INTRODUCTION

Non-alcoholic Fatty Liver Disease (NAFLD) is characterized by fat accumulation or steatosis in hepatocytes without secondary causes such as excessive alcohol consumption. The global prevalence of NAFLD from 1990 to 2019 is 30.05% and is expected to increase every year. NAFLD increases in individuals with Western diet consumption and lack of physical activity, resulting in metabolic syndrome. Metabolic syndrome increases a further form of NAFLD, namely Nonalcoholic Steatohepatitis (NASH).

The frequency of NASH among NAFLD patients who underwent biopsy was 15.9% to 68.3%, and in obese patients from 12.6% to 30.4%. NASH is characterized by steatosis and inflammation with hepatocyte damage (ballooning) with or without fibrosis. NASH increases the risk of liver diseases such as cirrhosis and hepatocellular carcinoma. Oxidative stress is important in forming and progressing from NAFLD to NASH.

Oxidative stress plays an important role in the progression of NAFLD, where fat accumulation causes an increase in ROS production and decreases in liver tissue's antioxidants. Fat accumulation occurs due to an imbalance between entry and exit rates of triglycerides in the liver. This can be caused by increased Free Fatty Acids (FFAs) from increased lipolysis or dietary fat intake, decreased FFA oxidation, increased de novo lipogenesis, and decreased triglyceride secretion. This accumulation causes an increase in ROS.
Table 1. Summary of ALA’s potential on stress oxidative in NAFLD

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<th>The first author (publication year)</th>
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<td>Cervera et al (2016)</td>
<td>Experimental</td>
<td>Sixty male rats were divided into 5 groups. Each group was given an iso-caloric diet, and the control group was assigned iso-caloric diet and sunflower oil/SFO (&lt;1% ALA), the experimental group was given canola oil/CO (10% ALA), rosa mosquenta oil/RMO (33% ALA), sacha inchi oil/SIO (48% ALA), and chia oil/ChO (63% ALA).</td>
<td>21 days</td>
<td>Increased GSH and GSH/GSSG ratio in SIO and ChO compared to control. Parameters of oxidative stress (protein carbonyl, F2-isoprostanes and TBARS) in the liver and parameters of oxidative stress in plasma (TBARS and antioxidant capacity) did not change with the ALA diet intervention. Antioxidant enzymatic activity in the liver (SOD, CAT, GPX, and GR) increased in the SIO and ChO groups.</td>
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<td>Han et al. (2017)</td>
<td>Experimental</td>
<td>Male rats were divided into 3 groups. The control group was given a normal diet, a high fat and high cholesterol diet group, a high fat and high cholesterol diet group, and a flaxseed oil group.</td>
<td>12 weeks</td>
<td>The increase in ROS levels in the liver induced by a high fat and cholesterol diet decreased after given flaxseed oil. Decreased MDA concentration and increased GSH and SOD levels in serum and liver.</td>
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<td>Ambulay et al (2020)</td>
<td>Experimental</td>
<td>Male rats were induced into obesity with a high-fat diet for 16 weeks, then divided into control groups: obese (OC, n=6), obese with sacha inchi 2.5 ml (OSI1, 0.25gr omega3/day, n=7), obese with sacha inchi 2.5 ml (OSI2, 0.5gr omega3/day, n=7), obese with 10 mg/kg atorvastatin (OAT, n=6), obese with 10 mg/kg atorvastatin plus 2.5 ml sacha inchi (OATS12, n=6), and a non-obese control group (NOC, n = 6) who were fed a standard diet.</td>
<td>27 weeks</td>
<td>Serum MDA in the OSI2 group decreased significantly by 27% compared to the OC group. AOPP serum also considerably decreased by 41% and 33% in the OSI1 and OSI2 groups compared to the OC group. Total antioxidant capacity (FRAP and ABTS) in the serum: There was no change in the group that was given sacha inchi. The decrease in hepatic MDA and SOD was not significant. Significantly increased hepatic antioxidant capacity (FRAP and ABTS) in the OSI2 group. Considerable increase in the enzyme activity from catalase (CAT) in OSI2 compared to the control group. The decrease in hepatic MDA and SOD was not significant. Significantly increased hepatic antioxidant capacity (FRAP and ABTS) in the OSI2 group. Considerable increase in the enzyme activity from catalase (CAT) in OSI2 compared to the control group.</td>
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<td>Moreira et al (2022)</td>
<td>Experimental</td>
<td>Forty male rats were divided into 2 groups; the group was given a standard diet (n = 10), and the group was induced on a high-fat, high-fructose diet (n = 30) for 8 weeks. Then, the HFHF group was divided into 3 groups: the HFHF group (n = 10), the HFHF + Chia Flour (CF) group (n = 10), the HFHF + Chia oil (CO) group (n = 10) for 10 weeks.</td>
<td>18 weeks</td>
<td>There was no significant difference in total antioxidant capacity in serum. In the liver, consumption of chia increases the full antioxidant capacity, SOD, and decreases NO.</td>
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<td>Xu et al. (2015)</td>
<td>Experimental</td>
<td>Forty male rats were divided into 4 groups (n=10), control group high-fat diet + refined rapeseed oil (RRO), high-fat diet + cold pressing oil (CPO), high-fat diet + dehulling-cold pressed rapeseed oil (DCPO), and high fat diet + microwave pretreatment cold pressing rapeseed oil (MPCPO).</td>
<td>10 weeks</td>
<td>There was no difference in hepatic SOD activity in all groups, but there was an increase in hepatic GPx in the DCPO and MPCPO groups and hepatic CAT in the MPCPO group compared to the control group. Increased hepatic GSH level in all groups compared to control. Hepatic TBARS decreased in the CPO and MPCPO groups.</td>
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and damages hepatocyte cells, resulting in mitochondrial and Endoplasmic Reticulum (ER) dysfunction, leading to impaired fatty acid oxidation and fat synthesis.\textsuperscript{12,14} Oxidative stress also makes Kupffer cells produce cytokines such as TNF-\textalpha, which increase inflammation. In addition, lipid peroxidation due to oxidative stress causes Stellate cells to proliferate and synthesize collagen, causing fibrosis.\textsuperscript{13–15}

In fighting ROS in the liver, nuclear factor E2-related factor 2 (Nrf2) plays an important role. Exposure to oxidative stress induces several antioxidant genes by activating the antioxidant response element (ARE). ARE gene expression is largely regulated by Nrf2. Nrf2 activation protects mitochondria from oxidative stress by increasing antioxidant levels and maintaining mitochondrial redox states. In addition, this activation also regulates fatty acid metabolism genes, such as CD36. If there is a disturbance in the expression of the ARE gene, it will cause an increase in cell sensitivity to oxidative stress.\textsuperscript{15,16}

Oxidative stress can damage macromolecules, such as fats, proteins, and DNA, resulting in oxidative damage products, such as Malondialdehyde (MDA), lipid peroxides, and 8-Isoprostane.\textsuperscript{13} Some antioxidants also play a role in fighting oxidative stress; both enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and non-enzymatic antioxidants, such as glutathione (GSH). These antioxidant levels can also be used to evaluate the level of oxidative stress.\textsuperscript{15,31}

In NAFLD, increased ROS can reduce antioxidant molecules and inhibit antioxidant enzymes, decreasing antioxidant capacity in liver cells.\textsuperscript{28} The most commonly used antioxidant markers are SOD, CAT, GPx, GR, and GSH, and the results usually show decreasing antioxidant levels in the liver.\textsuperscript{19} MDA is also one of the most frequently used markers in measuring oxidative stress and consistently increases in research models.\textsuperscript{12,23} Increased levels of MDA are also thought to be associated with increased levels of liver enzymes aspartate transaminase (AST) and alanine transaminase (ALT).\textsuperscript{33}

### ALA and oxidative stress in NAFLD

Alpha-linolenic acid (ALA) is an n-3 PUFA with high antioxidant anti-inflammatory and hypolipidemic effects.\textsuperscript{18} Consumption of ALA is predicted to have a protective effect on NAFLD, where its antioxidant effect can reduce oxidative stress and increase antioxidant levels. Without the delta 12 and 15 desaturase enzymes, the body cannot synthesize ALA.\textsuperscript{18,34,35} Therefore, ALA is only obtained from foods of plant origin, such as oil, nuts, and seeds.\textsuperscript{36}

Based on research by Han et al., flaxseed oil (57.82% ALA) administration increased GSH and SOD levels and reduced MDA in both serum and liver in rats induced by high fat and high-cholesterol diet.\textsuperscript{23} This could be due to ALA's ability to reduce free radical production or through increased free radical scavenging activity. This study also has similarity to the study of Moreira et al., using chia seed (31.8% ALA) in rats induced by high fat and high fructose diet.\textsuperscript{23} This study demonstrated the effect of chia as a hepatoprotective caused by high fat and high fructose diet.\textsuperscript{28,37}

In the study of Ambulay et al., obesity-induced rats were given sacha inchi oil (54.79% ALA), and an increase in the total antioxidant capacity in the liver was also found. Still, there was no change in the total antioxidant capacity in the serum.\textsuperscript{24} This protective role can be caused by the effect of ALA in restoring antioxidant enzyme activity and GSH levels. In addition, ALA also regulates the expression of HO-1 through Nrf2 activation. HO-1 is an antioxidant enzyme that plays a role in cytoprotection and tissue injury, which prevents oxidative stress.\textsuperscript{35}

Besides the effect of increasing antioxidant activity, ALA can also regulate Adipor2 gene expression. This gene improves glucose metabolism by lowering blood sugar, triglycerides, and body weight, thus reducing oxidative stress and fatty liver.\textsuperscript{25} The ratio of omega 6 : omega 3 also affects the protective effect of ALA, where if the ratio is closer to 1:1, it can provide a strong hepatoprotective effect.\textsuperscript{32}

In the study of Ambulay et al., there was also a significant increase in the activity of the hepatic enzyme CAT at a dose of 0.5 g (2.5 ml), where the results of this study were similar to those of Rojanaverawong et al., which compared administration of sacha inchi oil (ALA 41.29%) with different doses in DM male rats. There was an increase in GPx and CAT and a significant decrease in hepatic MDA only at a dose of 2 ml. This study demonstrated the effect of ALA as a hepatoprotective factor caused by high fat and high fructose diet.\textsuperscript{28,37}

### Experimental Study

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<td>Rojanaverawong et al.\textsuperscript{27}</td>
<td>Experimental</td>
<td>Male rats were induced on a high-fat diet for 2 weeks and then injected with streptozotocin (STZ) for 3; the control group was given a normal diet + citrate buffer injection. Then divided into 6 groups (n=6/group). Control group, DM, DM + sacha inchi / SI (0.5 ml/kg), DM + SI (1 ml/kg), DM + SI (2 ml/kg), and DM + the drug Pioglitazone hydrochloride (PZ) for 5 weeks.</td>
<td>7 weeks</td>
<td>Significant increase in hepatic SOD except for 0.5 ml dose. Increased GPx and CAT and a significant decrease in hepatic MDA only at a dose of 2ml.</td>
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Research Cervera et al. compared sunflower seed oil (<1% ALA), canola oil (10% ALA), rose mosqueta oil (RMO) (33% ALA), sacha inchi oil (48% ALA), and chia oil (63% ALA). There was an increase in GSH and activity of antioxidative enzymes in the liver (SOD, CAT, GPx, and GR) in the sacha inchi oil and chia oil groups.22

In Xu’s research, et al. showed that differences in the processing of rapeseed oil (9% ALA) also affect the nutrients it contains. In microwave pretreatment-cold pressed rapeseed oil (MPCPO), there was an increase in GSH, GPx, and CAT activity and a decrease in hepatic TBARS (MDA), where there was an increase in antioxidative capacity, which could reduce oxidative stress. This research shows that the processing process can also affect the antioxidative effect on ALA.26

Despite the potential benefits of ALA on stress oxidative in NAFLD, there are several limitations to consider in existing research. Firstly, the number of studies exploring the effect of ALA and stress oxidative in NAFLD is limited, indicating a research gap. The available studies also vary in sample size, duration, and intervention, making it difficult to draw definitive conclusions. The evidence suggests a potential effect between ALA and stress oxidative in NAFLD, but further well-designed studies are needed. This is to overcome limitations and provide a more comprehensive understanding of this effect.

**CONCLUSION**

ALA has an antioxidant effect that can reduce the impact of oxidative stress in NAFLD, especially in liver tissue, which provides a hepatoprotective effect and regulates gene expression that can reduce oxidative stress in NAFLD. However, several things can affect the antioxidative effect of ALA in reducing oxidative stress in NAFLD, such as the selection of plant levels containing ALA, the number of doses, the ratio of omega-6 compared to omega-3, and the processing process.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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**AUTHOR CONTRIBUTION**

All of the authors contributed to this study.
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