral observations as opposed to the open field test. It is based on the assumption, that head-dipping activity of the animals is inversely proportional to their anxiety state (Bilkei-Gorzo and Gyertyan, 1996). Increased hole-pokes in rats and mice, indicates a loss in behavioral inhibition.

1.7.4 For Seizures

(a) Pentylenetetrazol induces seizures: Pentylenetetrazol (PTZ)-induced seizures in rodents are amongst the most representative of human epileptic seizures. PTZ is a very potent and non-competitive GABA receptor antagonist and is characterized by quick distribution to all organs, high bioavailability and very short latency of action. Numerous studies revealed that the activities of antioxidant enzymes, superoxide dismutase (SOD), GPx (glutathione peroxidase), glutathione reductase (GR), and CAT (catalase), were reduced in the rat or mouse brains due to seizures induced by PTZ (Bloms-Funke *et al.,* 1996, Zienowicz *et al.,* 2005).

PTZ-induced seizures increase lipid peroxidation (LPO), decrease GSH (glutathione) in the hippocampus, and enhance ROS generation in the mitochondria isolated from the epileptic hippocampus, indicating an aggravation of oxidative stress in epileptic kindling (Zhen *et al.,* 2014).

Behavioral evaluation of respective rat was based on the 30 min observation of frequency in epileptic seizures right after each time of PTZ injection. (You *et al*., 2011). Seizures are commonly induced by a single systemic administration of PTZ, and the recovery is very fast, within 30 minutes (Kandratavicius *et al*., 2014).

1.8 Rationale for the study

Given the known antioxidant effects of vitamin C and its potential to mitigate oxidative stress-induced damage, there arises a compelling rationale to investigate its role in countering the development and progression of isoniazid-induced neuropathy. This study aimed to critically evaluate the attenuating property of vitamin C in the context of isoniazid induced neuropathy (IN), exploring its potential as an adjunct therapy to mitigate the neurological complications associated with isoniazid treatment.

The findings from this study are not only to shed light on the pathophysiology of isoniazid-induced neuropathy but also open new avenues for therapeutic interventions to protect against drug-induced neurotoxicity. Ultimately, the knowledge generated from this investigation could pave the way for more effective and safer treatment strategies for patients undergoing isoniazid therapy while also offering valuable insights into the broader field of neuropathy research.

1.9 Aim and Objectives

The aim of the study was to investigate the effect of vitamin C in some INH induced neuropathies. The objectives were:

(a) To induce neuropathies in rats using INH.

(b) To determine the effects of concurrent administration doses of vitamin C on induced neuropathies.

(c) To investigate the underlying mechanisms for any observed attenuating effect of vitamin C.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Animals

Adult male albino Wistar rats (150-200 g) were gotten from the animal house of the Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. The rats were kept in the animal house, they were provided allowed free access to pelleted feed and water which were kept in standard animal cages. The rats were exposed to natural lighting at room conditions and were properly handled following ethical guidelines for handling laboratory animals (Derek *et al*.,1997).

2.2 Drugs

Isoniazid tablets (Macleods Pharmaceuticals Industries Nigeria Ltd, pyridoxine tablets (PAUCO Pharmaceuticals Nigeria Ltd), pentylenetetrazol (Sigma-Aldrich, Germany), ascorbic acid (Sigma-Aldrich, Germany) were used. All drug solutions were freshly prepared before use

2.3 Experimental Groups and Protocols

Rats were randomly allotted to six groups (n=7) as follows:

Group 1: Treated with distilled water (10 ml/kg) and served as negative control.

Group 2: Treated with isoniazid (800 mg/kg)

Group 3: Treated with isoniazid (800 mg/kg) and ascorbic acid (7.5 mg/kg)

Group 4: Treated with isoniazid (800 mg/kg) and ascorbic acid (15 mg/kg)

Group 5: Treated with isoniazid (800 mg/kg) and ascorbic acid (30 mg/kg)

Group 6: Treated with isoniazid (800 mg/kg) and pyridoxine (50 mg/kg) which served as the positive control (standard).

All treatments were done orally for seven consecutive days using an orogastric tube. Noise was kept at its barest minimum in the laboratory while the following tests were carried out. The following tests were carried out on day 7 of treatment.

2.3.1 Evaluation of pain sensation

The tail immersion test was used to evaluated pain sensation.

The water bath was turned on and regulated to a fixed temperature at 55^{o}C to ensure the safety of the rat and to allow the rat to respond before tissue damage occurred. The rat was then gently restrained, and its tail was immersed whilst an observer monitored the animal closely. The tail flick latency was measured as the time it took for the animal to flick its tail away from the water bath. Timing started immediately the tail was immersed and stopped when the rat flicked its tail or moved (Otto *et al.,* 2011). The latency was measured in seconds.

2.3.2 Evaluation of skin sensitivity

The rat was gently placed in a suitable testing surface. The fur on the dorsal area of the rat's body was carefully shaved to expose a clean area of skin. This area was then cleaned with isopropyl alcohol to prevent contamination. A small, sterile needle was used to gently prick the shaved area.

The rat's behaviour was observed for signs of withdrawal, paw lifting, squeaking or other responses and then scored on a 11-point numeric rating scale which consists of numbers between 0 and 10 where 0 indicates ❽no pain❾and 10 indicates ❽maximum pain❾(Reed *et al*., 2017). A brisk withdrawal and strong reaction indicates sensitivity to the stimulus (Kennedy *et al.,* 1996).

2.3.3 Evaluation of motor coordination

The static rod test model was used to evaluate motor coordination. A horizontal metal rod of a specific diameter (2 cm) was secured in place from a support 30 cm apart The rod was elevated 60 cm above a surface, creating a challenging environment for the animal to maintain its balance. The animal was gently placed on the static rod, and its behaviour as it attempts to retain its position on the rod was observed, the duration the animal was able to stay on the rod before falling off or exhibiting unstable movements was recorded (Deacon, 2013).

2.3.4 Evaluation of anxiety

The hole board method was used. A square hole board arena was used measuring 60 × 60 × 45 cm (**Figure 2.1**). There were 16 round-shaped holes in the bottom of the arena, each 5 cm in diameter, distributed evenly at equal distances from each other. Each animal was left on the board for five minutes. Each rat was free to explore the experimental board without impediment. After completing the session, the animal was removed from the experimental arena and returned to its home cage. The hole board test was carried out once. The testing arena was cleaned after each rat using isopropyl alcohol and cotton wool (Pisula *et al*., 2021).

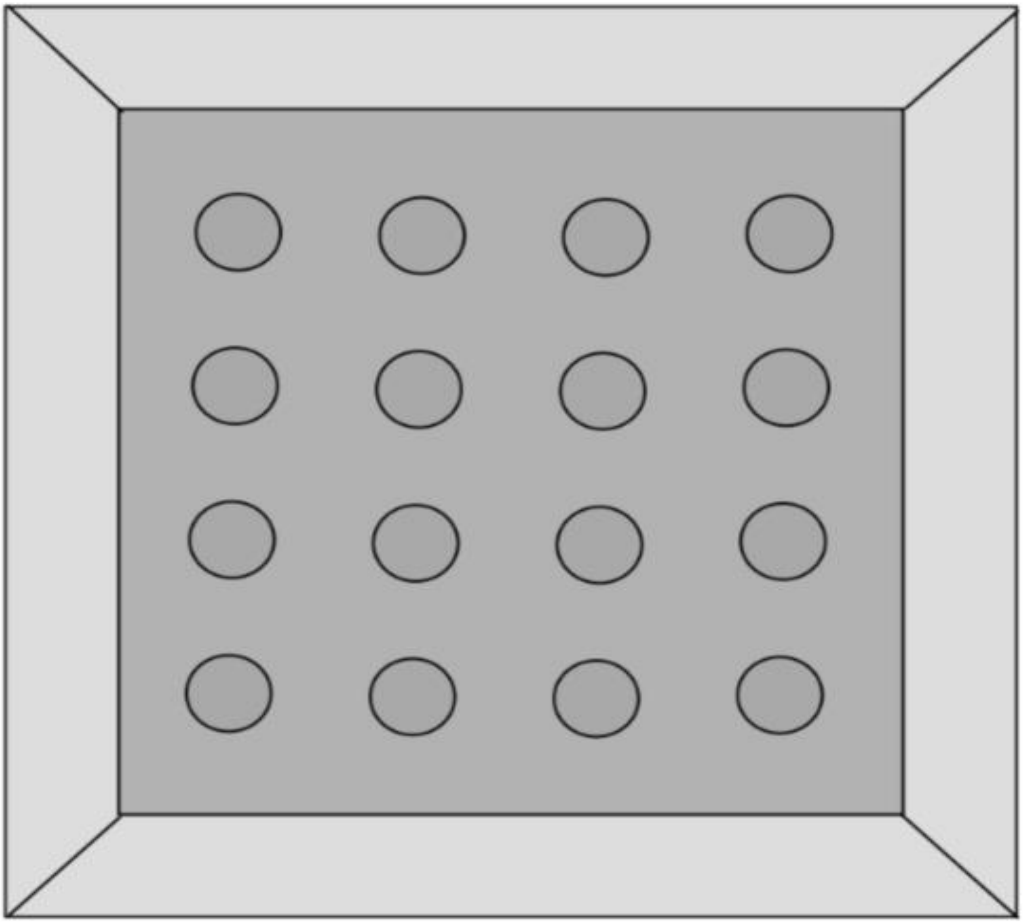

Figure 2.1: Hole board apparatus (view from above) (Pisula *et al*., 2021).

2.3.5 Evaluation of proneness to convulsion

Seizure was induced by the administration of pentylenetetrazol (PTZ) prepared in distilled water on the day of use and the animal's body weight was measured. The animal was then placed in an observation chamber for habituation (3 min) after which PTZ was administered at a dose of 70 mg/kg intraperitoneally. Animal behaviors for 30 min after PTZ administration were observed and noted (Shimada T, Yamagata K. 2018). The latency to the first seizure, duration of seizure, time of death (if observed) after the PTZ injection were all collected. Mild seizures or behavioral changes in the animals beyond the 30-min observation period were also noted. Additionally, the seizure duration of each observed seizure as changes in duration relate to the seizure severity (Kosobud *et al*., 1992; Shimada and Yamagata, 2018).

2.3.6 Evaluation Of Survival Rate

On the seventh day of administration, the mortality rate was determined as the sum of all animal death associated with INH induced neuropathies prior to and following the administration of PTZ and recorded as a percentage of the whole number of animals within the group.

2.4 Data Presentation and Statistical Analysis

The results are presented as mean ± SEM (standard error of mean) and ❻n❼signifies the number of animals (rats) used in the experiment. Inferential analysis was carried out using the one-way ANOVA followed by Tukey post hoc test. $P<0.05$ indicates statistically significant difference between compared data.

CHAPTER THREE

RESULTS

3.1 Effect of Vitamin C (ascorbic acid) on Latency to Tail Flick by Rats

Figure 3.1 shows that the difference between the ascorbic acid treated groups and the negative control group was statistically insignificant. There was no statistically significant reduction in the latency to tail flick at 7.5, 15, and 30 mg/kg dose of ascorbic acid when compared to the group given only isoniazid.

3.2 Effect of Vitamin C(ascorbic acid) on Rats' Skin Sensation

Figure 3.2 shows that the difference between the ascorbic acid treated groups and the negative control group was not statistically significant. There was no statistically significant improvement in sensory function at 7.5, 15, and 30 mg/kg of ascorbic acid when compared to the isoniazid treated group.

3.3 Effect of Vitamin C on the Time Spent on a Static Rod by Rats

Figure 3.3, INH significantly (*$P<0.05$) reduced the time spent on the static rod compared to the distilled water treated group. However, there was no significant difference between concurrent administration of 800mg/kg INH with 7.5,15 and 30 mg/kg ascorbic acid when compared with INH alone, i.e., the reduction in the time spent on the rod was not reversed by vitamin C. The time spent by the group given INH and pyridoxine was not significantly different from the distilled water group.

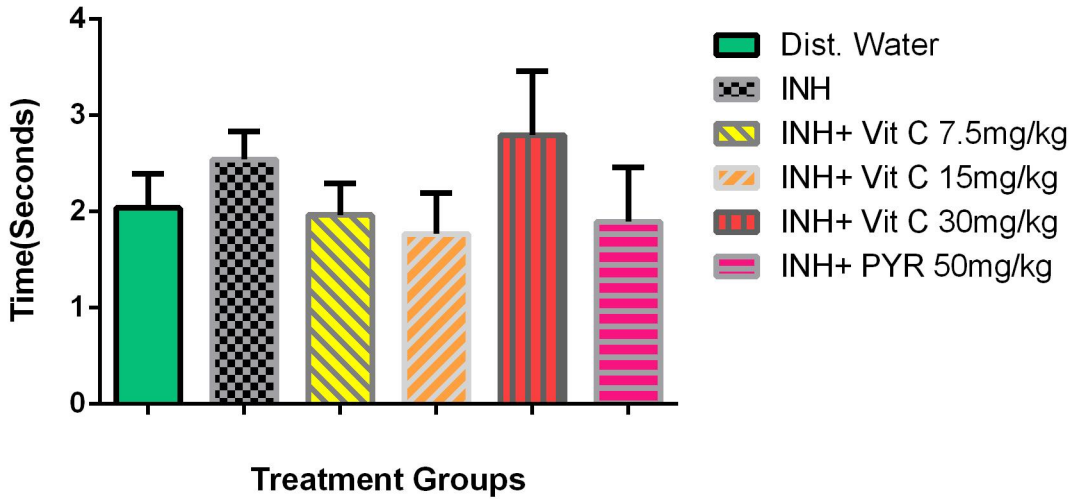

Figure 3.1: Effect of daily concurrent administration of 800 mg/kg of isoniazid (INH) vitamin C (vit C) or pyridoxine (PYR) for seven days on tail immersion test.

Data are not significantly different. n=7

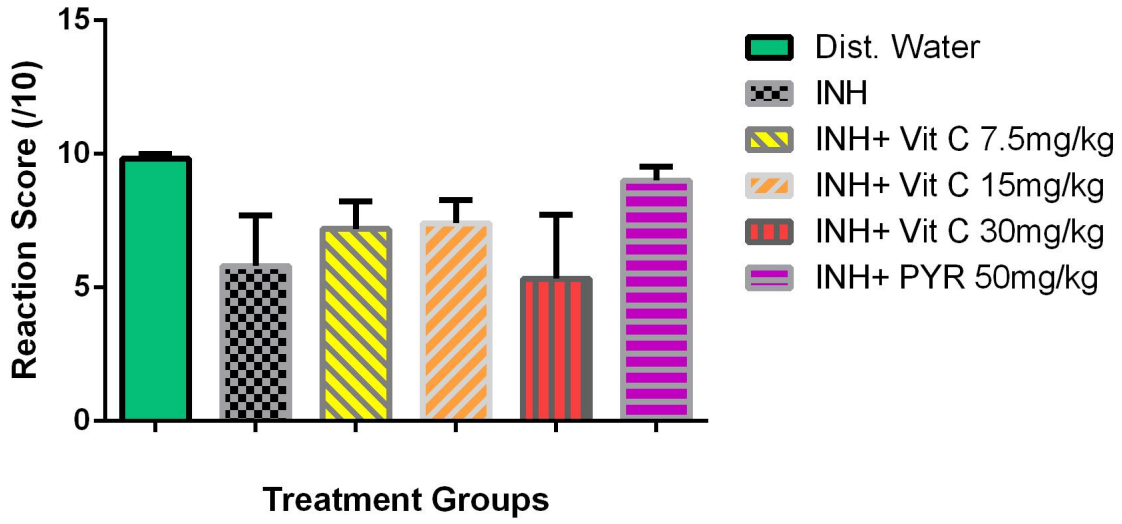

Figure 3.2: Effect of daily concurrent administration of 800 mg/kg of isoniazid (INH) vitamin C (vit C) or pyridoxine (PYR) for seven days on skin prick sensation.

Data are not significantly different. n=7

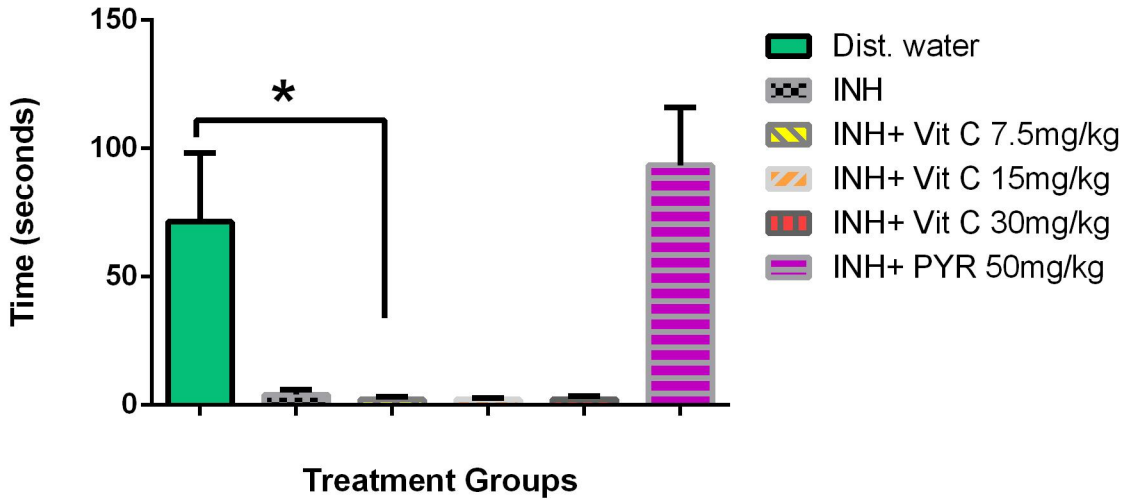

Figure 3 3: Effect of daily concurrent administration of 800 mg/kg of isoniazid (INH) vitamin C (vit C) or pyridoxine (PYR) for seven days on the time spent on a static rod.

***P< 0.05 versus control n=7**

3.4 Effect of Vitamin C(ascorbic acid) on the Number of Pokes into the Hole Board by Rats

Figure 3.4 shows that there was a statistically significant(*P<0.05) reduction in the number of pokes with concurrent administration of 800mg/kg isoniazid with 7.5, 15, and 30 mg/kg dose of ascorbic acid when compared to the control group and the group given isoniazid and pyridoxine.

3.5 Effect of Vitamin C on the Onset of PTZ-induced Seizures in Rats

Figure 3.5 shows that there was a statistically significant (***P<0.001) reduction in the onset of seizures in groups concurrently administered 800mg/kg isoniazid with 7.5,15,30mg/kg ascorbic acid or pyridoxine treatment groups when compared to the control group.

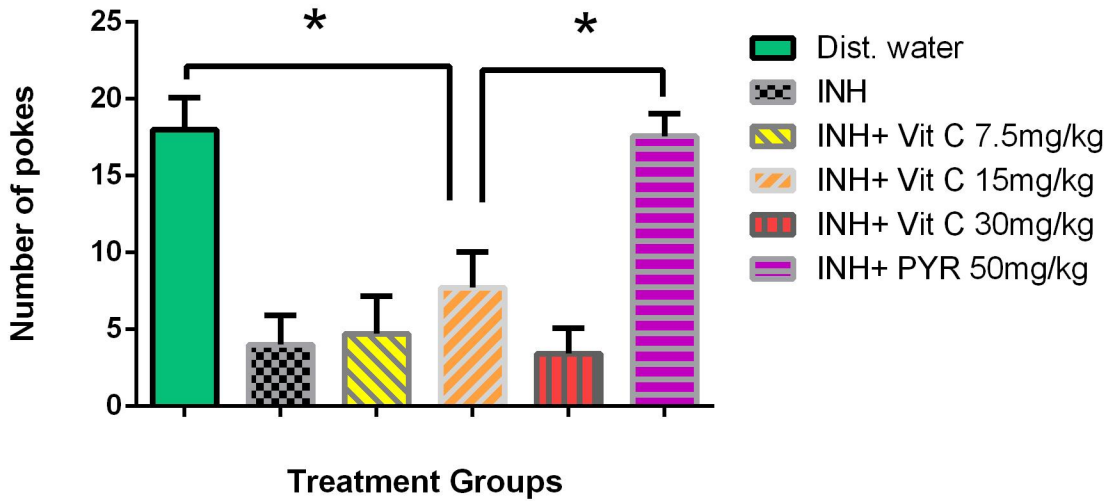

Figure 3.0.4: Effect of daily concurrent administration of 800 mg/kg of isoniazid (INH) vitamin C (vit C) or pyridoxine (PYR) for seven days on the number of pokes by the rat.

*** P < 0.05 ,n=7**

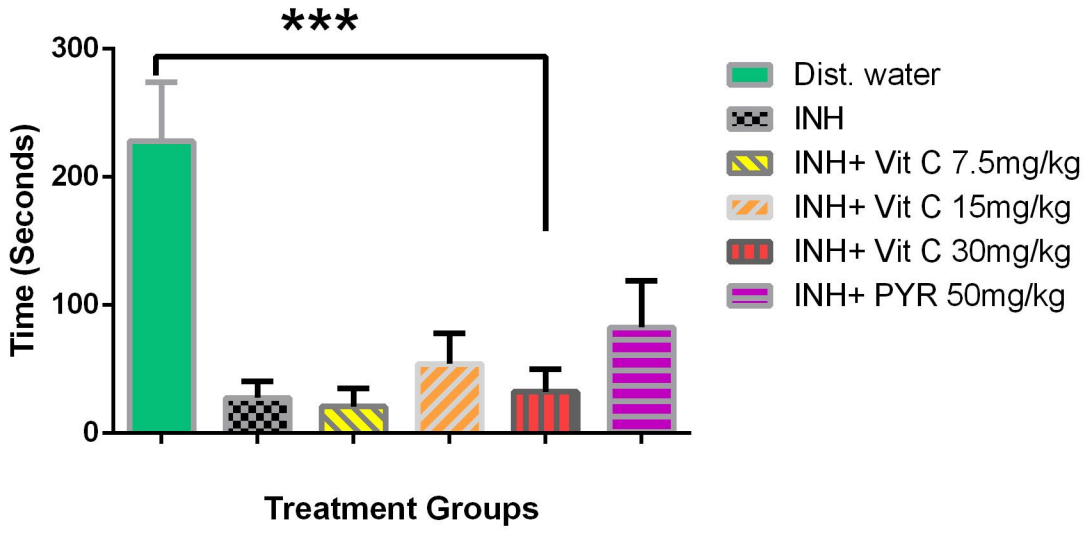

Figure3.5: Effect of daily concurrent administration of 800 mg/kg of isoniazid (INH) vitamin C (vit C) or pyridoxine (PYR) for seven days on the rat's onset of seizures.

***** P < 0.0001 ,n=7**

3.6 Effect of Vitamin C on the Survival of Rats

Table 3.1: Effect of vitamin C supplementation on convulsion and death associated with INH induced neuropathies

GROUP TREATMENT	PERCENTAGE CONVULSION (%)	PERCENTAGE DEATH (%)
Distilled Water	100.0	16.7
Isoniazid	100.0	75.0
Isoniazid + 7.5 mg/kg Vitamin C	100.0	100.0
Isoniazid + 15 mg/kg Vitamin C	100.0	100.0
Isoniazid + 30 mg/kg Vitamin C	100.0	71.4
Isoniazid +50 mg/kg Pyridoxine	100.0	42.9

CHAPTER FOUR

DISCUSSION

4.1 Effect of Vitamin C on INH induced Altered Sensation

In the course of this study, two models which are the **skin sensation** and t**ail immersion** tests were used to evaluate the protective properties of vitamin C against sensory impairment. In the isoniazid treated groups, diffuse hypoalgesia was found when compared with the control.

Aberrant peripheral nociceptor firing is implicated in promoting heightened pain, hypoalgesia or allodynia (Haroutounian *et al*., 2014). Also, damage to the C fibers and A fibers, which are tiny unmyelinated peripheral nerve fiber terminals, causes peripheral neuropathy (Valek *et al*., 2019). With acute noxious stimuli (e.g., mechanical stimuli as in the needle prick) nociceptors are triggered to allow the central nervous system (CNS) to avoid potentially damaging stimuli in both active and passive settings (Armstrong and Herr, 2023).

A noxious heat stimulus (if under physiologic conditions) or a sub-noxious heat stimulus (if sensitized by inflammatory markers such as prostaglandins or bradykinin) gets detected by TRPV2 (52 degrees Celsius) receptors (Woller *et al*., 2017). The heat produces a conformational change that opens the non-selective cation (Ca^{2+}) channel, which produces a local depolarization. The depolarization gets propagated by the opening of voltage-gated sodium channels, which generates an afferent action potential that travels to the brain (Armstrong and Herr, 2023). However, cases where the sensory fibers are damaged may have an impact on the terminals of somatosensory neurons, particularly the nociceptive neurons that innervate the skin, muscles, and

visceral organs (somatic fibers) (Valek *et al.*, 2019) and peripheral sensory loss may mask central ❻pain❼in behavioral tests thus resulting in the low values obtained amongst the isoniazid treated groups.

For the skin sensation test, the rating was based on an objective numerical pain rating scale which is a commonly used tool to assess and quantify pain experienced by the rat. It has been shown to have a low reproducibility (Van Tubergen *et al*., 2002). A possible error that may have arisen was the reliance of the score on measures that are observed by the experimenter, and this leaves room for a significant amount of variability in the determined value. Also, pain is inherently subjective, and ratings can be influenced by the immediate environment, pain intensity fluctuation with time due to factors such as movement, rest etc. A pain rating taken at one specific moment may not accurately represent an overall pain experience (Rosier *et al.,* 2002).

4.2 Effect of Vitamin C on INH Induced Altered Anxiety

In the isoniazid treated groups, reflexes and movements were reduced. The observed movements were often slow, dysfunctional in a patchy way whereas the control group had normal, even, and brisk movements. The control group exhibited typical exploratory behavior, as indicated by a higher number of hole entries and longer cumulative time spent in the apparatus. Rats in this group demonstrated increased curiosity by sniffing and poking their noses into the holes. The isoniazid group displayed a significant decrease in exploratory behavior, with fewer hole entries and reduced time spent exploring. This is consistent with previous research indicating that stress (chemical stress in this case) can lead to reduced exploratory behavior in rodents, potentially reflecting anhedonia or increased anxiety (Pisula *et al*., 2021).

When examining anxiety-related behavior, we observed that rats in the isoniazid group displayed a higher latency to the first hole entry, along with a decreased number of head dips, suggesting heightened anxiety. In contrast, the isoniazid with pyridoxine treatment group showed results similar to the control group, with no significant differences in latency to hole entry or head dipping behavior. This suggests that the pyridoxine treatment effectively mitigated the anxiety-like behaviors induced by chemical stress of the isoniazid. The decreased exploratory behavior and increased anxiety-like responses in the isoniazid group align with the well-established link to altered behavioral patterns due to the drug induced neuropathy (Pisula *et al*., 2021). The patchy, weakened movement may be as a result of weak and wasted muscles. These results also display the ability of the test to discriminate sensitive and subtle changes in rodents❼behavior due to acute drug induced neuropathy.

4.3 Effect of Vitamin C on altered Motor Function

There was a significant reduction in the claw clasping and duration spent on the rod for the isoniazid alone and the isoniazid with vitamin C groups. This indicates severe motor dysfunction and a general sign of neurological impairment (Deacon, 2013). Sensory loss in the limbs causes alterations to gait biomechanics, affecting balance, and this is a major falls risk factor (Handsaker *et al*., 2014). The severely increased rate of falls is possibly due to the complete absence of peripheral foot sensation and the delayed neuromuscular control (Reeves *et al*., 2021).

Insensate feet due to PN cause a slower speed of strength generation, reducing lower limb control over gait, leading to impaired balance (Handsaker *et al*., 2014).

During the period of administration, there was also an observed marked reduction in amount of food pellets consumed by the isoniazid treated groups when compared with the control. Drug-induced myopathy is fairly common amongst muscle diseases and can range from mild myalgia to chronic myopathy, with severe weakness, or massive rhabdomyolysis with acute renal failure (Shah and Venkatesan, 2015). Isoniazid is implicated in muscle atrophy and myopathy as seen in a case report where I'm electromyography confirmed the myopathy and isoniazid withdrawal was marked by the total and quick disappearance of the symptoms. While the reintroduction of a half-dose of isoniazid only induced a few transitional muscular fasciculations (Chaouch *et al*., 2011).

Weakness in these cases is generally distal and the arms are usually affected earlier and more severely than the legs (Nobile-Orazio, 1996). Severe motor-dominant neuropathy can be reversible with pyridoxine supplementation (Arsalan and Sabzwari, 2015). This is the possible underlying reason for the better performance of the animals in the pyridoxine group in comparison to the vitamin C group.

4.4 Effect of Vitamin C on INH Induced Pro-convulsion and Deaths

All rats were pentyleneteratzol (PTZ) treated and there was a 100% exhibition of the behaviors of immobility, facial clonus, head nodding, and facial clonus with scratching. However, the control and pyridoxine treated group possessed a lower death rate compared to the other treatment groups.

For latency to seizure onset, all the isoniazid treated groups displayed a shorter latency to the onset of convulsions compared to the water group. This rapid onset suggests a heightened

sensitivity to PTZ's proconvulsant effects and may indicate differences in the threshold for seizure initiation between the groups.

PTZ is a gamma aminobutyric acid (GABA)-A receptor antagonist. PTZ suppresses the function of inhibitory synapses, leading to increased neuronal activity. This regulation causes generalized seizures in animals (Squires *et al*., 1984). GABA (gamma-aminobutyric acid) is the major inhibitory neurotransmitter in the central nervous system and plays a critical role in regulating neuronal excitability. INH can affect GABAergic neurotransmission at 250 mg/kg indirectly through its impact on vitamin B6 metabolism, potentially leading to alterations in GABA receptor function (Horton *et al*., 1979).

When rats were pretreated with INH followed by PTZ, there was an increased severity and shorter latency to seizure onset compared to rats treated with PTZ alone. This supports the electrophysiological findings and suggests that the combination of INH and PTZ may exacerbate seizure susceptibility (You et al., 2011; Shimada and Yamagata 2018). The observed synergistic reduction in GABAergic currents and increased seizure susceptibility when INH and PTZ were administered together suggest a complex interaction between these compounds at the level of GABA receptors (Preziosi, 2007).

Understanding the interplay between genetic predisposition and environmental influences could provide a more comprehensive view of seizure susceptibility. This complex interplay of genetic and neurobiological factors results in variation in seizure susceptibility using the PTZ-induced convulsion model. Genetic factors likely play a significant role in determining proneness to PTZ-induced convulsions. The observed differences could reflect genetic variations in ion channels, neurotransmitters receptors, and other molecular targets implicated in seizure generation and propagation (Copping *et al*., 2019). The distinct responses to PTZ-induced convulsions may

arise from variations in neural circuitry and excitability. Hyper excitability of neurons and altered balance between excitatory and inhibitory neurotransmission e.g., nitric oxide, GABA, glutamate, connexin (Zhu *et al*., 2017; Gadja *et al*., 2005) could contribute to the increased susceptibility seen in the isoniazid treated groups.

CHAPTER FIVE

CONCLUSION

5.1 Conclusion

This study was carried out to evaluate the attenuating effects of vitamin C in isoniazid induced peripheral neuropathies. Rats were selected at random to eliminate bias. All evaluations were carried out using standard protocols. From the results obtained from study, it can be concluded that:

(a) The dose of INH was effective in inducing neuropathies.

(b) The doses of vitamin C used in the study were not effective in attenuating the symptoms and manifestations of isoniazid induced peripheral neuropathies.

5.2 Contribution to Knowledge

The study has contributed to knowledge in the following ways:

1. It has reinforced the neurotoxicity of isoniazid at 800 mg/kg on oral acute administration.
2. It has provided information on the non-ameliorating effect of the doses of vitamin C in isoniazid induced peripheral neuropathy at the specific treatment doses.

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APPENDIX I

DYSFUNCTIONAL RAT BEHAVIOR ON THE ARENA FOR HOLE BOARD TEST

APPENDIX II

TABLE SHOWING THE LATENCY TO TAIL FLICK SCORES (in seconds) ACROSS TREATMENT GROUPS

Animal ID	Distilled Water	Isoniazid 800mg/kg	INH 800mg/kg +Vitamin C 7.5mg/kg	INH 800mg/kg + Vitamin C 15 mg/kg	INH 800mg/kg +Vitamin C 30 mg/kg	INH 800mg/kg +Pyridoxine 50mg/kg
1	1.90			2.15	4.09	1.46
2	0.97	2.91	2.52	1.39	1.87	2.29
3	1.63	2.46				4.52
4	2.06	3.45	1.99			
5	3.60	1.86		0.81	2.43	0.78
6	2.07	2.03				1.31
7			1.38	2.73		1.01

APPENDIX III

TABLE SHOWING THE SKIN SENSATION SCORES ACROSS TREATMENT GROUPS

Animal ID	Distilled Water	Isoniazid 800mg/kg	INH 800mg/kg +Vitamin C 7.5mg/kg	INH 800mg/kg + Vitamin C 15 mg/kg	INH 800mg/kg +Vitamin C 30 mg/kg	INH 800mg/kg +Pyridoxine 50mg/kg
1	9.00		10.00	10.00	10.00	10.00
2	10.00	10.00	8.00	6.00	4.00	7.00
3	10.00	10.00				10.00
4	10.00	5.00	4.00	5.00		
5	10.00	4.00		8.00	2.00	10.00
6	10.00	0.00	8.00			9.00
7			6.00	8.00		8.00

APPENDIX IV

TABLE SHOWING THE NUMBER OF POKES MADE BY THE ANIMALS ACROSS TREATMENT GROUPS

Animal ID	Distilled Water	Isoniazid 800mg/kg	INH 800mg/kg +Vitamin C 7.5mg/kg	INH 800mg/kg + Vitamin C 15 mg/kg	INH 800mg/kg +Vitamin C 30 mg/kg	INH 800mg/kg +Pyridoxine 50mg/kg
1	10.00		10.00	9.00	10.00	25.00
2	18.00	8.00	12.00	11.00	8.00	13.00
3	19.00	9.00			.	15.00
4	15.00	6.00	6.00	5.00	.	19.00
5	25.00	6.00	.	14.00	6.00	18.00
6	21.00	3.00	2.00.	.	.	18.00
7			5.00	15.00	.	15.00

APPENDIX V

PICTURE OF EXPERIMENTER PERFORMING INTRAPERITONEAL ADMINISTRATION ON A RAT