REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Apoptosis and necroptosis in nonalcoholic steatohepatitis; New strategy for treatment

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Abstract

NASH is a kind of liver inflammation and damage caused by an accumulation of fat in the liver. It is one of several disorders known as nonalcoholic fatty liver disease. You may be diagnosed with a "fatty liver." NASH can progress to cirrhosis of the liver, which leads to cirrhosis. However, the condition does not always worsen. Recent research has also highlighted the risk of developing hepatocellular carcinoma because of NASH. Free fatty acids cause widespread apoptosis in hepatocytes, resulting in steatohepatitis. Necrosis and necro-inflammation are also histological features of human NASH, indicating that alternate cell death forms may play an important part in the disease's pathophysiology. Kaempferol (KP) is a flavonoid present in many fruits. Epidemiologic studies and clinical trials have demonstrated that a diet high in kaempferol has pharmacological benefits for liver diseases. Pentoxifylline (PTX) is a methylxanthine molecule that acts as a non-selective phosphodiesterase inhibitor, causing vasodilation. Several modest clinical trials have been conducted to confirm the efficacy of PTX in the treatment of NASH.

Keywords: NASH, Kaempferol, Pentoxifylline, Apoptosis, Necroptosis, Inflammation.

1. Non-alcoholic steatohepatitis (NASH)

Non-alcoholic steatohepatitis (NASH) develops from non-alcoholic fatty liver disease (NAFLD). Currently, around 25% of the population is estimated to have NAFLD, and 25% of NAFLD patients are estimated to have NASH. NASH is typically characterized by liver steatosis inflammation, and fibrosis driven by metabolic disruptions such as obesity, diabetes, and dyslipidemia. NASH patients with significant fibrosis have increased risk of developing cirrhosis and liver failure **(Peng et al., 2020)**.

2. Epidemiology of non-alcoholic steatohepatitis

NASH-induced cirrhosis has been recognized as one of the fastest-growing liver diseases and is the second greatest contributor to an indication for liver transplantation in the United States is projected to increase to 27 million by 2030 **(Burra et al., 2020).**

In parallel with the development and progression of obesity and type 2 diabetes a recent study highlighted that the annual healthcare cost associated with NAFLD in the United States was

approximately US$103 billion, and €35 billion in four European Epidemiological studies show that roughly 82% of NASH patients are obese, 83% exhibit hyperlipidemia and 48% are diagnosed with type 2 diabetes **(Peng et al., 2020)**.

3. Diagnosis and detection methods

NASH itself can often be asymptomatic, although patients with a high body mass index (>25 kg/m2) and T2DM features such as hyperglycemia and insulin resistance are encouraged to be screened for the presence of fatty liver disease **(Peng et al., 2020)**. Nevertheless, a recent population study has highlighted that NASH patients have a higher incidence of fatigue and abdominal discomfort which are shown to be correlated with hepatic lobular inflammation **(Mahjoubin-Tehran et al., 2021)**.

Elevation in the plasma of the liver enzymes alanine transaminase (ALT) and aspartate aminotransferase (AST) in a routine blood test is generally the first line of diagnosis. ALT and AST are highly expressed in hepatocytes. ALT and AST are thus insufficiently specific and sensitive enough to determine the presence or severity of NASH **(Paul, 2020)**.

To confirm the presence of fatty liver, computed tomography (CT) scan or magnetic resonance imaging (MRI) can potentially be used as a non-invasive diagnostic tool to assess the percentage of fat in the liver **(Chartampilas, 2018)**. However, using MRI as a diagnostic tool in the clinic may not be practical due to the high cost and limited availability **(Guo, 2018)**.

4. Causes and Risk Factors

NASH is most common in patients who are overweight or obese. Risk factors include:

4.1. Obesity and insulin resistance

Certainly, one of the factors contributing to the rise of NAFLD diffusion is obesity. In fact, paralleling the epidemic proportion of overweight and obesity in children and adolescents, in the last three decades it has been reported a doubled prevalence of hepatic steatosis with about 7 million children and adolescents affected by NAFLD in the USA **(Scapaticci et al., 2021)**.

According to data provided by the World Health Organization (WHO) in 2016, 18% of children and adolescents aged between 5 and 19 years are overweight or obese worldwide **(Figure 1)**, and the forecasts estimate a further increase over the years **(Hruby, 2015)**.

4.2. Metabolic syndrome

The rising of childhood obesity is accompanied also by an increased prevalence of Metabolic Syndrome (MetS) in children and adolescents. Nowadays, it is estimated that about 40.8% of children with NAFLD presented MetS **(Scapaticci et al., 2021)**.

In contrast to adults, it does not exist a universally accepted definition of MetS in the pediatric population. The most recent scientific evidence provided by the International Diabetes Federation (IDF) propose to consider central obesity as the main characteristic of this condition and hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C), hypertension, and alteration in glucose metabolism as additional factors **(Villalobos et al., 2014)**.

4.3. Genetic background

The extreme variability in the prevalence of NAFLD existing between different groups of the same region exposed to the similar risk factors suggest a possible role of a genetic predisposition. To support this hypothesis there is evidence of a certain degree of hereditability. In fact, 59% of siblings and 78%of parents of obese children with biopsy proven NAFLD develop hepatic steatosis with a rate of hereditability of 1.0 for fatty liver and 0.386 for liver fat fraction **(Scapaticci et al., 2021)**.

4.4. Lifestyle and diet quality influence

Age, sex, and ethnicity combined with genetic predisposition represent the main risk components which promote fat accumulation. However, environmental, and individual factors could affect the natural history of liver damage and metabolic dysregulation **(Scapaticci et al., 2021)**.

Certainly, high-fat diet and sedentary lifestyle, contributing to weight gain, result in increased body fat deposition and thus to an increased risk to develop NAFLD as well as to end-stage liver diseases **(Francque et al., 2021)**. Population studies in adult patients have identify a diet mainly based on carbohydrates, saturated fat (SFA) and cholesterol rich foods in patients with NAFLD **(Hydes et al., 2021)**.

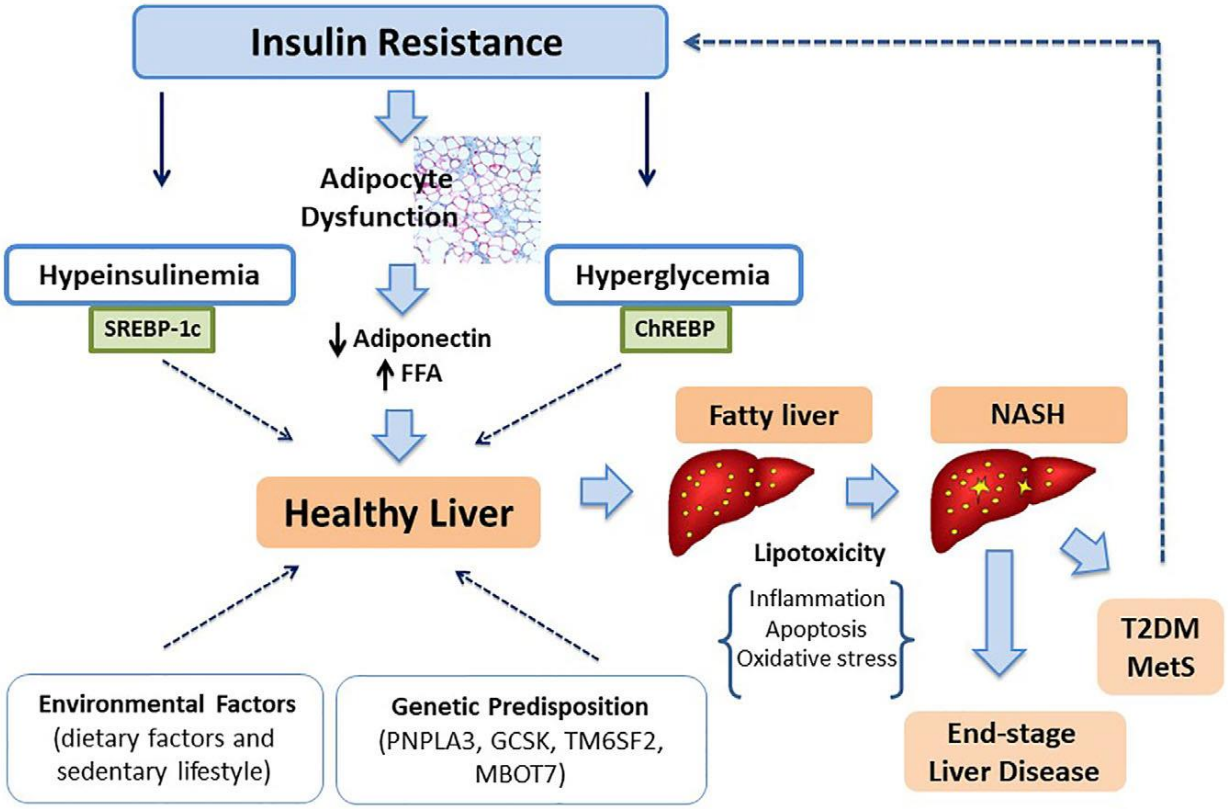

Figure 1: The role of IR in NAFLD development and progression (Scapaticci et al., 2021).

5. Apoptosis and necroptosis and lipogenesis and role in NASH

5.1. Apoptosis

The concept of apoptosis was first proposed Although it initially attracted little attention, excellent research progress has been made in the last ten years **(Zhao et al., 2021)**. Apoptosis is considered a basic biological and physiological process. Its dysregulation is involved in various diseases and pathologies, including damage from drug toxicity, immune responses, infections and tumors, and metabolic disorders **(Canovas, 2021)**.

Apoptosis has highly specific and recognizable morphological features. It is characterized by concentrated chromatin and cell shrinkage caused by DNA breakage. Under normal circumstances, the entire process of apoptosis is controllable, meaning that the integrity of the cell plasma membrane is maintained to prevent the leakage of cell contents, thus preventing inflammation **(Zhao et al., 2021)**.

5.2. Necroptosis

Necroptosis was first observed in experiments by Ray et al. in 1996, It is characterized by morphological changes like those observed in necrosis but is distinct in that it is a controllable form of death. Its morphological changes include organelle swelling, damage to the plasma membrane and release of cellular contents, which may lead to the occurrence of secondary inflammation **(Miller, 2017)**.

Key molecules for necroptosis include mixed lineage kinase domain-like (MLKL), and RIP protein kinase family members RIPK1 and RIPK3. TNF is a key cytokine in inflammation and other aspects of biology. TNFR1 has been widely studied in regulating cell survival, apoptosis and necroptosis **(Zhang et al., 2021)**. TNFR1-mediated signal transduction is an example of the molecular mechanism of necroptosis and the conversion between apoptosis and necroptosis **(Amstein et al., 2022)**.

5.3. Principle of apoptosis and necroptosis

Cell death can occur in distinct pathophysiological contexts. On one hand, in the setting of massive hypoxic or toxic injury, cells can reach a state of cellular stress and energy depletion in which they lose their ability to maintain basic homeostatic

functions, resulting in necrosis, a passive and uncontrolled autolytic loss of cellular integrity **(Galluzzi et al., 2018)**.

Morphologically, cells undergoing necrosis typically show a swelling and disruption of cell membranes and organelles without the picture of pyknosis (a reduction of cellular and nuclear volume) seen upon chromatin condensation in apoptosis **(Hu et al., 2021)**.

In the liver, necrosis is observed in multiple disease contexts and experimental models, such as ischemia–reperfusion injury or acute liver injury due to CCl_4 treatment or acetaminophen (also known as paracetamol) intoxication. Moreover, necrosis is believed to trigger a massive inflammatory response that can cause substantial collateral damage to neighboring cells **(Ezquer et al., 2022)**.

In contrast to passive necrosis that results from detrimental injury or stress, cell death can also be executed in an ordered, regulated fashion via a specific suicide program, termed apoptosis, which exerts only minimal effects on surrounding cells and thereby does not disrupt tissue homeostasis or organ development **(Montero et al., 2022)**.

Morphologically, apoptosis is characterized by cellular shrinkage, a dense cytoplasm with tightly packed organelles and pyknosis caused by the characteristic condensation and fragmentation of chromatin **(Miller, 2017)**.

The biochemical execution of apoptosis is mediated by the activation of initiator and executioner caspases, which kill cells through the cleavage of proteins and subsequent activation of nucleases that cleave DNA into short, regularly sized fragments **(Redza-Dutordoir, 2016)**.

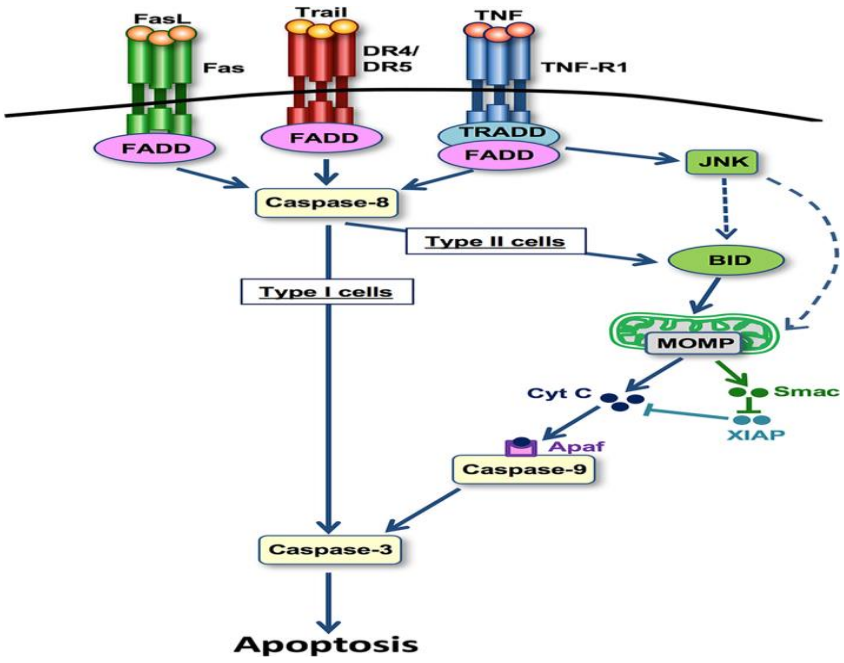

Figure 2: Mechanisms of death receptor-induced apoptosis. Following activation of apoptosis-mediating surface antigen FAS, TNF-related apoptosis-inducing ligand (TRAIL) receptors death receptor 4 (DR4; also known as TNFRSF10A) or DR5 (also known as TNFRSF10B) or TNF receptor 1 (TNFR1), caspase 8 becomes activated (Fig. 1). In type I cells (which induce apoptosis independent of mitochondria), caspase 8 activation is sufficient to trigger caspase 3 activation and apoptosis. In type II cells (in which apoptosis is mitochondria-dependent), caspase 8 cleaves BH3-interacting domain death agonist (BID), thereby triggering mitochondrial outer membrane permeabilization (MOMP) and release of cytochrome c and second mitochondria-derived activator of caspase (SMAC). SMAC neutralizes E3ubiquitin-protein ligase XIAP, thereby allowing the cytochrome c–apoptotic protease-activating factor 1 (APAF1) complex to trigger caspase 9 activation, which in turn triggers activation of caspase 8. JUN N-terminal kinase (JNK) activation amplifies this mitochondrial amplification pathway (dashed line) in TNFR1-induced apoptosis. FADD, FAS-associated death domain protein; FASL, FAS antigen ligand; TRADD, TNFRSF1A-associated via death domain **(Micheau, 2018).**

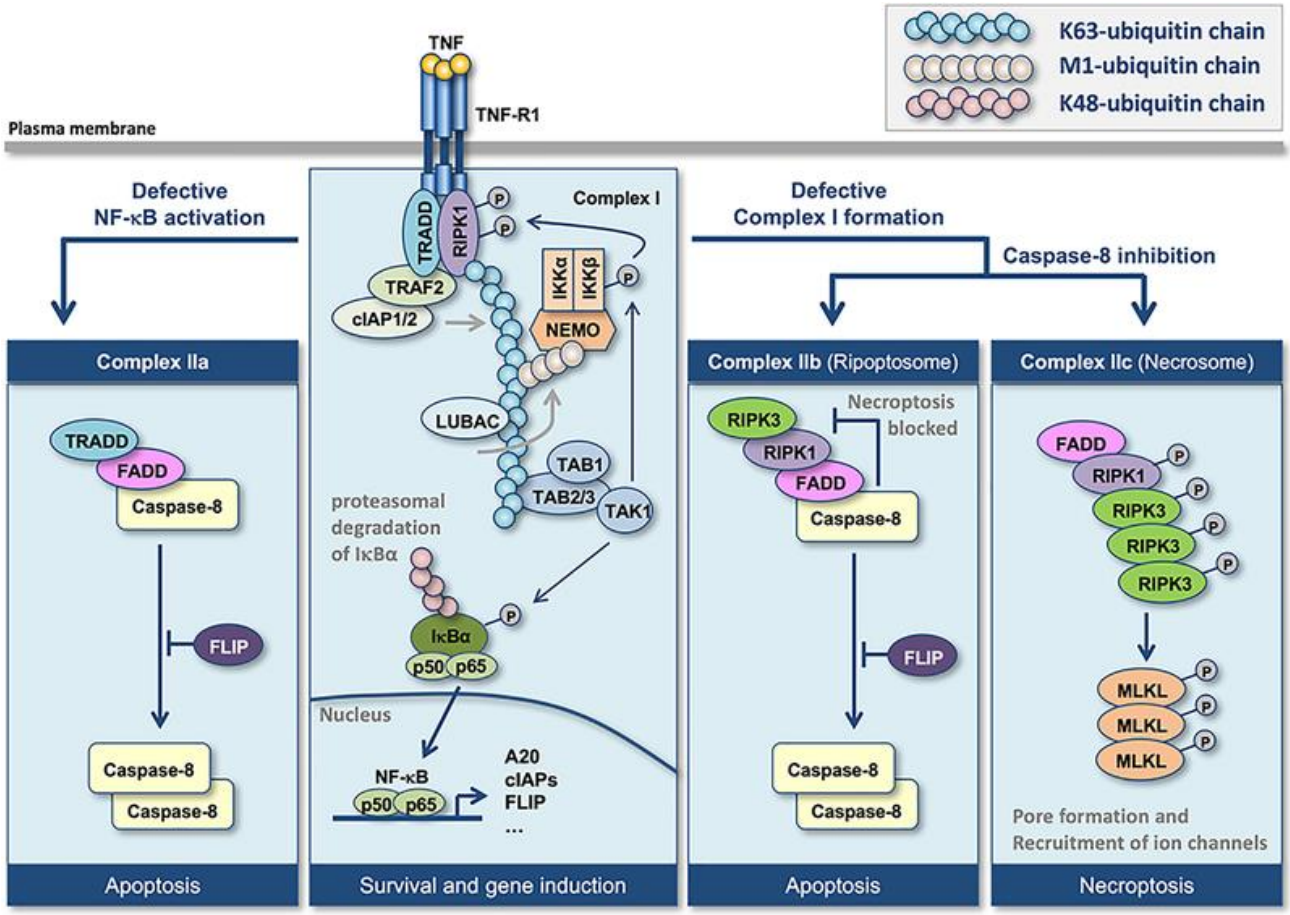

Figure 3: Mediators of TNF-dependent programmed cell death (simplified scheme). Activation of distinct cell death pathways in response to TNF signalling is regulated by diverse post-transcriptional modification steps, including phosphorylation and ubiquitylation. Upon ligation of TNF to its receptor, TNF receptor 1 (TNFR1), distinct signalling complexes can be formed, which is mainly orchestrated through ubiquitylation events that influence cell fate towards survival or cell death. The first complex that forms upon TNF stimulation is complex I, consisting of the adaptor protein TNFRSF1A-associated via death domain (TRADD), receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and the E3 ligases TNF receptor-associated factor 2 (TRAF2), cellular inhibitor of apoptosis 1 (CIAP1) and CIAP2, which together mediate the main ubiquitylation events (for example, ubiquitylation of K63 and K48), thereby enabling the further recruitment of the linear ubiquitin chain assembly complex (LUBAC), the inhibitor of NF-κB (IκB) kinase (IKK) complex (comprising IKK subunit-α (IKKα), IKKβ and NF-κB essential modulator (NEMO)), orphan nuclear receptor TAK1 and its adaptor proteins (TAK1-binding protein 1 (TAB1), TAB2 and TAB3) and subsequent activation of the pro-survival transcription factor nuclear factor-κB (NF-κB). Upon genetic or pharmacological inactivation of NF-κB or other factors that drive NF-κB activation (such as TAK1 or NEMO), deubiquitylation and release of RIPK1 from complex I can promote the formation of other complexes that tip the balance of TNF signalling towards cell death. Complexes containing FAS-associated death domain protein (FADD), caspase 8 and RIPK1 (complex IIb) or alternatively TRADD (complex IIa) typically trigger apoptosis. By contrast, complexes containing RIPK1 and RIPK3 (complex IIc) typically activate necroptosis, a form of programmed necrosis, via RIPK3-mediated phosphorylation of mixed lineage kinase domain-like protein (MLKL). Complex formation is further modified by deubiquitinases such as zinc-finger protein A20, ubiquitin carboxyl-terminal hydrolase CYLD or ubiquitin carboxyl-terminalhydrolase 2. c-FLIP, cellular FLICE-like inhibitory protein; p50, nuclea rfactor NF-κB subunit p50; p65, nuclear factor NF-κB subunit p65 **(Fischer, et al. 2020).**

5.4. Apoptosis and necroptosis in liver disease

5.4.1. NASH and alcoholic liver disease

Both alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) are serious health problems with a global rise of incidence and importance. Although patients with ALD consume excessive amounts of alcohol, NAFLD the most common liver disease in the Western world is typically defined by the presence of steatosis in >5% of hepatocytes in patients with little or no alcohol consumption. The term nonalcoholic steatohepatitis (NASH) defines a more severe form of NAFLD, characterized by hepatocyte steatosis, inflammation, hepatocyte cell death and often fibrosis **(Mitra et al., 2020)**.

Theories to explain the molecular and metabolic alterations driving the initiation and progression of NASH vary between a 'two hit hypothesis' and a 'multiple hit hypothesis'. In these concepts, the first hit constitutes rising hepatic lipid concentrations, which render hepatocytes sensitive to additional hits featuring oxidative stress and hepatocyte cell death that drives inflammation and fibrosis **(Wang et al., 2021)**.

In terms of hepatocyte cell death in NASH, most data point towards apoptosis as the key driver. Immunohistochemical tests assessing hepatocyte caspase 3 cleavage and TUNEL positivity were positive in both liver tissue from patients with NASH and in commonly used mouse models of NASH **(Shojaie et al., 2020)**.

Moreover, non-invasive markers of apoptosis such as circulating CK18 levels are increased in patients with NASH and can predict the presence of the disease. Finally, inhibition of apoptosis using the pan-caspase inhibitor VX-166 markedly reduced liver injury and liver fibrosis in db/db mice fed a methionine-choline-deficient (MCD) diet **(Huda et al., 2022)**.

In line with these findings, the oral caspase inhibitor GS-9450 — which inhibits caspases 1, 8 and 9 — led to a reduction of alanine aminotransferase levels in patients with NASH in a clinical trial. Other pan-caspase inhibitors such as emricasan are currently being tested in large, randomized trials in NASH **(Dhani et al., 2021)**.

Necrosis and necroinflammation are typical histological features of NASH suggesting the involvement of alternative cell death pathways. The initial publication of caspase 3 and caspase 7 cleavage in human NASH showed an immunohistochemical picture of almost 100% caspase 3 positive hepatocytes in patients with NASH, compared with no positivity in control cells. However, even in the most severe form of acute liver failure, apoptotic hepatocytes represent a low percentage of all hepatocytes, probably owing to rapid clearance **(Avrutsky, 2021)**.

6. Treatment strategies for of NASH

6.1. Lifestyle Modification

Management of fatty liver diseases has been addressed by lifestyle modifications, including regular physical exercise, and consuming a hypocaloric diet **(Hallsworth and Adams, 2019)**. More importantly, lifestyle changes alone are insufficient to stop disease progression, especially for patients who are at later stages of the disease where there are ongoing liver inflammation and fibrosis **(Li and Alazawi, 2020)**.

6.2. Bariatric Surgery

Bariatric surgery is another effective non-pharmacological weight-loss therapy indicated for patients with a BMI > 35 and severe comorbidities, such as T2DM and hypertension. Several studies have reported the resolution of steatosis, NASH, and fibrosis in patients who have undergone weight-loss surgery **(Hashem et al., 2021)**.

According to a meta-analysis of 21 studies, bariatric surgery results in histological or biochemical improvement of steatosis, NASH, and fibrosis in 88%, 59%, and 30% of NAFLD patients, respectively **(Hashem et al., 2021)**. Furthermore, patients with NAFLD who undergo bariatric surgery have a lower risk of progression to cirrhosis compared to matched controls without surgery **(Wirth et al., 2020)**.

6.3. Pharmacological Treatments

6.3.1. Vitamin E

vitamin E has been studied in the landmark PIVENS (adult patients) and TONIC trials (pediatric population), where the antioxidative and free radical scavenging property of vitamin E has been hypothesized to improve NASH **(Sharma et al., 2021)**.

Indeed, there was a significant improvement in

steatosis and inflammation in patients treated with vitamin E for 96 weeks compared to placebo. However, there was no improvement in fibrosis. Guidelines recommend vitamin E (α-tocopherol) at a daily dose of 800 IU/day in nondiabetic adults with biopsy-proven NASH, weighing the risk-benefit ratio before initiation of treatment **(El Hadi, et al., 2018)**.

6.3.2. Ursodeoxycholic acid (UDCA)

UDCA at a dose of 13–15 mg/kg body weight in patients with biopsy-proven NASH has not shown any benefit compared with a placebo and is not recommended for NASH **(Paul, 2020)**. A recent human study (phase 2 trial) reported significant improvement in serum ALT levels at 12 weeks with the use of UDCA at 1,500 mg per day compared to placebo **(Sharma et al., 2021)**.

6.3.3. Peroxisome proliferator-activated receptor agonists

Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptors that are expressed in the liver, adipose tissue, heart, skeletal muscle, and kidney and are responsible for the transcriptional regulation of processes such as fatty acid oxidation, lipid transport, and gluconeogenesis **(Fougerat et al., 2020)**. There are three PPARs (α, β/δ and γ) that bind the same target DNA sequence but differ in tissue distribution **(Hassan et al., 2021)**.

Several PPAR agonists are on the market for treating T2D (pioglitazone) and hypertriglyceridemia (gemfibrozil, fenofibrate) (Botta, et al., 2018). PPARα agonists or fibrates reduce TG but have not been shown to provide beneficial effects on liver histological endpoints in patients with NAFLD. Several PPAR single or dual receptor agonists have been evaluated or are currently being tested in clinical trials in NAFLD/NASH patients **(Lange et al., 2022)**.

6.3.4. Omega-3 fatty acids

Omega-3 fatty acids can reduce oxidative stress, lip toxicity, and inflammation in patients with NASH. There have been conflicting reports about the efficacy of omega-3 fatty acids in NAFLD.10, However, the benefits of omega-3 fatty acid supplementation have been noted with a dose of ≥0.83 g/day **(Yang et al., 2019)**.

6.3.5. Metformin

Early studies with metformin showed improved insulin resistance, liver chemistry, and a modest reduction in hepatic steatosis **(Sharma et al., 2021)**.

6.3.6. Obeticholic acid (OCA)

OCA, 6α-ethyl chenodeoxycholic acid, is a semi-synthetic derivative of chenodeoxycholic acid. It is a 100 times more potent agonist of Farnesoid X recptor (FXR) than chenodeoxycholic acid **(Đanić et al., 2018)**.

7. Kaempferol

Kaempferol (KP; chemicalname 3,5,7 trihydroxy 2 (4 hydroxyphenyl) 4H 1 benzopyran 4 one; also known as kaempferol 3, kaempferide or kaempferol flavonol) is a type of flavonoid. KP has a molecular weight of 286.23 and is a pure yellow crystalline powder with a melting point of 276 278˚C. It is soluble in hot ethanol, ether alkaline, and slightly soluble in water. KP has hydrophobic properties due to its diphenyl propane structure **(Ren al., 2019)**.

7.1. Role of Kaempferol

7.1.1. Anti-inflammatory effects

In vascular tissues, inflammation is an important biological response, as it reflects tissue damage, which is caused by different pathogens, irritants, or cell damage. KP is a common type of dietary flavonoid with antioxidative and anti-inflammatory properties **(Chen et al., 2017)**.

Studies also indicated that KP decreased lipopolysaccharide (LPS) induced tumor necrosis factor α (TNF α) and interleukin 1 (IL 1) expression by increasing the number of activated macrophages; suppression of TNF α mediates the translocation of NF κB p65 to the nucleus. As inflammation may be classified as acute or chronic, the inflammatory diseases in different categories were discussed separately to understand the role of KP in inflammatory diseases **(Hwangbo et al., 2021)**.

7.1.2. Chronic inflammation

KP slows IVD degeneration. IVDs are composed of nucleus pulposus, the annulus fibrosus and cartilage endplates, and IVD degeneration has been considered an irreversible process when cell viability decreases, type II collagen is synthesized

and the nucleus pulposus is dehydrated. It was demonstrated that KP inhibits LPS induced apoptosis by inhibiting LPS induced decreases in the levels of chondrogenic markers which means chondrogenic markers SOX 9, Collagen II and Aggrecan, and reducing the level of matrix degrading enzymes **(Feng et al., 2022)**.

7.1.3. Application of kaempferol in the liver and metabolic diseases

7.1.3.1. Kaempferol protects against hepatotoxicity

As the first organ that metabolizes foreign compounds, the liver is vulnerable to various diseases, including hepatitis, cirrhosis, or HCC. One of the most common liver diseases caused by drugs is hepatitis induced by anti-tuberculosis (TB) drugs **(Elbahrawy et al., 2021)**.

Drug-induced hepatotoxicity may be due to oxidative stress caused by the production of toxic metabolites or free radicals. The primary pathway of inhibitor of isoniazid (INH) metabolism by N acetyltransferase 2 generates acetyl INH. Acetyl INH undergoes hydrolysis to form acetyl hydrazine and the nontoxic substance iso nico¬tinic acid, which is oxidized by cytochrome P450 family 2 subfamily E member 1 (CYP2E1) to form reactive acylating hepatotoxins or its breakdown products **(Villanueva-Paz et al., 2021)**.

7.1.3.2. Kaempferol protects against alcoholic liver injury

Alcoholic liver injury is one of the major health problems in the world, accounting for ~4% of the total global death toll. High alcohol consumption leads to hepatotoxicity associated with oxidative stress due to the promotion of ROS production and reduction of antioxidant effects. Studies have indicated that CYP2E1 is an important factor in alcohol-induced liver injury, as it is highly inducible and has a high catalytic activity for alcohol **(Tan et al., 2020)**.

KP increased antioxidant effects and reduced ROS production by inhibiting CYP2E1. Although KP was demonstrated to reduce lipid accumulation in nonalcoholic fatty liver disease, its applica¬tion in the clinical treatment of alcoholic liver disease remains to be evaluated **(Harjumäki et al., 2021)**.

8. Pentoxifylline (PTX)

PTX is a methylated xanthine derivative that was approved for treating peripheral vascular disorders **(Wei, et al., 2021) (Figure 4)**.

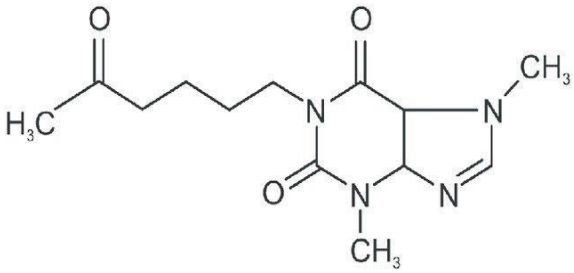

Figure 4: Chemical structure of pentoxifylline **(Hassan et al., 2014)**.

8.1. Metabolism of PTX

PTX is like other methyl-xanthine derivatives, which are primarily metabolized in the liver and red blood cells by redox and demethylation reactions **(Coelho et al., 2021)** as in **Figure 5**.

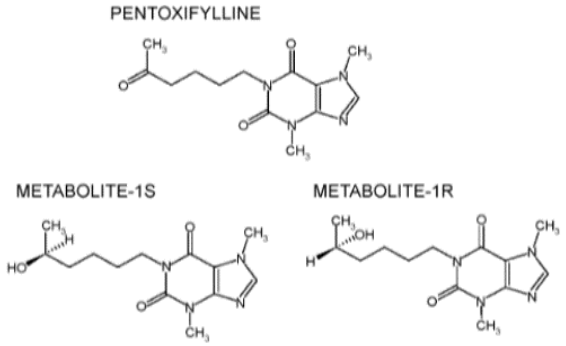

Figure 5: Metabolism of pentoxifylline. The chiral metabolite is formed from the reduction of pentoxifylline.

8.2. Pharmacological activities

8.2.1. Anticancer activity

PTX fights cancer through its apoptotic, anti-angiogenic, anti-metastatic, anti-inflammatory, and immunomodulatory properties **(Afroze et al., 2020)**.

PTX induces apoptosis by down-regulation of anti-apoptotic proteins together with upregulation of death receptors DR4 and DR5. Moreover, PTX impairs DNA-repair mechanisms and induces cell cycle arrest at G1/ S phase **(Michalkova et al., 2021)**.

8.2.2. Anti-inflammatory and immunomodulatory effects

PTX possess potent anti-inflammatory and immunomodulatory activity mainly through inhibition of pro-inflammatory cytokines. The anti-inflammatory action of PTX is probably related to

its ability to suppress oxygen radical production, scavenger ROS, and blockade of ERK phosphorylation and TNF-α production **(Salehi et al., 2020)**.

PTX attenuates the oxidative burst of polymorphonuclear leukocytes, inhibits proliferation of peripheral blood mononuclear cells, attenuates interleukin-12 (IL-12) release, and prevents adherence to the cell matrix and the endothelium **(Salehi et al., 2020)**.

Moreover, PTX has been shown to inhibit T-cell cytotoxicity with subsequent reduction of interferon gamma (IFN-γ), granulocyte-macrophage colony-stimulating factor (GMCSF), and IL-6. The anti-inflammatory and immunomodulatory effects of PTX have been proven to be beneficial in various clinical conditions associated with hyper-inflammation **(Debele, et al., 2020)**.

8.2.3. Role of Pentoxifylline and Kaempferol in Apoptosis and necroptosis

The recent study proved that pentoxifylline, alone or in association with Kaempferol, is effective. and promising in treating and preventing NASH by different mechanisms through downregulating caspase 8, pMLKL and RIPK3 which stimulate apoptosis and necroptosis pathways. Moreover, the reduction of cytokines like TNF-α, IL-6, NF-κB and oxidative stress, as well as acting through the decrease of lipogenesis genes such as AMPK and SREBP-1 all alleviates NASH **(Hamouda et al., 2022)**.

9. Conclusion

New strategy for treatment of NASH through modulation of apoptosis and necroptosis and related proteins and genes pathways and alleviation of meta-inflammation associated with NASH through inhibition of pro-inflammatory cytokines. Moreover, treatment with KP and PTX corrected the insulin resistance, obesity-associated dyslipidemia, hyperglycemia, hyperinsulinemia and HOMA IR and improved histopathology, considered a promising protective and treatment against NASH induced complications.

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