The Benefits and Risks of Antioxidant Treatment in Liver Diseases

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Chronic liver diseases are a public health issue, because of their high incidence and prevalence, the important impact on the quality of life and high mortality rates. From a pathogenic point of view, in almost all liver diseases there is an increase in oxidative stress. Oxidative stress represents as an imbalance between the production of oxidizing agents and antioxidants. This imbalance contributes to the initiation and progression of hepatic injury. Among the most important risk factors for increased oxidative stress in chronic liver diseases are alcohol, drugs, environmental pollutants and irradiation. For the restoration of the oxidant-antioxidant balance and reduction of the oxidative stress in chronic liver diseases, a promising role may have the antioxidants. This hypothesis is now based on experimental evidence of their efficacy in animal models. In low concentrations, antioxidants improve liver function by preventing the oxidation of an oxidizable substrate, but in high doses, they may cause adverse reactions, such as the pro-oxidant effect, glutathione S transferase inhibition and thus the inhibition of detoxification and interference with coagulation. The objective of the article is to review the benefits of antioxidant treatment in chronic diseases of the liver, in order to put them in balance with their adverse reactions.

Keywords: chronic liver diseases, antioxidant treatment, silymarin, resveratrol, vitamin A.

The liver is the main organ involved in the intermediate metabolism and detoxification of the body. Liver diseases are very common all throughout the world. Cirrhosis represents the final stage of evolution of chronic liver diseases, being pathologically defined by the association of fibrosis with a nodular transformation of the liver parenchyma. Fibrosis is a constant component of cirrhosis, but it is not synonymous with it. It could be present, even in the absence of nodular aspects, in conditions such as right heart failure, biliary tract obstruction, and congenital fibrosis. Simultaneously, the presence of nodules without the presence of fibrosis characterizes nodular regenerative hyperplasia, which it not a form of cirrhosis [1]. Liver cirrhosis may appear in all age groups, races, and both sexes. In the last few decades, it has been established as the ninth cause of death worldwide and the fifth cause in the 45-65 y age group [2].

The etiology of cirrhosis varies with geographical region. Thus, the most common cause in Asia and Africa is viral hepatitis, while in Europe and America it is alcohol abuse [3]. Among other etiological causes, there are hereditary and metabolic diseases, such as nonalcoholic steatohepatitis, Wilson's disease, hemochromatosis, Gaucher disease, porphyria, alpha-1 antitrypsin deficiency; autoimmune diseases, like chronic autoimmune hepatitis and primary biliary cirrhosis; drug-induced toxicity, such as methotrexate, isoniazide, amiodarone and alpha methyldopa; venous congestion because of right heart failure, Budd-Chiari syndrome, constrictive pericarditis, veno-occlusive disease and others [3-6].

An essential element in the pathophysiology of the chronic liver disease is the increase in oxidative stress, which activates stellate cells and stimulates the production of extracellular matrix, resulting in the appearance and progression of fibrosis [7-9].

New treatments that may slow or stop the progression of fibrosis are continuously searched. A promising role is played by antioxidant agents.

Oxidative stress

Oxidative stress is defined as the accumulation of oxidative damage of human cells, as a result of the imbalance between the oxidizing systems (with an overproduction of ROS) and the anti-oxidizing systems (through their capacity to decrease the first ones' function) [10] (table 1).

The factors that are involved in the development of oxidative stress are grouped into two categories: endogenous and exogenous. They are briefly explained in table 2.

Free radicals have unpaired electrons in their atoms, thus presenting a high grade of reactivity with molecules such as proteins, lipids or DNA. Oxygen radicals and nitrogen radicals are among the free radicals that are responsible for oxidative stress. They mainly come from cellular metabolism. This, however, is not the only mechanism. The environment plays a critical role in the production of free radicals such as ROS (reactive oxygen species) and RNS (reactive nitrogen species), by air pollution, UV, X and gamma rays [12].

Free oxygen radicals, known as reactive oxygen species, are produced during physiological metabolic reactions, in intact cells. The most important ROS are superoxide, hydroxyl radicals and peroxide radicals. Besides these, there are also non-radical species like ozone, hydrogen peroxide and acid hypochlorous acid. Reactive nitrogen species

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Table 1

THE OXIDIZING AND ANTI-OXIDIZING SYSTEMS [MODIFIED AFTER 11]

Oxidizing systems	Anti-oxidizing systems	
1. Physiological • physical effort • pregnancy • senescence • modulation of gene expression • cell growth and differentiation • mitochondrial function • structure and membrane function **2. Pathological** • inflammation • apoptosis • producing the immune response • carcinogenesis • atherosclerosis • diabetes mellitus, etc.	**1. Enzymatic** • superoxide dismutase (SOD) • catalase (CAT) • glutathione peroxidase (GSH-Px) • glutathione transferase (GSH-ST)	**Nonenzymatic** • glutathione • vitaminaC • vitamin E • vitamin A • carotenes • selenium • uric acid • bilirubin • albumin • porphyrin • polyamine • estrogens • saturated or unsaturated fatty acids • chelating agents
	2. Liposolubles • Tocopherols • Carotenoids • Quinones • Bilirubin • 2-Hydroxyestrone • 2-Hydroxyestradiol	**Hidrosolubles** • glutathione •vitamin C • uric acid • cysteine • creatinine • chelators of metals • Hem fixative proteins
	3. Naturals •superoxide dismutase (SOD) • glutathione (GSH) • tocopherol •vitamin C • adenosine • lactoferrin • nicotinamide • carotenoids	**Synthetics** • thiols • xanthine oxidase inhibitors • iron chelators

Table 2

THE ENDOGENOUS AND EXOGENOUS FACTORS INVOLVED IN THE DEVELOPMENT OF OXIDATIVE STRESS

Endogenous factors	Exogenous factors
• nutritional deficiency • stress (physical and mental) • surgery • diabetes mellitus • dysfunction of lipid metabolism • high level of homocysteine • chronic kidney dysfunction • diseases associated with inflammatory reactions	• ionizing and UV radiation • pollution of the ozone layer, nitrogen oxides • consumption of alcohol and nicotine • certain drugs (cytostatic drugs)

include both radical and non-radical species, like peroxynitrite, nitric oxide radicals or nitrogen dioxide. They are derived from nitric oxide and superoxide under the influence of NOS (nitric oxide synthase) and nicotinamide adenine dinucleotide phosphate (NADPH), respectively. Under the influence of NOS, L-arginine is transformed in L-citrulline and NO (nitric oxide) [13,14].

Both ROS and NOS can initiate lipid peroxidation, alter DNA (deoxyribonucleic acid) and oxidize every cell of the biological tissues. Though, the human organism is capable of disposing of ROS and RNS, effectively preventing the negative effects of these radicals. The cellular injury appears when there is an imbalance between the forming of these reactive species and their disposing of. This phenomenon can also happen when the anti-oxidant production is low [1].

The forming of ROS is a physiological process during aerobic respiration, influencing the cellular functions such as signal transduction, immunity and the expression of the genes that promote proliferation and apoptosis [2]. ROS have other beneficial effects: they contribute to the defense mechanisms against pathogenic microorganisms and they consolidate biological defense mechanisms that protect the body during physical activities. ROS excess is, however,

toxic for the cells, as these radicals could potentially react with lipids, proteins or DNA, altering their functions. ROS destroy cellular membranes, resulting in necrosis, by oxidizing the unsaturated fatty acids from the cell membrane, a process which is known as lipid peroxidation. They also react with proteins, through the oxidizing of the –SH group of the cysteine residues, leading to the appearance of disulfides, sulfonic acid and sulfoxide. DNA and RNA (*ribonucleic acid)* reactions cause structural changes, with the development of mutations. Oxidative stress determines upregulation of the cytokines, such as transforming growth factor-β (TGF-β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), contributing to fibrogenesis. TGF-β increases the production of ROS in fibroblasts, endothelial, epithelial and smooth muscle cells [3,15].

The major cell antioxidants include glutathione (GSH), tocopherol (vitamin E) and vitamin C. The key participating enzymes that remove ROS from the system are superoxide dismutase, glutathione peroxidase and catalase [2].

The liver is among the main affected organs by the increased oxidative stress. In hepatocytes, mitochondria and cytochrome P450 enzymes are major sources of free oxygen radicals. Additionally, the activation of Kupffer cells and the inflammatory infiltrate, especially neutrophils, are ROS sources for hepatic injury, as well. Nitric oxide has also a role in inflammation. ROS might react with nitric oxide, generating peroxynitrite, a very potent oxidizing agent that contributes to lipid peroxidation. Numerous other nitrogen species can also promote nitration, altering protein structure and function, beyond the standard ROS effects [2].

Regarding the immune cells, components of the body's defense mechanisms, the phagocytes act through the cytotoxic effects generated by some of the oxidants. Thus, when the phagocytes encounter a microorganism, the latter is surrounded by a fraction of the phagocyte's membrane, which folds, forming a phagosome. This process leads to the increased oxygen consumption by the phagocytes and initiates a complex biochemical signaling system, which in turn activates a unique oxidizing membrane complex, that is dependent on NADPH. In the phagosome, under the influence of NADPH, O_2 is reduced to O_2-, causing the formation of H_2O_2, and ROS [10].

There are some well-documented pieces of evidence, according to which ROS are major pathogenic factors in acute or chronic hepatic diseases. Thus, both an increase in the production of ROS, as well as a decrease in their specific elimination may be involved. High levels of ROS can cause cellular death through apoptosis and necrosis, promote fibrogenesis and carcinogenesis and modify certain biomolecules that, by their antigenic similarity, generate an autoimmune disease [16,17].

Antioxidant treatment in chronic liver disease

Substances that have anti-oxidizing properties can impede on or even prevent oxidizing. By donating a few electrons to the free radicals, they reduce the latter's reactivity, being able to maintain the balance between oxidants and anti-oxidants. Such compounds are either an intrinsic part of the body or can be obtained through certain foods. Their efficacy was proven in a multitude of conditions, including chronic liver diseases, such as alcoholic liver disease, non-alcoholic steatohepatitis, chronic viral hepatitis, hemochromatosis and Wilson's disease [18,19].

Given the absence of curative therapies for these diseases, it is necessary to examine the therapeutical benefits of anti-oxidizing agents that might slow down the evolution of such diseases through their multiple effects, such as anti-apoptotic, anti-inflammatory and membrane stabilization properties.

Even though animal studies have certainly proven the antioxidants' efficacy, this could not be recreated in humans. For example, a clinical prospective randomized trial, with 789 patients who suffered from alcoholic hepatic disease, failed to show any benefits on the progression of fibrosis, neither from a biochemical standpoint nor from a histological one. In conclusion, there is a need for well-designed studies to investigate the benefits of such compounds and simultaneously clarify their mechanism in relation to the disease's physiopathology [20].

Another problem insufficiently studied is related to the adverse reactions of anti-oxidizing agents.

Patients with chronic hepatic diseases frequently self-administer anti-oxidants. United States of America study reports that 39% of these patients use such agents, while a Germany study shows that 65% fall in this category [21]. This number can be underestimated because other studies proved that between 31-40% of patients do not report these substances to the doctor. Beyond the cellular effects, anti-oxidants have an effect on the patient's psyche. Generally, the users believe in the compounds' efficacy, which in turn helps to improve their image of the disease [22].

Silimarin

One of the antioxidant agents that has been used on a large scale in chronic hepatic illnesses is Silymarin, an active compound extracted from Silbyummarianum. Experimental studies showed that Silymarin has anti-oxidizing properties by neutralizing ROS and by intensifying antioxidant defense mechanisms. Moreover, it intervenes in modulating the cytokine balance, suppressing pro-inflammatory cytokines production, while increasing anti-inflammatory cytokine synthesis (Fig. 1). It has also a role in delaying the evolution of hepatic fibrosis. It was used in acute and chronic viral hepatitis, alcoholic liver disease, drug-induced hepatic injury and primary biliary cirrhosis, although the benefits were inconclusive. A possible explanation can be the heterogeneous patient cohorts included in these trials, with high abandonment rates and short-term follow-ups. Secondary effects are insignificant and, despite the absence of any relevant benefits in humans, it is continued to be used on a large scale [2, 22, 23].

Resveratrol

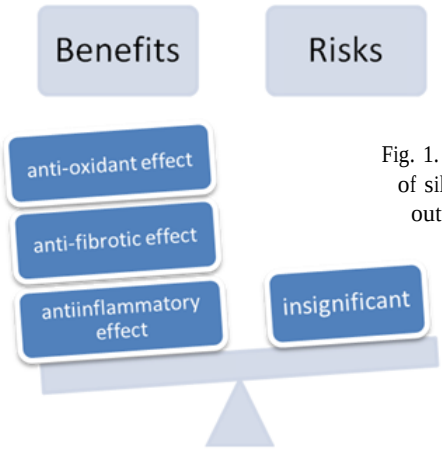

Fig. 1. Benefits and risks of silymarin. Benefits outweigh the risks

Resveratrol or 3,5,4' trans-trihydroxystilbene can be found in peanuts, berries, red grapes and wine, as a polyphenol. [9,24,25]. This compound is metabolized under the influence of sulfotransferase to resveratrol sulfate, and only in small quantities, under the influence of UDP-

glucuronosyltransferase, to resveratol glucuronide [9]. It has a 75% absorption rate when orally administered. The evidence regarding the benefits of this compound comes from hepatocyte cultures experiments and from animal trials. Among the beneficial effects, there are: anti-oxidizing, anti-inflammatory, anti-carcinogenic effects, anti-fibrogenic properties, insulin levels regulation and obesity prevention [26].

In high doses (3000 mg/kg body weight/day for 4 weeks), an animal study reported renal toxicity, a decrease in body weight and in the consumption of food, among other tissue lesions evidence. These effects are, however, dose-dependent, and as such, non-existent at a dose of 700 mg/kg body weight. Another risk is represented by their interaction with anti-coagulant and nonsteroidal anti-inflammatory medication, which leads to a higher risk of hemorrhage. Starting from these data, further clinical studies are necessary to certify resveratrol's liver protecting effect [3].

Coffee

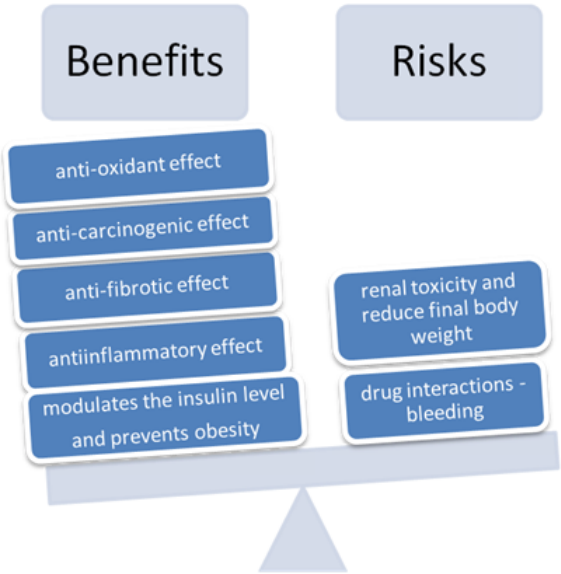

Fig. 2. Benefits and risks of resveratrol. Benefits outweigh the risks

Coffee represents a combination of molecules, such as carbohydrates, alkaloids, vitamins, lipids, nitrogenous molecules and phenolic compounds [26]. Coffee consumption was associated with a decrease in the frequency of certain chronic diseases, due to its beneficial effects such as: antioxidant, anti-fibrotic, anti-necrotic, anti-cholestatic and chemoprotective effect [27]. The most active compound of the coffee is caffeine, which is rapidly absorbed in 5 minutes after oral ingestion, reaching its peak concentration in 30 minutes. High quantities can result in adverse reactions. Thus, a quantity of over 200 mg can lead to tachycardia and different arrhythmias [28]. Furthermore, various studies showed a rise in blood pressure and a negative impact on cognitive function and memory [29]. A dose of 300 mg of caffeine per day can lead to hallucinations [30].

Most of the caffeine is metabolized in the liver. Over time, researchers formulated multiple hypotheses that discuss the existence of an inversely proportional relationship between coffee intake and liver cirrhosis.

Vitamins E, A, C

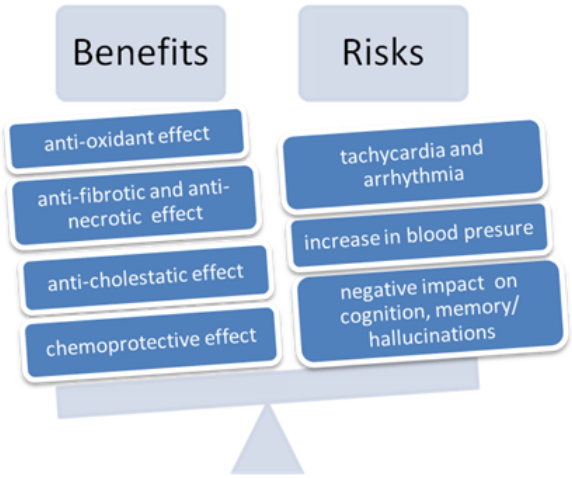

Fig. 3. Benefits and risks of coffee. Benefits outweigh the risks

Vitamin E was tested in NASH (nonalcoholic steatohepatitis), alcoholic liver disease and viral hepatitis. The results of the use of Vitamin E were mixed. Many of the clinical trials that used Vitamin E included a small number of patients, used different doses and the patients were followed for different periods of time. Despite the anti-oxidizing and anti-fibrotic effects, there is no evidence that vitamin E contributes to slowing the evolution of liver diseases. Moreover, it has been proved that in high doses (higher than 400 IU/day), vitamin E acts as an oxidizing agent, it inhibits the GSH S-transferase (glutathione S-transferase), thus inhibiting detoxification and interfering with coagulation (it raises the risk of a hemorrhagic stroke) [30]. Similarly to vitamin E, high doses of vitamin A have also oxidizing effect, while chronic usage of both vitamins E and A is associated with an increase in the risk for developing pulmonary cancer [30, 31].

Vitamin C could potentially enhance iron-mediated toxicity and should be avoided in patients with an iron excess in the liver [2].

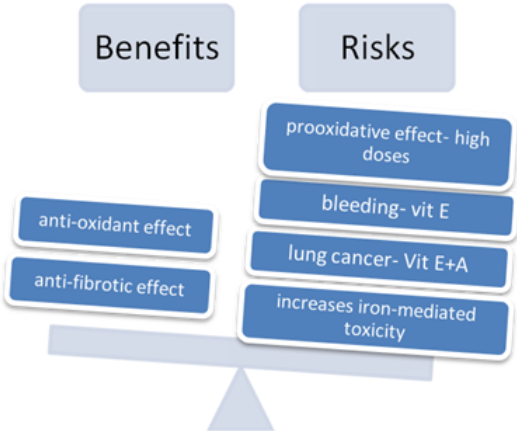

Fig. 4. Benefits and risks of Vitamin E and Vitamin A.The risks outweigh the benefits in chronic hepatic disease

Green tea

Green tea or Camellia sinensis is a world-wide known beverage. Among the beneficial effects of tea consumption, there are anti-oxidizing, anti-inflammatory, anti-arthritic and anti-angiogenic effects. In contrast, among green tea components, there is also a primary amino acid, theanine. It has a chemical structure similar

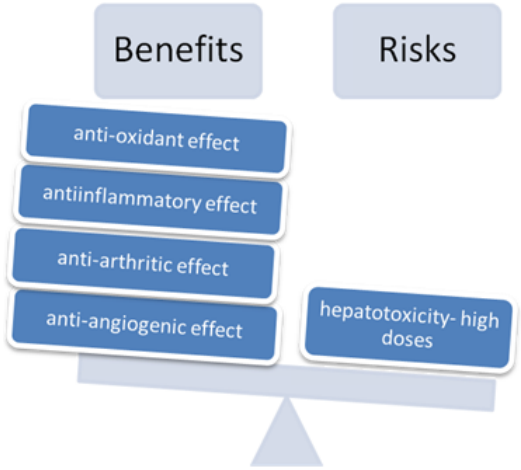

Fig 5. Benefits and risks of green tea. The benefits surpass the risks when taken in correct dosages

to that of glutamic acid, a glutathione precursor. Studies have shown that this amino acid contributes to maintaining glutathione levels, hence preventing the development of cancer and neurotoxicity. In different clinical studies, green tea proved to be protective against various cancers including prostate, esophageal, colonic, rectal and pancreatic cancer [32]. However, in hepatic cancer, it was proven ineffective [33]. A study conducted by de Halegoua-De Marzio et al [34] showed that green tea, taken by cirrhotic patients infected with hepatitis C virus, in a single 400 mg dose, is safe and well-tolerated. However, newer clinical studies regarding the positive effects on liver pathologies are necessary. Green tea intake should be consumed in a dose of 1-2 cups per day [35]. When consumed in high quantity, for body weight control, it can have a hepatotoxic effect [36, 37].

Conclusions

The available data regarding the benefits of antioxidant treatment in patients with chronic liver diseases is insufficient. A differentiation between antioxidants regarding their liver-protective effects is not possible until now, as they have both different chemical structures and different anti-oxidizing potency. The results of many studies have suggested that antioxidants may be used like as adjuvants in numerous diseases, especially in those where an increase of oxidative stress is involved, including chronic liver diseases. In this review, we tried to weigh the risks and benefits of antioxidant use in hepatic diseases. After a careful analysis, we can conclude that in most cases, the benefits outweigh the risks. Even so, the adverse reactions should not be ignored and patients need to be informed about such effects. The evidence is, however, weakly supported by the studies conducted on humans, if not contradictory. Most positive outcomes come from animal experiments. In conclusion, it is necessary that, in the future, more well-designed studies are conducted, to prove the effects of antioxidants in chronic hepatic diseases, to identify the compounds with the highest protective effects and the optimal doses, at which the benefits outweigh the risks.

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