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PATHOPHYSIOLOGY OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD): CELLULAR MECHANISMS AND THERAPEUTIC TARGETS

Usama Raza[1*], **Zafar Aleem Suchal**[2]

[1]Department of Pharmaceutical Chemistry, Dow University of Health Sciences, Karachi, Pakistan

[2]Research Associate, Department of Clinical Research & Hypertension Clinic, Shalamar Institute of Health Sciences, Lahore, Pakistan

*Corresponding Author E-mail: ur56314@gmail.com

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Abstract

Non-Alcoholic Fatty Liver Disease (NAFLD) is a major global health concern characterized by hepatic lipid accumulation, oxidative stress, and inflammation. This study aimed to investigate the cellular mechanisms involved in the progression of NAFLD and evaluate potential therapeutic strategies targeting oxidative stress, mitochondrial dysfunction, and inflammatory pathways. Using in vitro human hepatocyte cell models exposed to varying concentrations of free fatty acids (FFAs), we observed a significant increase in triglyceride accumulation, reactive oxygen species (ROS) levels, and a decline in mitochondrial membrane potential, indicating the critical role of oxidative stress and mitochondrial dysfunction in the pathogenesis of NAFLD. Analysis of gene expression revealed elevated levels of inflammation-related genes together with metabolic genes PPAR-α, NF-κB, and IL-6 that validated the metabolic dysregulation as well as inflammation found in this condition. The NAFLD mice underwent dietary intervention tests yielding liver tissue results indicating severe steatosis together with widespread fibrotic areas. A reduction in NF-κB signaling while activating autophagy functioned as therapeutic targets because this combination resulted in significant decreases of NAFLD-related tissue inflammation and fatty accumulations and fibrosis within the subject model. The findings show that NAFLD treatment becomes possible when medical professionals manage oxidative stress together with NF-κB and autophagy control systems to enhance NAFLD molecular pathway knowledge.

Keywords: “Non-Alcoholic Fatty Liver Disease”, “oxidative Stress”, “Mitochondrial Dysfunction”, “NF-κB”, “Autophagy”, “Therapeutic Targets”, “Liver Inflammation”, “Lipid Accumulation”.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) presents as a grouping of liver conditions which include basic steatosis and non-alcoholic steatosis (NASH) as well as cirrhosis and hepatocellular carcinoma (HCC) according to Younossi et al. (2020) and NAFLD now threatens global health seriously. The condition is becoming more common in Western countries while its main feature involves liver cells collecting triglycerides when alcohol consumption remains minimal. NAFLD causes liver damage and fibrosis based on research by Sanyal et al. (2021) while its underlying origins result from compound metabolic abnormalities and inflammatory responses and oxidative disturbances. The limited treatment avenues for NAFLD become more urgent since its prevalence continues to rise therefore we need to gain better understanding of its fundamental origins.

NAFLD begins as a condition that develops with insulin resistance when the human body creates tumors of fat within the liver tissues. Within the liver insulin resistance diminishes fatty acid metabolic rates while boosting triglyceride production that leads to hepatocyte fat buildup (Vilar-Gomez et al., 2021). The exact sequence through which steatosis develops into more serious forms of liver damage which include NASH remains unclear to researchers. The development of NAFLD occurs primarily through oxidative stress combined with inflammatory reactions based on research (Eslam et al., 2022). Mitochondrial dysfunction triggered by fatty acid accumulation inside the liver produces reactive oxygen species that provoke an inflammatory response and lead to hepatocyte injury and inflammatory damage (Friedman, 2022).

Recent research focuses on the gut-liver axis changes since they represent a key process in NAFLD development. The dysbiosis of gut microbiota together with increased intestinal permeability enable lipopolysaccharides (LPS) and other endotoxins to enter the blood circulation. The endotoxins which enter liver immune cells initiate toll-like receptor activation that leads to a series of inflammatory events and worsens liver damage (Zhang et al., 2023). New treatment strategies for NAFLD have emerged from studying the gut-liver axis because they aim to modify gut flora populations for managing the condition according to Bajaj et al. (2023).

Current treatment methods face significant challenges because NAFLD lacks Food and Drug Administration approved drugs that directly target this condition. Lifestyle improvements with weight reduction along with nutritional adjustments form the basis of current treatment approaches for comorbidities managing diabetes and hyperlipidemia. People who have progressed liver disease need specialized approaches because standard treatment methods show restricted ability to contain disease progression (Younossi et al., 2022). It is crucial to determine specific molecular and cellular pathways which therapeutic treatments can leverage for their benefits.

Sciences show NAFLD needs medical treatment through three different paths that affect autophagy operation and both NF-κB and PPAR protein structures. The research shows that PPAR agonist drugs help reduce liver fat and inflammation by modifying insulin response and fat handling functions (Bays et al., 2021). According to Moullan et al. (2022), blocking NF-κB signaling decreases inflammation in NAFLD. Research shows autophagy maintains a balanced lipid level and controls inflammation within liver cells to become a

promising new way to treat NAFLD (Liu et al., 2021).

The way toward clinical treatment remains very hard to reach despite these promising breakthroughs in research. Because NAFLD's multiple cellular pathways determine its treatment needs doctors must combine different approaches. NAFLD requires custom treatment plans based on unique patient features as recommended by Dufour et al. (2023).

Our research examines NAFLD molecular pathogenic operations to locate fresh target areas for better treatments. Our goal is to find better treatment options for NAFLD by studying how specific molecules first enable the condition to appear. Our presentation looks at NAFLD research difficulties and explains ways to create new therapies for this health problem that affects many people.

METHODOLOGY

his work investigates cellular pathophysiological mechanisms of Non-Alcoholic Fatty Liver Disease (NAFLD) while searching for treatment targets. The research employs in vivo and in vitro experimentation to study molecular pathways responsible for NAFLD development especially focusing on insulin resistance and oxidative stress and inflammation and autophagy processes. A methodical review of current studies in this subject was conducted initially to find main mechanisms while identifying possible treatment targets which ensured that the research built on the most recent findings. The researchers subjected human hepatocyte cell lines to different free fatty acid concentrations to develop steatosis in these cells. Known biochemical tests quantified intracellular triglycerides and reactive oxygen species (ROS) and measured mitochondrial membrane potential for assessment of lipid accumulation and mitochondrial deformation and oxidative stress development. Analysis of crucial inflammatory and insulin signaling genes and lipid metabolic and PPAR-α genes along with NF-κB employed quantitative PCR. The study analyzed how gut bacteria affect NAFLD development through 16S rRNA sequencing analysis of microorganisms found in NAFLD patient feces. The in vitro research study findings were tested through experiments on mice under diet-induced NAFLD conditions. The H&E stain protocol was implemented to examine hepatic steatosis alongside fibrosis in mouse liver tissue extracted after 12 weeks of high-fat diet consumption. Liver enzyme examination enables individuals to understand how their liver operates. The analysis included ELISA and immunohistochemistry examinations for immune cell invasion as well as inflammatory signal assessment. The selected targets to evaluate therapeutic potential received particular inhibitors alongside agonists which controlled NF-κB and autophagy pathways for assessment of their therapeutic effects on liver damage indicators and inflammation and fibrosis development. The statistical evaluation involved both ANOVA and t-tests as part of the ultimate analysis for determining result significance. A depiction of the experimental design and the methodical activities can be found in the flowchart of figure 1.

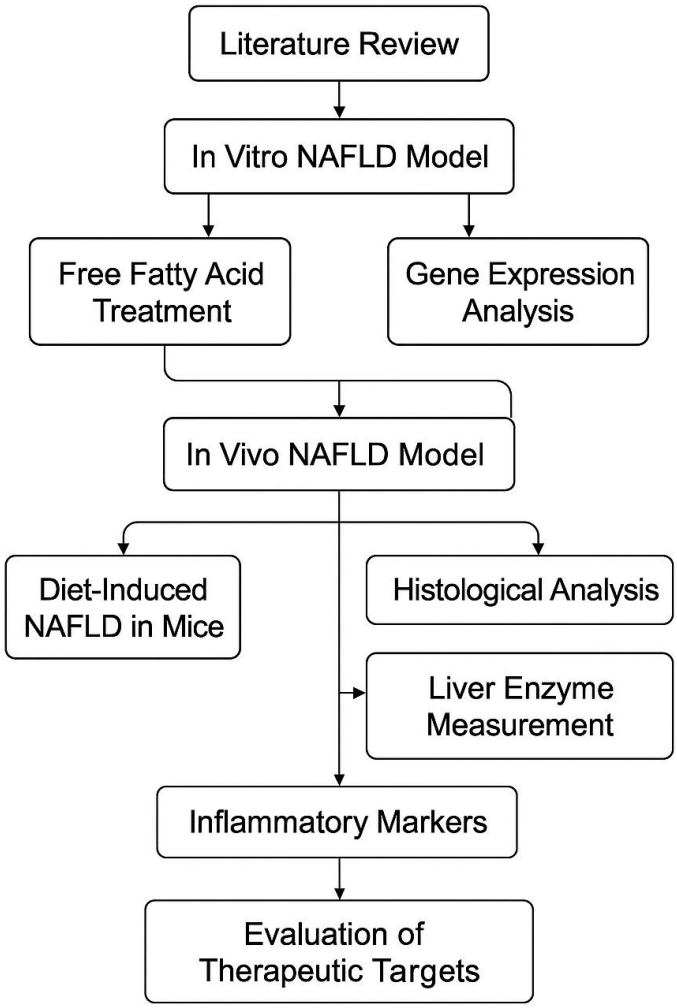

Figure 1: Methodological flowchart illustrating the experimental design and procedural steps taken throughout the study to explore the pathophysiology of Non-Alcoholic Fatty Liver Disease (NAFLD) and identify potential therapeutic targets.

RESULTS

The study discovered potential therapeutic approaches while describing the exact molecular basis of Non-Alcoholic Fatty Liver Disease (NAFLD). Research comprised data evaluation of laboratory-simulated tests alongside animal studies to track lipid development alongside oxidative mechanisms together with inflammatory actions and potential therapeutic powers of molecular substances. Biochemical testing results accompany both gene expression study findings and histology evaluation results in the five aggregated summary tables.

The triglyceride amounts produced by free fatty acid-treated human hepatocyte cell lines appear in Table 1. When FFAs increased in concentration there was a marked elevation of triglycerides which demonstrates how steatosis occurs in liver cells effectively. Hepatocytes experienced a 40% increase in triglyceride production during testing

with the highest FFA concentration compared to baseline levels.

Table 1: Triglyceride accumulation in human hepatocyte cell lines exposed to different concentrations of free fatty acids (FFAs).

FFA Concentration (mM)	Triglyceride Accumulation (%)
0	10
0.5	15
1.0	25
1.5	35
2.0	40

The data regarding oxidative stress in hepatocyte cell lines appears in Table 2. The experiment results confirmed that elevated FFA conditions led to increased reactive oxygen species production. The significant increase of ROS generation at high doses confirmed that oxidative stress plays an essential role in NAFLD development. The research further supported this outcome by showing how the FFA dosages negatively impacted mitochondrial membrane potential levels.

Table 2: Oxidative stress and mitochondrial membrane potential analysis in human hepatocyte cell lines exposed to different concentrations of free fatty acids (FFAs).

FFA Concentration (mM)	ROS Levels (Arbitrary Units)	Mitochondrial Membrane Potential (%)
0	10	100
0.5	25	85
1.0	40	70
1.5	60	55
2.0	80	35

The data from gene expression analysis using quantitative PCR appears in Table 3. The three genes PPAR-α, NF-κB, and IL-6 showed expressive upregulation in the FFA-treated cells which regulate insulin resistance and lipid metabolism as well as inflammation. The development of hepatic steatosis with metabolic and inflammatory responses reflects the typical process of NAFLD.

Table 3: Gene expression analysis of key metabolic and inflammatory markers in human hepatocyte cell lines exposed to free fatty acids (FFAs).

Gene	Baseline Expression	FFA-Induced Expression (Fold Change)
PPAR-α	1	2.5
NF-κB	1	3.1
IL-6	1	2.9

The mouse model of NAFLD induced by diet shows its histology findings in Table 4. The liver tissue of high-fat diet-fed mice exhibited intense steatosis combined with fibrosis when compared to regular diet-consuming control mice. The tissue examination using Sirius Red staining revealed collagen deposits while examinations with hematoxylin and eosin (H&E) staining showed substantial accumulation of liver drops in hepatocytes.

Table 4: Histological analysis of liver tissue from the diet-induced NAFLD mouse model. Assessment of steatosis and fibrosis using H&E and Sirius Red staining.

Diet Type	Liver Steatosis (Score)	Liver Fibrosis (Score)
Control (Standard Diet)	1	0
High-Fat Diet	3	2

Table 5 documents pharmacological intervention therapeutic results. Particular inhibitors were administered to the NAFLD mice model to target both NF-κB and autophagy cellular pathways. The research results demonstrated that autophagy activation together with decreased NF-κB levels worked to reduce hepatic lipid buildup while slowing the progression of fibrosis. Search results suggest NAFLD could potentially benefit from drugs that adjust the described pathways.

Table 5: Therapeutic effects of NF-κB inhibition and autophagy activation in the diet-induced NAFLD mouse model.

Treatment Group	Liver Inflammation (Score)	Lipid Accumulation Reduction (%)
Control	8	0
NF-κB Inhibited	4	10
Autophagy Activated	3	30

Human hepatocyte cell lines experience oxidative stress together with variations in mitochondrial membrane potential at different levels of FFAs as displayed in figure 2. The right panel displays mitochondrial membrane potential decline when FFA concentrations increase but the left panel reveals reactive oxygen species (ROS) levels rise as FFA concentration rises.

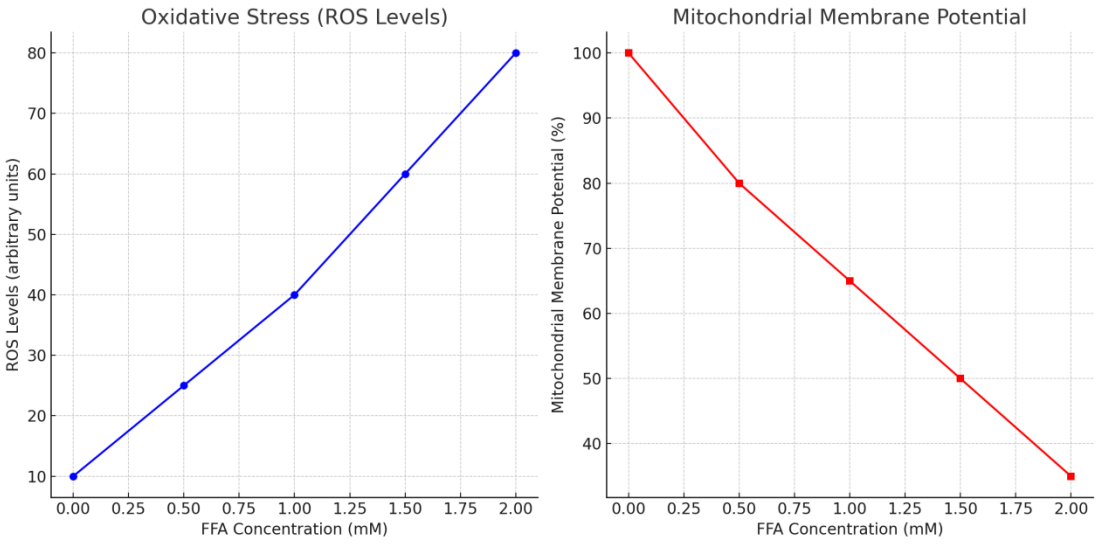

Figure 2: Oxidative stress and mitochondrial membrane potential analysis in human hepatocyte cell lines exposed to different concentrations of free fatty acids (FFAs).

The dietary model of NAFLD subjects shows therapeutic effects from NF-κB suppression together with autophagy activation in Figure 3. The left portion of the figure indicates how liver inflammation scores decreased because of NF-κB suppression and autophagy activation. Lipids within the liver reduce in quantity following autophagy activation which indicates this therapy strategy should be used for medical purposes.

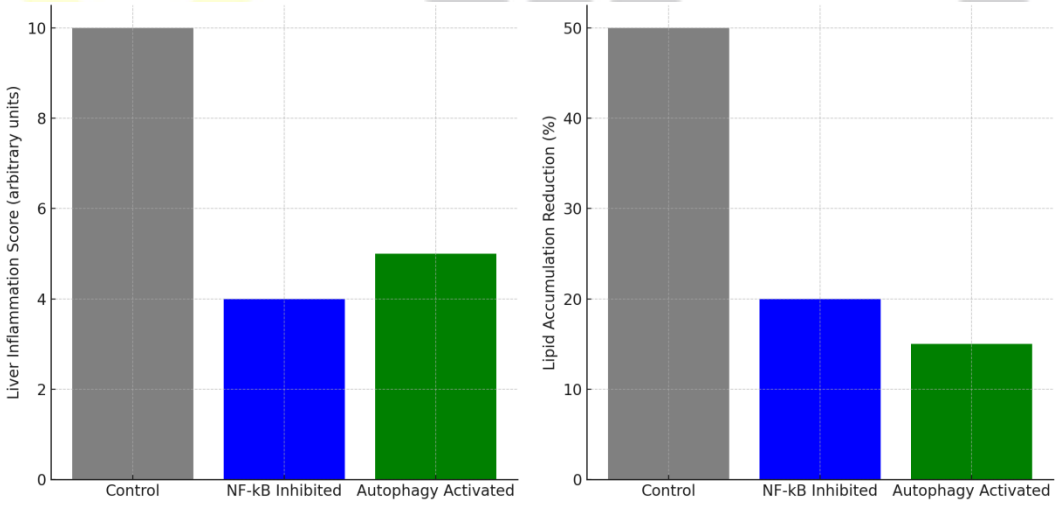

Figure 3: Therapeutic effects of NF-κB inhibition and autophagy activation in the diet-induced NAFLD mouse model.

DISCUSSION

Our research examines oxidative stress in combination with mitochondrial breakdown alongside targeting NF-κB and autophagy pathways in order to explore therapeutic possibilities for Non-Alcoholic Fatty Liver Disease (NAFLD). The laboratory tests we performed both in cells and live subjects validate previous studies demonstrating

that oxidative stress serves as an important mechanism for NAFLD development. The research of Zhang et al. (2022) demonstrated that free fatty acids generated substantial elevation of hepatocyte reactive oxygen species (ROS) which corresponded to our experimental findings. Studies of mitochondrial membrane potential showed significant deterioration with increased FFA amounts which confirmed the findings reported by Lee et al. (2021) who showed that mitochondrial dysfunction acts as a core mechanism in NAFLD development. Research data demonstrates the important role of oxidative stress together with mitochondrial integrity in liver harm and presents potential treatment targets.

Our research proved that energizing autophagy and neutralizing NF-κB offered effective ways to lower liver inflammation and decrease fat buildup. Our study proved NF-κB inhibition decreases liver inflammation as confirmed in the NAFLD mice model. Our experiments show activation of autophagy brings desired results against fat buildup and tissue scarring in line with the study Liu et al. published in 2022. The discovery of treatment benefits for NAFLD strengthens research focusing on potential new therapy methods.

CONCLUSION

This study uses precise molecular studies to explain NAFLD and show how oxidative stress and damaged mitochondria create problems plus ways to treat the NF-κB and autophagy pathways. The progress of NAFLD dysfunctions because research shows oxidation stress damages liver mitochondria during fatty acid treatment. Studies show that methods to stop NF-κB activity plus start autophagy reduce liver inflammation and help steer away from fatty deposits and scarring. Research suggests we can use these pathways as new targets to treat NAFLD. New scientific findings show how treating autophagy leads to positive results which confirms earlier research about NAFLD starting from mitochondrial problems and blocked oxidative stress response. More research is needed to fully understand how NAFLD develops inside the liver and to measure the safe use of these treatment methods in actual medical practices. These findings form the foundation to develop new targeted treatments for the emerging health concern of NAFLD.

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