UNIVERSITI KEBANGSAAN MALAYSIA

The National University of Malaysia

Title: The Effect of Palm Tocotrienol Rich Fraction on Alcoholic Fatty Liver Disease (AFLD): A Phase II Clinical Trial

Version 2 (Date: 30/11/2023)

Researchers:

Dr. Siti Norain Azahar (M.D.)

Principal Investigator

Professor Dr. Nur Azlina Mohd Fahami (DVM, MMed Sc, PhD) (UKM)

Co-investigators

Dr. Zaida Zainal (PhD)

Associate Professor Dr. Chin Kok Yong (PhD) (UKM)

Investigator (Clinical)

Dr. Deborah Chew Chia Hsin (MBChb (Aberdeen, UK) MRCP) (UKM)

Industrial Collaborators:

1. Malaysian Palm Oil Board

No.6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang Selangor

2. ExcelVite Sdn Bhd

Lot 56442, 7½ Mile

Jalan Ipoh/Chemor 31200, Chemor, Perak

3. Hovid Berhad

121, Jalan Tunku Abdul Rahman,

30010 Ipoh, Perak.

Research Locations:

1. Hospital Canselor Tuanku Muhriz Universiti Kebangsaan Malaysia
2. Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia

THE EFFECT OF PALM TOCOTRIENOL RICH FRACTION ON ALCOHOLIC FATTY LIVER DISEASE (AFLD): A PHASE II CLINICAL TRIA

ABSTRACT

Background: Alcohol abuse is a serious public health concern that is associated with many diseases, including alcoholic liver disease (ALD). The World Health Organization (WHO) reported that the use of alcohol has resulted in more than 3 million deaths every year and accounting for 5.9% of all deaths worldwide. As the metabolism of alcohol in individuals mainly depends on the liver, long-term and excessive alcohol consumption damages the liver leading to alcoholic fatty liver disease (AFLD). Since tocotrienols have been proven for their hepatoprotective effects in animal models of NAFLD, the aim of the study is to explore the potential role of tocotrienol rich fraction, in patients with AFLD.

Methodology: 26 patients with AFLD aged 18 to 65 years old, will be randomized into treatment (n=13) and placebo group (n=13). The treatment group will be prescribed with palm tocotrienol soft gel (200 mg twice daily) while the placebo group will receive refined, bleached, and deodorised (RBD) palm olein for six months. The subjects will be answering questionnaires related to alcohol consumption and their blood will be collected for the determination of liver function test, redox, haematological profile and inflammatory biomarkers. Fibroscan and FibroTest will be done twice during the 3-monthly follow-ups.

Significance: This study will validate the efficacy of tocotrienol in AFLD and its related morbidity, thereby producing data for subsequent phases of clinical trials. The findings of this study will also enhance the medicinal values of palm tocotrienol and diversify the usage of palm-based products in sustaining the Malaysian palm industry.

General Information

Protocol title: The Effect of Palm Tocotrienol Rich Fraction on Alcoholic Fatty Liver Disease (AFLD): A Phase II Clinical Trial

Protocol identifying number and date: PT3_AFLD; Version Number: 02; 30/11/2023

Amendment number(s): Version 2

Name and address of the sponsors: Malaysian Palm Oil Board
No.6, Persiaran Institusi, Bandar Baru Bangi, 43000
Kajang Selangor

ExcelVite Sdn Bhd (Ms. Chan Yuen Teng)
Lot 56442, 7½ Mile
Jalan Ipoh/Chemor
31200, Chemor, Perak

Hovid Berhad (Mr David Ho, Ms Michelle Ho)
121, Jalan Tunku Abdul Rahman,
30010 Ipoh, Perak.

Name and title of the person authorized to sign the protocol and the amendment(s):
Professor Dr. Nur Azlina Mohd Fahami (DVM, MMed Sc, PhD) (UKM)

Name and title of the investigator(s):
Project Principal Investigator: Professor Dr. Nur Azlina Mohd Fahami (DVM, MMed Sc, PhD) (UKM)
Co-investigators:
Dr. Zaida Zainal (PhD)
Associate Professor Dr. Chin Kok Yong (PhD) (UKM)
Dr. Deborah Chew Chia Hsin (MBChb (Aberdeen, UK) MRCP) (UKM)

PhD Candidate: Dr. Siti Norain Azahar

Trial site:
Address: Hospital Canselor Tuanku Muhriz
Universiti Kebangsaan Malaysia
Jalan Yaacob Latif, Bandar Tun Razak,
56000, Cheras, Kuala Lumpur
Telephone Number: 03-91455555

Other department(s) and/or institutions involved in the trial:
Name: Department of Pharmacology
Address: Faculty of Medicine, Universiti Kebangsaan Malaysia
Jalan Yaacob Latif, Bandar Tun Razak,
56000, Cheras, Kuala Lumpur

PROJECT SYNOPSIS

Alcoholic misuse has resulted in 3.3 million deaths every year according to the World Health Organization (WHO) report on noncommunicable diseases [1]. Meanwhile, the National Health and Morbidity Survey (NHMS) 2019 data shows that the prevalence of current drinkers among Malaysian adults was 11.8%, of which 17.6% were risky drinkers [2]. Alcoholic liver disease has caused an estimated 48% of deaths from cirrhosis. The most common health problem associated with chronic alcoholism is alcoholic liver disease (ALD) [1]. Chronic alcohol abuse can result in a spectrum of liver injury that ranges from mild fatty infiltration to cirrhosis and hepatocellular carcinoma [3]. Fat accumulation in the liver cells is the earliest and most predictable response to alcoholic ingestion and is seen in 90% of heavy drinkers. The development of necroinflammation and fibrosis (alcoholic hepatitis or steatohepatitis) occurs in 10 to 35% of heavy drinkers [4]. It is important to recognize alcoholic hepatitis as patients with severe disease have extremely high short-term mortality rates, they can also develop portal hypertension in the absence of cirrhosis and the development of alcoholic hepatitis is a well-documented precursor for cirrhosis with long term risk 9 times higher than that for patients with fatty liver alone [1].

Pathogenesis of alcoholic hepatitis:

The liver is the main organ responsible for ethanol metabolism. Ethanol is metabolized by 3 major systems in the liver: alcohol dehydrogenase (ADH), cytochrome p450 2EI (CYP2EI) and finally catalase. ADH and CYP2EI convert ethanol to acetyldehyde which is then converted by aldehyde dehydrogenase (ALDH) to acetate. Acetyldehyde is a highly reactive and potentially toxic compound that is responsible for the many toxic effects of alcohol such as nausea, headaches and flushing.

Acetyldehyde is also postulated to play an etiologic role in alcoholic liver disease as it forms adducts with reactive residues on proteins or small molecules like cysteine [5]. These chemical modifications can alter or interfere with normal biological processes resulting in formation of substances such as acetyldehyde and malondialdehyde (MAA). The formation of MAA antigens during alcohol-induced liver disease likely contributed to the immunologic reaction associated with alcohol related liver damage [6]. Acetyldehyde has also been shown to impair mitochondrial glutathione transport and to sensitize hepatocytes to TNF-mediated killing. Lastly, acetyldehyde distrupts intestinal barrier function leading to endotoxemia and proinflammatory cytokine production.

Oxidative stress in the liver also increases after alcohol consumption. In hepatocytes, CYP2EI activity increases after alcohol consumption [7]. The CYP2EI system leaks electrons which initiate the oxidative stress [8]. Other sources of oxidative stress are infiltrating inflammatory cells in the liver. Oxidative stress in alcoholic liver disease is documented by detection of protein oeixation (eg: protein thiol or carbonyl products), lipid oxidation (eg: MAA), DNA oxidation (eg: oxodeoxyguanosine) or depletion of antioxidant defences (eg: vitamin E, glutathione, thioredoxin) [8]. Therefore, antioxidant supplementation could be a useful adjunct in the treatment of alcoholic hepatitis.

Diagnosis of alcoholic liver disease:

AFLD is currently defined by steatosis with a range of hepatic histopathologic changes including the presence of inflammatory infiltrates and varying degrees of fibrosis and cirrhosis that develop in the presence of chronic alcohol consumption, which is a known trigger factor that causes fat accumulation. Despite having comparable pathophysiological spectrum, the distinction between AFLD and NAFLD is determined by the amount of alcohol ingested, with AFLD being diagnosed based on the patient's history of regular alcohol usage, defined as >30 g/day in males and >20 g/day in females (according to clinical practice guidelines from the scientific associations for the study of liver diseases) [9, 10].

Apart from a reliable history of significant alcohol intake, no single laboratory or imaging testing can definitively confirm the diagnosis of ALD. Hence, its diagnosis is based on the compilation of clinical findings of hepatomegaly with signs of chronic liver disease, radiographic evidence of liver steatosis, fibrosis or cirrhosis, combined with abnormal biochemical results suggestive of liver injury [11].

Meanwhile, NAFLD is closely linked to obesity, caused by excessive food intake with a high-calorie, high-fat diet combined with a sedentary lifestyle [12, 13]. Most often, it is also associated with metabolic dysfunction [11, 14]. Obese people are more likely to develop

metabolic syndrome, and nonalcoholic fatty liver disease (NAFLD) has been identified as the hepatic manifestation of metabolic syndrome [15].

Therefore, in the context of this study, we propose to exclude the subjects who are obese (a body mass index (BMI) of 30 kg/ m^2 or higher) and with metabolic syndrome (based on the harmonised criteria) to minimise the risk of misclassification between AFLD and NAFLD.

Clinical evidence of decompensated liver disease such as ascites and its severity, hepatic encephalopathy; severity according to West Haven criteria, jaundice and coagulopathy must be assessed for. Evidence of alcoholic hepatitis as demonstrated by hepatocellular transaminitis of AST:ALT > 2:1 due to deficiency of pyridoxal 5'phosphate level in alcoholic patients, raised gamma glutamyl transpeptidase (GGT) raised mean corpuscular volume (MCV), raised total bilirubin with severely ill patients having coagulopathy as demonstrated by raised international normalised ratio (INR) and prothrombin time (PT). Patients with other forms of bleeding disorder, as well as those on anticoagulant or antiaggregant treatments are not eligible to participate in the trial. Patients who have been taking vitamin E, herbal supplements or other investigational products are also excluded.

Other types of liver disease, such as hepatitis B, hepatitis C and autoimmune hepatitis, hereditary hemochromatosis and non-alcoholic fatty liver disease will also need to be excluded. Assessment of the presence of advanced fibrosis or cirrhosis can be accessed via Fibroscan as well as FibroTest (FT) which is a serum marker used to calculate the degree of liver fibrosis. Patients should also be screened for hepatocellular carcinoma (HCC), a complication that can occur in alcoholic cirrhosis. Patients who are found to have HCC should be excluded from the study. Patients who have other coexisting chronic liver diseases (CLD) will not be eligible for recruitment as the presence of the CLD can confound the prognosis of alcoholic hepatitis. Once other chronic liver diseases have been ruled out, the severity of alcoholic liver disease will then be graded according to Maddrey's discriminant function.

The pathogenesis of ALFD involves oxidative stress and disruptions of lipid metabolism [7]. It also has been demonstrated that the response to hypoxia plays an essential role in the development of ALFD [8] and this in turn has been linked to mitochondrial radical oxygen species (ROS) generation. Currently, the most effective AFLD treatment is alcohol abstinence and there are still few effective pharmacological treatments for patients afflicted with this disease. Therefore, new therapies are urgently needed to prevent the progression of AFLD.

Background Information of the investigational product

Vitamin E are well known for its potent antioxidant capabilities, while studies had also shown its positive effects on inflammation. Animal study had also shown the protective effect of palm tocotrienol on the liver [16]. The most common homologue of the vitamin E family investigated in treating liver disease is alpha tocopherol. Other homologues of vitamin E, namely tocotrienols, also exist in isomers designated as alpha, beta, gamma and delta, and they differentiate from analogous isomers of tocopherol by the presence of an unsaturated phytyl chain ('tail') as opposed to a saturated one in tocopherols [17]. Tocotrienols are preferentially distributed to the liver [18, 19] and alpha-tocotrienol has been reported to be 40-60 times more potent than alpha-tocopherol against lipid peroxidation in rat liver microsomes [20].

Several clinical studies have been conducted to evaluate the tocotrienol effects on the liver. All these studies have demonstrated the hepatoprotective effects of palm tocotrienols in their adult subjects. According to Patel et al. (2012) [29], tocotrienol attenuated the time-dependent rise in the Model for End Stage Liver Disease (MELD) score vs the alpha-tocopherol group in liver transplant subjects with end-stage liver disease. Magosso et al. (2013) [30] have demonstrated that in subjects with mild untreated hypercholesterolemia and ultrasound-proven non-alcoholic fatty liver (NAFLD), there is a normalization of the hepatic echogenic response in the tocotrienol-treated group vs. the placebo group.

There is also a significant reduction in aspartate aminotransferase (AST), alanine aminotransferase (ALT), malondialdehyde (MDA), and C-reactive protein (CRP) among subjects with ultrasound-proven non-alcoholic fatty liver (NAFLD) in the tocotrienol group vs. the placebo group, as reported by Pervez et al. (2018) [31]. These findings are in agreement with the outcomes of following study by Pervez et al. (2020) [21]. In their study subjects with NAFLD, tocotrienol significantly reduced AST, ALT, MDA, CRP, fatty liver index (FLI), and improved grading of hepatic steatosis on ultrasound, compared to the placebo group.

Tocotrienols are generally safe and are usually well tolerated. By participating in this research, it is possible that the treatment given to the subjects may or may not help improve their liver function. There is a risk that their condition will not get better and that the intervention won't work. The treatment given to them are usually in low doses and are unlikely to cause serious side-effects. However, in patients with bleeding disorders, tocotrienols might slow blood clotting. Tocotrienols may also interact with "blood thinners" such as warfarin and antiaggregants like aspirin, increasing the risk of bleeding. Thus, we advise against taking the treatment with these medications. Symptoms like nausea, diarrhea, stomach cramps, tiredness, dizziness, and blurred vision may rarely occur in cases of overdose. Allergic reactions to this supplement are rare; however, if they develop any symptoms of a serious allergic reaction, including rash, itching or swelling (especially of the face, tongue, or throat), dizziness, or trouble breathing, they should tell the doctor and get medical help right away. Venous blood taking would require a needle to pierce through their skin, which might also result in bruising and mild pain.

This trial will be conducted in compliance with the protocol, GCP and the applicable regulatory requirement(s). The population to be studied is adult patients aged 18-65 years, with a history of alcohol use disorder, who could comply with alcohol abstinence, and those with diagnosis of AFLD with lab evidence of Alcoholic Steatohepatitis (AST:ALT >2.0, elevated GGT) who come for follow up in Gastroenterology & Hepatology clinic in Hospital Canselor Tuanku Muhriz UKM (HCTM). Additionally, our research subjects were also recruited from the general public who responded to our recruitment poster on social media platforms.

Research Gap

Concerns about the high prevalence of AFLD globally and the not fully understood reasons for its progression to more severe forms of liver diseases have led to the initiation of this interventional, double-blind placebo-controlled study. The aim of the investigation was to

evaluate the activity of tocotrienols in normalising hepatic echogenic response in adults with AFLD.

Currently, there are no effective treatment for alcoholic fatty liver disease apart from alcohol abstinence and making some lifestyle changes to control or reverse the fat build-up in the liver. An antioxidant and anti-inflammatory agent are speculated to enhance the healing of liver after alcohol abstinence, but this concept has not been effectively demonstrated in a clinical trial. Therefore, new therapies are urgently needed to prevent the progression of AFLD. Tocotrienol are well established for its potent antioxidant capabilities, while evidence had also shown its positive effects on inflammation. Thus, this study will validate the efficacy of tocotrienol in AFDL and its related morbidity, thereby producing data for subsequent phases of clinical trials. The findings of this study will also enhance the medicinal values of palm tocotrienol and diversify the usage of palm-based products in sustaining the Malaysian palm industry.

SCIENTIFIC IMPACT

The health benefits of TRF in relation to liver health have been reported in vitro, in vivo and even human clinical studies. However, these clinical studies were conducted on subjects with liver cirrhosis and non-alcoholic fatty liver disease (NAFLD). To date, no data is available on human intervention studies involving TRF in alcoholic fatty liver disease (AFLD). It's important to carry out this clinical trial to find out if TRF would show the similar protective effect on subjects with AFLD, as it did with NAFLD.

In addition, the findings from this study could serve as the basis for future clinical studies related to liver diseases.

SOCIAL IMPACT

This project outcome will give a significant impact to social and economy, locally and worldwide. Alcohol consumption is a major risk factor for chronic disease. Based on 58 studies from 17 Global Burden of Diseases (GBD) regions, alcohol use disorders accounted for 9.6% (7.7%-11.18%) of age-standardized disability-adjusted life years (DALYs) worldwide in 2010 [26]. Alcohol-induced liver cirrhosis was responsible for 0.9% of all global deaths and 47.9% of all liver cirrhosis deaths in 2010 [27].

Palm Oil is the largest agricultural contributor to Malaysia's gross domestic product (GDP) with a total of RM44. 8 billion or 3.8 per cent of the GDP contribution in 2017 [28]. Malaysia is the largest tocotrienols manufacturer and the second largest palm oil producer in the world. The beneficial effect of Palm TRF in AFLD would encourage the local and global usage of Palm TRF and Palm Oil (the richest source of TRF) in pharmaceutical, dietary supplement, and functional food and drinks.

Meantime, there is no drug or treatment for AFLD at the moment. Palm TRF can be the potential adjuvant therapy to help the AFLD subjects in potentially maintaining healthy liver function. This project is also expected to produce a postgraduate student who will develop a strong background in clinical trial.

REFERENCES:

1. Liver EAftSot. EASL Clinical Practice Guidelines: Management of alcohol-related liver disease. Journal of hepatology 2018;69: 154-181.

2. Institute for Public Health (IPH), National Institutes of Health, Ministry of Health Malaysia. 2020. National Health and Morbidity Survey (NHMS) 2019: Vol. I: NCDs – Non-Communicable Diseases: Risk Factors and other Health Problems.

3. Chacko KR, Reinus J. Spectrum of alcoholic liver disease. Clinics in liver disease 2016;20: 419-427.

4. Deleuran T, Grønbaek H, Vilstrup H, Jepsen P. Cirrhosis and mortality risks of biopsy-verified alcoholic pure steatosis and steatohepatitis: a nationwide registry-based study. Alimentary pharmacology & therapeutics 2012;35: 1336-1342.

5. Setshedi M, Wands JR, de la Monte SM. Acetaldehyde adducts in alcoholic liver disease. Oxidative medicine and cellular longevity 2010;3.

6. Rolla R, Vay D, Mottaran E, et al. Detection of circulating antibodies against malondialdehyde-acetaldehyde adducts in patients with alcohol-induced liver disease. Hepatology 2000;31: 878-884.

7. Nagata K, Suzuki H, Sakaguchi S. Common pathogenic mechanism in development progression of liver injury caused by non-alcoholic or alcoholic steatohepatitis. J Toxicol Sci. 2007;32(5):453–68.

8. Nath B, Levin I, Csak T, Petrasek J, Mueller C, Kodys K, et al. Hepatocyte-specific hypoxia- inducible factor-1α is a determinant of lipid accumulation and liver injury in alcohol-induced steatosis in mice. Hepatology. 2011;53(5):1526–37.

9. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. (2012) 55:2005–23.

10. European Association for the Study of the Liver, European Association for the Study of Diabetes, European Association for the Study of Obesity. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. (2016) 64:1388–402.

11. Sharma, P., & Arora, A. (2020). Clinical presentation of alcoholic liver disease and non-alcoholic fatty liver disease: spectrum and diagnosis. Translational Gastroenterology and Hepatology, 5: 19–19.

12. Sullivan S. Implications of diet on nonalcoholic fatty liver disease. *Curr Opin Gastroenterol* 2010; 26: 160-164

13 Zelber-Sagi S, Nitzan-Kaluski D, Goldsmith R, Webb M, Blendis L, Halpern Z, Oren R. Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. J Hepatol 2007; 47: 711-717

14. Cotter TG, Rinella M. Nonalcoholic fatty liver disease 2020: the state of the disease. Gastroenterology. (2020) 158:1851–64.

15. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003; 37: 917-923

16. Nur Azlina Mohd Fahami & Muharani Tajudin. 2011. Kesan suplimentasi tokotrienol dan tokoferol ke atas perubahan status oksidatif hepar dan aktiviti enzim antioksidan tikus aruhan stres (Effect of tocotrienol and tocopherol on oxidative status and antioxidant enzymes activities in stress-induced rat's liver). Sains Malaysiana 40(5): 481-487.

17. Ahsan H, Ahad A, Siddiqui WA. A review of characterization of tocotrienols from plant oils and foods. J Chem Biol. 2015 Jan 20;8(2):45-59.

18. Lluis JM, Colell A, García–Ruiz C, Kaplowitz N, Fernández–Checa JC. Acetaldehyde impairs mitochondrial glutathione transport in HepG2 cells through endoplasmic reticulum stress. Gastroenterology 2003;124: 708-724.

19. Arteel GE. Oxidants and antioxidants in alcohol-induced liver disease. Gastroenterology 2003;124: 778-790.

20. Serbinova E, Kagan V, Han D, Packer L. Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol and alpha-tocotrienol. Free Radic Biol Med. 1991;10(5):263–275.

21. Pervez et al. Delta-tocotrienol supplementation improves biochemical markers of hepatocellular injury and steatosis in patients with nonalcoholic fatty liver disease: A randomized, placebo-controlled trial. Complementary Therapies in Medicine 52 (2020) 102494

22. Sakpal TV. Sample Size Estimation in Clinical Trial. Perspect Clin Res. 2010;1(2):67-9.

23. Retrieved from https://nida.nih.gov/sites/default/files/audit.pdf

24. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharma 2016;7:27-31.

25. Yap SP, Yuen KH. Influence of lipolysis and droplet size on tocotrienol absorption from self-emulsifying formulations. Int J Pharm. 2004;281(1–2):67–78.

26. Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, Charlson FJ, Norman RE, Flaxman AD, Johns N, et al. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. Lancet. 2013;382: 1575–1586.

27. Rehm J, Samokhvalov AV, Shield KD. Global burden of alcoholic liver diseases. Journal of Hepatology. 2013; 59:160–168.

28. Retrieved from https://www.nst.com.my/business/2019/03/466143/malaysia-cap-65m-ha-oil-palm-plantations-2023 on 28th August 2020

29. Patel, V., et al. Oral Tocotrienols Are Transported to Human Tissues and Delay the Progression of the Model for End-Stage Liver Disease Score in Patients. The Journal of Nutrition. 2012; 142(3): 513–519.

30. Magosso, E., et al. Tocotrienols for normalisation of hepatic echogenic response in nonalcoholic fatty liver: a randomised placebo-controlled clinical trial. Nutrition Journal. 2013; 12(1):166.

31. Pervez et al. Effects of Delta-tocotrienol Supplementation on Liver Enzymes, Inflammation, Oxidative stress and Hepatic Steatosis in Patients with Nonalcoholic Fatty Liver Disease. Turk J Gastroenterol. 2018; 29(2):170-176.

STUDY OBJECTIVES

General:

To determine the effects of palm tocotrienol rich fraction (TRF) on the progression of alcoholic fatty liver disease.

Specific:

1. To determine the effects of TRF on fatty liver index and FibroScan score of patients with alcoholic fatty liver disease.

2. To determine the effects of TRF on the liver enzyme profile of patients with alcoholic fatty liver disease.

3. To determine the effects of TRF on metabolic status of patients with alcoholic fatty liver disease.

4. To determine the effect of TRF on inflammatory cytokines and redox status of patients with alcoholic fatty liver disease.

HYPOTHESIS

1. TRF reduces fatty liver index and improves FibroScan score of patients with alcoholic fatty liver disease.

2. TRF normalises the liver enzyme profile of patients with alcoholic fatty liver disease.

3. TRF normalises the metabolic profile of the patients with alcoholic fatty liver disease.

4. TRF normalises the cytokines level and redox status of patients with alcoholic fatty liver disease.

METHODOLOGY (STUDY DESIGN, WORK PLAN, MILESTONES)

This study would be a randomized, double-blind, placebo-controlled, phase 2 Study on the Effect of Palm Tocotrienol Rich Fraction on Alcoholic Fatty Liver Disease (AFLD). Patients who come for follow up in Gastroenterology & Hepatology clinic in PPUKM, with diagnosis of AFLD would be recruited in the study. Secondary data obtained from Casemix unit would also be screened to look for potential patients for the study.

Primary Outcome Measures:

1.Change from baseline in Liver Enzyme Profile (Aspartate Aminotransferase [AST], Alanine Aminotransferase [ALT], and Gamma-Glutamyl Transferase [GGT]) at 3 and 6 months. Measured in units per liter (U/L) and reported as absolute and percentage change. Lower values indicate improved liver function.

2.Between-group difference in Liver Enzyme Profile (AST, ALT, GGT) at 3 and 6 months (Tocotrienol-Rich Fraction vs placebo). Measured in U/L. Lower values indicate improvement.

3.Change from baseline in Fatty Liver Index (FLI) at 3 and 6 months. Unitless score (range 0–100). Higher scores indicate greater hepatic steatosis (worse outcome). Reported as absolute and percentage change.

4.Between-group difference in Fatty Liver Index (FLI) at 3 and 6 months (Tocotrienol-Rich Fraction vs placebo). Range 0–100. Higher scores indicate worse steatosis.

5.Change from baseline in Liver Stiffness Measurement using Transient Elastography (FibroScan® score) at 3 and 6 months. Measured in kilopascals (kPa; typical range 2–75). Higher values indicate greater fibrosis. Reported as absolute and percentage change.

6.Between-group difference in Liver Stiffness Measurement (FibroScan® score) at 3 and 6 months (Tocotrienol-Rich Fraction vs placebo). Measured in kPa. Higher values indicate worse fibrosis.

*Aspartate aminotransferase (AST) is usually used to detect liver damage. It is often ordered in conjunction with other liver enzymes, alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT), or as part of a liver panel or comprehensive metabolic panel (CMP) to screen for and/or help diagnose liver disorders. The changes in these liver enzymes can also be used to monitor the compliance of patients toward alcohol abstinence.

Secondary Outcome Measures:

1. Change From Baseline in Plasma Cytokine Levels (Anti-inflammatory Effect of Tocotrienols) at baseline, 3 months, and 6 months.

•Cytokines measured: Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 beta (IL-1β) in plasma; Method: Multiplex Enzyme-Linked Immunosorbent Assay (ELISA); Unit: picograms per milliliter (pg/mL); Typical range: IL-6 (0–50 pg/mL), TNF-α (0–100 pg/mL), IL-1β (0–50 pg/mL); Higher values indicate greater inflammation (worse outcome).

2. Change From Baseline in Metabolic Profile (Fasting Blood Glucose [FBG] and Lipid Panel) at baseline, 3 months, and 6 months.

•Parameters measured: FBG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG); Unit: milligrams per deciliter (mg/dL); Typical ranges: Fasting glucose (70–100 mg/dL), TC (125–200 mg/dL), LDL-C (0–130 mg/dL), HDL-C (40–60 mg/dL), TG (0–150 mg/dL); Higher values indicate worse metabolic profile.

SAMPLE SIZE CALCULATION

Sample size calculation was based on the comparison of two means derived from the effects of tocotrienol on fatty liver index (FLI) in patients with non-alcoholic fatty liver disease (NAFLD) (21). This is because the effectiveness of tocotrienol in alcoholic hepatitis has not been evaluated previously.

Formula of calculating sample size in clinical trial (22):

$$n = [(Z\alpha/2 + Z\beta)^2 \times \{2(ó)^2\}]/ (\mu1 - \mu2)^2$$

Where:

n = sample size required in each group

μ1 = mean of FLI for tocotrienol group = 12.82 μ2 = mean of FLI for placebo group = 3.86

ó = standard deviation = 7.0

Zα: This depends on level of significance, for 5% this is 1.96 Zβ: This depends on power, for 80% this is 0.84

$$n = [(1.96 + 0.84)^2 \times \{2(7.0)^2\}]/ (12.82 – 3.86)^2$$

The sample size calculated is 10 per group. Taking into account 20% of subjects might be lost to follow up, the number of each group is adjusted to 13. Hence, the total sample size required for this study is 26 individuals.

SCREENING

Primarily we will be recruiting adult patients who fulfil all the inclusion criteria of this study, who come for follow up in Gastroenterology & Hepatology clinic in Hospital Canselor Tuanku Muhriz UKM (HCTM). Additionally, our research subjects were also recruited from the general public who responded to our recruitment poster in the hospital and on social media platforms. Subjects will answer a questionnaire on their demographic and lifestyle details, medical and medication history. Their alcohol consumption behaviour will be surveyed using the AUDIT questionnaire. A qualified physician will perform physical examination on the subjects. The AUDIT questionnaire (23) is a 10-item screening tool used to identify individuals with harmful drinking behaviour. A score of 8 or more indicates harmful or hazardous drinking behaviour. In addition, patients will be screened for clinical (signs and symptoms of alcoholic liver disease such as parotid swelling, hepatomegaly and clinical evidence of cirrhosis) and biochemical evidence of alcoholic liver disease (transaminitis, AST:AST ratio>2.0, raised gamma glutamyl transferase (GGT), mean corpuscular volume (MCV) and total bilirubin).

Body anthropometry

Body anthropometry will be performed on every visit. Height of the subjects will be measured using a stadiometer. Body weight of the subjects will be measured using a weighing scale. Body composition of the subjects will be analysed using a 4-point bioimpedance machine. Body mass index of the subjects will be calculated as per the convention.

Blood collection

Blood collection will be performed on every visit. Subjects will be requested to fast for 8 hours before blood withdrawal. Venepuncture will be performed by a qualified phlebotomist. 5 ml of fasting blood will be drawn in the morning.

Determination of plasma tocotrienol level (HPLC)

A portion of the blood will be collected using heparin tubes. Plasma will be extracted and aliquoted into 200 µL. The plasma will be stored in - 70°C until analysis. Upon analysis, the plasma will be thawed, added with 50 ul 95% ethanol containing 10 ug/mL bytylated hydroxytoluene, and vortexed vigorously for 5 seconds. Then, 1 mL absolute alcohol will be added to the mixture, which will be remixed before being centrifuged at 1500 g for 15 minutes at 18°C. The pellet will be removed, and the supernatant will be added with 3 mL hexane and mixed vigorously for 5 minutes. The samples will be centrifuged again at 1500 g for 15 minutes at 18°C. The upper layer (2.5 mL) of the supernatant will be removed, placed in new Eppendorf tubes and vacuum evaporated for 40 minutes. The dried residues will be reconstituted in 100 uL HPLC grade hexane, vortexed, and passed through a 0.45 uM filter to remove residual particles. The samples will be collected using an amber vial and analysed by an HPLC machine (Shidmadzu RF-10A XL with fluorescence detector) at the excitation wavelength of 294 nm and the emission wavelength of 330 nm. The tocotrienol isomers and alpha- tocopherols will be separated on a 250 mm x 4.6 mm, 5 um silica column and eluted with a mobile phase of 99:1 (v/v) hexane-isopropanol at 1.5 mL per minutes flow rate. The identity of each compound will be confirmed by co-elution with spiked standard obtained from Excelvite Sdn. Bhd. The peaks will be quantified and integrated using Shimadzu Workstation software.

Biochemical assay

Full blood count, liver profile (AST, ALT, GGT), lipid profile and fasting glucose test will be sent to an accredited laboratory for analysis using automated biochemistry analyser. HepatitisB and C status, as well as antibodies for autoimmune hepatitis will be assayed using an automated immunoassay machine. A portal of the blood will be centrifuged to isolate serum for acetyldehyde, malondialdehyde and inflammatory cytokines. These parameters will be assayed using enzyme-linked immunoassay based on manufacturer protocols.

Ultrasound

Ultrasound of the hepatobiliary system will be performed during initial recruitment to rule out the presence of hepatocellular carcinoma (HCC) which can occur in patients with alcoholic liver cirrhosis. During the screening an ultrasound probe will be placed in the patients' right hypochondrium to assess for any abnormal lesions representative of HCC. It is a non-invasive procedure which takes approximately 20 minutes. Patients with hepatocellular carcinoma will be excluded from the study.

Fibroscan

This is a non-invasive test to measure liver stiffness, kPa (indicator of liver fibrosis) and to detect degree of fat accumulation (CAP range value of 100-400 dB/m). Liver stiffness of > 8kPa indicates advanced fibrosis while CAP of > 263 indicates fatty liver. During the screening, the patient will lie down on the examination bed with his/her right hand behind

his/her head. Investigator will place the probe between the right ribs of the patient. Measurements will be recorded into the machine (FibroScan® 502 Touch). The screening is quick, easy and non-invasive and usually takes less than 15 minutes.

ALGORITHM OF DIAGNOSIS OF ALCOHOLIC HEPATITIS:

Alcohol misuse disorder:

Regular alcohol consumption > 20g/day in female and >30g/day in males

Clinical parameters:

Ascites (Degree)

Hepatic encephalopathy (West haven criteria)

Biochemistry:

Raised ALT and AST ratio AST: ALT>2.0

Raised mean corpuscular volume (MCV)

Raised gamma glutamyl transpeptidase (GGT)

Raised total bilirubin

Platelets

Coagulopathy: Deranged INR and PT

Oxidative markers:

Acetyldehyde

Malondialdehyde

Exclusion of:

Obese patients (a BMI of a BMI of 30 kg/m^2 or more) and with metabolic syndrome

Patients with bleeding disorders, and on anticoagulant or antiaggregant treatments

Hepatitis B (HBsAg)

Hepatitis C (Anti-HCV)

Autoimmune hepatitis (ANA, AMA, ASMA, IgG)

NAFLD (BMI, fasting glucose, blood pressure, fasting total cholesterol, triglyceride and HDL)

Hereditary hemochromatosis (Ferritin, Tsat)

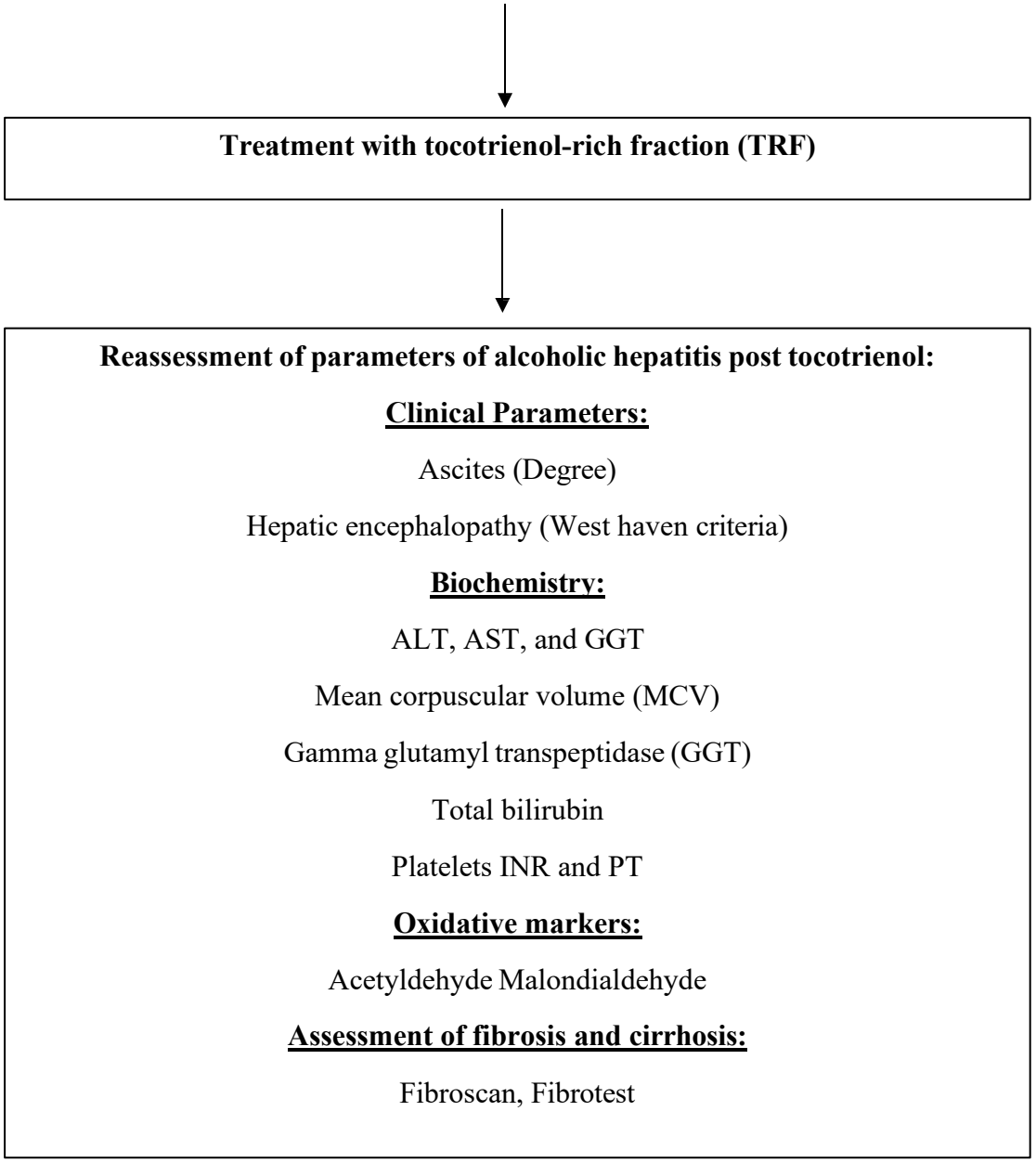
Severe alcoholic hepatitis (Maddrey's discriminant score)

Assessment of advanced fibrosis/cirrhosis:

Fibroscan, FibroTest (FT)

Radiology:

Ultrasound of the hepatobiliary system TRO hepatocellular carcinoma



Randomisation and Treatment

The treatment assigned to participants for 6 months was either mixed tocotrienols 200 mg twice daily or placebo. A treatment period of 6 months will be adopted because only patients with mild alcohol steatohepatitis are recruited, whose condition would resolve within months with alcohol abstinence alone. The content in each capsule of the mixed tocotrienols preparation was 61.5 mg, 112.8 mg and 25.7 mg for alpha-, gamma- and delta-tocotrienol, respectively and 61.1 mg of alpha-tocopherol. Both placebo and tocotrienols, were soft gel capsules and were similar in terms of colour, size, shape and surface texture. Participants were randomised using a computer-generated random allocation sequence. A permuted block design was employed. Each block of specified size contained an allocation ratio of 1:1 (placebo:tocotrienols). The

random allocation sequence would select the next block and determine the next allocations. Sequence was not made known to researchers who enrolled participants. For the purpose of assigning participants to interventions, a subject number was used. The researcher who generated the random allocation sequence and assigned participants was blinded to subjects' clinical data and was independent from the persons who enrolled participants. The mixed tocotrienols preparation and the placebo capsules were purchased from Hovid (Ipoh, Malaysia). Researchers and volunteers were blinded to the assigned treatment.

Source and composition of tocotrienol

Tocotrienol mixture derived from palm oil will be used. It will be sponsored by Hovid Sdn Bhd (Ipoh, Malaysia). The composition of the tocotrienol mixture is 24.7% α-tocotrienol, 4.5% β-tocotrienol, 36.9% γ-tocotrienol, 12.0% σ-tocotrieno l and 21.6% α-tocopherol. It is formulated with a self-emulsifying system (SES) to enhance absorption of tocotrienol.

The gelatin sheet used in the manufacturing of tocotrienol soft gel capsules is prepared using gelatin derived from a bovine source.

Dosing of tocotrienol

Animal studies reported that a dose of 60 mg/kg tocotrienol could be effective in maintaining liver health [16]. To extrapolate this to human equivalent dose, body surface conversion (24) is used:

Human equivalent dose (HED)

= [Km rat/Km human] x [dose in rat] x [expected body weight of humans]

= [6 / 37] x [60 mg/kg] x [60 kg]

= 584 mg ≈ 600 mg

However, this calculation does not consider the bioavailability-enhancing effect of SES. A previous study showed that the use of SES enhanced the bioavailability of tocotrienol by 4-fold [25]. Notwithstanding that, the effects of SES on the tissue distribution of tocotrienol, especially in the musculoskeletal system are not well-known. Therefore, we propose to use 400 mg (a decrease of 33.3% from the original calculated dose) in the trial subjects.

It will be packaged into two soft gels, each containing 200 mg palm tocotrienol. One soft gel will be taken orally, daily after breakfast and dinner to complete the 400 mg daily dose. The treatment period will be 6 months. The placebo will consist of equivolume refined, bleached, and deodorised (RBD) palm olein. Both tocotrienol and placebo capsules will be manufactured in accordance with any applicable GMP, and is coded and labeled in a manner that protects the blinding. In addition, the labeling should comply with applicable regulatory requirement(s).

Dispense of TRF

Since the study activity for each subject will last for six months, either TRF or placebo with identical appearance and packaging will be dispensed to the subjects twice, which is every three months (during baseline and visit 1). There is a ±7-day allowable window period for visit 1 as some subjects might not be able to turn up on the exact visit date. Softgel counting will be

performed at visit 1 and 2 to determine the compliance of the subjects. Subjects will be requested to consume the softgel after meal.

“Stopping rules" or "discontinuation criteria

The participation of the trial subjects will be terminated if they cannot adhere to alcohol abstinence and cannot comply with the treatment regimes. Failure to adhere is a serious problem that not only affects the subjects but also the overall conduct and outcome of the research. They will also be stopped from participating in situations where the risks outweigh the anticipated benefits, in cases of severe allergic reactions or any adverse events that occurred in individual trial subjects. Subjects in these trials should be particularly closely monitored and should be withdrawn if they appear to be unduly distressed.

Accountability procedures for TRF product and placebo

We will take the responsibility for TRF product and placebo accountability at the trial site. The records of the product's delivery to the trial site, the inventory at the site, the use by each subject, and the return to the sponsor or alternative disposition of unused product will be maintained. These records should include dates, quantities, batch/serial numbers, expiration dates and the unique code numbers assigned to the TRF and placebo products and to trial subjects. We will maintain the records that document adequately that the subjects were provided the doses specified by the protocol and reconcile all investigational product received from the sponsor. The products will be stored in accordance with applicable regulatory requirements, and will be ensured that they are used only in accordance with the approved protocol. We will explain the correct use of the products to each subject and will check, at intervals appropriate for the trial, that each subject is following the instructions properly.

Maintenance of trial treatment randomization codes and procedures for breaking code

We will follow the trial's randomization procedures, and will ensure that the code is broken only in accordance with the protocol. Given that the trial is blinded, we will promptly document and explain to the sponsor any premature unblinding (e.g., accidental unblinding, unblinding due to a serious adverse event) of the investigational product. We will have a written procedure for rapidly identifying a ‘blinded’ IP in case of an emergency. The procedure will be secure, readily available at all times during the trial, and not allow breaks of the blinding to go undetected.

Data recorded directly on the CRFs is to be considered the source data.

We will maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site’s trial subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (via an audit trail). We will ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports. Data reported on the CRF, that are derived from source documents, should be consistent with the source documents or the discrepancies should be explained. Any change or correction to a CRF will be dated, initialed, and explained (if necessary) and will not obscure the original entry (i.e. an audit trail should be maintained); this applies to both written and electronic changes or corrections. Records of the changes and corrections will be retained.

We will maintain the trial documents as specified in Essential Documents for the Conduct of a Clinical Trial in the Malaysian Guideline for Good Clinical Practice and as required by the applicable regulatory requirement(s). Measures that prevent accidental or premature destruction of these documents will be taken. All the essential documents will be retained until at least 2 years or for a longer period, if required by the applicable regulatory requirements. We will make available for direct access all requested trial-related records, upon request of the monitor, auditor, IRB/IEC, or regulatory authority.

SELECTION AND WITHDRAWAL OF SUBJECTS

Inclusion criteria:

1. Patients with history of alcoholic use disorder with clinical and biochemical evidence of alcoholic steatohepatitis.

2. Patients with Maddrey's discriminant function ≤ 32, and do not require the treatment of corticosteroid therapy or pentoxifylline.

3. Patients aged 18 to 65

4. Patients who could comply with alcohol abstinence.

Exclusion criteria:

1. Severe alcoholic hepatitis defined as Maddrey's discriminant function >32

2. Patients with other concomitant liver diseases:

    a. Hepatitis B
    b. Hepatitis C
    c. Non-alcoholic fatty liver disease (NAFLD)
    d. Autoimmune hepatitis (AIH)
    e. Hereditary hemochromatosis

3. Patients who are obese (a BMI of 30 kg/ m^2 or more) and with metabolic syndromes

4. Patients with bleeding disorders and who have been on anticoagulant or antiaggregant treatments

5. Patients who have been on corticosteroid therapy or pentoxifylline for alcoholic hepatitis

6. Patients with hepatocellular carcinoma

7. Pregnant patients

8. Patients who are breastfeeding

9. Patients with Childs C liver cirrhosis

10. Patients who have pyridoxine allergy or history

11. Patients who are judged by investigator that participation of the study is difficult due to disease as follow; hepatic cirrhosis, Wilson's disease, malignant tumor, serious metabolic disease, severe renal disease, severe pulmonary disease, severe cardiovascular disease, severe nervous disease/psychiatric disorder, muscle disease and etc

12. Patients taking vitamin E, herbal supplements, or other investigational products within 90 days prior to the participation in the study.

13. Patients who have been taken any medications that could affect the treatment: hypoglycemic agents, colchicine, penicillamine, corticosteroids, ursodeoxycholic acid, pentoxifylline, long-term use of NSAIDs, statins, neuroleptics, anticonvulsant medications, high-dose acetaminophen(>=2.5g/day)

14. Patients who have received treatment that may affect liver function within 1 month prior to the participation in the study

15. Patients who could not comply with alcohol abstinence.

16. Patient who considered ineligible for participation in the study as Investigator's judgment

Note: Patients enrolled in this study MUST abstain from alcohol consumption throughout the study period

Withdrawal criteria

During the course of the trial a subject may choose to withdraw early from the trial treatment at any time. This may happen for a number of reasons, including but not limited to:

- The occurrence of what the participant perceives as an intolerable adverse event (AE).
- Inability to comply with trial procedures
- Participant decision

Subjects may choose to stop their participation, and it will not affect their treatment at UKM-affiliated hospitals and clinics in any way. Without losing any of their rights as patients, they will not be penalised and can still continue with the standard treatment available and for follow-up visits at UKMMC. The data and samples already collected would not be used in the final study analysis and would be discarded.

In addition, we may discontinue a participant from the trial treatment at any time if we consider it necessary for any reason including, but not limited to:

- Pregnancy
- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening)
- Significant non-compliance with treatment regimen or trial requirements
- An adverse event which requires discontinuation of the trial medication or results in inability to continue to comply with trial procedures

- Disease progression which requires discontinuation of the trial medication or results in inability to continue to comply with trial procedures

We have adjusted our sample size calculation for this study by considering the risk of dropout among the subjects. Hence, the withdrawn subjects will not be replaced while ensuring that the minimal sample size of this study is achieved. The type of withdrawal and reason for withdrawal will be recorded in the CRF. If the participant is withdrawn due to an adverse event, we will arrange them for follow-up visits at our clinic until the adverse event has resolved or stabilised.

Treatment of Subjects

The research takes place over six months in total. It will be necessary for each subject to come to the clinic at HCTM for an initial screening and three-monthly follow-ups. During these visits, they will undergo a routine screening which involves blood collection procedure, a liver fibrosis scan, and a dual-energy absorptiometry scan and they will be informed of the results of the tests conducted. They will also be given either the test supplement or the placebo for the management of AFLD. They will be required to take 2 softgels containing 200 mg TRF or placebo orally, each after breakfast (one softgel) or dinner (one softgel). The assignment of TRF and placebo will be random, and the physician and enumerator attending you will not be aware of your assigned treatment. At the end of six months, all data will be collected and analysed, and the research will be completed.

Identifying specific barriers for each subject and adopting suitable techniques to overcome them will be necessary to improve the subject's compliance and medication adherence. Health care professionals involved in this study such as physicians and nurses have significant role in their daily practice to improve subject medication adherence. We will give medication reminders via text or email to our study subjects. The subjects s may keep a diary, or they can bring all unused or part-used medication/vials and packaging from used medication at each follow-up visits for soft gel capsule counting and to discuss their compliance. Another method is to keep track of their medications with a "pill card" or "medication calendar." The subjects will be required to bring their "pill cards" to every appointment, and/or we may ask them to keep a monthly calendar on which they will mark each day when they took their capsules.

Subjects' non-adherence to alcohol abstinence during the duration of this study might also pose a risk to this study. We will try to minimise this risk by closely monitoring all study subjects to detect any withdrawal symptoms and offering them comprehensive care and the necessary psychological counseling or pharmacotherapy.

Adverse Events (AEs), Serious Adverse Events (SAEs), and Suspected Unexpected Serious Adverse Reactions (SUSARs)

While the possibility of having some unwanted side effects and unexpected events related to the tocotrienol supplementation and the treatment procedures is very low, the enrolled subjects should still be aware of the possibility. We will try to decrease the chances of this event occurring through close monitoring of all subjects but if something unexpected happens, we will provide them with proper care and appropriate treatment. We may use some other medicines to decrease the symptoms of the side effects or reactions. Or we may stop the use of this supplement and end their participation. If this is necessary, we will discuss it with the

subjects, and they will always be consulted before we move on to the next step. Given that the subjects must adhere to alcohol abstinence throughout the course of this study, if they experience any withdrawal symptoms, we will give them supportive care and medications as part of the treatment regimens used in alcohol withdrawal states to minimise the symptoms and prevent complications.

Our qualified physician, who is a co-investigator for the trial, will be responsible for all trial-related medical decisions. During and following a subject's participation in this trial, we will ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the trial. We will inform a subject when medical care is needed for intercurrent illness (es) of which we become aware. We will also recommend the subjects to inform their primary physician about their participation in this trial if the subject has a primary physician and if the subject agrees to the primary physician being informed, while fully respecting the subject's rights.

Reporting Procedures for Adverse Events

Adverse events and/or laboratory abnormalities identified as critical to safety evaluations will be reported to the sponsor according to the reporting requirements and within the specified time periods.

All AEs occurring during the trial that are observed by the Investigator or reported by the trial subject will be reported on the trial case report form (CRF), whether or not attributed to trial medication. The following information will be reported on the CRF: description, date of onset and end date, severity, assessment of relatedness to trial medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

Non-serious AEs considered related to the trial medication as judged by a medically qualified investigator or the Sponsor will be followed up either until resolution, or the event is considered stable.

It will be left to the clinical judgment of our medical officer to decide whether or not an AE is of sufficient severity to require the participant’s removal from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant will be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable.

Reporting Procedures for Serious Adverse Events

All SAEs must be reported on the SAE Reporting Form to the Sponsor or delegate immediately or within 24 hours of Site Study Team becoming aware of the event being defined as serious. The immediate reports will be followed promptly by detailed, written reports. The immediate and follow-up reports should identify subjects by unique code numbers assigned to the trial subjects rather than by the subjects' names, personal identification numbers, and/or addresses, in accordance with the applicable regulatory requirement(s) related to the reporting of unexpected serious adverse drug reactions to the regulatory authority(ies) and the IRB/IEC.

Review of SAEs must be timely, taking into account the reporting timeline for a potential SUSAR. SAEs that are reported late must be accompanied by an explanation for this.

SUSAR Reporting

All SUSARs will be reported by the sponsor delegate to the relevant Regulatory Authority and to the REC and other parties as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

Treatment codes will be un-blinded for specific participants. Additionally, principal investigator will be informed of all SUSARs for the relevant IMP for all studies with the same sponsor, whether or not the event occurred in the current trial.

STATISTICAL ANALYSIS

Normality of the data will be assessed through Shapiro-Wilk test. Since the study adopts a time x group design, mixed-design ANOVA will be used to compare the effects across time (within group) and between treatment groups. Small effect analysis will be used as the pairwise post hoc analysis. If the data is skewed, the data will be analysed with Kruskal Wallis test and Mann Whitney U-test with Bonferroni correction for type-1 error. All analyses will be performed using Statistical Package for Social Sciences version 23 (IBM, Armonk, USA). The data will be presented as mean (standard deviation). A p-value less than 0.05 is considered statistical significance.

Direct Access to Source Data/Documents

Direct access to the subjects' original medical records and any data pertaining to the research study will not be shared with or given to anyone. Such access is only authorised to the research team, the monitor(s), the auditor(s), the IRB/IEC including the REC UKM, and the regulatory authority(ies) for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations.

We will permit trial related monitoring, audits, IRB/IEC review, and regulatory inspection(s), providing direct access to source data/documents.

Quality Control and Quality Assurance

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities.

Ethical and Regulatory Considerations

We will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki, with relevant regulations and with Good Clinical Practice.

This research protocol was approved by the Research Ethics Committee, from the Research and Ethics Review Board, UKMMC (reference number: UKM PPI/111/8/JEP-2021-694)

Other Ethical Considerations

The tocotrienol preparation used in this trial were encapsulated in soft gel capsules. The main component of these capsules sheet is gelatin, derived from a bovine source, which may be culturally unacceptable for certain groups of people.

Data Handling and Record Keeping

All trial data will be entered on to paper CRFs and/or an electronic data entry system which have been validated by the standard operating procedures.

The participants will be identified by a unique trial specific number and/or code in any database. The name and any other identifying detail will not be included in any trial data electronic file.

If identifiable personal data may be transferred during or after the study, it is necessary to assure against the risks that are presented by this processing, i.e., risks of accidental or unlawful destruction, loss, alteration, unauthorised disclosure of, or access to the personal data transferred and stored or otherwise processed by the recipient. This is true for paper based and electronic data transfers.

If some samples collected in the study are intended for further use beyond the study, the consent form will need to be retained for the life of the sample to meet the regulatory requirements.

If participants are given the option to be approached for future research, it is necessary to retain the consent form as the basis for retention of details and future approach. Those contact details should be held securely, separately from the research data, and kept updated.

Compliance with the relevant sponsor organisation's data policy should be ensured.

FINANCE AND INSURANCE

Funding

This study is being conducted with a UKM Research Grant, co-sponsored by the Malaysian Palm Oil Board, ExcelVite Sdn. Bhd and Hovid Sdn. Bhd.

Insurance

This study has yet to receive its trial insurance. However, we have started the purchasing process and are still waiting for the details and updates from the insurance agency.

Publication Policy

The publication policy which cover authorship, acknowledgements, and review procedures for scientific publications should comply to department or institution policy, or trial agreement.

We will ensure that the publication policy stated here is consistent with any contract applicable to the trial. We will inform the trial subjects that the data from this study will be made into a report, which may be published. If the trial results are published, the subject's identity will remain confidential.

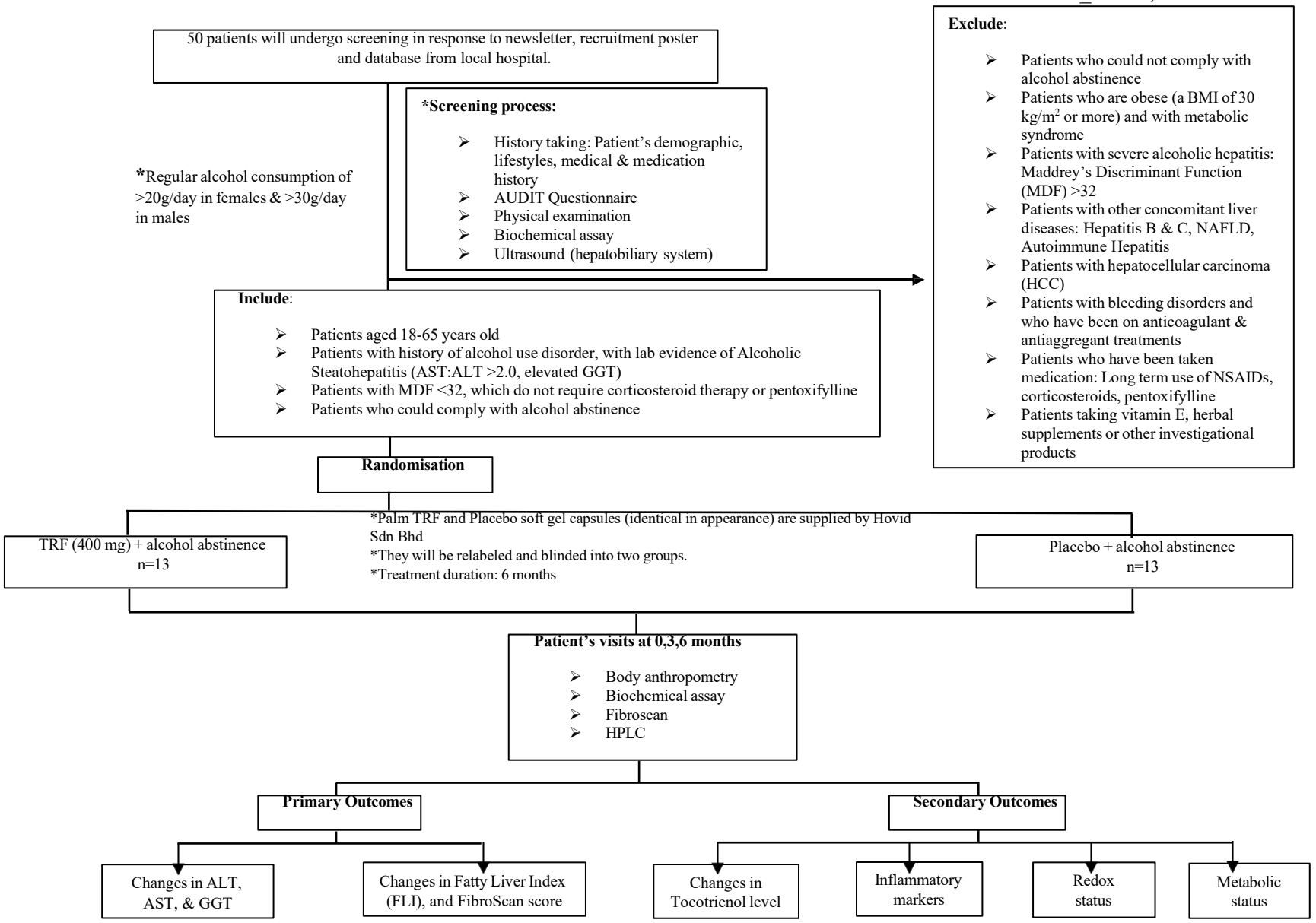


Figure 1: Flow chart of the summary of the study intervention

GANTT CHART

Year	Year 1							Year 2							Year 3						
Semester	Semester 1			Semester 2				Semester 3			Semester 4				Semester 5			Semester 6			
Activities (in Month)	April-June	July-Aug	Sept	Oct	Nov	Dec-Jan	Feb-March	April-May	June-July	Aug-Sept	Oct	Nov	Dec-Jan	Feb-March	April-June	July-Aug	Sept	Oct	Nov	Dec-Jan	Feb-March
Literature review																					
Research proposal writing																					
Presentation for Ethics approval																					
Obtained Ethics approval																					
Preparation and submission of documents for CTX application																					
Presentation of research proposal at the department																					
Data retrieval from the Casemix unit and A&E department to look for potential AFLD patients																					

Revision of sample size calculation																				
Preparation and dissemination of recruitment materials (poster/flyer, bunting, Facebook page, Facebook ads, etc)																				
Recruitment of subjects																				
Tocotrienol and placebo samples arrival																				
Progress report presentation at the department																				
Subjects' Visit 1																				
Subjects' visit 2																				
Subjects' visit 3																				
Final data collection																				
Data analysis																				
Final report writing																				
Final (result) presentation at the department																				
Submission of final manuscript																				

INFRASTRUCTURE OF TRIAL

Facility	Site
Subject screening, and consultation	Laboratory of Pharmacology Department, Faculty of Medicine
Body anthropometry measurements	Laboratory of Pharmacology Department, Faculty of Medicine
Dual-energy X-ray absorptiometry	Laboratory of Pharmacology Department, Faculty of Medicine
Milliplex reader	Prima Nexus Sdn. Bhd
ELISA reader	Laboratory of Anatomy Department, Faculty of Medicine
Automated blood biochemistry analyser	Pathlab Sdn. Bhd
High performance liquid chromatography	Laboratory of Pharmacology Department, Faculty of Medicine
Fibroscan	Hospital Canselor Tuanku Mukriz

DETAILED BUDGET

Description	Price/unit (RM)	Unit	Total price (RM)
Research materials/ Consumables			
Tocovid SupraBio™ 200mg capsules: 6 months x RM 4 x 2 x 13 subjects	18720	1	18720
Placebo capsules: 6 months x RM 1 x 2 x 13 subjects	4680	1	4680
Multiplex immunoassay kits for biomarkers: 3 sampling points for all subjects (26 subjects) Human Cytokines Profiling Selected Platform: M Sample type: Serum / Plasma sCD40L, EGF, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1α, MIP-1β, PDGF-AB/BB, RANTES, TGF-α, TNF-α, TNF-β, VEGF, Eotaxin/CCL11, PDGF-AA	2160	26	56160
Immunoassay kits: 3 sampling points for all subjects (26 subjects). 6 kits × 3 sampling points (Each sample = RM 80) Technical triplicate	1250	26	32500

Acetyldehyde, malondialdehyde, total antioxidant capacity, total superoxide dismutase, glutathione peroxidase, glutathione reductase			
HPLC Analysis: 3 sampling points for all subjects (26 subjects). (RM 150 per Analysis)	450	26	11700
Plastics consumables - Blood tubes (heparin, EDTA, plain tubes), gloves, syringes and surgical mask, eppendorf tubes and tips • Cyrovials, 1.5 ml microcentrifuge tubes • 15 ml and 50 ml centrifuge tube • 10 ul, 100 ul, 200 ul and 1,000 ul pipette tips	8000	1	8000
Cleaning consumables • Glove, hand soap, cleaning liquid, wipe, trash bag	5000	1	5000
Stationary • All stationaries needed for the whole period of trial • Photocopy of questionnaires, advertisements, case report forms and subject diaries	2000	1	2000
Total			**138760**
Services			
Clinician Fee	200	26	5200
Phlebotomy: 5 sessions × 5 sampling points x 2 phlebotomists	5600	1	5600
Blood biochemistry: 26 samples × 3 sampling points x RM 300 • Full Blood Count o Differential count: polymorphs, lymphocytes, monocytes, eosinophils, basophils o Absolute count: polymorphs, lymphocytes, monocytes, eosinophils, basophils, ESR and platelet count o Film: RBC, WBC, platelet • Blood Glucose • Lipid Profile (total cholesterol, LDL, HDL, triglycerides, non-HDL cholesterol) • Liver function: bilirubin, ALP, AST, ALT, GGT, albumin, total protein • INR and PT	630	26	16380
Fibroscan: 3 sampling points x RM350	550	26	14300
Clinic Registration: 3 Times x RM 25 each	75	26	1950

CRO service: • Pre-study set-up activities, trial master file – RM 10,000 • Protocol set-up, SOP optimization, SOP revision and review – RM 10,000 • Study familiarization, in house briefing, site initiation visit, site monitoring visit, site closeout visit – RM 15,000 • Project management according to the project timeline (from site initiation visit until site closeout visit) and monitoring activities for 24 months – RM 25,000	60000	1	60000
Full body Dexa Scan Lipid scan 3 sampling points for all subjects (26 subjects)	650	26	16900
Insurance	20000	1	20000
Statistician	10000	1	10000
Questionnaire platform	60	26	1560
Total			**151890**
Manpower			
Study coordinator/student (RM2300/month) x 24 months	55200	1	55200
Total			**55200**
Miscellaneous			
Publication Fee	8000	3	24000
Conference Registration	5000	2	10000
Total			**34000**
SUBTOTAL (RM)			**379850**
UKM & ExcelVite Sdn Bhd (134640+172240)			**181948**
GRANT TOTAL (MPOB)			**197902**