

Effectiveness of Low Molecular Weight Fucoidan and High Stability Fucoxanthin (LMF- HSFx) in Phaeophyta as Bioactivity in Emergency Therapy of Non-Alcoholic Fatty Liver Disease (NAFLD)

Norina Agatri^{1*}, Nimas Adiani Sekar Laranti¹, Hanifa Nur Shabrina¹, Fatmanur Hurulhanifa¹

¹Faculty of Medicine. University of Muhammadiyah Purwokerto. Indonesia

*) Correspondence author

Norina Agatri

Faculty of Medicine

University of Muhammadiyah Purwokerto. Indonesia

Email Address: agatrinorina87@gmail.com

Abstract

Indonesia is a maritime nation with a rich marine ecosystem and biodiversity. The waters are abundant in Phaeophyta (brown algae), which are a significant source of bioactive compounds such as fucoidan and fucoxanthin. These compounds have shown potential in treating Non-Alcoholic Fatty Liver Disease (NAFLD), a metabolic disorder characterized by excessive fat accumulation in the liver, leading to complications such as fibrosis, cirrhosis, and hepatocellular carcinoma. The objective of this study is to evaluate the effectiveness of Low Molecular Weight Fucoidan (LMF) and High Stability Fucoxanthin (HSFx) in the curative treatment of NAFLD. Specifically, the study aims to assess the bioactivity and biocompatibility of LMF-HSFx and compare its efficacy with a placebo group in the treatment of NAFLD. The study used a non-invasive Fibroscan to test Controlled Attenuation Parameter (CAP) and Transient Elastography on NAFLD patients. It included 42 NAFLD patients, divided into two groups: the LMF-HSFx group and the placebo group. The LMF-HSFx group received oral supplementation of 825 mg LMF fucoidan and 825 mg HSFx twice daily for six months. The effectiveness of the treatment was measured through changes in hepatic lipotoxicity, serum markers, and pro-inflammatory cytokines. The results showed significant reductions in hepatic lipotoxicity, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, and triglycerides in the LMF-HSFx group. The study also observed significant reductions in pro-inflammatory cytokines (IL-6 and IFN- γ) and improvements in adiponectin and leptin levels. These findings suggest that the LMF-HSFx combination effectively reduces hepatic inflammation and improves metabolic parameters in NAFLD patients.

Keywords: Phaeophyta; Low Molecular Weight Fucoidan; High Stability Fucoxanthin; Non-Alcoholic Fatty Liver Disease; Bioactivity; Biocompatibility; Hepatic lipotoxicity

INTRODUCTION

Indonesia is a maritime country with abundant marine ecosystems and biodiversity. Indonesian waters are rich in *Phaeophyta* or often referred to as brown algae or seaweed. Various types of *Phaeophyta* are widely distributed throughout Indonesia. The brown algae are biological source that produce fucoxanthin and fucoidan which are beneficial for health.⁽¹⁾

Phaeophyta belongs to the category of the kingdom of protists with a talus structure, which consists of holdfast, stipe, and blade included in macro algae.⁽²⁾ The structure of *Phaeophyta* consists

of carbohydrates, lipids, and proteins so that it can be used as a potential food source and medicinal raw material. One of the benefits of *Phaeophyta* in the health sector is as a curative therapy for Non-Alcoholic Fatty Liver Disease (NAFLD). The curative therapy uses polysaccharide content in *Phaeophyta*, such as fucoidan (FU), fucoxanthin, alginate (ALG), and laminarin (LAM). Based on research conducted by Ping-Hsiao Shih *et al*, fucoidan and fucoxanthin are the most influential polysaccharides in the curative therapy of Non-Alcoholic Fatty Liver Disease (NAFLD).⁽³⁾

Low Molecular Weight Fucoidan (LMF) is a *Phaeophyta* polysaccharide with a class of L-fucose-enriched sulfated polysaccharides that can be used as an anti-inflammatory by inhibiting lipid metabolism and reducing dyslipidemia and repairing liver damage caused by NAFLD.⁽⁴⁾ In addition, flavonoids contain anti-oxidants that are useful for inhibiting free radicals.⁽⁵⁾

Fucoxanthin is a carotenoid present in the chloroplasts of brown algae. Previous studies have shown that the compound has strong anti-inflammatory, anti-cancer and anti-obesity activities with high stability. High Stability Fucoxanthin (HSFx) has great potential in the treatment of NAFLD.

Non-Alcoholic Fatty Liver Disease (NAFLD) is a metabolic disorder that causes accumulation of fat in the liver. NAFLD can lead to various pathological complications, namely Non-Alcoholic Steatohepatitis (NASH), fibrosis, hepatitis cirrhosis, and even hepatocellular carcinoma. Factors that influence a person to develop NAFLD include lifestyle, environment, genetic predisposition and diet.

NAFLD is one of the silent burden diseases with the classification of noncommunicable diseases. Based on meta-analysis research, it is estimated that the global prevalence of NAFLD is 30% among the human population during 1990-2019. Overall, NAFLD cases are 40% more prevalent in men compared to 26% in women and continue to increase with age. In addition, in 2019, Asia accounted for 46.2% of the total global NAFLD cases including Indonesia.⁽⁶⁾ NAFLD complications such as hepatic cirrhosis can reduce the productivity of patients and contribute to the mortality rate of the Indonesian

population. Based on this, NAFLD therapy can be done using *Phaeophyta* as an anti-oxidant and anti-inflammatory.

METHODS

Phaeophyta has Low Molecular Weight Fucoidan (LMF) (<10 kDa), Middle Molecular Weight Fucoidan (MMWF) (10-10,000 kDa), and High Molecular Weight Fucoidan (HMWF) (>10,000 kDa) and a composition of 44% fucose and 26% sulfate.⁽¹⁷⁾ Fucoidan has a complex chemical structure of (1 → 3)- α -l-fucose-(1 → 4)- α -l-fucose. Low Molecular Weight Fucoidan (LMF) combined with High Stability Fucoxanthin (HSFx) has various functions such as anti-oxidant, anti-inflammatory and anti-lipemic which have great potential in NAFLD therapy. Based on research, the therapeutic effect of LMF-HSFx is used for insulin resistance and inflammation in NAFLD patients.

The method used was Non-Invasive Fibroscan to test Controlled Attenuation Parameter (CAP) and Transient Elastography. Furthermore, testing the mechanism of LMF-HSFx was carried out in rats with High Fat Diet (HFD) or high fat diet and HepaRG cells.

Hepatic lipotoxicity was tested in two groups, the LMF-HSFx group and the placebo group. There were 42 patients with NAFLD who were orally administered LMF-HSFx (825 mg LMF fucoidan + 825 mg HSFx twice daily) and placebo group (1650 mg cellulose powder twice daily) for 6 months. After testing, the results of hepatic lipotoxicity in NAFLD patients decreased with oral supplementation of LMF-HSFx. There was a significant decrease in serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), total cholesterol, and triglycerides.

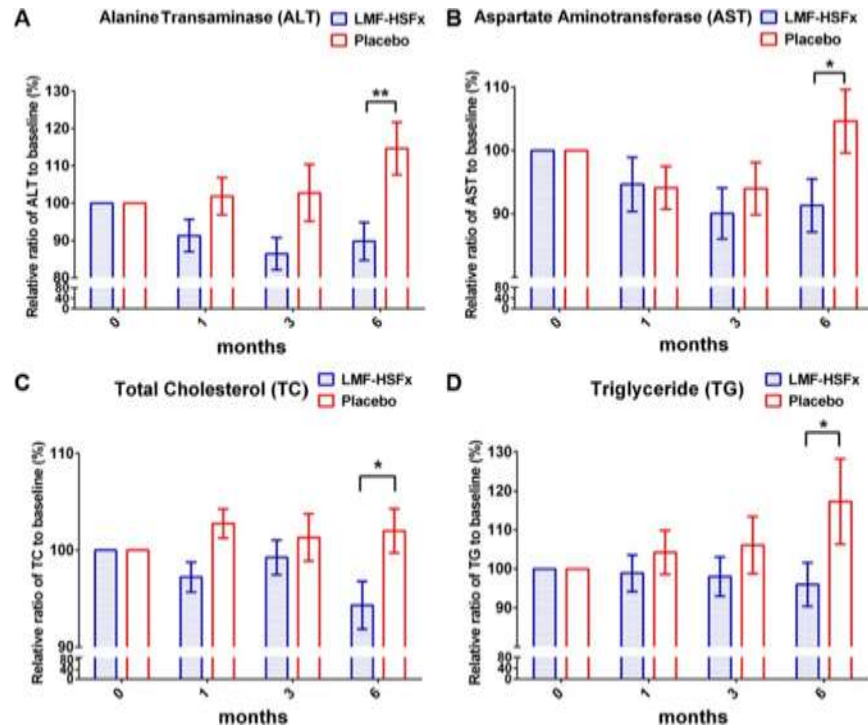


Figure 1: Reduction of hepatic lipotoxicity using LMF-HSFx in NAFLD patients. A) Alanine Transaminase (ALT); B) Aspartate Aminotransferase (AST); C) Total Cholesterol (TC); D) Triglyceride (TG); Significant reduction in ALT, AST, TC, and TG observed for 6 months.

Testing hepatic steatosis and fibrosis in NAFLD patients using LMF-HSFx shows a significant decrease. It can be seen in the following diagram.⁽³⁾

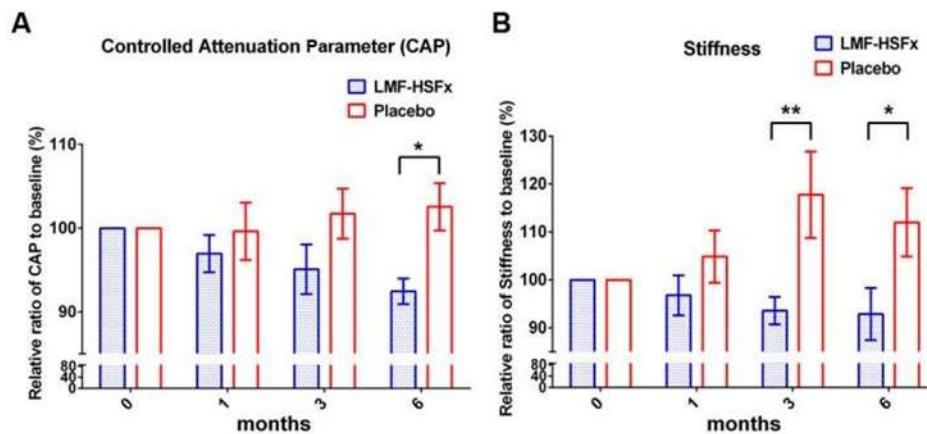


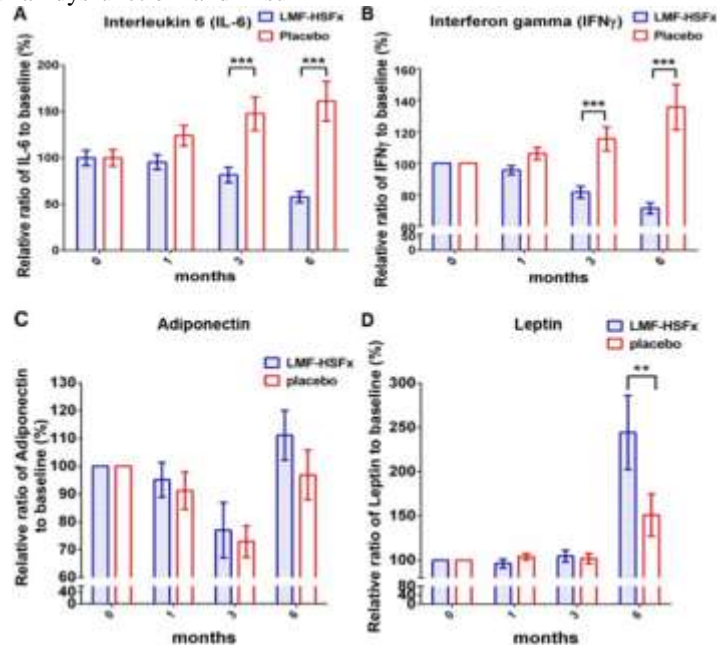
Figure 2: Reduction of hepatic steatosis and fibrosis using LMF-HSFx in NAFLD patients. A) Controlled Attenuates Parameter (CAP); B) Liver stiffness of patients with LMF-HSFx or placebo group.

Main mediators of the pathogenesis of NAFLD are pro-inflammatory cytokines (IL-6 and IFN- γ) and adipokines (adiponectin and leptin).

Studies have shown that there is a significant reduction of IL-6 and IFN- γ in the third and sixth months of LMF-HSFx use. Adiponectin is an anti-

diabetic adipokine that can stimulate lipid and glucose metabolism, including fatty acid oxidation and glucose utilization and suppression of hepatic gluconeogenesis, and reduce adiponectin levels in obesity with mitochondrial dysfunction and insulin

resistance. One of the other adipokines is leptin which can reduce appetite, stimulate thermogenesis, increase fatty acid oxidation, reduce glucose levels and control body weight.



LMF-HSFx decreased the inflammatory process triggered by NAFLD and increased adipogenesis. A) IL-6; B) IFN- γ ; C) Adiponectin; D) Leptin from patients with LMF-HSFx and placebo group. In the LMF-HSFx combination, there was a significant decrease of IL-6 and IFN- γ observed in the third and sixth months and an increase of leptin in the sixth month compared to the placebo group.

The results of the investigation showed an increase in insulin in the LMF-HSFx group accompanied by a significant decrease in fasting blood sugar and HbA1c within six months. After calculating Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and insulin secretion index as indicators of insulin resistance and beta cell

function. There was a lower HOMA-IR, but the insulin secretion index in the LMF-HSFx combination group compared to the placebo group which means LMF-HSFx has the potential for insulin resistance and improving beta cell function in NAFLD.

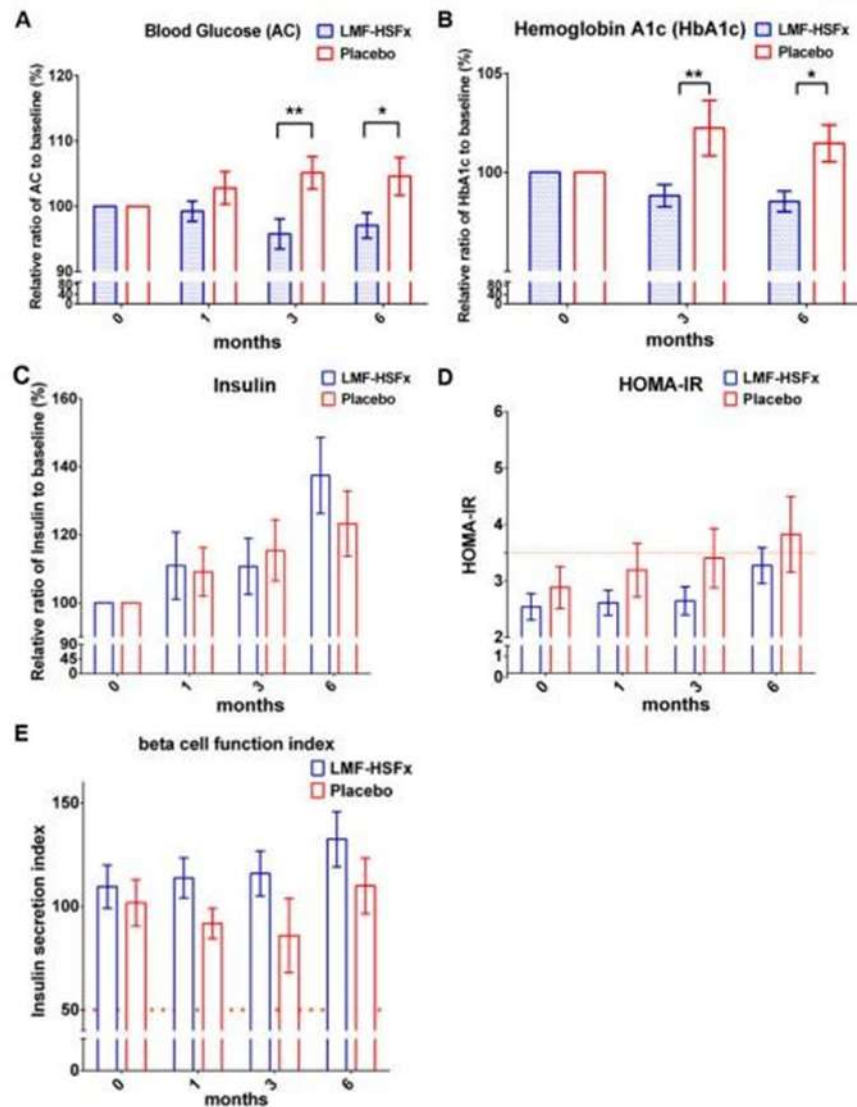


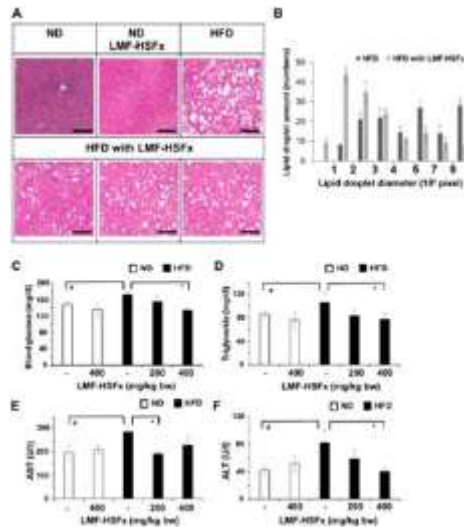
Figure 4: Decrease in insulin resistance in NAFLD patients. The graph shows the baseline changes of A) AC, B) HbA1c, C) Insulin, D) HOMA-IR, and E) Insulin secretion index (beta cell function index) performed in patients with LMF-HSFx treatment or placebo group. Significant decreases in AC, HbA1c changes in the third and sixth months in the LMF-HSFx group. D) The mean HOMA-IR of the placebo group was not higher than 3.5 at month six (LMF-HSFx). E) Improved beta cell function index in the LMF-HSFx group over six months (compared to the placebo group).

Furthermore, the effects of the LMF-HSFx combination on hepatic lipotoxicity and modulation of adipogenesis in mice with a high-fat diet were investigated. Tests were conducted by administering LMF-HSFx orally or not (400 mg/kg/BW/day) for four months. Hematoxylin Eosin (HE) staining of rat liver tissue showed abundant lipid droplet accumulation and hepatocyte necrosis (Figure 5A,

upper right panel), but the combination significantly inhibited lipid accumulation in the liver (Figure 5A, lower panel). Further analysis on the number and size of lipid droplets in the liver tissue of high-fat diet mice showed that LMF-HSFx supplementation decreased the volume of lipid droplets in the liver of high-fat diet mice (Figure 5B). Figure 5C shows, Fasting Blood Sugar (GDP) level was significantly

increased in high-fat diet rats compared to Normal Diet (ND) rats, while treatment with 400 mg/kg/BB/day LMF-HSFx significantly decreased serum glucose level in high-fat diet rats. Triglyceride (TG) levels in high-fat diet rats in the LMF-HSFx group also decreased compared to high-fat diet rats (Figure 5D). Furthermore, serum AST and ALT levels were significantly increased in high-fat diet rats compared to Normal Diet (ND) rats, whereas the

LMF-HSFx combination significantly reduced hepatic lipotoxicity in the *in vivo* assay (Figure 5E,F). Next, the effect of LMF-HSFx on the dysregulation of adipogenesis in high-fat diet mice was evaluated. In adipose tissue and pro-chocolate adipose tissue, LMF-HSFx also significantly upregulated the gene expression of adiponectin, adipoq, adig, and leptin gene expression. (Table 1)



LMF-HSFx modulates the PGC-1 treatment pathway in HepaRG hepatocytes with Palmitic Acid (PA).

In HepaRG cells PA induced hepatic lipotoxicity resulted in a decrease of SIRT family protein expression (Figure 6A,B), which is modulated by the leptin- adiponectin axis. In addition, it was found that PA treatment specifically induced cleavage or decreased SIRT expression in a dose-dependent manner (cells without PA, with PA200, with PA400), whereas LMF-HSFx significantly restored PA-induced SIRT2, 3, 6 degradations (Figure 6A). SIRT1-3, 6 levels were significantly increased in the LMF-HSFx group compared to the control group (Figure 6B). Furthermore, PGC-1 α increased but PGC-1 β decreased in hepatocytes treated with PA in a dose-dependent manner (cells without PA, with PA200, with PA400), whereas LMF-HSFx significantly restored the effect (Figure 6C,D). Coordinately, the level of the lipid metabolism- related protein, Adipose Triglyceride Lipase (ATGL), was also significantly restored by 25 μ g/mL LMF-HSFx treatment (Figure 6C,D).

Biocompatibility testing inhibited the growth of various fucoidans determined by MTT cell proliferation and cytotoxic assays for L929 cells

(Mouse fibroblast cells). The test method began with incubation of L929 cells for 24 hours-37°C-at 5% CO₂ with a density of 6×10^4 . Next, the culture medium was replaced with 100 μ L of new medium containing fucoidan with various concentrations. After the cells were incubated, for 48 hours, the media was washed and 10 μ L MTT reagent was added. Then, after the incubation process is complete, the culture medium is discarded and 150 μ L of DMSO solution is added. Finally, cell survival can be calculated by following the following formula.⁽¹⁸⁾ The results of fucoidan biocompatibility testing showed that there was no significant pathological effect on >90% of hepatocytes.

Fucoanthin has gone through several experiments, including in animals and humans. In ICR rats, Fucoanthin did not cause death and abnormalities in single dose studies of 1000 and 2000 mg/kg, equivalent to 500 and 1000 mg/kg in repeated dose studies for 30 days. Oral single-dose studies in rats also showed no toxicity with the administration of Fucoanthin 200 mg/kg BW and can be used on human skin. Therefore, administration of

Fucoanthin is considered safe and has great potential.⁽¹⁹⁾

The mechanism of action of LMF-HSFx for NAFLD starts with targeting adipocytes and hepatocytes. In hepatocytes, LMF-HSFx directly activates the SIRT-PGC1 axis and PGC-1 family expression.^(20,21) LMF-HSFx also decreased proinflammatory cytokines (IL6 and INF γ) released from hepatocytes and suppressed fibrosis in NAFLD.

RESULT AND DISCUSSION

Non-Alcoholic Fatty Liver Disease (NAFLD)

Chronic liver disease (CLD) is the leading cause of death in the world. There were approximately 1.5 billion cases of CLD worldwide in 2020 with 1.32 million deaths, which is more prevalent in men than women with a ratio of two to one.^(7,8) Non-Alcoholic Fatty Liver Disease (NAFLD) is more than 5% hepatocyte fat accumulation. The diagnosis of NAFLD remains a major challenge until now. Metabolic dysregulation can cause obesity and result in liver dysfunction. NAFLD results from fat accumulation caused by a combination of increased de novo lipogenesis from nonlipid sources such as carbohydrates and increased free fatty acids (FFA). This is due to lipolysis and an increase in dietary-derived fat, decreased FFA oxidation, and triglycerides (TG) as Very Low-Density Lipoproteins (VLDL). A recent concept on the pathogenesis of NAFLD suggests the involvement of the hepatic-intestine axis which has complex interactions. An unbalanced diet can disrupt the activity of the gut microbiota and the activation of Kupffer cells. Kupffer cells in the liver release pro-inflammatory cytokines such as TNF- α , IL-6, and IL-1 β .⁽⁹⁾

Other pathophysiologists explain that NAFLD is a very complex disease. There are several things associated with it, including insulin resistance, obesity, type 2 diabetes, dyslipidemia that can increase LDL, decrease HDL, and cardiovascular disorders.⁽¹⁰⁾ *Phaeophyta* is a type of seaweed that has been developed into food and medicine, due to its bioactivity. Polysaccharides from *Phaeophyta* have health functions, namely anti-inflammatory and anti-oxidant. The hydroethanol extract of *Phaeophyta* also showed anti-cholesterol activity and α -glucosidase enzyme help. Natural ingredients from *Phaeophyta* contribute to the discovery of modern medicine and the development of new drugs, as they have been proven safe to consume and used as traditional

medicine in several countries. Consumption of *Phaeophyta* can reduce the incidence of chronic diseases such as cancer, hyperlipidemia, coronary heart disease, and obesity.⁽¹¹⁾

Phaeophyta has fucoxanthin and fucoidan which have the most significant potential in curative therapy of NAFLD.⁽¹²⁾ Fucoxanthin is a rubberonoid found mainly in *Phaeophyta* with a molecular weight of 65.89 g/mol, the compound has a chemical structure (C₄₂H₅₈O₆) located in chloroplasts and is responsible for photochemical events.^(13,14) *Phaeophyta* cell walls are rich in sulfated polysaccharides called fucoidan.

Low Molecular Weight Fucoidan (LMF) - etHigh Stability Fucoxanthin (HSFx)

Based on the research of J.Wang *et al.* noted that high anti-oxidant activity comes from LMF (4.58 - 8.5 kDa) which has higher solubility and contributes to their penetration into cells. LMF has high anti-inflammatory activity even in the lowest concentration (50 mg/ml).⁽¹⁵⁾ HSFx is known to reduce oxidative stress induced by palmitic acid without affecting cell survival, it can limit lipid accumulation induced by palmitic acid at high concentrations through long non-coding RNA (lncRNA).⁽¹⁶⁾

CONCLUSIONS

LMF-HSFx combination in *Phaeophyta* showed success in curative therapy of Non-Alcoholic Fatty Liver Disease (NAFLD). LMF-HSFx treats liver damage and prevents its complications. LMF-HSFx *Phaeophyta* is a hope for the development of modern medicine that is tested preclinically and clinically.

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None

CONFLICT OF INTEREST

None

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