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Review Article

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Drug-Induced Fatty Liver

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ABSTRACT

Drug-induced fatty liver disease is defined as the accumulation of fat in the liver due to exposure to some drugs. This condition is called non-alcoholic fatty liver disease (NAFLD). Fatty liver can be progressed to inflammation called non-alcoholic steatohepatitis or NASH, which can be progressed into fibrosis and eventually liver failure. This condition is frequently associated with long-term intake of the potentially harmful drug. Differents mechanisms have been postulated to illustrate how these drugs could induce fatty liver. Due to current lifestyle, the fatty liver rate is increasing, however, some drugs can induce this condition even in non-obese persons. This review focuses on drug-induced fatty liver and the possible role for some antioxidants in reversing this condition.

Key words: *DILI, liver, drug, toxicity, fatty liver, NAFLD, NASH, oxidative stress, steatosis.*

INTRODUCTION

Drug-induced liver injury (DILI) is very common and remains the main reason for drugs withdrawal. Drug-induced fatty liver is a type of DILI, and is associated with the type, duration, and dose of the drug used. The increase in medicines taken can be the reason for the occurance of various clinicopathologic forms of liver disorders. Steatosis and steatohepatitis are well-documented in clinical practices. The liver is the main organ in which the metabolism of xenobiotics is carried out and this explains the susceptibility of the liver to drug-induced injuries. It has been proved that different mechanisms can lead to liver steatosis (Fig. 1). During the past decades, some medicines have been known to induce steatosis, through prevention of mitochondrial beta-oxidation, lowering VLDL secretion, insulin resistance, elevated liver absorption of fatty acids, and other unknown pathophysiological pathways. [1] Some medicines can cause a rapid onset by inhibiting the synthesis of adenosine triphosphate (ATP) by mitochondria, which results in micro-vesicular steatosis. [2]

Common drugs responsible for fatty liver

Amiodarone

Amiodarone is an antiarrhythmic medicine and is a positive cation with both hydrophilic and hydrophobic moieties, which can cross the mitochondrial membrane and affecting its functions, thereby causing fatty liver [3]Amiodaronecan be accumulated in the mitochondria resulting in high intra-mitochondrial concentration which impairs beta-oxidation and electron transport chain, causing increased lipid accumulationand ROS production [4] Amiodarone can prevent microsomal triglyceride transfer protein (MTP) activity, which physiologically plays an important role in VLDL regulation. Some drugs have a comparable structure to amiodarone and may, therefore, have the same influences on hepatocytes. [5]Amiodarone hepatotoxicity ocurrs due to direct damage to lipid bilayers and impairment of lysosomal and/or mitochondrial function[6].

Dronedarone is a ‘second generation’ anti-arrhythmic medicine. In recent years, it has been found that there are high levels of liver injury associated with the use of dronedarone and it can cause serious acute hepatic failure.

[7]. Dronedarone can prevent beta-oxidation of fatty acids *in vivo*[8] without damaging the electron transport chain possibly due to its short half-life that makes it hard to reach a high concentration inside the mitochondria. [5]

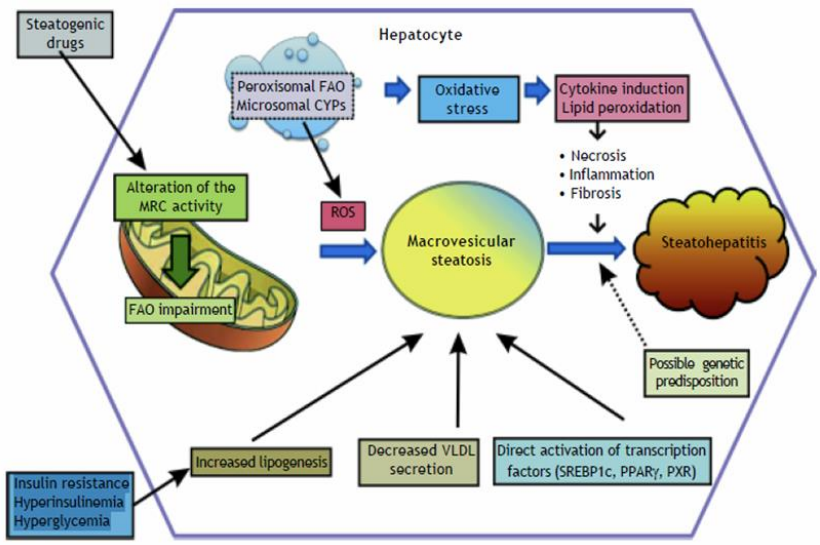

Fig. 1: Schematic representation of drug-induced hepatic steatosis. [11]

Tamoxifen & raloxifene

Tamoxifen is efficiently used in breast cancer therapy, especially in the treatment of the molecular subtypes that express estrogen receptors (ER). Alpha and beta as 2 subtypes of ER are found in the mitochondrial membrane. [10]**Satapathy*et al.***confirmed that the use of tamoxifen can cause fatty liver disease with reduced beta-oxidation and increased fatty acid synthesis. [11, 12] Tamoxifen is a positive cationic amphiphilic compound and its acquisition is the leading cause of hepatotoxicity. [5]

Steatosis improves in about thirty percent of the patients, generally during two years after starting the anti-estrogentreatment**,**[13]Tamoxifen can promote the development of NAFLD.However, tamoxifendiscontinuation can ameliorate steatosis and steatohepatitis. [14] Preventing the progression of NASH due to tamoxifen treatment in patients with breast cancer can be carried out using bezafibrate treatment. A comprehensive research needs to be undertaken before any recommendations on the best course of treatment in these cases. [15] Raloxifene is another important drug used to prevent and treat osteoporosis in postmenopausal women.There is evidence that it may worsen the pre-existing fatty liver and some studies have shown that raloxifene can prevent beta-oxidation of fatty acids. [11, 16]

Valproate

Valproate, or valproic acid, is a fatty acid and widely prescribed antipsychotic drug. Therefore, it competes with another fatty acid in the metabolic pathways of liver cells. [5]

The free acid form of valproate enters the cell and then is transported into the mitochondria, combined with coenzyme A (CoA). This results in CoA deficiency and inhibition of the catabolism of fatty acids to triglycerides for storage, leading to steatosis. Moreover, the mitochondrial valproate toxicity occurs due to its ability to release protons that leads to damaging the electron transport chain and ATP generation. Moreover, constantly treatment with valproate can cause obesity, which increases the risk of progressing the pre-existing fatty liver disease. [17]

Many clinical studies have investigated valproate liver toxicity. Hepatic steatosis estimated by ultrasound scan was reported in more than 60% of patients treated with valproate comparing to group treated with different epilyptic drug. [18] Mild elevation of aminotransferase levels without cholestasis is common among patients treated with this drug. [19] Histologically, valproate generally causes microvesicular steatosis. Moreover, valproate-induced steatosis can produce mitochondrial impairment**.** [20]It is highly recommended that patients treated with valproate should be closesly monitored to follow the progress of NAFLD and another medication might be recommended inorder to prevent further liver damage.

Tetracycline

Many antibiotics are hepatotoxic, among which tetracycline is recognized with the major potential of causing fatty liver disease, mostly if delivered intravenously. [21]

Microvesicular steatosis is the common histological type of liver damage caused by tetracycline-based treatment. [20] Studies have shown that tetracycline prevents beta-oxidation of fatty acids and MTP, an enzyme with the main role in cholesterol regulation. [22]

Transcriptomic analysis conducted by**Szalowska*et al*.** [23]found that tetracycline can down regulategenes involved in beta-oxidationincluding peroxisome proliferator-activated receptor alpha (PPARα), carnitine palmitoyltransferase I (CPT-I), and fatty acid-binding protein 1 (FABP- 1). Furthermore, doxycycline, and minocycline, which are tetracycline-based compounds,can promote ROS production in hepatocytes. [24] This is dependent on the activation of activating transcription factor 4 (ATF4), which adjusts CYP2E1 that is involved in some metabolic pathways leading to the production of ROS. [5]

The possible molecular mechanisms of hepatocyte damage have been investigated by the proteomic profiling of a tetracycline-treated murine model. The results showed that the highestaffected mitochondrial proteins are those found in beta-oxidation of fatty acids, especially acyl-CoA dehydrogenase, which may cause a reduction in their enzymatic activity.[25]

Chemotherapeutic agents

'Chemotherapy-associated steatohepatitis' (CASH) is the name given to chemotherapeutic liver injury. Irinotecan, 5-fluorouracil (5-FU), and oxaliplatin are the commonchemotherapeuticcompounds related to steatohepatitis. Many therapeutic regimens contain these medicines, especially those for patients with metastatic colon cancer. [26]

A research was conducted to estimate the level of liver damage induced by chemotherapy in patients undergoing neoadjuvant chemotherapy for colorectal liver metastasis, and the results showed that steatosis is a common histological finding, with different possible periods of injury.[26]Another histological estimation of non-tumoral liver parenchyma investigated 384 patients, undergoing liver resection for metastatic colorectal cancer. The results showed that high BMI, irinotecan treatment, and elevated levels of glucose in the blood were all related to steatosis of hepatocytes. [27]Some studies have suggested that these drugs may influence mitochondrial DNA and it is possible that the mitochondrial toxicity plays the vital role in drug-induced steatosis.

Methotrexate (MTX)

Methotrexate (MTX) is a chemotherapeutic and immunosuppressant drug. [28]It is believed that TMX-induced liver damage due to direct toxicity. **Kremer *et al*.** [29] observed that the polyglutamated metabolite of MTX is gradually accumulated in the liver cells and may lead to hepatotoxicity. Itcan abnormally alter the role of mitochondria by reducing mitochondrial folate stores; it does this not by directly affecting the intra-mitochondrial stored folate, but by inhibiting folate entry into the mitochondria, which means there is no replenishment of the mitochondrial folate stocks. It has been demonstrated that MTX-caused mitochondrial abnormalities can lead to ROS generation and the induction of caspase-dependent apoptosis. [30, 31]

MTX can cause damage to the epithelial barrier, drive to leaky intestines syndrome, which is related to the initiation and progression of fatty liver disease. [32]

MTX-induced liver damage can also be detected by the occurrence of low to moderate aminotransferase increases in up to 50% of patients. [33] This biochemical change is generally temporary; occasionally needs to be investigated or treated. [34] Steatohepatitis, extensive fibrosis, and cirrhosis can be caused by MTX and it has been found that these diseases only occur in 4-5% of patients. [35]Steatohepatitis by MTX can be increased by the presence of pre-existingsteatosis, which can result in advanced liver injury.Studies have shown that other confounding factors related to MTX-induced hepatotoxicity include NAFLD, alcohol consumption, chronic hepatitis B or C, overweight, and hyperglycemia. [14]

Possible management of drug-induced fatty liver disease

The therapeutic strategies for fatty liver disease are firstly aimed at improving the metabolic parameters that lead to pathogenesis, including weight loss, exercise, and lipid and glycaemic controls. [36] These lifestyle modification strategies will have a useful function in the treatment of drug-induced fatty liver.

Vitamin E (alfa-tocopherol) is a potential treatment to improve liver histology in healthy adults with biopsy-confirmed non-alcoholic steatohepatitis (NASH). [37]Vitamin E is fairly safe and easy to use. The reasons for utilizing vitamin E in patients with NASH is based on its antioxidant characteristics, which means it could potentially be beneficial in drug-induced steatohepatitis.

The potential use of other antioxidants including S-adenosylmethionine (SAMe) [38] and Silymarin,[39]has not been fully investigated as treatments.

Current studies have created enormous interest and high expectations for production of therapies for non-alcoholic fatty liver disease (NAFLD), NASH and drug-induced steatohepatitis. [40] One of these drugs is

obeticholic acid (6α-ethyl-chenodeoxycholic acid), which is a semi-synthetic form of the primary human bile acid that is a nuclear hormone receptor that controls the metabolismof lipid and glucose. It wasshown to improve the histologicalfeatures of non-alcoholic steatohepatitis, however, further studies are being undertaken. [40]

Antioxidant defense system

The natural antioxidant systems are divided into two main groups, enzymatic and non-enzymatic. Enzymatic antioxidants had contained a limited number of proteins with some supporting enzymes. These include catalase for H_2O_2, superoxide dismutase (SOD) for superoxide, and glutathione peroxidase (GPx) for H_2O_2 and lipid peroxide, though; there are no specific defense mechanisms against hydroxyl radicals, which are the most potent ROS. Non-enzymatic antioxidants include the direct-acting antioxidants, such as GSH, metallothionein (MT), caeruloplasmin, and transferrin. [41] Chelating agents also act as antioxidants by binding to redox metals to prevent the free radical generation, which makes them extremely important in the defense against ROS. [42, 43] Most of the antioxidants are derived from dietary sources, [44] but the cell itself can synthesize a small number of these molecules, for example,ascorbic acid. Importantly, these systems are found to be impaired in patients with NAFLD/NASH, which can potentially further increase the levels of oxidative stress. [45]

1- Hepatic Glutathione

Glutathione (γ-glutamyl-cysteinyl glycine) is an important intracellular antioxidant that scavenges the common ROS (*e.g.* hydroxyl radicalsand superoxide anion) and other free radical species, such as peroxynitrite, lipid peroxyl radicals, and H_2O_2. [46] Glutathione protects cells against endogenous and exogenous toxins; for example, it protects against free radicals produced by xenobiotics or their metabolites. It is capable of binding to potentially harmful electrophilic compounds through two mechanisms: first, it can protect protein thiols against ROS/RNS and secondly, it can reverse oxidative effects through the removal of the disulfide bond and nitrothiols. [47] Such radical species are removed by glutathione (non-enzymatic reduction), whereas the removal of hydroperoxides needs glutathione peroxidase (GPx) (enzymatic catalysis). In cells, GSH interacts with electrophilic compounds/metabolites and with free radicals during detoxification, and depletion of the reduced form of glutathione was reported to be as a hepatotoxicity marker. [48]GSH conjugation is catalyzed by the glutathione-*S*-transferase family of cytoplasmic enzymes, which are of high importance in protecting the cells from ROS produced through normal processes, such as drug biotransformation. Reduced GSH levels have been implicated in NAFLD pathogenesis; **Videla*et al.*** [49] reported that hepatic GSH is significantly depleted in patients with hepatic steatosis and steatohepatitis.

2- Metallothionein

Metallothionein (MT) was first discovered in 1957 and is a ubiquitous, highly inducible, and low molecular weight protein, which contains high amounts of heavy metals. [50]MT exists in four isoforms (MT-1-4) in mammals, all of which have metal-binding sites; MT-1 and MT-2 are ubiquitously expressed, MT-3 is mainly expressed in the brain and MT-4 is found in squamous epithelia. [51]

MT synthesis can be induced by various stimuli, including metals such as zinc and cadmium, but importantly, non-metallic compounds including oxidative stress can also induce its synthesis. [52]

The induction of MTs under oxidative conditions has led to speculation that MTs can act as radical scavengers, and this is backed up by a considerable amount of data showing the ability of MT to function as strong free radical scavengers. Moreover, *in vivo* studies have shown the important role of MT against free radicals [53] (Fig. 2).

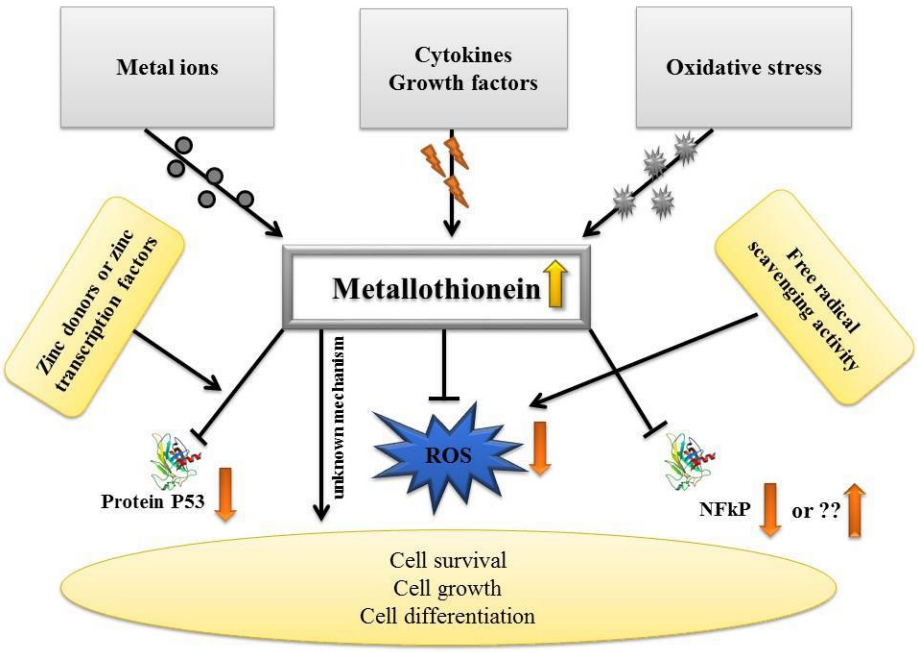

Fig. 2: Stimuli of MT expression and possible downstream effects [54]

In addition to the potential role of MT in oxidative stress, it also plays 2 main roles in apoptosis: regulation of intracellular zinc content and the interaction of MT with some proteins that are involved in apoptosis. Zinc depletion is an intracellular mediator of apoptosis in different cell lines through activation of caspases. [55, 56]MT protects against apoptosis by distributing cellular zinc. [57] The tumor suppressor protein is a metal-binding transcription factor that binds DNA through a complex domain stabilized by a zinc atom. [58] The nuclear accumulation of MT is probably involved in supplying metals such as zinc to target molecules including tumor suppressor gene products, zinc-finger transcription factors, and enzymes. [58] The regulation of NF-κB activity one of the most important MT interaction with proteins that are involved in apoptosis. NF-κBis a transcription factor that is involved in gene activation and various cellular activities associated with development, growth, and cell death regulation. [59] Hence, while the potential for MT-mediated protection from ROS is established, its modulation by disease conditions and the physiological consequences of this are unclear and need further examination.

Micronutrients as a therapeutic strategy

Micronutrients are substances that provide nourishment essential for growth and maintenance of life and are required in very small amounts for physiological functions. [60, 61]Micronutrients include electrolytes (sodium, chloride, and potassium), minerals (calcium, phosphorus, and zinc), vitamins, and carotenoids. These substances are inorganic compounds needed for tissue structure, pH regulation, neuronal signaling, muscle contraction, and enzymatic activities. Electrolytes may bind to minerals to neutralize their charge. The current function of electrolyte homeostasis in NAFLD is notyet fully understood, but there is a strong epidemiologic relation among rising sodium diets and NAFLD. [62, 63]

Micronutrient has only limited consideration for NAFLD therapeutics. For example, carotenoid supplementation has not been approved in NAFLD patients despite there being proof that alanine aminotransferase (ALT) activity is improved in runners provided with the carotenoid-containing fruit pulp oil. [64]Moreover, there is evidence that NASH patients fail to normalize vitamin D3 level or liver histology in response to six months of a daily dose of 2000 IU of vitamin D3 supplementation. [65] The results of daily 1000 mg vitamin C and 1000 IU vitamin E for 6 months are more promising, which modestly improved fibrosis scores in biopsy-proven NAFLD patients. [66]

In addition to the curative effects of micronutrients, vitamin E is an effective model of how substance required in trace amounts for the normal growth and development of living organisms can be utilized to help control NAFLD.Also, its supplementation has been shown to improve liver enzyme levels and health. There have been some suggestions that vitamin E has an antifibrotic activity,but studies have shown that there was no advantage of vitamin E on liver cell fibrosis in adults. [67]

In children who were given 800 IU vitamin E daily, the results showed an improved NASH histology.[68]These results have been supported by another study. [69] In adults, no antifibrotic influence of vitamin E has been found. [68]

CONCLUSIONS

Several drugs are known to induce fatty liver and therefore may increase the risk of DILI. The most commonly used medicines with possibility of inducing hepatotoxic effects need to be closely monitored as this can lead to the identification of clinical markers relevant to measuring liver damage. Studies have shown micronutrient intake can help to restore tissue damage and energy homeostasis in NAFLD patients. These substances are required in trace amounts for the normal growth and development of living organisms and the beneficial effect toward hepatotoxicity might be due to regulating lipid metabolism and their antioxidant properties.

REFERENCES

1. Miele, L., Liguori, A., Marrone, G., Biolato, M., Araneo, C., Vaccaro, F. G., Gasbarrini, A. and Grieco, A. Fattyliver and drugs: the twosides of the same coin. EuropeanReview for Medical and Pharmacological Sciences, 2017; 21 (1 Suppl): 86-94.
2. Stravitz RT, Sanyal AJ. Drug-induced steatohepatitis. Clinics in liverdisease. 2003 May 1;7(2):435-51.
3. Pessayre D, Fromenty B, Berson A, Robin MA, Lettéron P, Moreau R, Mansouri A. Central role of mitochondria in drug-inducedliverinjury. Drug metabolismreviews. 2012 Feb 1;44(1):34-87.
4. Berson A, De Beco V, Lettéron P, Robin MA, Moreau C, El Kahwaji J, Verthier N, Feldmann G, FromentyB, Pessayre D. Steatohepatitis-inducingdrugs cause mitochondrial dysfunction and lipidperoxidationin rat hepatocytes. Gastroenterology. 1998 Apr 1;114(4):764-74.
5. Schumacher JD, Guo GL. Mechanisticreview of drug-induced steatohepatitis. Toxicology and appliedpharmacology. 2015 Nov15;289(1):40-7.
6. Grieco A, Forgione A, Miele L, Vero V, Greco AV, Gasbarrini A, Gasbarrini G. Fattyliver and drugs.EurRev Med PharmacolSci. 2005;9(5):261-3
7. Joghetaei N, Weirich G, Huber W, Büchler P, Estner H. Acute liverfailureassociatedwithdronedarone. Circulation: Arrhythmia and Electrophysiology. 2011 Aug;4(4):592-3.
8. Felser A, Stoller A, Morand R, Schnell D, Donzelli M, Terracciano L, Bouitbir J, Krähenbühl S. Hepatictoxicity of dronedarone in mice: Role of mitochondrial β-oxidation. Toxicology. 2014 Sep 2;323:1-9.
9. Pessayre D, Berson A, Fromenty B, Mansouri A. Mitochondria in steatohepatitis. InSeminars in liverdisease 2001 (Vol. 21, No. 01, pp. 057-070). Copyright© 2001 by ThiemeMedical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel.:+ 1 (212) 584-4662.
10. Chen JQ, Delannoy M, Cooke C, Yager JD. Mitochondrial localization of ERalpha and ERbeta in humanMCF7 cells. Am J PhysiolEndocrinolMetab 2004; 286: E1011-1022.
11. Satapathy SK, Kuwajima V, Nadelson J, Atiq O, Sanyal AJ. Drug-inducedfattyliverdisease: An overviewof pathogenesis and management. Annals of hepatology. 2015 Nov 1;14(6):789-806.
12. Cole LK, Jacobs RL, Vance DE. Tamoxifen inducestriacylglycerol accumulation in the mouse liver by activation of fattyacidsynthesis. Hepatology. 2010 Oct;52(4):1258-65.
13. Bruno S, Maisonneuve P, Castellana P, Rotmensz N, Rossi S, Maggioni M, Persico M, Colombo A, Monasterolo F, Casadei-Giunchi D, Desiderio F. Incidence and riskfactors for non-alcoholicsteatohepatitis: prospective study of 5408 womenenrolled in Italian tamoxifen chemopreventiontrial. bmj. 2005 Apr 21;330(7497):932.
14. Patel V, Sanyal AJ. Drug-induced steatohepatitis. Clin Liver Dis 2013; 17: 533-546.
15. Ogawa Y, Murata Y, Saibara T, Nishioka A, Kariya S, Yoshida S. Follow-up CT findings of tamoxifen-induced non-alcoholic steatohepatitis (NASH) of breast cancer patients treatedwithbezafibrate. Oncology reports. 2003 Sep 1;10(5):1473-8.
16. Martins-Maciel ER, Campos LB, Salgueiro-Pagadigorria CL, Bracht A, Ishii-Iwamoto EL. Raloxifeneaffects fattyacidoxidation in liversfromovariectomized rats by acting as a pro-oxidant agent. Toxicologyletters. 2013 Feb 13;217(1):82-9.

17. Luef G, Rauchenzauner M, Waldmann M, Sturm W, Sandhofer A, Seppi K, Trinka E, Unterberger I, Ebenbichler CF, Joannidis M, Walser G. Non-alcoholicfattyliverdisease (NAFLD), insulinresistance and lipid profile in antiepilepticdrugtreatment. Epilepsyresearch. 2009 Sep 1;86(1):42-7.
18. Luef GJ, Waldmann M, Sturm W, Naser A, Trinka E, Unterberger I, Bauer G, Lechleitner M. Valproate therapy and nonalcoholicfattyliverdisease. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society. 2004 May;55(5):729-32.
19. Rabinowich L, Shibolet O. Drug induced steatohepatitis: an uncommonculprit of a commondisease. BioMedresearch international. 2015;2015.:168905.
20. Ramachandran R, Kakar S. Histological patterns in drug-inducedliverdisease. Journal of clinicalpathology. 2009 Jun 1;62(6):481-92.
21. Leitner JM, Graninger W, Thalhammer F. Hepatotoxicity of antibacterials: pathomechanisms and clinicaldata. Infection. 2010 Feb 1;38(1):3-11.
22. Lettéron P, Sutton A, Mansouri A, Fromenty B, Pessayre D. Inhibition of microsomal triglyceridetransferprotein: anothermechanism for drug-induced steatosis in mice. Hepatology. 2003 Jul;38(1):133-40.
23. Szalowska E, van der Burg B, Man HY, Hendriksen PJ, Peijnenburg AA. Model steatogenic compounds (amiodarone, valproicacid, and tetracycline) alter lipidmetabolism by differentmechanisms in mouse liver slices. PloS one. 2014 Jan 29;9(1):e86795.
24. Brüning A, Brem GJ, Vogel M, Mylonas I. Tetracyclines cause cell stress-dependent ATF4 activation and mTOR inhibition. Experimentalcellresearch. 2014 Jan 15;320(2):281-9.
25. Deng Z, Yan S, Hu H, Duan Z, Yin L, Liao S, Sun Y, Yin D, Li G. Proteomic profile of carbonylatedproteins in rat liver: Discovering possible mechanisms for tetracycline-induced steatosis. Proteomics. 2015 Jan;15(1):148-59.
26. Brouquet A, Benoist S, Julie C, Penna C, Beauchet A, Rougier P, Nordlinger B. Risk factors for chemotherapy-associatedliver injuries: amultivariateanalysis of a group of 146 patients withcolorectal metastases. Surgery. 2009 Apr 1;145(4):362-71.
27. Wolf PS, Park JO, Bao F, Allen PJ, DeMatteo RP, Fong Y, Jarnagin WR, Kingham TP, Gönen M, Kemeny N, Shia J. Preoperativechemotherapy and the risk of hepatotoxicity and morbidityafterliverresection for metastatic colorectal cancer: a single institution experience. Journal of the American College of Surgeons. 2013 Jan 1;216(1):41-9.
28. Manal M.S. Mansoury; Goji Berry (Lyciumbarbarum L.) ExtractAlleviate Acute HepatotoxicityInducedby Methotrexate in Rats, EntomolApplSciLett, 2018, 5 (4):119-126.
29. Kremer JM, Galivan J, Streckfuss A, Kamen B. Methotrexatemetabolismanalysis in blood and liver of rheumatoidarthritis patients: association withhepatic folate deficiency and formation of polyglutamates. Arthritis&Rheumatism: Official Journal of the American College of Rheumatology. 1986 Jul;29(7):832-5.
30. Dalaklioglu S, Genc GE, Aksoy NH, Akcit F, Gumuslu S. Resveratrolamelioratesmethotrexate-inducedhepatotoxicity in rats via inhibition of lipidperoxidation. Human &experimentaltoxicology. 2013 Jun;32(6):662-71.
31. Di Martino S, Rainone A, Marotta G, Mazzarella N, Pugliese S, Rinaldi L. Nutraceutical agents withhepatoprotectiveeffects in cancer patients. World Cancer Research Journal. 2016 Jan 1;3(4).
32. Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Masciana R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM. Increased intestinal permeability and tightjunctionalterations in nonalcoholicfattyliverdisease. Hepatology. 2009 Jun;49(6):1877-87.
33. Bath RK, Brar NK, Forouhar FA, Wu GY. A review of methotrexate-associatedhepatotoxicity. Journal of digestive diseases. 2014 Oct;15(10):517-24.
34. Fournier MR, Klein J, Minuk GY, Bernstein CN. Changes in liverbiochemistryduringmethotrexate use for inflammatoryboweldisease. The American journal of gastroenterology. 2010 Jul;105(7):1620.
35. Whiting-O'Keefe QE, Fye KH, Sack KD. Methotrexate and histologichepaticabnormalities: a meta-analysis. The American journal of medicine. 1991 Jun 1;90(6):711-6.
36. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholicfattyliverdisease. QJM: An International Journal of Medicine. 2009 Nov 13;103(2):71-83.
37. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. American College of Gastroenterology. The diagnosis and management of non-alcoholicfattyliverdisease: practice

guideline by the American Gastroenterological Association, American Association for the Study of LiverDiseases, and American College of Gastroenterology. Gastroenterology. 2012 Jun;142(7):1592-609.

38. Anstee QM, Day CP. S-adenosylmethionine (SAMe) therapy in liverdisease: areview of currentevidenceand clinical utility. Journal of Hepatology. 2012 Nov 1;57(5):1097-109.
39. Wellington K, Jarvis B. Silymarin: areview of itsclinicalproperties in the management of hepaticdisorders. BioDrugs. 2001 Jul 1;15(7):465-89.
40. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV. Farnesoid X nuclearreceptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): amulticentre, randomised, placebo-controlled trial. The Lancet. 2015 Mar 14;385(9972):956-65.
41. Fridovich I. Superoxide anion radical (O• 2), superoxide dismutases, and relatedmatters. Journal of Biological Chemistry. 1997 Jul 25;272(30):18515-7.
42. Mates JM. Effects of antioxidant enzymes in the molecular control of reactiveoxygenspeciestoxicology. Toxicology. 2000 Nov 16;153(1-3):83-104.
43. Noureldeen AF, Qusti SY, Al-shammari EM, Ramadan RM. Antitumor and AntioxidantActivities of a Novel Platinum (II)-PyrazineCarboxamideComplex Against Ehrlich Ascites CarcinomaInduced in Mice. International Journal of Pharmaceutical and PhytopharmacologicalResearch. 2017 Dec 1;7(6):1-0.
44. Alsanie WF, El-Hallous EI, Dessoky ES, Ismail IA. Viper'sBugloss (EchiumVulgare L) Extract as A Natural Antioxidant and ItsEffect on Hyperlipidemia. International Journal of Pharmaceutical and PhytopharmacologicalResearch (eIJPPR). 2018 Feb 1;8(1):81-9.
45. Madan K, Bhardwaj P, Thareja S, Gupta SD, Saraya A. Oxidant stress and antioxidantstatusamongpatients withnonalcoholicfattyliverdisease (NAFLD). Journal of clinicalgastroenterology. 2006 Nov 1;40(10):930-5.
46. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002 Oct 1;18(10):872-9.
47. Han Y, Zhang J, Chen X, Gao Z, Xuan W, Xu S, Ding X, Shen W. Carbon monoxidealleviates cadmium-inducedoxidative damage by modulatingglutathionemetabolism in the roots of Medicago sativa. New Phytologist. 2008 Jan;177(1):155-66.
48. DeLeve LD, Kaplowitz N. Glutathionemetabolism and itsrole in hepatotoxicity. Pharmacology&therapeutics. 1991 Dec 1;52(3):287-305.
49. VIDELA LA, Rodrigo R, Orellana M, Fernandez V, Tapia G, Quinones L, Varela N, Contreras J, Lazarte R, Csendes A, Rojas J. Oxidative stress-relatedparameters in the liver of non-alcoholicfattyliverdisease patients. Clinical science. 2004 Mar 1;106(3):261-8.
50. Carpene E, Andreani G, Isani G. Metallothionein functions and structural characteristics. Journal of Trace Elements in Medicine and Biology. 2007 Dec 11;21:35-9.
51. Quaife CJ, Findley SD, Erickson JC, Froelick GJ, Kelly EJ, Zambrowicz BP, Palmiter RD. Induction of a new metallothionein isoform (MT-IV) occursduringdifferentiation of stratifiedsquamousepithelia. Biochemistry. 1994 Jun;33(23):7250-9.
52. Iszard MB, Liu J, Klaassen CD. Effect of several metallothionein inducers on oxidative stress defensemechanisms in rats. Toxicology. 1995 Dec 15;104(1-3):25-33.
53. Matsubara J, Shida T, Ishioka K, Egawa S, Inada T, Machida K. Protective effect of zinc againstlethalityin irradiatedmice. Environmentalresearch. 1986 Dec 1;41(2):558-67.
54. Ruttkay-Nedecky B, Nejdl L, Gumulec J, Zitka O, Masarik M, Eckschlager T, Stiborova M, Adam V, KizekR. The role of metallothionein in oxidative stress. International journal of molecularsciences. 2013;14(3):6044-66.
55. Ho LH, Ruffin RE, Murgia C, Li L, Krilis SA, Zalewski PD. Labile zinc and zinc transporter ZnT4 in mastcell granules: role in regulation of caspase activation and NF-κB translocation. The Journal of Immunology. 2004 Jun 15;172(12):7750-60.
56. Rudolf E, Rudolf K, Cervinka M. Zinc inducedapoptosis in HEP-2 cancer cells: the role of oxidativestress and mitochondria. Biofactors. 2005 Jan 1;23(2):107-20.
57. Maret W, Jacob C, Vallee BL, Fischer EH. Inhibitory sites in enzymes: zinc removal and reactivation by thionein. Proceedings of the National Academy of Sciences. 1999 Mar 2;96(5):1936-40.

58. Méplan C, Verhaegh G, Richard MJ, Hainaut P. Metal ions as regulators of the conformation and functionof the tumoursuppressorprotein p53: implications for carcinogenesis. Proceedings of the Nutrition Society. 1999 Aug;58(3):565-71.
59. Gilmore TD. Introduction to NF-κB: players, pathways, perspectives. Oncogene. 2006 Oct;25(51):6680-4.
60. Gernand AD, Schulze KJ, Stewart CP, West Jr KP, Christian P. Micronutrientdeficiencies in pregnancyworldwide: healtheffects and prevention. Nature ReviewsEndocrinology. 2016 May;12(5):274.
61. Joint FA. WHO Expert Consultation on Human Vitamin and MineralRequirements and World HealthOrganization Dept of Nutrition for Health and DevelopmentVitamin and mineralrequirements in human nutrition. Vitamin and mineralrequirements in human nutrition. 2nded., Geneva: World HealthOrganization. 2005:341.
62. Shenkin A. The key role of micronutrients. Clinical nutrition. 2006 Feb 1;25(1):1-3.
63. Choi Y, Lee JE, Chang Y, Kim MK, Sung E, Shin H, Ryu S. Dietary sodium and potassium intake in relation to non-alcoholicfattyliverdisease. British Journal of Nutrition. 2016 Oct;116(8):1447-56.
64. Miranda-Vilela AL, Akimoto AK, Alves PC, Pereira LC, Gonçalves CA, Klautau-Guimarães MN, GrisoliaCK. Dietarycarotenoid-richpequioilreduces plasma lipidperoxidation and DNA damage in runnersand evidence for an association withMnSODgenetic variant-Val9Ala. Genet Mol Res. 2009 Dec15;8(4):1481-95.
65. Dasarathy J, Varghese R, Feldman A, Khiyami A, McCullough AJ, Dasarathy S. Patients withnonalcoholicfattyliverdisease have a lowresponse rate to vitamin D supplementation. The Journal of nutrition. 2017 Aug 16;147(10):1938-46.
66. Harrison SA, Torgerson S, Hayashi P, Ward J, Schenker S. Vitamin E and vitamin C treatmentimprovesfibrosis in patients withnonalcoholic steatohepatitis. The American journal of gastroenterology. 2003 Nov 1;98(11):2485-90.
67. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M. Pioglitazone, vitamin E, or placebo for nonalcoholicsteatohepatitis. New England Journal of Medicine. 2010 May 6;362(18):1675-85.
68. Lavine JE, Schwimmer JB, Van Natta ML, Molleston JP, Murray KF, Rosenthal P, Abrams SH, ScheimannAO, Sanyal AJ, Chalasani N, Tonascia J. Effect of vitamin E or metformin for treatment of nonalcoholicfattyliverdisease in children and adolescents: the TONIC randomizedcontrolled trial. Jama. 2011 Apr 27;305(16):1659-68.
69. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholicfattyliverdisease: practice guidance from the American Association for the Study of LiverDiseases. Hepatology. 2018 Jan 1;67(1):328-57.