# The Effectiveness of Fixed Dose Combination of Alpha Lipoic Acid and Vitamin B Preparation for Treatment of Diabetic Polyneuropathy in Type 2 diabetes mellitus patients : A randomized placebo-controlled trial.

## DATE: 31ST MARCH 2024

## RESEARCH TITLE

The Effectiveness of Fixed Dose Combination of Alpha Lipoic Acid and Vitamin B Preparations for Treatment of Diabetic Polyneuropathy in Type 2 diabetes mellitus patients: A randomized placebo-controlled trial

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## 1. INTRODUCTION

Diabetes poses a significant non-communicable health challenge in both developed and developing nations, including Malaysia. Individuals with diabetes are experiencing longer lifespans but they are susceptible to microvascular and macrovascular complications.

Diabetic peripheral neuropathy (DPN) stands as one of the most prevalent and debilitating complications of type 2 diabetes mellitus (T2DM), attributed to chronic hyperglycaemia and is characterized by peripheral nerve dysfunction following the elimination of other potential causes (Darivemula *et al.*, 2019). Diabetes mellitus (DM) is an escalating global epidemic that leads to an increase in prevalence of peripheral neuropathy affecting 50.7% of patients (Mimi *et al.*, 2003). The collective occurrence of DPN varied across various countries, amounting to 26.71% (Lu *et al.*, 2020). In Malaysia, a study conducted in Selangor found that, the proportion of patients with positive neuropathy symptoms score (NSS) was 49.4%. However, only 0.2% were diagnosed with positive DPN in their clinical foot examination record (Lee *et al.*, 2022).

This neuropathic condition arises from chronic hyperglycaemia-induced damage to peripheral nerves and is characterized by a symmetrical, distal, and length-dependent pattern of sensory and motor deficits. This diverse range of conditions impacts various aspects of the nervous system and manifests with various clinical symptoms.

The most prevalent form of diabetic neuropathies is chronic distal symmetric polyneuropathy (DSPN), constituting approximately 75% of all cases of diabetic neuropathies. Painful DSPN is encountered in 13–26% of diabetes patients (Pop-Busui *et al.*, 2017b).

While the exact pathophysiological mechanisms underlying DPN remain multifaceted and not entirely elucidated, the cumulative effect of metabolic abnormalities, neurovascular dysfunction, oxidative stress, and inflammatory processes play pivotal roles in its development and progression. The reason remains unidentified in as many as 40% of individuals experiencing neuropathy (Jensen *et al.*, 2021).

Clinically, DPN manifests with a spectrum of symptoms, reduced or abolished sensation to touch, pinprick, temperature, vibration, and, more rarely, proprioception. Most of the patient is

asymptomatic and abnormalities may only be revealed during the clinical examination (Jensen *et al.*, 2021).

To diagnose painful DPN, a neurological examination with socks and shoes off is a basic prerequisite for symptom assessment (Tesfaye *et al.*, 2011). One of the methods to access the presence and severity of neuropathy symptoms and signs by using the modified Neuropathy Symptom Score (NSS) and Neuropathy Disability Score (NDS) which has 82% sensitivity and 67% specificity (Lee *et al.*, 2022).

DPN exerting a profound impact on patients' quality of life and overall health outcomes. This chronic neuropathic pain has been linked to poor sleep, anxiety and depressive symptoms, and a lower quality of life (QoL) in diabetic patients (Gylfadottir *et al.*, 2020). This will also increase risk of falls, foot ulceration and amputation (Callaghan *et al.*, 2020). So early detection and comprehensive management strategies are paramount in mitigating the impact of DPN and preventing its severe complications (Debele *et al.*, 2023).

## 2. PROBLEM STATEMENT AND RATIONALE

As the incidence of T2DM continues to rise globally, the prevalence of DPN is also escalating, representing a significant healthcare burden (Jadhao *et al.*, 2024). Although initially the patient may have no symptoms, it can cause significant morbidity later as DPN primarily changes symmetrical sensory function, leading to abnormal sensations and numbness (Debele *et al.*, 2023). This resulting in amputations, foot ulcers, incontinence, and sexual dysfunction (Juster-Switlyk and Smith, 2016). Furthermore, the distressing symptoms of DPN frequently result in sleep disturbances, feelings of anxiety and depression, and a low quality of life (Kioskli *et al.*, 2019).

Diabetic peripheral neuropathy frequently under treated, and the impact of enhancing glycaemic control, particularly in type-2 diabetes, is still uncertain. First-line pharmacotherapy options for painful diabetic neuropathy are Tricyclic antidepressants (imipramine and amitriptyline). But because of cholinergic side effects such dry mouth, orthostatic hypotension, constipation and urinary retention their usage has been restricted (Khdour, 2020). While research has also shown that opioids are effective in treating the neuropathic pain linked to DPN, their limited effectiveness, tendency for misuse, and long-term safety issues make them unsuitable for routine usage (Zhu *et al.*, 2023).

A combination of alpha lipoic acid and vitamin B preparations is increasingly favoured for the treatment of diabetic polyneuropathy in patients with type 2 diabetes mellitus as its showed decreased incidence of adverse effects. The main adverse effect associated with alpha-lipoic acid is predominantly nausea (Reljanovic *et al.*, 1999; Papanas and Ziegler, 2014).

A systematic review on alpha-lipoic acid's effect in treating diabetic neuropathy found the results were incongruent regarding the efficacy of α-lipoic acid in treating diabetic neuropathy (Abubaker *et al.*, 2022). Four trials observed a significant improvement in symptoms, including a reduction in the total symptom score (TSS), a reduction in the symptoms of autonomic neuropathy, and improvements in measures of nerve conduction (Ziegler and Gries, 1997; Tankova *et al.*, 2004; Liu *et al.*, 2007; El-Nahas *et al.*, 2020). On the contrary, the remaining four trials did not detect any significant outcomes (Reljanovic *et al.*, 1999; Ziegler *et al.*, 1999; Won *et al.*, 2020b; Gilron *et al.*, 2021).

All studies revealed alpha-lipoic acid was deemed a safe and well-tolerated intervention, with no adverse effects reported. Its administration may lead to symptom reduction, providing a safe and tolerable treatment alternative (Abubaker *et al.*, 2022).

Therefore, more research is demanded to evaluate the efficacy of a fixed-dose combination of vitamin B and alpha lipoic acid formulations for the treatment of diabetic polyneuropathy in individuals with type 2 diabetes mellitus.

Hence, this study aims to determine the effectiveness of a fixed dose combination of alpha lipoic acid and vitamin B preparations in comparison with placebo for treatment of diabetic polyneuropathy (DPN) in type 2 diabetes mellitus patients.

# 3. OBJECTIVES AND HYPOTHESIS

## 3.1 RESEARCH QUESTIONS

1. Are there any significant differences in mean total symptom score (TSS) and neuropathic symptoms score (NSS) over 12 weeks between diabetic polyneuropathy patients taking a fixed dose combination of alpha lipoic acid and vitamin B Preparations and diabetic polyneuropathy patients taking a placebo?
2. Are there any significant differences in total symptom score (TSS) and neuropathic symptoms score (NSS) among diabetic polyneuropathy patients at baseline and 12 weeks after taking a fixed dose combination of alpha lipoic acid and vitamin B Preparations?
3. Are there any significant differences in fasting plasma glucose, HbA1C level, fasting lipid profile, body mass index (BMI), blood pressure and diabetes quality of life over 12 a week between diabetic polyneuropathy patients taking a fixed dose combination of alpha lipoic acid and vitamin B preparations and diabetic patients taking placebo?
4. Are there any significant differences in the safety parameters of renal function and liver function tests among diabetic polyneuropathy patients at baseline and 12 weeks after taking a fixed dose combination of alpha lipoic acid and vitamin B preparations?
5. Are there any significant differences in fasting plasma glucose, HbA1C level, fasting lipid profile, body mass index (BMI), blood pressure and diabetes quality of life among diabetic polyneuropathy patients at baseline and 12 weeks after taking a fixed dose combination of alpha lipoic acid and vitamin B preparations?

## 3.2 OBJECTIVES

To determine the effectiveness of a fixed dose combination of alpha lipoic acid and vitamin B preparations in comparison with placebo for treatment of diabetic polyneuropathy (DPN) in type 2 diabetes mellitus patients

### 3.2.1 Primary endpoint

1. To compare the mean change in total symptom score (TSS) and neuropathic symptoms score (NSS) over 12 a week period between diabetic polyneuropathy patients taking a

fixed dose combination of alpha lipoic acid and vitamin B preparations versus diabetic polyneuropathy patients taking placebo (inter-group comparison)

2. To compare the mean change in total symptom score (TSS) and neuropathic symptoms score (NSS) among diabetic polyneuropathy patients at baseline and 12 weeks after taking a fixed dose combination of alpha lipoic acid and vitamin B preparations (intra-group comparison)

### 3.2.2 Secondary endpoint

1. To compare changes and differences in fasting plasma glucose, HbA1C level, fasting lipid profile, body mass index (BMI), blood pressure and diabetes quality of life over 12 a week period between diabetic polyneuropathy patients taking a fixed dose combination of alpha lipoic acid and vitamin B preparations and in diabetic polyneuropathy patients taking placebo (inter-group comparison).
2. To compare the changes in the safety parameters of the renal function and liver function tests among diabetic polyneuropathy patients at baseline and 12 weeks after taking a fixed dose combination of alpha lipoic acid and vitamin B preparations (intra-group comparison).
3. To compare changes and differences of fasting plasma glucose, HbA1C level, fasting lipid profile, body mass index (BMI), blood pressure and diabetes quality of life among diabetic polyneuropathy patients at baseline and 12 weeks after taking a fixed dose combination of alpha lipoic acid and vitamin B preparations (intra-group comparison).

## 3.3 HYPOTHESIS

1. There are significant improvements in mean total symptom score (TSS) and neuropathic symptoms score (NSS) in diabetic polyneuropathy patients taking a fixed dose combination of alpha lipoic acid and vitamin B preparations as compared to diabetic polyneuropathy patients taking a placebo.
2. There are significant improvements in total symptom score (TSS) and neuropathic symptoms score (NSS) among diabetic polyneuropathy patients taking a fixed dose combination of alpha lipoic acid and vitamin B preparations for 12 weeks.
3. There are significant improvements in fasting plasma glucose, HbA1C level, fasting lipid profile, body mass index (BMI), and diabetes quality of life in diabetic patients taking a fixed dose combination of alpha lipoic acid and vitamin B preparations as compared to diabetic patients taking placebo.
4. There are no significant differences in the safety parameters of renal function and liver function tests among diabetic polyneuropathy patients at baseline and 12 weeks after taking a fixed dose combination of alpha lipoic acid and vitamin B preparations.
5. There are significant improvements in fasting plasma glucose, HbA1C level, fasting lipid profile, body mass index (BMI), and diabetes quality of life among diabetic patients taking a fixed dose combination of alpha lipoic acid and vitamin B preparations for 12 weeks.

# 4. LITERATURE REVIEW

## 4.1 Diabetic peripheral neuropathy (DPN)

Diabetic polyneuropathy (DPN) is a serious diabetes complication and one of the most prevalent complications of both type 1 and type 2 diabetes. Around half of patient with diabetes experience neuropathy during the progression of their condition, with neuropathic pain occurring in 30–40% of cases (Jensen *et al.*, 2021).

The peripheral nerve damage can present as progressive distal symmetric polyneuropathy, autonomic neuropathy, radiculo-plexopathies, and mononeuropathies. The prevalent form, distal symmetric polyneuropathy (DSPN), typically exhibits a glove-and-stocking-like pattern of distal sensory or motor function loss (Jensen *et al.*, 2021). Patients can present with numbness, tingling, pain, or a combination of these that typically starts in their toes and slowly spreads proximally (Callaghan *et al.*, 2020). Typically, symptoms manifest in the knees before extending to the fingertips. Weakness in DPN is a late sign and often initially detected as a lack of strength in toe extension, followed by ankle dorsiflexion (Callaghan *et al.*, 2020).

## 4.2 Screening and diagnosis of distal symmetric polyneuropathy (DSPN)

American Diabetes Association recommends regular examination of people with diabetes mellitus for the diagnosis of DPN and loss of protective sensation using simple standard tests to identify those at risk for diabetic foot ulcer(Association, 2020). The screening should be considered for people with prediabetes/T2DM who have symptoms of peripheral neuropathy. The guidelines stated that, all type 2 diabetes should be assessed for DSPN starting at diagnosis and for type 1 diabetes it needs to be started 5 years after the diagnosis. These bedside tests should be performed at least annually (Pop-Busui *et al.*, 2017a; Association, 2020).

More commonly, screening for DSPN involves history taking for neuropathic symptoms and examination of the feet, along with a screening test(Yang *et al.*, 2018). In most cases, DSPN can be diagnosed clinically, and electrophysiological tests are rarely required. Electrophysiological tests can be considered if there are atypical features such as rapid onset or progression of neuropathy, asymmetrical neuropathy, predominantly motor neuropathy or in cases of diagnostic uncertainty (Pop-Busui *et al.*, 2017a).

DSPN should be assessed with a 10-g monofilament (starting from the dorsum of hallux, then moving proximally); and one other modality including pin prick, vibration sense using a 128-Hz tuning fork, ankle reflexes or vibration perception threshold testing using a biothesiometer. These increase the sensitivity of detecting DSPN by 87%(Perkins *et al.*, 2001; Herman *et al.*, 2012; Malaysia, 2020). The 10-g monofilament is a useful clinical tool mainly for detecting more advanced neuropathy and identifying patients at increased risk of ulceration and amputation (Tan, 2010). Monofilament test is a simply performed office test to diagnose patients at risk for ulcer formation due to peripheral sensory neuropathy. The test is abnormal

if the patient cannot sense the touch of the monofilament when it is pressed against the foot with just enough pressure to bend the filament (Figure 1) (Armstrong and Lavery, 1998)

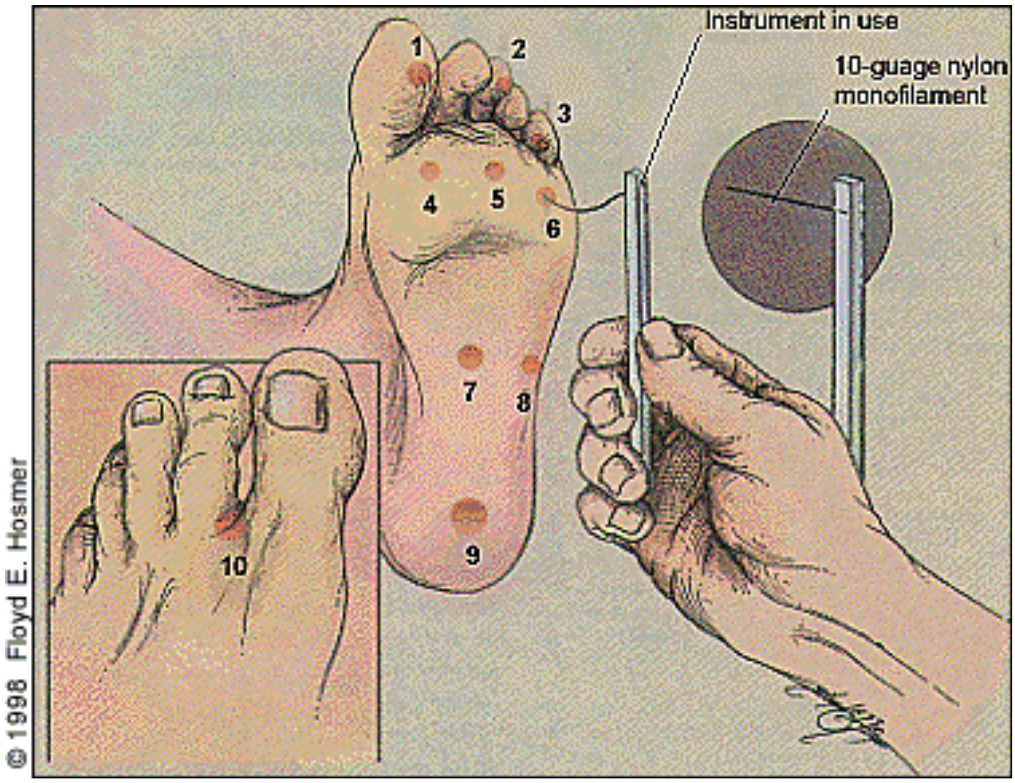



Figure 1: Nylon monofilament test. There is a risk of ulcer formation if the patient is unable to feel the monofilament when it is pressed against the foot with just enough pressure to bend the fi lament. The patient is asked to say "yes"each time he or she feels the filament. Failure to feel the filament at four of 10 sites is 97 percent sensitive and 83 percent specific for identifying loss of protective sensation(Armstrong and Lavery, 1998)

### 4.2.1 Diagnostic Tests For DSPN

The diagnosis of DSPN is principally based on clinical findings. A combination of typical symptomatology and symmetrical distal sensory loss or typical signs in the absence of symptoms in a patient with diabetes is highly suggestive of DSPN and may not require additional evaluation or referral. As up to half of the patients may be asymptomatic, a diagnosis may only be made on examination or, in some cases, when the patient presents with a painless foot ulcer(Pop-Busui *et al.*, 2017a).

Based on this, the diagnostic tests are focused on assessing the symptoms and signs of nerve dysfunctions. Numerous testing methods are available to assess the peripheral nervous system's structure and function, with each test having its advantages and disadvantages. Bedside tests aid DSPN diagnosis—including the 10g monofilament, the Ipswich Touch Test, and vibration perception threshold testing with the Vibratip and a 128-Hz tuning fork. However, these tests tend to diagnose DSPN when it is already well established. Late diagnosis hampers the potential benefits of intensified multifactorial intervention at an early stage of the disease, which could prevent the sequelae of DSPN. Unfortunately, when DPN is detected with

the current crude tests, it is often very well established and consequently impossible to reverse or halt the inexorable neuropathic process. Early diagnosis and timely intervention are thus essential in preventing the development of DSPN(Carmichael *et al.*, 2021).

### 4.2.2: Scoring systems for DSPN (Symptoms)

Various clinical scoring systems available for DPN screening involve symptom scoring, sign scoring or both. These systems may enhance diagnostic accuracy through a composite score of different combined tests and are useful tools for aiding the diagnosis of DPN, along with quantitative measures. Each questionnaire has a scoring system that can diagnose and, in some, stratify disease severity (Carmichael *et al.*, 2021). The commonly used screening tests to assess important symptoms and signs of DSPN are described below.

#### 1. Neurological Symptom Score (NSS) and Neuropathy Disability Score (NDS)

The NSS and NDS criteria were first used in a study on the prevalence of diabetic peripheral neuropathy in hospital-based among UK population (Young *et al.*, 1993). The NSS and NDS questionnaire is a validated instrument used to aid in the diagnosis of DPN. The NSS and NDS have 71.1% sensitivity (indicating positive predictive value) and 90% specificity (referring to negative predictive value)(Chawla *et al.*, 2013). Hence, applying NDS+NSS >10 as per " Young et al criterion " could pick up early DPN in 96 out of 135 (sensitivity of 71.1% & specificity of 90% ). This has a +ve predictive value of 57.14% & negative predictive value of 94.32% as validated & documented(Chawla *et al.*, 2013).

The sensitivity, specificity and diagnostic efficacy of NSS and NDS score was checked in a study conducted by Asad et al., 2010, taking nerve conduction studies as the gold standard. It was found that, NSS and NDS had 82.05%, 92.31% sensitivity and 66.67%, 47.62% specificity, respectively. The diagnostic efficacy of NSS and NDS was 77%(Asad *et al.*, 2010). Studies that have been conducted in Brazil, Germany, United Kingdom, Uganda, Egypt and Malaysia used NSS and NDS scores as a criterion to establish the diagnosis of distal symmetric polyneuropathy (DSP)(Abbott *et al.*, 2011; Kamel *et al.*, 2015; Kisozi *et al.*, 2017; Li-Ying *et al.*, 2017; André Pfannkuche *et al.*, 2020; Reis de Matos *et al.*, 2020)

#### 2. Michigan Neuropathy Screening Instrument (MNSI)

The Michigan neuropathy screening instrument (MNSI) is another commonly used composite scoring system that includes a questionnaire and a foot examination. The MNSI was designed by Feldman et al., 1994 to diagnose diabetic polyneuropathy in outpatient clinics (Feldman *et al.*, 1994). The MNSI can be administered by any health care professional involved in the treatment of diabetic patients.

The MNSI is used widely to evaluate distal symmetrical peripheral neuropathy (DSPN) in diabetes. The MNSI assesses both the key symptoms and signs of diabetic polyneuropathy in two separate parts (Feldman *et al.*, 1994). The first part is on the history/symptom portion of

the MNSI that consists of a 15-items. It can be self-administered by the respondents by answering "Yes" or "No" for each item (Feldman *et al.*, 1994). The responses are added to obtain a total score. 'Yes' responses to questions 1,2,3,4 5,6, 8,9,10,11,12,14,15 are each counted as one point. Questions 7 and 13 were reversed and scored so that 'no' responses indicated an abnormality and counted as one point in the scoring algorithms. All items on the questionnaire were coded as 0 for a negative response and 1 for a positive response (negative responses on items 7 and 13 counted as 1 point) (Feldman *et al.*, 1994; Herman *et al.*, 2012). Original cut of point to detect neuropathy can be defined as seven or more positive responses to this symptoms section alone (Feldman *et al.*, 1994). However, in 2012, Herman et al., altered the cut point to define an abnormal test from ≥ 7 abnormal to ≥ 4 abnormal items to improves the performance of the MNSI questionnaire. When the threshold to define an abnormal test was set at ≥ 4, the questionnaire was 40% sensitive and 92% specific and had a positive predictive value of 69% and a negative predictive value of 78%. However, when the threshold to define an abnormal test for the MNSI questionnaire was ≥ 7, the questionnaire was 13% sensitive and 99% specific in identifying confirmed clinical neuropathy. Positive and negative predictive values were 84 and 73%, respectively(Herman *et al.*, 2012).

The second part of MNSI was lower extremity examination that includes inspection for appearance and ulcer, assessment of vibratory sensation and ankle reflexes and is scored by assigning points for abnormal findings. During the MNSI examination, a health professional need to inspects each foot for deformities, dry skin, calluses, infections and fissures. Each foot with any abnormality receives a score of 1. Each foot is also inspected for ulcers and each foot with an ulcer receives a score of 1. The ankle reflexes are also elicited. If the reflex is absent, the patient is asked to perform the Jendrassic manoeuver and, if present, the reflex is designated as present with reinforcement and is scored as 0.5. If the reflex is absent with the Jendrassic manoeuver, the reflex is designated as absent and is scored as 1. Vibration sensation is then tested in the great toe using a 128-Hz tuning fork. In general, the examiner should be able to feel vibration in his or her hand for 5 s longer than a normal subject can at the great toe. Vibration is scored as present if the examiner senses the vibration on his or her finger for < 10 s longer than the subject feels it in the great toe, decreased if sensed for  10 second (scored as 0.5) or absent (scored as 1). The total possible score is 8 points and, in the published scoring algorithm, a score ≥ 2.5 is considered abnormal (Feldman *et al.*, 1994; Herman *et al.*, 2012). When the threshold to define an abnormal MNSI examination was set at ≥ 2.5, the MNSI examination was 61% sensitive and 79% specific in defining confirmed clinical neuropathy and had a positive predictive value of 55% and a negative predictive value of 83%(Herman *et al.*, 2012).

### 3. Total symptoms score (TSS)

The total symptoms score (TSS) was used by Ziegler et al. (1995) to evaluate the effects of the antioxidant alpha-lipoic acid in a 3-week multicentre, randomized, double-blind placebo-controlled trial (Alpha-Lipoic Acid in Diabetic Neuropathy: ALADIN) study among 328 non-insulin-dependent diabetic patients with symptomatic peripheral neuropathy(Ziegler *et al.*, 1995). The TSS is a summation of symptom scores for stabbing pain, burning pain, paresthesia,

and numbness by symptoms frequency (occasionally, often, or continuous) and intensity (not present, mild, moderate, or severe) on which scores range from 0 to 14.64 (table 1)(Ziegler *et al.*, 1995; Won *et al.*, 2020a).The severity score needs to be conducted by the physician or a trained nurse.

### 4.3 Alpha-lipoic acid (ALA) in treating Diabetic peripheral neuropathy

Alpha-lipoic acid (ALA) is a potent antioxidant that might be effective in the treatment of diabetic peripheral neuropathy. ALA could potentially serve as both a pain-relieving therapy and an enhancer of nerve function by mitigating the harmful effects of hyperglycemia (Bartkoski and Day, 2016).

Studies revealed that ALA enhanced endothelium-dependent vasodilation mediated by nitric oxide in diabetic patients and enhanced microcirculation in patient suffering from diabetic polyneuropathy (Vallianou *et al.*, 2009). An animal model study done proposed that ALA exhibits effectiveness against moderate ischemia-reperfusion injury, particularly in cases where the distal sensory nerves suffer damage (Khan *et al.*, 2022). Additionally, ALA has been shown to lower plasma levels of plasminogen activator 1 and interleukin 6, which may indicate that ALA improves endothelial dysfunction by acting as an anti-thrombotic and anti-inflammatory agent (Sola *et al.*, 2005).

Ziegler et al. conducted a meta-analysis of four trials—ALADIN I, ALADIN III, SYDNEY, and NATHAN II—to assess the effectiveness and safety of intravenous administration of 600 mg of ALA over a three-week period. Their findings demonstrated that the treatment was well-tolerated and effectively reduced positive neuropathic symptom. Alpha-lipoic acid (ALA) 600 mg daily is recommended for individuals who are resistive to or intolerant of first-line pharmacotherapies and interested in a nutritional supplement approach as it is safe and significantly improves both positive neuropathic symptoms and neuropathic deficits to a clinically meaningful degree in diabetic patients with symptomatic polyneuropathy (Ziegler *et al.*, 2004).

Another study done in the Oral Pilot (ORPIL) trial revealed that administering oral 600 mg ALA three times daily for three weeks could improve symptoms and deficits caused by polyneuropathy in type 2 diabetes patients, despite the trial's smaller sample size (Ruhnau *et al.*, 1999).

While a systematic review conducted by Abubaker et al. revealed that the use of ALA alone did not significantly improve neuropathic pain in patients with diabetes but still played a role in reducing neuropathic symptoms (Abubaker *et al.*, 2022).

### 4.4 Alpha lipoic acid and different metabolic parameters

ALA, also known as thioctic acid, is a naturally occurring compound found in various foods and synthesized in small amounts by the human body. It serves as a cofactor for several mitochondrial enzymes involved in energy metabolism, making it a vital component in cellular energy production (Agathos *et al.*, 2018).

ALA is a potent antioxidant that can scavenge a variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS). By reducing oxidative stress, ALA may help to mitigate inflammation and cellular damage associated with metabolic disorders such as diabetes and obesity (Nagamatsu *et al.*, 1995). In one study done by (Papanas and Ziegler, 2014) in addition to reduces oxidative stress in diabetic neuropathy, ALA also improve nerve blood flow and nerve conduction velocity.

Study done by (Salehi *et al.*, 2019) ALA has been shown to improve glucose uptake and utilization in skeletal muscle cells by enhancing insulin sensitivity. This effect is attributed to ALA's ability to activate AMP-activated protein kinase (AMPK), a key regulator of cellular energy metabolism.

ALA has also been reported to have beneficial effects on lipid metabolism by reducing levels of triglycerides and LDL cholesterol (Mousavi *et al.*, 2019). These effects may be mediated in part by ALA's antioxidant properties, which help to prevent lipid peroxidation and inflammation.

### 4.5 Quality of life in patients with Diabetic peripheral neuropathy

The impact of DPN on the quality of life (QoL) of affected individuals can be profound and multifaceted as symptoms such as pain, numbness, tingling, and weakness in the extremities may interfere with patient daily activities which lead to poor quality of life (Pop-Busui *et al.*, 2017b).

Despite advancements in therapy, diabetic neuropathy continues to be linked with significant morbidity, elevated mortality rates, and diminished quality of life (QoL) when compared to diabetic patients without neuropathy (Agathos *et al.*, 2018).

One of the most significant ways DPN affects QoL is through its impact on physical functioning. Pain and discomfort associated with DPN can limit mobility, impair balance, and decrease overall physical activity levels (Chiles *et al.*, 2014). This can lead to a reduced ability to perform daily tasks, such as walking, standing, and even simple activities like dressing or cooking.

Chronic pain and discomfort from DPN can also take a toll on psychological well-being. Many patients experience anxiety, depression, or frustration due to the constant presence of symptoms and the limitations they impose on daily life which usually lead to diabetes distress

(Feldman *et al.*, 2019). Diabetes distress refers to the emotional and psychological burden experienced by individuals living with diabetes. It encompasses feelings of stress, anxiety, frustration, and burnout related to the daily management of the condition (Fisher *et al.*, 2012). Sleep disturbances are also common, further exacerbating mental health issues and contributing to a lower QoL (Gylfadottir *et al.*, 2020).

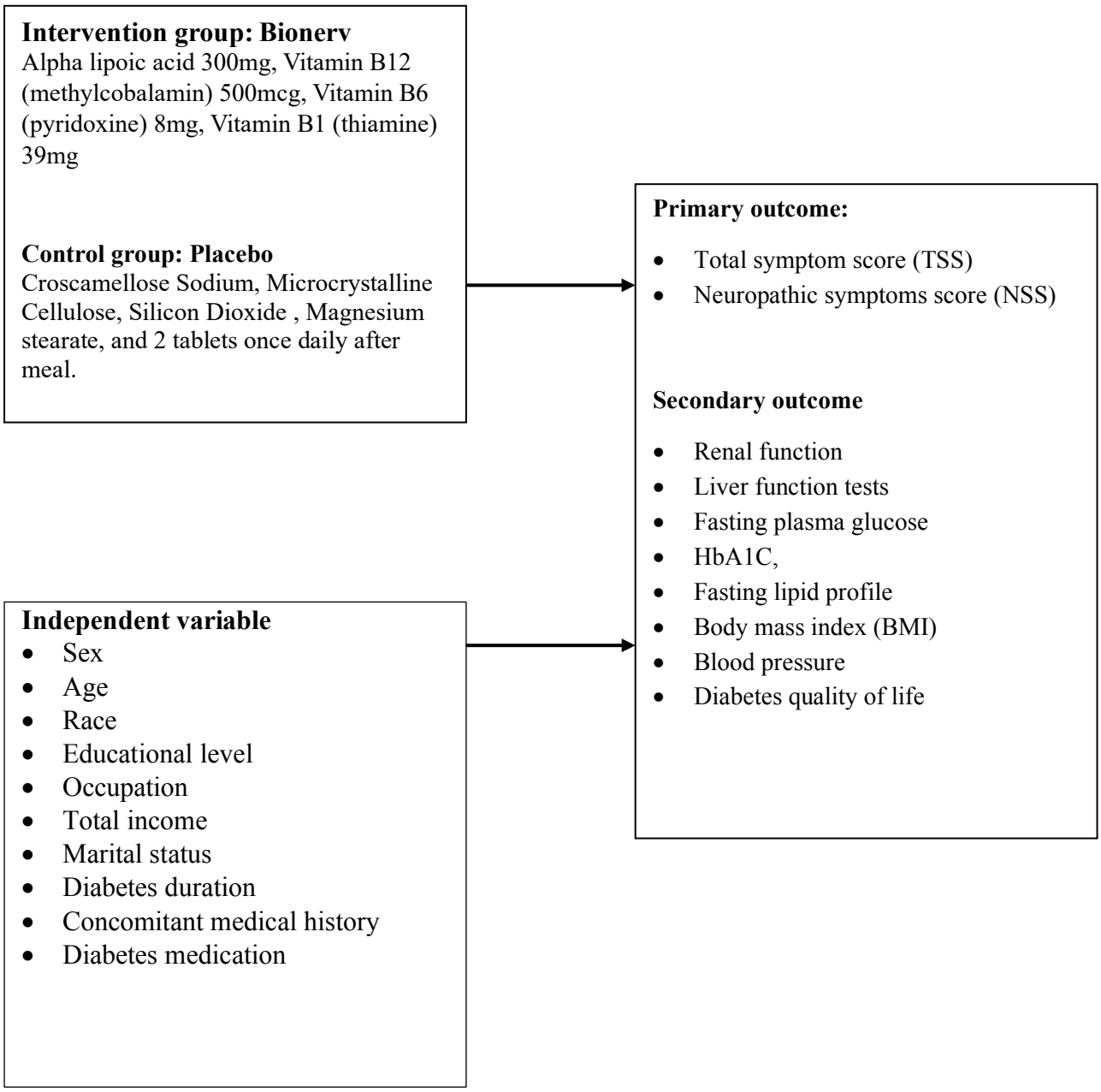
### 4.6 Vitamin B in treating diabetic peripheral neuropathy.

Vitamin B, particularly certain B vitamins such as B1 (thiamine), B6 (pyridoxine), and B12 (cobalamin), has been studied for its potential role in treating diabetic peripheral neuropathy (DPN). There is growing evidence in the literature indicating that these vitamins play a role in promoting nerve repair by accelerate nerve tissue regeneration and aid in the recovery of nerve function through various mechanisms (Altun and Kurutas, 2016).

Thiamine plays a crucial role in nerve function and may be deficient in individuals with diabetes, contributing to neuropathic symptoms. Some studies have suggested that thiamine supplementation may help improve symptoms of DPN, particularly when the neuropathy is associated with thiamine deficiency (Farah and Yammine, 2022). However, more research is needed to confirm its effectiveness in broader populations of individuals with DPN.

Vitamin B12 deficiency is also relatively common in individuals with diabetes and has been linked with neurological complications such as neuropathy. Study done by (Altun and Kurutas, 2016) the use of vitamin B complex or specifically vitamin B12 has been demonstrated to enhance the quantity of Schwann cells and myelinated nerve fibers as well as the diameter of axons. This promotes the regeneration of myelinated nerve fibers and the proliferation of Schwann cells which are essential for the structural integrity and maintenance of proper nervous system functions.

## 5. CONCEPTUAL FRAMEWORK



# 6. METHODOLOGY

## 6.1 Study design

This is a single-center, randomized, double-blind, placebo-controlled trial study.

## 6.2 Study area

This study will be conducted at the Klinik Rawatan Keluarga and diabetes clinic Hospital Universiti Sains Malaysia.

## 6.3 Study duration

This study will be conducted from February 2024 to January 2025 (1 year).

## 6.4. Population and sample

### 6.4.1 Reference population

Patients with type 2 diabetes mellitus attending the Hospital Universiti Sains Malaysia.

### 6.4.2 Source population

Patients with type 2 diabetes mellitus attended Klinik Rawatan Keluarga and diabetes clinic Hospital Universiti Sains Malaysia during the study period.

### 6.4.3 Sampling frame

Patients with type 2 diabetes mellitus attended and registered Klinik Rawatan Keluarga and diabetes clinic Hospital Universiti Sains Malaysia, who fulfilled the criteria.

**Inclusion criteria**

- Aged 18 years and over
- Diagnosed with type 2 diabetes mellitus based on WHO diagnostic criteria for diabetes (Organization, 2020). Diabetes patients refer to those who have fulfilled the WHO diagnostic criteria for diabetes where:1) plasma glucose concentration measured after an overnight fast above 7.0mmol/l and/or 2) plasma glucose concentration measured two hours after a 75g oral glucose load above 11.1 mmol/l(Organization, 2020).
- Diagnose with diabetic polyneuropathy by Neurological Symptom Score (NSS) and Neuropathy Disability Score (NDS). Presence of moderate signs (NDS >6) regardless of symptoms or mild signs (NDS > 3) with moderate symptoms (NSS > 5) (Young *et al.*, 1993).

**Exclusion Criteria**

- Those with a documented mental impairment which impacted on their ability to answer questions independently.
- Patients with peripheral vascular disease (non-palpable foot pulses, intermittent claudication)
- Patients with an amputated foot or leg
- Aspartate aminotransferase or alanine aminotransferase levels >3 times normal levels
- Patients with renal impairment - CKD stage IV and V
- Patients using drugs with possible influence on the study results (antidepressants, anticonvulsants, opiates, neuroleptics, antioxidants, and particularly methylcobalamin, pyridoxine and other B complex preparations)
- Pregnancy, lactation, or childbearing age without safe contraception
- History of allergy with vitamin B complex preparations (i.e. Vitamin B12, B6 and B1) and alpha lipoic acid

### 6.5 Sample size estimation

The sample size for this study was determined based on the primary study endpoint (change in TSS and NSS from the beginning of treatment to week 12 after treatment) and the sample-sized requirements were calculated using G*Power Software version 3.1.9.7. Since the intended statistical analysis (Repeated measures ANOVA) investigates between-factor effect, within-factor effect, and within-between interaction effect, the sample sizes were calculated using these three options. The sample size calculations were summarized in the table below.

Table 1: Summary of sample size calculation.

| Calculation Option in G*Power Software | Parameter used for calculation | Total sample size |
|---|---|---|
| Test family: F tests<br>Statistical test: ANOVA, Repeated Measures, between factors | Effect size f = 0.28 (Medium effect size)<br>Type I error probability: 5%<br>Power: 80%<br>Number of groups: 2<br>Number of measurements: 3<br>Correlation among repeated measures: 0.5 | n=70<br>(35 per group) |
| Test family: F tests<br>Statistical test: ANOVA, Repeated Measures, within factors | Effect size f = 0.28 (Medium effect size)<br>Type I error probability: 5%<br>Power: 80%<br>Number of groups: 2<br>Number of measurements: 3<br>Correlation among repeated measures: 0.5 | n=24<br>(12 per group) |

| | | |
|---|---|---|
| Test family: F tests<br>Statistical test: ANOVA, Repeated Measures, within-between interaction | Effect size f = 0.28 (Medium effect size)<br>Type I error probability: 5%<br>Power: 80%<br>Number of groups: 2<br>Number of measurements: 3<br>Correlation among repeated measures: 0.5 | n=24<br>(12 per group) |

In summary, the largest sample size was obtained with calculation for between subject effect (35 participants per group). Anticipating a 10% dropout, the corrected sample size was 38 participants per group. The total number of participants required for this study was 76 participants.

### 6.5.1: Interim Analysis

No interim analysis is planned.

### 6.5.2: Determination of end of clinical trial (all patients)

The end of the study is defined as being the "last patient last visit" planned with the protocol, including follow-up visit.

## 6.6 Sampling method

Purposive sampling method will be used in this study. The total number of participants required for this study is 76 participants, which is 38 participants for intervention and 38 participants for control group. The sample will be selected purposely based on inclusion and exclusion criteria.

## 6.7 Research tools

### 6.7.1 Questionnaire

A questionnaire guided the participants' interviews will be used to collect the variables of interest in this study. Participants will be interviewed in person by researchers who will be trained before starting data collection. The case report form will consist of 2 parts:

A. The first part is a neurological symptom score (NSS) and neuropathy disability score (NDS). It will be used to screen the patients whether they have diabetes polyneuropathy or not. If the patient fulfils the criteria for diagnosis of diabetes polyneuropathy with the presence of moderate signs (NDS >6) regardless of symptoms or mild signs (NDS > 3) with moderate symptoms (NSS > 5)(Young et al., 1993), and other inclusion and exclusion criteria are fulfilled, they will be recruited in the study. Neurological Symptom Score

(NSS) will also be used to measure the severity of neuropathy symptoms and as a primary outcome of the study.

B. The second part will consist of 5 sections as follows:

(a) Sociodemographic characteristics
(b) Medical and diabetes profile
(c) Total symptoms score (TSS)
(d) The revised version of Diabetes Quality of Life (RV-DQoL)
(e) Physical examination (height, weight, BMI and blood pressure)

**Part A: Neurological Symptom Score (NSS) and neuropathy disability score (NDS)**

Neurological Symptom Score consists of 4 components. The first component is on reported discomfort symptoms: burning/numbness/tingling (2 points), fatigue/cramping and aching feelings (1point) and 0 point for none. Second component is on symptom location whether the symptom occurs in the feet (2 points), calves (1 point) and 0 points for elsewhere. The third component is on timing of symptoms, either nocturnal exacerbation (2 points), symptoms present both day and night (1 point) and 0 for symptoms present during daytime only. A score of 1 was added if the symptoms had ever woken the patient from sleep. The fourth component is on a manoeuvre to reduce the symptoms by either walking (2 points) or standing (1pointand and 0 for sitting or lying down. The maximum symptom score was 9 (Young *et al.*, 1993; André Pfannkuche *et al.*, 2020).

The NDS was derived from the neurological examination testing sensory modalities such as vibration, temperature, pin prick and Achilles tendon reflex on both feet. Each test was assessed with points to calculate the total disability score. Vibration sense was elicited using a 128 Hz turning fork at the hallux of the big toe (Kisozi *et al.*, 2017). The vibration was scored as either present = 0 or reduced/absent = 1 for each side. The temperature perception on the dorsum of the foot was determined by using the tip of cold tuning fork with scored as either present = 0 or reduced/absent = 1 for each side(Kamel *et al.*, 2015). Pain perception was assessed by the application of a pin prick at the proximal end of the big toe nail to distinguish sharp/blunt. The results will scored as either present = 0 (when the patient could distinguish sharpness) or reduced/absent = 1(when the patient was unable to distinguish it) for each side (Kisozi *et al.*, 2017). The ankle reflex was tested both-sided using a standard tendon hammer. By this, the patient´s foot was in a relaxed position. The reflex was scored as normal = 0, present with reinforcement = 1 or absent = 2 per side(Young *et al.*, 1993; André Pfannkuche *et al.*, 2020).

Neuropathy symptoms were classified into mild (NSS 3-4), moderate (NSS 5-6) and severe (NSS 7-9) while neuropathy signs were also classified mild (NDS 3-5), moderate (NDS 6-8) and severe (NDS 9-10)(Young *et al.*, 1993).

**Part B: Second Part of Proforma**

**(a) Sociodemographic characteristics**

The first part will obtain the participants' demographic characteristics, including sex, age, race, educational level, occupation, total income, and marital status.

**(b) Medical and diabetes profile**

The second part will obtain a diabetes profile including diabetes duration, concomitant medical history (presence of hypertension, dyslipidaemia, retinopathy, IHD/MI), list of diabetes medications are taken, and drug allergies. This part will be obtained from patients' medical records by the researcher.

**(c)Total symptoms score (TSS)**

Total Symptom Score is the sum of 4 neuropathic symptoms (stabbing pain, burning pain, paraesthesia, and numbness) reported as symptoms frequency (occasionally, frequent, or continuous) and symptom intensity (absent, slight, moderate, or severe) on which scores range from 0 to 14.64(table 1)(Ziegler *et al.*, 1995; Won *et al.*, 2020a).

Table 1: Scoring approach for the neuropathic symptoms included in the total symptom score (stabbing pain, burning pain, paraesthesia, and numbness)

| Symptom frequency | Symptom intensity | | | |
|---|---|---|---|---|
| | Absent | Slight | Moderate | Severe |
| Occasional | 0 | 1.00 | 2.00 | 3.00 |
| Frequent | 0 | 1.33 | 2.33 | 3.33 |
| (Almost) continuous | 0 | 1.66 | 2.66 | 3.66 |

**(d) The revised version of Diabetes Quality of Life (RV-DQoL)**

The revised version of Diabetes Quality of Life (Rv-DQoL) is adapted from the Diabetes Quality of Life (DQoL) questionnaire (Jacobson *et al.*, 1994). DQoL was intended to be used for evaluating the quality of life specifically related to T2DM and was made up of three major domains, namely, (i) Diabetes Life Satisfaction (QoL Satisfy), 18 items; (ii) Disease Impact Scale (QoL Impact), 27 items; and (iii) Disease-Related Worries Scale (QoL Worry), 14 items and one general question to reflect self-rating of overall general health. All items in the QoL Satisfy domain are scored on a five-point scale, ranging from 1 (very satisfied) to 5 (very dissatisfied), whereas the items in the QoL Impact and QoL Worry are scored on a five-point scale, ranging from 1 (never) to 5 (all the time); the score was presented as the total of the items of each scale divided by the number of items. A higher average score indicates poorer QoL (79).

A Malay version of the instrument was developed, keeping the three main domains of 'satisfaction', 'impact' and 'worry' with similar score scale. Each domain's redundant questions were removed, leaving the newly revised DQoL (Rv-DQoL) with a total of 13 questions. Cronbach's α values for each domain ranged from 0.75 to 0.93, indicating good internal consistency, and it was validated for use among adult T2DM patients in Malaysia.

It has a 5-point Likert scale from "no impact/no worries" to "always satisfied/always affected". A higher score indicates a lower quality of life (Bujang *et al.*, 2018). The questionnaires used had permission from the authors.

**(e) Physical examination (height, weight, BMI and blood pressure)**

Respondents will be given instructions on how to measure weight and height correctly. BMI is calculated by dividing a respondent's body weight in kilograms by their height in meters squared [weight (kg) / height (m2)]. Blood pressure will be measured using an OMRON automated blood pressure machine.

### 6.7.2: Laboratory Evaluation (laboratory assessment)

Baseline blood investigations will be measured at baseline (visit 1), and weeks 12 (last visit). The blood investigations include:

1. HbA1c
2. Fasting Blood Sugar
3. Fasting Lipid profile
4. Renal function test
5. Liver function test

Fasting blood samples will be collected in heparinized tubes. Six ml will be drawn from each subject and will be sent to a private lab: Pantai Premier Pathology (PPP) lab, for analysis.

### 6.8 Study procedure

Participants will be recruited from Klinik Rawatan Keluarga and diabetes clinic Hospital USM via convenience sampling. Potential participants will be identified from the case notes of patients. Then, the participants will be approached individually. Those who are willing to participate in this study will be given information regarding the study. The participants will be screened to determine their eligibility criteria, including performing neurological symptom score (NSS) and neuropathy disability score (NDS) to determine diabetes polyneuropathy diagnosis. If all the inclusion and exclusion criteria are fulfilled, informed consent will be obtained from all patients who agree to participate in this study. The consent will be obtained by a study team that recruited the patient. Upon consented, the participant's socio-demographic data will be collected, and the medical record will be assessed to fill in their medical and diabetes profiles. They will also answer the total symptoms score (TSS), and revised version

of the Diabetes Quality of Life (Rv-DQoL) questionnaire. The physical examination includes measurement of height, weight, calculated Body Mass Index (BMI), and blood pressure during sitting will be done. Then 6ml of fasting venous blood will be taken for measurement of HbA1c, fasting blood glucose, RFT, LFT and fasting lipid profile as baseline. All participants were advised not to consume any special supplement or other replacement meal throughout the study.

The patients then will be randomized to either intervention or control group. The intervention group will receive a fixed dose combination of alpha lipoic acid and vitamin B preparations, and the control group will receive a placebo.

The patients will be seen 6 weeks after taking the product. During this visit, they will be assessed for any side effects or adverse events and compliance with the product supplied (compliance form). The total symptoms score (TSS), neurological symptom score (NSS), a revised version of Diabetes Quality of Life (Rv-DQoL), blood pressure, weight, height, calculated Body Mass Index (BMI) will also be measured. Another 6-week supply of the product will be given.

Lastly, the patient will be assessed in week 12. During this visit, the same measurement will be taken as visit 1, including answering the questionnaires. Adverse events, blood taking, and compliance will also be assessed.

The duration for visit 1 and visit 3 (at 12 weeks) is about 50-60 minutes for each visit since it will involve blood taking procedure. Duration for visit 2 (at 6 weeks) is about 30 minutes since it will not involve blood taking.

### 6.8.1 Baseline screening and assessment

Vital signs (supine blood pressure, pulse rate), weight, height, and calculated body mass index (BMI) will be recorded on the baseline and every visit.
The weight of subjects will measure in light clothing without belts and shoes. All contents will be removed from their pockets. Height will be measured to the nearest 0.1 cm from a scale marked in cm. A standardized weighing machine and a height measuring scale will measure both height and weight.

Blood pressure will be measured using the OMRON automated blood pressure set. Systolic and diastolic blood pressure will measure once while sitting on the chair.

Then a qualified staff nurse will take 6 ml of venous blood to measure HbA1c, fasting blood glucose, fasting lipid profile and safety parameters (RFT, LFT). All respondents need to fast for 8 hours before blood taking. All the blood will be sent to the private lab Pantai Premier Pathology (PPP) lab to process the blood sample.

### 6.9 Study flow chart

Eligible and consenting participants will be randomized into two groups: An intervention group will receive a fixed dose combination of alpha lipoic acid and vitamin B and the control group will receive a placebo.

Outcome measurements will be assessed at the baseline and after the completion of the 12-week intervention.

Sociodemographic data, diabetes and medical profile, total symptoms score (TSS), neurological symptom score (NSS), a score of the revised version of Diabetes Quality of Life (RV-DQoL), BMI, blood pressure, blood result HbA1c, FLP, FBS and safety parameters blood (RFT, LFT) will be recorded in case report form. Hence, the total duration of the study is 12 weeks. A brief research flow chart is shown in Figure 1.

**Flow chart of the study**

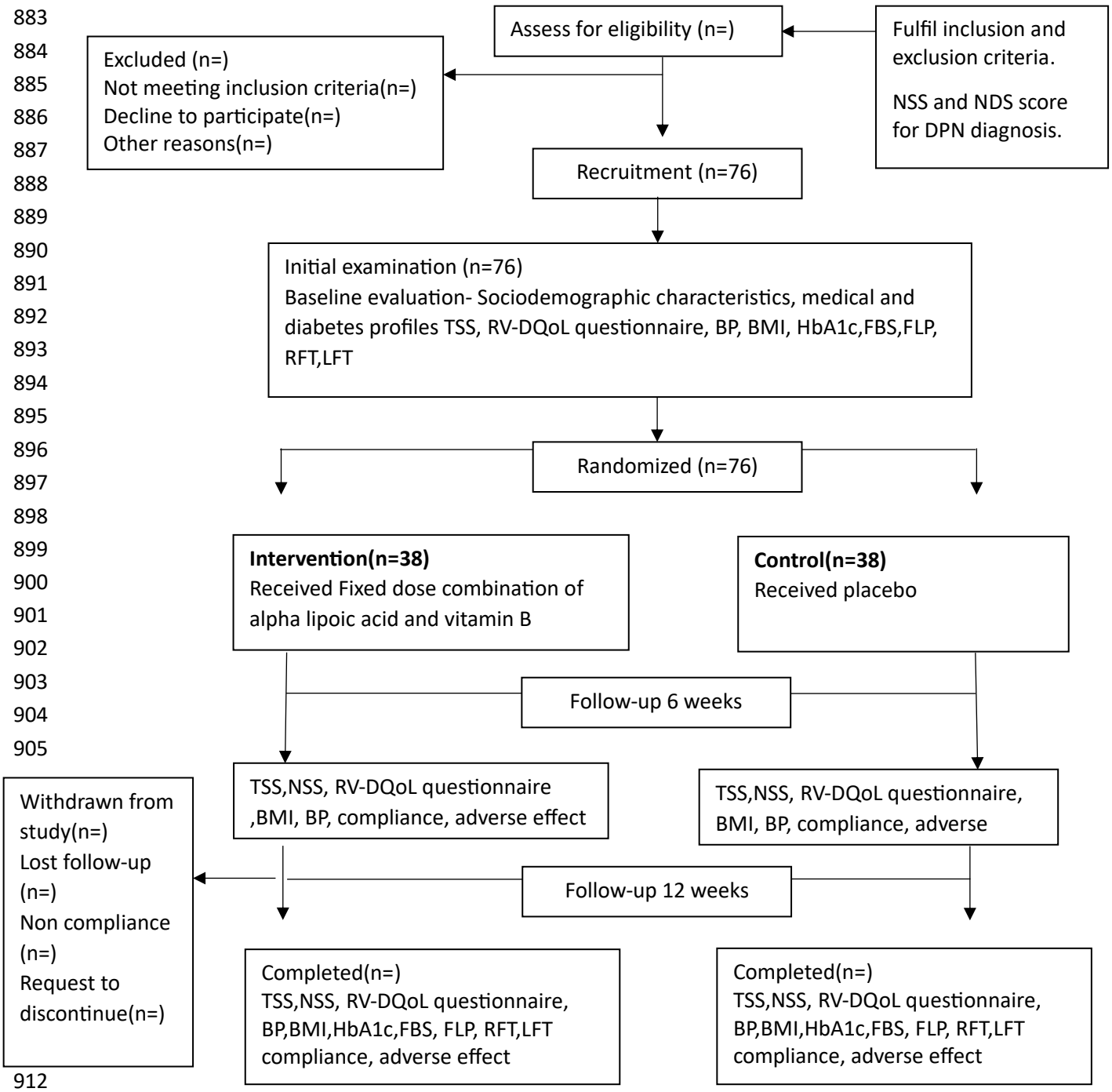



Figure 1: Flow chart of the study

### 6.10.1 Randomization methods

Using mixed block randomization at a 1:1 ratio, independent statisticians randomly assign patients to treatment and placebo groups using IBM SPSS software (group A and group B). An independent statistician will code treatment and placebo into groups A and B. In a brown opaque envelope, each participant received a written assignment with a code. After participant recruitment, the researcher will open this envelope. The independent statistician will reveal the allocation key after the study. An independent statistician tags the treatment and placebo groups as ‘A' or ‘B'.

### 6.10.2 Blinding and emergency unblinding procedures

This study will be conducted as a double-blind, placebo-controlled trial study. Both the participants and the assessor will not know the intervention received. The study's participants and outcome assessor are blinded to allocation status. The medical monitor may break the subject's sealed emergency code key and identify the test drug in the event of an adverse event (AE). The medical monitor will have sealed emergency code keys (one per subject) in a secure location and be always available by phone. Medical monitor is a hospital medical officer. He/she knows hospital system and study protocol. If emergency unblinding is needed, the investigator will call the medical monitor, who will break the subject's emergency code key, identify the test coil, and notify the investigator. The medical monitor will attach a detailed report to the case report form with the date and reason for identifying the study drug. The medical monitor and investigator must sign this report. All unused sealed code keys will be counted after the study. Except in emergencies, the treatment blind will remain until all subjects have completed treatment and the database is cleaned and locked. A comment on the case report form will justify and explain broken code, along with the date.

### 6.10.3 Intervention group: Fixed dose combination of alpha lipoic acid and vitamin B preparations

At baseline, the intervention group will receive a fixed dose combination of alpha lipoic acid and vitamin B preparations. This product is packaged and manufactured by BREGO Life Sciences Sdn Bhd company with a brand name of Bionerv®. BIONERV® is the first combination of alpha lipoic acid 300mg and vitamin B complex. Bionerv® is in oral film-coated, orange colour, oblong shape and no marking and embossing on the tablet. Each tablet contains 4 active ingredients as follows: Alpha lipoic acid 300mg, Vitamin B12 (methylcobalamin) 500mcg, Vitamin B6 (pyridoxine) 8mg, Vitamin B1 (thiamine) 39mg. All the above ingredients are synthetic. The shelf-life is 2 years. Storage condition is keeping in dry place below 300$^{o}$C and protecting from light and moisture. It has no bovine-gelatin capsule. There are no precautions on drug-drug interaction, but it is not suitable for children (<18 years). There is insufficient reliable data for pregnancy and breast feeding. It has JAKIM Halal Certification with reference number JAKIM.700-2/3/5 017-07/2005. One bottle consists of 60 tablets.

BIONERV® is approved and commercially marketed in Malaysia in 2018. BIONERV® is prescribed as adjuvant therapy for the treatment of peripheral neuropathy i.e. carpal tunnel syndrome, diabetic polyneuropathy and neuritis.

BIONERV® is registered as Health Supplement by National Pharmaceutical Regulatory Agency (NPRA), Ministry of Health Malaysia. Malaysia.

BIONERV® is available in government teaching institution hospitals including University Malaya Medical Centre (PPUM), Universiti Putra Malaysia Teaching Hospital (HPUPM), Universiti Kebangsaan Malaysia Medical Centre (PPUKM), Hospital Universiti Sains Malaysia (HUSM), Sultan Ahmad Shah Medical Centre (SASMEC @IIUM) and Universiti Teknologi MARA Hospital (HUiTM). BIONERV® is also available in major private hospitals such as KPJ Group Hospitals and IHH Malaysia (Pantai Hospitals, Prince Court Medical Centre & Gleneagles Hospitals), Columbia Asia Hospitals, Subang Jaya Medical Centre, Sunway Medical Group etc.

This medication needs to take 2 tablets a day after a meal and need to drink more water. In this study, the participants need to take 2 tablets once daily after breakfast. The duration of the intervention will be 12 weeks. All participants are required to return to the clinic in the 6th and 12th week for a post-intervention assessment.

### 6.10.4 Control group

At baseline, the control group will receive a placebo drug consisting of Croscamellose Sodium, Microcrystalline Cellulose, Silicon Dioxide and Magnesium stearate. The placebo was manufactured by Yanling Natural Hygiene Sdn Bhd. The formulation of the placebo tablet is derived from the excipients used in the Bionerv tablet, which are already approved with Halal Certification, which means the excipients have already been evaluated by JAKIM earlier with no issue in complying with the Halal requirements.

The participants need to take 2 tablets once daily after breakfast. The placebo drugs will share the same appearance and color as intervention drug.

The duration of the intervention will be 12 weeks. All participants are required to return to the clinic in the 6th and 12th week for a post-intervention assessment.

### 6.10.5 Follow up group

There are two follow-ups during this study. 1st follow-up will be on the 6th week and 2nd follow-up (final follow-up) will be during 12th week of the study.
During the 6th week follow-up, participants will be checked for their weight, height, calculated Body Mass Index (BMI), TSS, NSS, RV-DQoL questionnaire, compliance, and adverse effects.

A final visit (12th week) to the clinic will be conducted on the 12th week. During these visits, the outcome measures will be assessed to determine the effect of the fixed-dose combination of alpha lipoic acid and vitamin B. During this visit, the same measurement will be taken as visit 1.

### 6.10.6 Compliance and adverse effect monitoring.

A daily checklist form will be handed out to the participants to monitor their compliance with the treatment product. After taking the fixed dose combination of alpha lipoic acid and vitamin B or placebo, they need to tick on the form.

Monthly reminders will be sent through the WhatsApp application to remind the participants to ensure compliance with the medication given. Nonresponsive participants will be contacted through a phone call for an additional reminder.

Participants will be considered non-compliant if they miss more than 30% of the medication given throughout the intervention period.

Participants will also be asked to report any complications or adverse effects, and this information will be recorded on an adverse effect sheet. Any adverse events due to trial intervention will be reported to the clinic and attended to by a medical doctor from the research team if any treatment is needed.

### 6.10.7 Patient's withdrawal.

The investigator may cease study treatment and withdraw the participants, or the participant may withdraw herself from participation in the study at any time. The reason for withdrawal of a patient will be recorded in the case report form (CRF).

Reasons for patient withdrawal include:

a. The need to take medication, which may interfere with study measurement.
b. Patient experiences an intolerable/ unacceptable adverse event.
c. Patient exhibits non-compliance with the protocol.
d. Patient unwilling to proceed and/ or consent is withdrawn.
e. Investigator withdrawn patient for reasons unrelated to the study drug (e.g undercurrent illness).

## 6.11 Statistical Analysis

Data entry and analyses will be conducted using SPSS for windows version 27. Prior to analysis, the data will be explored to examine the pattern of missing data and appropriate method of missing data imputation will be executed. Both Intention-to-treat and per-protocol analysis will be conducted.

The numerical variables' shape of distribution will be examined both quantitatively (by examining the coefficient of variation, skewness value, kurtosis value, Kolmogorov-Smirnov test, and Shapiro-Wilk test) and qualitatively (by examining the histogram with overlaid normal curve, Q-Q plot, and the Box and Whisker plot). Numerical variables with gaussian (normal) distribution will be described as mean and standard deviation (SD), whereas median and interquartile range (IQR) will be used to describe variables with non-gaussian distribution.

Comparison of the participants' baseline characteristics (between the intervention and control group) will be made using independent sample t-test or Mann-Whitney U test (for numerical variables depending on the shape of distribution) and Chi-squared test or Fisher exact test (for categorical variables depending on the presence of small cells).
For all the study outcome (primary and secondary outcomes), independent T- test (inter-group comparison, Paired T-test (intra group-comparison) and repeated measures analysis of variances (ANOVA) will be conducted to determine whether there is any significant 1) within group changes over time (time effect), 2) overall between group differences (treatment effect), and 3) between group differences at each time level (time-treatment interaction effects). All the estimated will be reported as the estimated marginal means with its adjusted 95% Confidence Intervals. The 2-tailed p-values of less than 0.05 will be considered as significant.

### 6.11.1 Approaches to Deal with Missing Data

In this study, the Expectation and Maximization (EM) algorithm will be used to handle the missing data. EM is commonly used in various applications especially when dealing with data that has missing values. The SPSS software through the Expectation and Maximization (EM) algorithm will be utilized to impute good values for missing data.

## 7.0 SAFETY MEASUREMENTS.

The safety evaluation will be determined by the incidence of adverse events and the definition of adverse events (AE)s and serious adverse events (SAEs) are as follows: An adverse event (AE) is defined as any untoward medical occurrence (including clinically significant laboratory findings) in a patient or clinical investigation subject administered a pharmaceutical drug, and which did not necessarily have a causal relationship to the treatment. Adverse events may include:

1. The significant worsening of the disease or symptoms of the disease under investigation following the administration of the drug.

2. Any undercurrent illness with an onset after administration of the drug.

3. Exacerbation (i.e increase in frequency or intensity) of a pre-existing condition or event.

A serious adverse event (SAE) is defined as any untoward medical occurrence that at any dose of the investigational product, that fulfills one or more of the following criteria:

1. Results in death

2. Is life threatening. A 'life-threatening' adverse event refers to an event, which puts the patient at risk of death. It does no refer to an event, which hypothetically might cause death if it is more severe.

3. Requires in-patient hospitalization or prolongation of existing hospitalization. Hospitalization is defined as the patient being hospitalized overnight, or the patient's hospital stay being prolonged for at least an additional overnight stay. Hospital admissions for elective surgery, for social reasons or for normal disease management procedures that are not the result of worsening an underlying condition will not be considered a serious adverse event.

4. Results in persistent or significant disability/incapacity

5. Is a congenital anomaly / birth defect.

6. Is a malignancy.

7. Is the result of an overdose? An important medical event jeopardizes the patient and may require medical or surgical intervention to prevent one of the above outcomes from occurring.

**6.11 Statistical Analysis**

Data entry and analyses will be conducted using SPSS for windows version 27. Prior to analysis, the data will be explored to examine the pattern of missing data and appropriate method of missing data imputation will be executed. Both Intention-to-treat and per-protocol analysis will be conducted.

The numerical variables' shape of distribution will be examined both quantitatively (by examining the coefficient of variation, skewness value, kurtosis value, Kolmogorov-Smirnov test, and Shapiro-Wilk test) and qualitatively (by examining the histogram with overlaid normal curve, Q-Q plot, and the Box and Whisker plot). Numerical variables with gaussian (normal) distribution will be described as mean and standard deviation (SD), whereas median and interquartile range (IQR) will be used to describe variables with non-gaussian distribution.

Comparison of the participants' baseline characteristics (between the intervention and control group) will be made using independent sample t-test or Mann-Whitney U test (for numerical variables depending on the shape of distribution) and Chi-squared test or Fisher exact test (for categorical variables depending on the presence of small cells).

For all the study outcome (primary and secondary outcomes), independent T- test (inter-group comparison, Paired T-test (intra group-comparison) and repeated measures analysis of variances (ANOVA) will be conducted to determine whether there is any significant 1) within group changes over time (time effect), 2) overall between group differences (treatment effect),

and 3) between group differences at each time level (time-treatment interaction effects). All the estimated will be reported as the estimated marginal means with its adjusted 95% Confidence Intervals. The 2-tailed p-values of less than 0.05 will be considered as significant.

### 6.11.1 Approaches to Deal with Missing Data

In this study, the Expectation and Maximization (EM) algorithm will be used to handle the missing data. EM is commonly used in various applications especially when dealing with data that has missing values. The SPSS software through the Expectation and Maximization (EM) algorithm will be utilized to impute good values for missing data.

## 7.0 SAFETY MEASUREMENTS.

## 8.0 ETHICAL ISSUE

The information regarding this study will be provided to all subjects involved. Informed consent will be taken from the respondents. The confidentiality of the subject will be maintained throughout this study. This study will obtain ethical approval from an ethic committee of Universiti Sains Malaysia.

## 9.0 BUDGET

This study will be sponsored by the BREGO life sciences Sdn. Bhd. Company

## 10.0 ETHICAL STATEMENT

### 10.1 Vulnerability

This group of participants is considered a vulnerable group. However, participation in this study is entirely voluntary. Participants may refuse to take part in this study, or they may stop participation in this study at any time, without a penalty or loss of benefits to which the participant is otherwise entitled. Their participation in this study also will be anonymous. They also will be brought to a separate room to answer the questionnaires.

### 10.2 Declaration of absence of conflict of interest

Researcher declared that one has no conflict of interest in this study in any form. The treating physician is not a part of the research team. If the patient refused to participate in this study, it will not affect the standard of care of the patient.

### 10.3 Privacy and confidentiality

Respondents will be told that the information provided is confidential and their identities are not revealed in association with the information they provided. All forms are anonymous and

will be entered into SPSS software. Only research team members can enter the data. Data will be presented as grouped data and will not identify the respondents individually.

**10.4 Possible Benefit (Community Sensitives & Benefits)**

Findings of the study are very important to determine the effectiveness of fixed dose combination of alpha lipoic acid and vitamin B in comparison placebo for treatment of diabetic polyneuropathy in type 2 diabetes mellitus patients. It will help in managing diabetes polyneuropathy by giving more effective options to the physicians and patients to choose the best treatment in treating diabetic polyneuropathy.

**10.5 Honorarium and incentives:**

Honorarium will be given to both group with a value of RM50 for each visit.

**10.6 Other Ethical review board approval**

Not applicable

**10.7 Data destruction will be done at the end of the study.**

**10.8 Conflict of interest**

There is no conflict of interest related to sponsorship, data analysis and publication

**10.9 Collaborative study terms of reference**

This study is an industry sponsored research by BREGO Life Sciences Sdn Bhd company.

**10.10 Ethical approval will be obtained from approval by the Jawatankuasa Etika Penyelidikan Manusia (JEPeM) Universiti Sains Malaysia**

**10.11 The study already get approval from National Medical Research Register (NMRR) on 18.12.2023**

**11.0 EXPECTED RESULTS**

**Table 1: Sociodemographic data of the respondents (*n*=76)**

| Variables | Frequency (%) | Mean |
|---|---|---|
| Age (year) | | |
| Gender | | |
| Male | | |
| Female | | |

| | | |
|---|---|---|
| Race | | |
| Malay | | |
| Chinese | | |
| Indian | | |
| Others | | |
| Education | | |
| No formal education/primary school | | |
| Secondary school | | |
| Tertiary educational level | | |
| Occupation | | |
| Employed | | |
| Unemployed | | |
| Monthly Income (RM) | | |
| Marital status | | |
| Single/divorce/widow | | |
| Married | | |
| Diagnosis of DM (year) | | |
| Medication | | |
| Metformin | | |
| Glicazide | | |
| SGLT2i | | |
| DPP4I | | |
| Insulin | | |
| others | | |
| Comorbidities | | |
| Retinopathy | | |
| IHD/MI | | |
| Hyperlipidemia | | |
| Hypertension | | |
| Others | | |

**Table 2: Baseline biochemical and clinical characteristics of participants(*n*=76)**

| Variable | Mean (SD) | | | | |
|---|---|---|---|---|---|
| | Control | Intervention | Mean diff(95%CI) | t-stat (df) | *p*-value* |
| Height | | | | | |
| Weight (kg) | | | | | |
| BMI | | | | | |
| SBP (mmHg) | | | | | |
| DBP (mmHg) | | | | | |
| FBS (mmol/L) | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| HbA1c (%) | | | | | |
| TC (mmol/L) | | | | | |
| LDL (mmol/L) | | | | | |
| HDL (mmol/L) | | | | | |
| TG (mmol/L) | | | | | |
| Creatinine | | | | | |
| Urea | | | | | |
| AST | | | | | |
| ALT | | | | | |
| ALP | | | | | |
| Score DQoL | | | | | |

*Independent T-test

**Expected result for objective 1:** To compare the mean change in total symptom score (TSS) and neuropathic symptoms score (NSS) over 12 a week period between diabetic polyneuropathy patients taking a fixed dose combination of alpha lipoic acid and vitamin B preparations versus diabetic polyneuropathy patients taking placebo (inter-group comparison)

**Table 3: Comparison of total symptom score (TSS) score between control and intervention group(n=76)**

| **Comparison between TSS versus Group: F-Stat (df); *p-value*** [a] | | | | |
|---|---|---|---|---|
| | Mean (SD) | Mean diff (95%CI) | T-Statistic (df) | *p*-value[b] |
| Total symptom score at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| Total symptom score at 6 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| Total symptom score at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| | | | | |

*Significant at 0.05
a: Repeated measures ANOVA was applied
b: Independent Sample T-test

**Table 4: Comparison of neuropathic symptoms score (NSS) score between control and intervention group(*n*=76)**

| **Comparison between NSS versus Group: F-Stat (df); *p-value* [a]** | | | | |
|---|---|---|---|---|
| | Mean (SD) | Mean diff (95%CI) | T-Statistic (df) | *p*-value[b] |
| Neuropathic symptoms score at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| Neuropathic symptoms score at 6 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| Neuropathic symptoms score at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |

*Significant at 0.05
a: Repeated measures ANOVA was applied
b: Independent Sample T-test

**Expected result for objective 2:** To compare the mean change in total symptom score (TSS) and neuropathic symptoms score (NSS) among diabetic polyneuropathy patients at baseline and 12 weeks after taking a fixed dose combination of alpha lipoic acid and vitamin B preparations (intra-group comparison)

**Table 5: Comparison of TSS and NSS score within the intervention group (*n*=38)**

| | Mean (SD) | | | *F* -statistic *(df)* | *p*- value[a,*] |
|---|---|---|---|---|---|
| | Baseline (TSS_b) | 6 weeks (TSS_6) | 12 weeks (TSS_12) | | |
| **TSS** | | | | | |
| Total score | | | | | |
| Pain | | | | | |
| Burning | | | | | |
| Paraesthesia | | | | | |
| Numbness | | | | | |
| Multiple Comparison [TSS_b(mean(SD)]vs. [TSS_6(mean(SD)] :p-value [b]<br>[TSS_b(mean(SD)]vs. [TSS_12(mean(SD)] : p-value[b]<br>[TSS_6(mean(SD)]vs. [TSS_12(mean(SD)]: p-value[b] | | | | | |
| | Mean (SD) | | | *F* -statistic *(df)* | *p*- value[a,*] |
| | Baseline (NSS_b) | 6 weeks (NSS_6) | 12 weeks (NSS_12) | | |
| **NSS score** | | | | | |
| Multiple Comparison [NSS_b(mean(SD)]vs. [NSS_6(mean(SD)] :*p*-value[b]<br>[NSS_b(mean(SD)]vs. [NSS_12(mean(SD)] : *p*-value[b]<br>[NSS_6(mean(SD)]vs. [NSS_12(mean(SD)]: *p*-value[b] | | | | | |

*Significant at 0.05
a: Repeated measures ANOVA was applied.

b: Paired samples T Test
MulivariateNormality Assumption is fulfilled.

**Expected result for objective 3:** To compare changes and differences in fasting plasma glucose, HbA1C level, fasting lipid profile, body mass index (BMI), blood pressure and diabetes quality of life over 12 a week period between diabetic polyneuropathy patients taking a fixed dose combination of alpha lipoic acid and vitamin B preparations and in diabetic polyneuropathy patients taking placebo (inter-group comparison).

**Table 6: Comparison of fasting plasma glucose (FBS) and HbA1C level and fasting lipid profile between control and intervention group (*n*=76)**

| | Mean (SD) | Mean diff (95%CI) | T-Statistic (df) | *p*-value[a] |
|---|---|---|---|---|
| FBS level at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| FBS level at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| | | | | |
| HbA1C level at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| HbA1C level at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| | | | | |
| TC (mmol/L) level at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| TC (mmol/L) level at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| | | | | |
| LDL (mmol/L) level at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| LDL (mmol/L) level at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| | | | | |
| HDL (mmol/L)level at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| HDL (mmol/L) level at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| | | | | |

| | | | | |
|---|---|---|---|---|
| TG (mmol/L)level at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| TG (mmol/L)level at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| | | | | |
| BMI level at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| BMI level at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |

*Significant at 0.05
a: Independent Sample T-test

**Table 7: Comparison of diabetes quality of life between control and intervention group (*n*=76)**

| **Comparison between diabetes quality of life versus Group: F-Stat (df); *p-value*** [a] | | | | |
|---|---|---|---|---|
| | Mean (SD) | Mean diff (95%CI) | t-Statistic (df) | *p*-value[b] |
| Diabetes quality of life at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| Diabetes quality of life at 6 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| Diabetes quality of life at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |

*Significant at 0.05
a: Repeated measures ANOVA was applied.
b: Independent Sample T-test

**Table 8: Comparison of body mass index between control and intervention group (*n*=76)**

| **Comparison between diabetes quality of life versus Group: F-Stat (df); *p-value*** [a] | | | | |
|---|---|---|---|---|
| | Mean (SD) | Mean diff (95%CI) | t-Statistic (df) | *p*-value[b] |
| BMI level at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| BMI level at 6 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| BMI level at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |

*Significant at 0.05
a: Repeated measures ANOVA was applied.
b: Independent Sample T-test

**Table 9: Comparison of blood pressure between control and intervention group (*n*=76)**

| **Comparison between diabetes quality of life versus Group: F-Stat (df); *p-value*** [a] | | | | |
|---|---|---|---|---|
| | Mean (SD) | Mean diff (95%CI) | t-Statistic (df) | *p*-value[b] |
| BP level at baseline | | | | |
| Control | | | | |
| Intervention | | | | |
| BP level at 6 weeks | | | | |
| Control | | | | |
| Intervention | | | | |
| BP level at 12 weeks | | | | |
| Control | | | | |
| Intervention | | | | |

*Significant at 0.05
a: Repeated measures ANOVA was applied.
b: Independent Sample T-test

**Expected result for Objective 4:** To compare the changes in the safety parameters of the renal function and liver function tests among diabetic polyneuropathy patients at baseline and 12 weeks after taking a fixed dose combination of alpha lipoic acid and vitamin B preparations (intra-group comparison).

**Table 10: Comparison of renal function and liver function tests within intervention group (*n*=38)**

| | Mean (SD) | | t-statistic *(df)* | *p*- value[a,*] |
|---|---|---|---|---|
| | Baseline | 12 weeks | | |
| Creatinine | | | | |
| Urea | | | | |
| AST | | | | |
| ALT | | | | |
| ALP | | | | |

*Significant at 0.05
a: Paired samples T Test

**Expected result for Objective 5:** To compare changes and differences of fasting plasma glucose, HbA1C level, fasting lipid profile, body mass index (BMI), blood pressure and diabetes quality of life among diabetic polyneuropathy patients at baseline and 12 weeks after taking a fixed dose combination of alpha lipoic acid and vitamin B preparations (intra-group comparison).

**Table 11: Comparison of fasting plasma glucose, HbA1C level, and fasting lipid profile within intervention group (*n*=38)**

| | Mean (SD) | | t-statistic *(df)* | *p*- value[a,*] |
|---|---|---|---|---|
| | Baseline | 12 weeks | | |
| Fasting plasma glucose | | | | |
| HbA1C level | | | | |
| Fasting lipid profile | | | | |
| TC (mmol/L) | | | | |
| LDL (mmol/L) | | | | |
| HDL (mmol/L) | | | | |
| TG (mmol/L) | | | | |

*Significant at 0.05
a: Paired samples T Test

**Table 12: Comparison of diabetes quality of life within intervention group(*n*=38)**

| | Mean (SD) | | | *F* -statistic *(df)* | *p*- value[a,*] |
|---|---|---|---|---|---|
| | Baseline (DQoL_b) | **13** weeks (DqoL_6) | 12 weeks (DqoL_12) | | |
| **Diabetes quality of life (DqoL)** | | | | | |
| Multiple Comparison [DqoL_b (mean(SD)]vs. [DqoL_6)(mean(SD)] :p-value [b]<br>[DqoL_b (mean(SD)]vs. [DqoL_12 (mean(SD)] : p-value[b]<br>[DqoL_6 (mean(SD)]vs. [DqoL_12 (mean(SD)]: p-value[b] | | | | | |

*Significant at 0.05
a: Repeated measures ANOVA was applied
b: Paired samples T Test
MulivariateNormality Assumption is fulfilled.

**Table 13: Comparison of body mass index within intervention group(*n*=38)**

| | Mean (SD) | | | *F* -statistic *(df)* | *p*- value[a,*] |
|---|---|---|---|---|---|
| | Baseline (BMI_b) | **13** weeks (BMI _6) | 12 weeks (_BMI 12) | | |
| **Body Mass Index (BMI)** | | | | | |
| Multiple Comparison [BMI _b (mean(SD)]vs. [BMI_6)(mean(SD)] :p-value [b]<br>[BMI _b (mean(SD)]vs. [BMI _12 (mean(SD)] : p-value[b]<br>[BMI _6 (mean(SD)]vs. [BMI _12 (mean(SD)]: p-value[b] | | | | | |

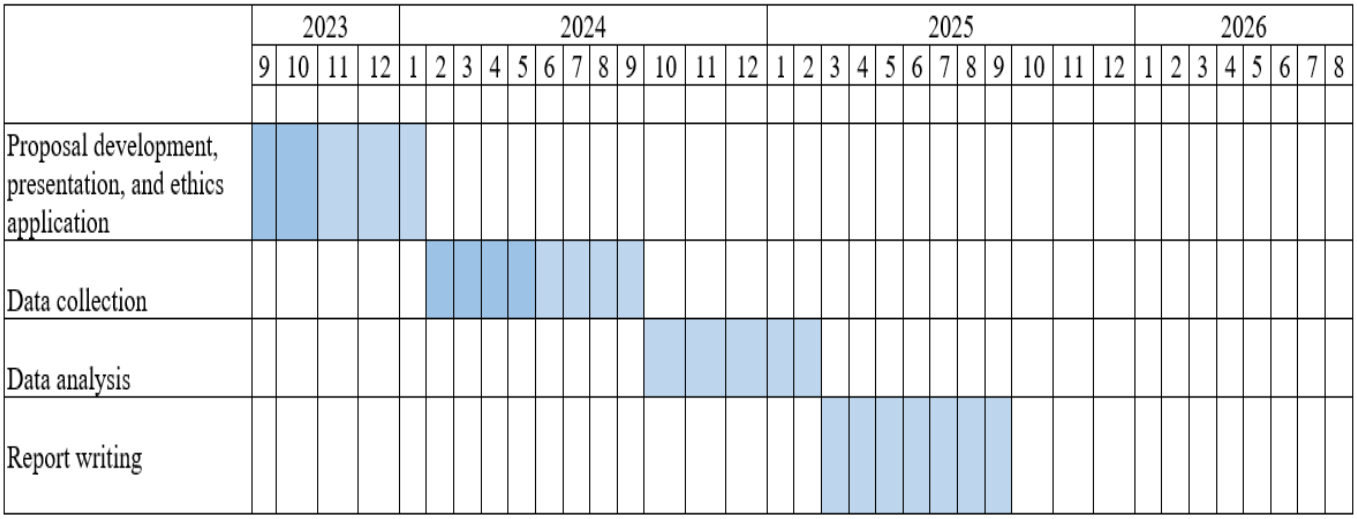
*Significant at 0.05
a: Repeated measures ANOVA was applied
b: Paired samples T Test
MulivariateNormality Assumption is fulfilled.

**Table 14: Comparison of blood pressure within intervention group(*n*=38)**

| | Mean (SD) | | | *F* -statistic *(df)* | *p*- value[a,*] |
|---|---|---|---|---|---|
| | Baseline (BP _b) | **13** weeks (BP_6) | 12 weeks (_BP 12) | | |
| **Blood pressure (BP)** | | | | | |
| Multiple Comparison [BP _b (mean(SD)]vs. [BP_6)(mean(SD)] :p-value [b]<br>[BP _b (mean(SD)]vs. [BP _12 (mean(SD)] : p-value[b]<br>[BP _6 (mean(SD)]vs. [BP _12 (mean(SD)]: p-value[b] | | | | | |

*Significant at 0.05
a: Repeated measures ANOVA was applied.
b: Paired samples T Test
MulivariateNormality Assumption is fulfilled.

## 12.0 GANTT CHART




## 13.0 MILESTONE

| | Plan | Date |
|---|---|---|
| 1 | Proposal development | August – November 2023 |
| 2 | Ethic submission | Nov 2023 – Jan 2024 |
| 3 | Data collection | Feb – Sept 2024 |
| 4 | Data analysis | Oct – Feb 2025 |
| 5 | Report writing | Mac – September 2025 |

## 14.0 BUDJET

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