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Review

Leflunomide an immunomodulator with antineoplastic and antiviral potentials but drug-induced liver injury: A comprehensive review

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ABSTRACT

Leflunomide (LF) represents the prototype member of dihydroorotate dehydrogenase (DHODH) enzyme inhibitors. DHODH is a mitochondrial inner membrane enzyme responsible for catalytic conversion of dihydroorotate into orotate, a rate-limiting step in the de novo synthesis of the pyrimidine nucleotides. LF produces cellular depletion of pyrimidine nucleotides required for cell growth and proliferation. Based on the affected cells the outcome can be attainable as immunosuppression, antiproliferative, and/or the recently gained attention of the antiviral potentials of LF and its new congeners. Also, protein tyrosine kinase inhibition is an additional mechanistic benefit of LF, which inhibits immunological events such as cellular expansion and immunoglobulin production with an enhanced release of immunosuppressant cytokines.

LF is approved for the treatment of autoimmune arthritis of rheumatoid and psoriatic pathogenesis. Also, LF has been used off-label for the treatment of relapsing-remitting multiple sclerosis. However, LF antiviral activity is repurposed and under investigation with related compounds under a phase-I trial as a SARS CoV-2 antiviral in cases with COVID-19.

Despite success in improving patients’ mobility and reducing joint destruction, reported events of LF-induced liver injury necessitated regulatory precautions. LF should not be used in patients with hepatic impairment or in combination with drugs elaborating a burden on the liver without regular monitoring of liver enzymes and serum bilirubin as safety biomarkers.

This study aims to review the pharmacological and safety profile of LF with a focus on the LF-induced hepatic injury from the perspective of pathophysiology and possible protective agents.

1. Introduction

Leflunomide (LF) is an immunomodulator and a member of the disease-modifying antirheumatic family of drugs (DMARDs). LF is used effectively in solo or as combined therapy in autoimmune arthritis [1]. Basic studies reported immunosuppressant, antirheumatic, antineoplastic, and antiviral potentials of LF [2–6]. While clinical studies secured an antirheumatic approval of LF in autoimmune arthritis, further studies on antineoplastic effectiveness have so far been inconclusive [7–9]. Additionally, clinical studies on the benefit of LF in treating refractory viral infections such as cytomegalovirus [10] and BK polyomavirus [11–14] have also been undertaken without yielding a

Abbreviations: TAK1, transforming growth factor β-activated kinase 1; AMPK, adenosine monophosphate-activated protein kinase; ULK1, Unc-51-like autophagy activating kinase; PIM1 kinase, proto-oncogene serine/threonine protein kinase Pim-1; P56lk, T-lymphotocyte-specific protein tyrosine kinase; Ephrin-A1, soluble ephrin-A1; Eph-A2, ephrin-A2; JNK, c-Jun N-terminal kinases; LDH, lactate dehydrogenase; ERK1/2, extracellular signal-regulated kinases; Bcl2, B-cell lymphoma 2 protein; Bax, Bcl-2-associated X protein; PCNA, proliferating cell nuclear antigen; CCL4, carbon tetrachloride; COX2, cyclo-oxygenase 2; NO, nitric oxide; iNOS, inducible nitric oxide synthase; PGE2, prostaglandin E2; AMP, adenosine monophosphate.

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final decision to certify. Currently, a clinical trial is underway to study the effectiveness of LF in mild cases with COVID-19 [15].

Leflunomide was first launched at the end of 1998 with alarming events of drug-induced liver injury (DILI) ranging from a mild elevation of serum transaminases to life-threatening hepatitis [16–24]. In the early 2000s, the FDA had labelled LF with a precautionary regular hepatic function monitoring throughout the therapeutic regimen [25]. Studies-driving this decision involved professional and community concerns of LF-induced detrimental effects on the liver [16,22,24,26,27].

2. Chemistry and pharmacokinetics

LF is an isoxazole antirheumatic and immunosuppressant approved medication with antineoplastic and antimicrobial investigational situation [5,9,28–39]. LF is used through the oral route in a formulation of 10, 20, or 100 mg per tablet. It is well absorbed, and upon exposure to first-pass intestinal and hepatic metabolism, LF is almost totally transformed into its active metabolite teriflunomide (A77-17226). In the liver, LF is a substrate of the hepatic microsomal enzymes CYP2C9, CYP3A4, and CYP2A1 [1,40]. Cytochrome P450 enzymes are responsible for the opening of the LF isoxazole ring and the production of teriflunomide in two forms (E & Z), with the former having a higher potency [41]. Drugs that have an inducing or inhibitory effect on these hepatic microsomal enzymes carry the risk of adverse drug interactions with LF [1]. LF has a short plasma half-life (t1/2) of 3.5 h; however, teriflunomide t1/2 is ~360 h. The steady-state concentration (Css) of LF/teriflunomide is attainable after ~2.5 months. To hasten LF Css, a loading dose regimen of 100 mg/day for three days is followed by a maintenance dose of 10–20 mg/day onward. Teriflunomide is eliminated through the hepatobiliary route in unchanged form. In the case of LF toxicity, or the need for abrupt LF withdrawal, an accelerated drug elimination procedure using cholestyramine or activated charcoal should be followed to lower the teriflunomide plasma concentration to 0.02 mg/L, which would take two years to be accomplished without this procedure [1,42,43]. Teriflunomide has a 99% plasma protein-binding capacity and 11 L volume of distribution with an incapability of being dialyzed [44,45].

3. Pharmacodynamics of LF

Teriflunomide is the biologically active mediator of LF actions [35,46,47]. LF acts through the inhibition of dihydroorotate dehydrogenase (DHODH), an inner mitochondrial membrane enzyme that catalyzes the rate-limiting step of the de novo pathway of pyrimidine biosynthesis [48]. Cellular regeneration and growth can be fulfilled through a salvage pathway with a two-fold coverage of pyrimidine nucleotide cellular requirements; however, the active proliferation of cells such as lymphocytes clonal expansion requires up to eightfold increase of pyrimidine nucleotides with a mandated dependence on the de novo pathway [49] Fig. 1.

3.1. Immunomodulator anti-inflammatory and antirheumatic

Immune-mediated disorders are associated with the active expansion of autoimmune lymphocytes and other innate immune cells such as monocytes and macrophages. The main and early characterized mode of action of LF is the cellular depletion of the pyrimidine nucleic acid building blocks with a milieu-dependent outcome such as inhibition of autoimmune lymphocyte expansion and, consequently, inhibition of immunokine and immunoglobulin production [2,50]. Additionally, LF acts through the inhibition of the tyrosine kinase activity responsible for the signal transduction of many vital pathways in the immune response [3]. For instance, the inhibition of immunoglobulin class switching of IgM to IgG1, which is mediated through IL-4-activated JAK3/STAT6 and immunoglobulin class switching.

![Fig. 1. Leflunomide inhibits de novo synthesis of pyrimidine through inhibition of the mitochondrial enzyme DHODH.](image1)

![Fig. 2. Leflunomide inhibits the immune system through inhibition of tyrosine kinase phosphorylation of the JAK3/STAT6 and immunoglobulin class switching.](image2)
Leflunomide main actions and their mechanisms.

| Clinical use                                  | MOA/action                                                                 | Action                                                                                           | References                  |
|----------------------------------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-----------------------------|
| Immunomodulator and antirheumatic            | - Inhibition of dihydroorotate dehydrogenase enzyme                         | - Depletion of pyrimidine nucleotide → inhibition of immune cell expansion.                      | [54,73,106,148-152]        |
|                                              | - Aryl hydrocarbon receptor agonist                                         | - Suppression of cell response to cytokines such as IL2.                                         | [4,153-157]                |
|                                              | - Tyrosine kinase inhibition                                                | - Inhibition of antibody production and antibody class switch                                    | [71]                       |
|                                              | - Anti-inflammatory                                                         | - Inhibition of graft rejection and graft-versus host disease                                    |                             |
|                                              | - Inhibition of epidermal growth factor receptors                           | - Decrease C-reactive protein → inhibition of bone erosion                                        | [3,52,83,158,159]         |
| Antineoplastic                               | - Inhibition of the canonical WNT/β-catenin signaling                       | - Inhibit IL2 signaling through JAK1 & JAK3.                                                    |                             |
|                                              | - Inhibition of Akt and its downstream pathway                             | - Inhibition of cell response to inflammatory cytokines                                         | [61,81,82,160]             |
| Antiangiogenic                               | - Inhibition of soluble ephrin-A1/EphA2 pathway                            | - Inhibition of cell proliferation and induction of apoptosis                                     | [32,35,161,162]           |
| Antiviral                                    | - DHODH inhibition                                                          | - Inhibition of viral assembly                                                                   |                             |
|                                              |                                                                            | - Inhibition of viral load                                                                       |                             |

Studies have highlighted the immunosuppressant activity of LF through the inhibition of the PI3K/Akt/mTOR [55]. Mammalian target of rapamycin (mTOR) is a serine/threonine kinase activated by the upstream effector phosphoinositol 3 kinase (PI3K). Downstream, mTOR induces cellular translational machinery in favor of cell growth, survival, and proliferation [55]. Akt and mTOR are important determinants of activated B-cell expansion and fate [56]. S6 Kinase 1 (S6K1)—a serine/threonine kinase—is a predominant downstream translational effecter in the Akt/mTOR cell growth/survival pathway. LF and its metabolite teriflunomide inhibit S6K1 activity with the arrest of the cell cycle in the S phase and, hence inhibit cell proliferation [57]. Additionally, mast cells undertake major and versatile immune defense roles. Mast cells play a crucial role in the pathogenesis of autoimmune and inflammatory pathological conditions [58]. Mast cells are abundantly detected in the synovial membrane of joints in patients with RA [59]. LF is reported to inhibit PI3K/Akt stimulation with the induction of mast cell apoptosis [60].

Early in the development of LF, the anti-inflammatory benefits were reported [61,62]. Manipulating innate immune responses is considered the anti-inflammatory gateway of LF. In a mouse model of lupus nephritis, LF inhibited the destructive tissue inflammatory pathway mediated through Toll-like receptor 9 (TLR9) signaling pathway with a reduction in the autoantibody production and immune complex deposit in the renal tissue [63]. LF anti-inflammatory activity is reported in a clinical study of patients with active rheumatoid arthritis. The pathological findings in this study are the reduction of the inflammatory joint destruction. IL1β and matrix metalloproteinases (MMP) such as MMP1 are reduced upon treatment with LF [64]. This may be explained by the inhibition of the TNF-α-dependent activation of NF-κB [65]. Furthermore, teriflunomide, the functioning metabolite of LF, was reported as being an inhibitor of neuroinflammatory events associated with HIV infection independent of viral replication which is attributed to the inhibition of the secretion of the proinflammatory mediators IL6, CXCL10, and CCL2 [66-68]. In a rat model of lung fibrosis-induced by bleomycin, LF reduced lung tissue expression of the inflammatory cytokines IL6, TNF-α, and NF-κB [69]. Additionally, LF anti-inflammation can be undertaken through the suppression of the trans-endothelial migration of blood mononuclear cells and the inhibition of the expression of adhesion molecule CD44 [70].

In patients with rheumatoid arthritis, increased levels of C-reactive protein (CRP) are correlated with joint destruction. Aryl hydrocarbon genomic activity induces a negative control on CRP expression. LF is an aryl hydrocarbon receptor agonist, which attenuates CRP expression and hence saves the structural integrity of the joints [71,72] Table 1.

3.2. Antiproliferative activity

LF at low doses has a reversible antiproliferative action upon cell replenishment with pyrimidine [73,74]. Meanwhile higher doses of LF showed irreversible antiproliferative activity [49]. Indeed, this action may carry a promising antineoplastic potential. In vitro studies reported dose- and time-dependent cytostatic effects of LF in transformed prostate epithelial cells through pyrimidine depletion, mitochondrial bioenergetic disruption, and cytochrome c release with an apoptotic sequence [32]. In neuroblastoma cells, LF induced cytostatic and apoptotic cellular fate attributed to the reduced expression of the DHODH enzyme at the transcriptional and translational levels [75]. In a melanoma cell line (A375), Dosacas and colleagues unveiled the inhibitory effect of LF/ A77-1726 (teriflunomide) on S6K1 with a resultant inhibition of its substrates S6, insulin receptor substrate-1 (IRS-1), and carbamoyl phosphate synthase 2, concluding an inhibition of cell proliferation [57]. Meanwhile, Xu et al, demonstrated the A77-1726-induced autophagy in vitro through releasing TAK1/AMPK/ULK1 pathway from the inhibitory effect of S6K1 [76]. In contrast, Cheng and colleagues (2020), reported the autophagy inhibitory action of LF, which is considered an enhancer of the cytotoxic effects on human bladder cancer cells. This was attributed to the inhibition of cancer cell escape mechanisms and the survival tendency through autophagy [35]. Furthermore, teriflunomide synergized Gemicitabine-induced growth inhibition of pancreatic cancer cells through PIM kinase-dependent inhibition of the c-myc tumorigenic signaling pathway [77]. Teriflunomide directly inhibits the entire PIM family, especially PIM-3 and PIM-1 [78]. Likewise, teriflunomide suppressed the proliferation of the murine leukemia cell line LSTRA through inhibition of the tyrosine kinase activity of pS6Kα [79]. Moreover, LF inhibited angiogenesis in an N-buty1-N-(4-hydroxybutyl)-nitrosamine (BBN)-induced bladder carcinogenesis animal model and a tumor xenograft model, as well as in bladder cancer cells in vitro via significant inhibition of the sEphrin-A1/ EphA2 system [80] Table 1.

In contrast, low doses of leflunomide exhibited cell survival potential through the activation of PI3K/Akt signaling and reduced apoptosis, which was induced by anticancer agents in erythroleukemia cells. Also, LF inhibited p38/MAPK/JNK basal activity with the reduced apoptotic activity of caspase-3 [30].

3.3. Antiviral activity

LF has antiviral properties through the inhibition of viral nuclear material replication within the host cells, and the inhibition of protein tyrosine-kine activity leading to inhibition of the phosphorylation of
cellular proteins required for vital processes [81–83].

LF shows antiviral activity against many viruses such as Cytomegalovirus (CMV), Polyomavirus type BK, Herpes simplex virus, Respiratory syncytial virus (RSV), and SARS CoV2. LF suppressed CMV infection by inhibiting virion assembly rather than the synthesis of viral DNA [84]. With promising therapeutic potential, LF restrained CMV infection in vitro [85], in vivo [86], and in clinical research [10] Table 1 & Fig. 3.

Also, LF can inhibit the replication of the BK polyomavirus in renal tubular epithelial cells through nonspecific pyrimidine depletion [12–14, 38]. Further, studies showed that the active metabolite of LF leads to the inhibition of RSV production in vitro and a reduction in viral load in vivo; this mechanism is uridine independent [87] Table 1 & Fig. 3.

Multiple sclerosis patients under treatment regimen with teriflunomide, and who developed COVID-19 showed better disease outcomes, which may be attributed to the immunosuppressant and antiviral activity of the drug [88].

The active form of LF was also found to be effective against Junín Virus. It can inhibit virus replication by inhibiting viral RNA synthesis through pyrimidine depletion in a dose-dependent manner. However, the addition of uridine or orotate reverses the inhibitory effect of LF [39].

Blocking of DHODH results in pyrimidine depletion which is very effective against rotavirus (responsible for dehydrating diarrhea). For that reason, LF anti-microbial activity was investigated against other organisms such as Helicobacter pylori, Plasmodium falciparum, and Schistosoma mansoni [89].

4. Clinical importance of LF

LF is FDA approved for the treatment of autoimmune arthritis of rheumatoid and psoriatic pathogenesis. LF can be used in solo, while in refractory cases it should be combined with other immunosuppressants. LF shows efficacy after four weeks of treatment with improved physical activity comparable to that of methotrexate. After two years, patients who received LF had no further increase in joint damage [90] Table 2.

4.1. Autoimmune and rheumatic conditions

LF has been initially approved for the treatment of autoimmune arthritis, mainly rheumatoid [1] and psoriatic types [91]. The target of therapy is to reduce the number of joints affected, which is scored clinically for pain and functionality [92]. The use of LF in other autoimmune conditions, such as systemic lupus and multiple sclerosis, is investigational but without an approval decision [93–95]. In multiple sclerosis, the use of LF active metabolite teriflunomide was approved in 2016 [96,97]. This approval did not compare the efficacy of LF to teriflunomide in a clinical context and, thus, may require further clinical and pharmacoeconomic investigations. The approval of teriflunomide, which is >60 times as expensive as LF, has set aside the alternative off-label use of LF in multiple sclerosis. Revising the clinical profile of teriflunomide showed that an LF dose of 20 mg/tablet is equivalent to the

Table 2

| Clinical indication | Disease                                      | Phase          | Outcome                                 | Reference |
|---------------------|----------------------------------------------|----------------|-----------------------------------------|-----------|
| **Antiviral**        | Mild COVID-19 patients                      | Phase I        | Ongoing                                 | [15]      |
|                     | HIV-1                                        | Phase I        | Promising with safety concerns limited progress to further studies | [167]     |
|                     | BK viremia associate nephropathy             | Phase IV       | Serum creatinine                        | [168,169] |
| **Cancer**           | Smoldering multiple myeloma                  | Early phase I  | Recruiting (to end June 2021)           | [109]     |
|                     | Relapsed/refractory Multiple myeloma         | Phase I/II     | Completed                               | [110]     |
|                     | Anaplastic astrocytoma                       | Phase II       | NA                                      | [7]       |
|                     | Glioblastoma multiforms                      | Phase III      | NA                                      | [8]       |
|                     | Advanced refractory prostate cancer          | Phase II/III   | NA                                      | [9,107]   |
|                     | Mutant metastatic melanoma                   | Phase I        | NA                                      | [170]     |
|                     | Metastatic triple negative breast cancer      | Phase I/II     | NA                                      | [171]     |
| **Autoimmune diseases** | IgG4-related sclerosing disease              | Phase IV       | LF + glucocorticoid is significantly superior to glucocorticoids monotherapy | [172]     |
|                     | Lupus nephritis                              | Phase III      | Low dose LF + prednisone effectiveness and safety | [173]     |
|                     | Non-randomized                               |                |                                         |           |
|                     | Open label                                   |                |                                         |           |
17 mg/tablet dose of teriflunomide [95–97].

The use of LF in systemic lupus erythematosus is still under investigation, some of these clinical trials have been suspended with no clinical data justifying this trial decision Table 2.

LF was also tried in other joint conditions such as ankylosing spondylitis. However, it did not show clinical promise except in patients with associating poly-arthritis who did show an improvement. While it was reported that the spinal manifestations did not significantly improve, this may be attributed to the pathogenic mechanisms which are different from the joint ones [98].

4.2. Transplant rejection and graft versus host disease

LF is used for the prevention of graft rejection and graft-versus-host disease (GVHD). Studies reported the benefit of LF in different animal models of graft rejection such as cardiac allograft [4,100], intestinal transplantation [101], renal transplantation [102], corneal allograft [103], and fish-to-mouse pancreatic islets xenograft [104]. Also, LF is found to be of benefit in chronic musculoskeletal graft versus the host disease following an allogeneic hematopoietic stem cell transplant [105] Table 2.

4.3. Cancer

Recently, LF has been flagged as a promising antineoplastic drug through its pyrimidine nucleotide cellular deprivation activity [73,106]. The limitation of LF antineoplastic clinical application may be attributed to the doubts surrounding its clinical efficacy due to the action reversal by uridine replenishment [73]. This may be true despite the protein tyrosine kinase inhibitory action, which was found to be of additional benefit. LF and its congeners are investigated in different in vivo and in vivo models of cancer including prostate [9,32,107], breast [34], neuroblastoma [8,108], multiple myeloma [78,109,110], thyroid [33], leukemia [111,112], and lymphoma [113] Table 2.

4.4. Antiviral

Recently, with the worldwide SARS CoV-2 pandemic, LF antiviral activity has been an appealing candidate fitting COVID-19 pathogenesis with a dual benefit. The first is the immunomodulatory and anti-inflammatory activity which may help to reduce the raged immune responses and cytokine storm. Secondly, the antiviral activity of the drug may have merit for handling the task [15]. A congener of LF, vidofludimus is currently under investigation in two phase-II/III trials in patients with COVID-19 [114] Table 2.

5. Common adverse effects

Antirheumatic agents are among the most commonly used drugs associated with hepatotoxic effects ranging from acute drug-induced liver injury (DILI) to chronic hepatic ailments and even drug-induced autoimmune hepatitis. Based on the National Data Bank for Rheumatic Diseases it has been estimated that leflunomide-related events leading to hospitalization (without formal causality assessment) occur in 2 out of 1000 patients per year [115].

LF is generally considered to be a safe drug with respect to the reported adverse effects. The main adverse effects of LF are gastrointestinal disturbances such as diarrhea (17%), nausea (9%), abdominal pain (5%), and increased hepatic enzymes (5–10%) [116,117]. Hepatic injury represents a serious drawback of LF based on the issued report by the European Agency for the Evaluation of Medicinal Products (EMEA) in 2001. In this report, 129 cases of LF-induced hepatotoxicity were reported, which included two patients with hepatic cirrhosis, 15 presented with acute hepatic failure, and a 60% fatality rate [118]. Accordingly, a community petition was addressed to the FDA, advocating for the withdrawal of LF from the U.S. market [119,120]. The FDA declined this petition based on the fact that the benefits outweighed the hazards with imposing a black-boxed warning on the pack of LF stating the need for a regular monitoring of hepatic enzymes and a restriction of use in patients with advanced hepatic diseases [1].

Furthermore, some cases with severe liver injury were reported with fatality outcomes [16,19,121]. This has been documented as occurring within six months of the start of LF in patients with risk factors for developing hepatotoxicity [122,123]. LF combined with methotrexate enhanced hepatic damage with elevated liver enzymes reaching > 3 times the upper limits of normal (ULN). One case of liver injury was reported in a patient with liver cirrhosis who had received a combination of both LF and methotrexate [90]. Liver damage associated with LF therapy is commonly noted as alimentary tract symptoms, including nausea and abdominal distention; hence, liver transaminases must be monitored throughout the therapeutic regimen [124].

On the other hand, doses of LF (4, 12, 36 mg/kg) were found to significantly decrease the serum transaminase (ALT, AST) activity and improve antioxidant and anti-inflammatory mediated hepatic injury [125].

6. Studies reporting the drawbacks of leflunomide on the liver

The use of LF can be applied in solo or as a combined regimen with other immune-suppressing drugs like methotrexate. Liver toxicity is rare in rheumatoid arthritis patients using combination therapy with LF (20 mg/day) and methotrexate (20–25 mg/week) [126]. Meanwhile, animal studies using the combined therapy of LF and methotrexate showed high antiarthritic benefit but with the possibility of a hepatotoxic effect. In the same study, LF (10 mg/kg/day) and an LF/methotrexate combination showed the greatest degree of liver fibrosis [127]. Accordingly, in any patient with hepatic impairment, this combination is contra-indicated. Furthermore, LF clinical guidelines recommend monthly monitoring of hepatic enzymes within the first six months of therapy with further trimonthly monitoring later. ALT levels greater than three times ULN without an increase in bilirubin have been identified as sensitive, but are not necessarily a specific signal of liver toxicity [128].

Clinical studies and basic research reported on the hepatotoxicity of LF, which was found to be dose and time-dependent [129–132]. For the former, the use of LF doses of higher than 20 mg/day is associated with a higher incidence of hepatic injury, which may be asymptomatic or of a fulminating nature with a life-threatening hazard [1,16,126].

LF causes hepatotoxicity, which is presented as increased liver

### Table 3

| Pathogenesis               | Mechanism                                                                 |
|----------------------------|---------------------------------------------------------------------------|
| Mitochondrial stress       | Leflunomide > teriflunomide preferentially inhibits mitochondrial OXPHOS complex V (F1F0 ATP synthase) → ATP depletion and the collapse of mitochondrial membrane potential [131]. |
| Endoplasmic reticulum stress | MAPK signaling cascade, through inhibiting JNK and enhancing ERK1/2 pathways [130]. |
| Metabolic stress and inflammatory pathway | TLR4-induced apoptosis through activation of PI3K/mTOR/NFκB pathway [129]. |
| Hepatic fibrosis           | Increased hepatic expression of TGF-β [127,129]. |
| CYP450 polymorphism        | CYP2C9*3 allele may be associated with hepatic toxicity of LF in rheumatoid arthritis patients [16,138]. |
|                            | Genetic polymorphism of CYP1A2*1F may be associate with hepatic toxicity of LF in rheumatoid arthritis patients [137]. |
enlargement of liver enzymes within 4-6 weeks afterward [26]. In the case of proof of LF-induced liver injury, a withdrawal maneuver is recommended with the use of cholestryamine, or activated charcoal for a faster washout of LF [1,118].

LF is considered a therapeutic choice in patients with autoimmune hepatitis while this may be cautiously considered due to the hepatotoxic nature of the drug itself. However, the idiosyncratic nature of LF hepatotoxicity supports the metabolic idiosyncratic notion [133]. For instance, in APAP-induced hepatotoxicity, LF inhibited JNK1/2 activation and prevented mitochondrial permeability transition pore opening, thus offering protection from cell death induced by toxic concentrations of APAP [134]. While, in immune-mediated hepatitis induced by concanavalin A, LF inhibited T-cell mediated hepatic injury through the inhibition of NF-κB, TNF-α, and caspase-mediated apoptosis [135].

6.1. Pathogenesis of LF-induced liver injury (Table 3)

The liver is the main detoxifying tissue in the body with its exposure to chemical and toxicant liabilities, it requires stable energy resources and continuously replenished tissue antioxidant mechanisms. The metabolic products of xenobiotics represent a major threat with population variations based on their environmental and genetic makeup [136]. The main pathogenesis in LF hepatotoxicity involves the hepatic oxidant burden and metabolic and tissue energy derangements leading to cellular damage. LF-induced DILI is mediated through different mechanisms including an inflammatory pathway ending with tissue damage.

In animal studies, LF-induced-inflammatory liver injury occurs through dose-dependent upregulation of the TLR4/P3K/mTOR pathway and the cellular apoptotic marker caspase 3 [129]. Also, LF and its active metabolite teriflunomide exhibited mitochondrial toxicity in human hepatic HepG2 cells. LF caused dose-dependent depletion in cellular ATP through the inhibition of mitochondrial oxidative phosphorylation complexes mainly complex V (FIFO ATP synthase), LDH leakage, and cell death [131]. Furthermore, LF-induced cytotoxicity in HepG2 cells was mediated by endoplasmic reticulum stress and the enhancement of the JNK and ERK1/2 of the MAPK signaling pathways [130].

Genetic polymorphic cytochrome P450 enzymes were investigated in 105 patients with rheumatic arthritis to examine the relationship between patients’ CYP1A2*1F, CYP2C9*17, CYP2C9*2, and CYP2C9*3 alleles and LF toxicity. Forty-three patients discontinued LF therapy within the first year due to toxicity. Patients with CYP1A2*1F were at a 9.7-fold higher risk than patients who only carried the allele. However, patients with CYP2C19 and CYP2C9 had no relationship [137]. Other genetic studies showed a correlation between the slow CYP2C9*3 allele and LF-induced hepatitis in rheumatoid arthritis patients [16,138].

6.2. Preventive and therapeutic agents for LF-induced liver injury

Drug-induced liver injury (DILI) anticipation and prevention represent an elusive target for health and pharmaceutical bodies. DILI is classified as intrinsic such as paracetamol- and alcohol-induced DILI. On the other hand, idiosyncratic DILI is unpredictable and, hence, difficult to avoid. Without an understanding of the molecular pathogenesis of idiosyncratic DILI, it will be difficult to prevent or specifically manage. The possible protective agents for minimizing LF-induced liver injury may rely on antagonizing the oxidant stress, metabolic derangements, and inflammatory character of its pathogenesis.

6.2.1. Using antioxidant hepatoprotective agents

Rheum Palmarum L. showed hepatoprotective effects through anti-inflammatory, antioxidant and antiapoptotic mechanisms in mice by inhibiting NF-κB, nitric oxide, IL-1β, Caspase 3 and Caspase 8 in the liver tissue. Also, it reduced iNOS, COX-2, and Bax and enhanced the expression of Bel-2 and PCNA [139]. Further, the silymarin and propolis hepatoprotective effect in CCL4 hepatotoxicity in rats is mediated through antioxidant properties [140]. In vivo studies on rats, showed that LF causes portal fibrosis, sinusoidal congestion, and infiltration with periducal inflammatory cells. Meanwhile, the use of LF combined with β-caryophyllene reduced the hepatotoxic effect of LF. β-carophyllene acts through antioxidant activity that leads to the inhibition of hydroxyl anions, lipid peroxides, and superoxide anions [141]. Oenanthi Javanica—a Chinese medicinal herb—is an aquatic perennial herb cultivated in East Asian countries. Total phenolics from Oenanthi Javanica in 125, 250, and 500 mg/kg doses showed hepatoprotective effects in D-galactosamine-induced liver injury in mice through anti-oxidant and anti-inflammatory actions detected as decreased iNOS, COX2, NO, and PGE2 [142].

6.2.2. Using mitochondrial and metabolic equilibrating agents

5-Aminimidazole-4-carboxamide ribonucleotide (AICAR) is an analog of AMP and an activator of AMP-dependent protein kinase (AMPK). AMPK prevents/reverses drug-induced mitochondrial and hepatocellular damage by regulating mitochondrial fusion and mitophagy in aceterminophen and diclofenac-induced hepatic cell toxicity of murine and human origin [143].

6.2.3. Using anti-inflammatory agents

The hepatoprotective effect of vitamin D3 (1,25(OH)2D3) [low-dose 0.025 μg/kg/day, moderate dose 0.15 μg/kg/day, and high dose 0.3 μg/kg/day] was investigated in diabetic-induced hepatic injury in rats. After four months of therapy, the high dose of vitamin D3 downregulated TLR4, NF-κB, ALT, and LDL and improved hepatic tissue architecture with reduced inflammatory cell infiltrates [144].

Aqueous extract of the Chinese herb Aconitum Carmichaelii Debeaux showed anti-inflammatory and antiapoptotic potential in the treatment of acute liver failure induced by D-galactosamine in rats. The hepatoprotective effects are presented as a decrease of hepatic pathological scores, reduced expressions of TLR4, NF-κB, HMGBl, and caspase-3, and increased PCNA cell regeneration marker [145].

Total glucoside of peony, which is a Chinese herb, was used as an adjuvant with methotrexate and lefunomide in doses of 0.6 and 1.8 g/ day for 12–24 weeks. This combination showed the increased anti-rheumatic effectiveness of the LF/methotrexate combination in rheumatoid arthritis patients with hepatoprotective advantages. This combination ameliorates liver fibrosis and decreases the progression of liver disease in a non-alcoholic patient through IL13 regulation. [146].

In support, assessment of the therapeutic effect of total glucosides of peony for juvenile idiopathic arthritis was detected through the extraction of data from eight electronic databases that concluded total glucosides of peony as a unique nonbiologic disease-modifying anti-rheumatic drug (nonbiologic DMARD) with good efficacy and minimal adverse effects [147].

In conclusion, the current study represents a comprehensive revision of the clinical importance of LF as a multitask therapeutic agent not only in the approved application in autoimmune arthritis but also as an antineoplastic and antimicrobial candidate. With the ongoing dilemma of the SARS CoV2 pandemic and the life-threatening conditions of COVID-19 patients, LF should be investigated in patients with COVID-19 as an anti-inflammatory immunomodulator due to its advantageous antiviral activity. LF adverse effects are highlighted with an emphasis on LF-induced hepatotoxicity molecular pathogenesis and the possible hepatoprotective agents which can be considered as a supportive treatment during long-term therapeutic regimen on LF.
Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors’ contribution
RDA, MAE, GAA, SMA, SSA & SHE all contributed to the conceptualization, database searches, article collection, revision, and data extraction. SMA prepared the illustrating figures of the pathways. MAE & SHG prepared the tables. SHG supervised the work and prepared the final manuscript.

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