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Case report

FREE RADICALS, ANTIOXIDANTS, AND LIVER DISORDERS: SPECIAL EMPHASIS ON SILYBIN

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ABSTRACT

Free radicals, or reactive oxygen species (ROS), are unstable molecules that contain at least one unpaired electron in their outer orbit. They can cause chain reactions that damage cells and organs. In the presence of metals, the free radical superoxide anion O2 and hydrogen peroxide (H2O2) form the hydroxyl radical OH- that is responsible for lipid peroxidation, which is very toxic to the body. Free radicals cause oxidative stress and are generated in various conditions including chemical-physical environmental pollution, the intake of drugs, or a dysregulated metabolism. ROS transform DNA, resulting in ageing of the body, activation of carcinogenesis, neurodegeneration, and autoimmune, cardiovascular, and muscular diseases. Oxidative stress can be linked to various liver disorders as well, such as alcoholic and non-alcoholic fatty liver disease, liver intoxication due to free radicals, and hepatitis, including hepatitis C, which is very tedious and difficult to treat. Dysregulation of the balance between antioxidants and oxidants in the liver causes oxidative stress; antioxidants oppose the oxidation process and contrast the effects of free radicals. They are beneficial to human health, slowing down the ageing process and reducing the incidence of cancer, inflammation, neurological disorders, diabetes, and autoimmune, cardiovascular, and liver diseases. Pollutants can lead to liver disorders, while antioxidants have shown preventative and therapeutic effects in these diseases. In *in vivo* and *in vitro* studies, some drugs have been shown to have a protective effect on the liver by limiting chemical damage and fibrosis in the organ, as well as improving the lipid status. Many antioxidant substances such as silybin, resveratrol, curcumin, naringenin green tea, quercetin, and flavonoids in general, are taken with the diet by virtue of their benefits for the body, including the liver. Silybin is an active

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compound of Silybum marianum and has several interesting biological effects, including ones against cancer and liver diseases. When administered to mice, silybin is rapidly absorbed in both plasma and tissue and turns has an antioxidant and anti-inflammatory effect. It has been reported that this antioxidant has been used to prevent and combat liver damage. In light of this scientific evidence, it can be deduced that free radicals can be reduced with antioxidants, including silybin, which have a therapeutic effect on the whole organism and protect the liver.

KEYWORDS: *antioxidant, free radical, liver, flavonoids, therapy, natural compounds*

INTRODUCTION

Oxygen is important for life, but when it turns into free radicals it becomes toxic. Free radicals, or reactive oxygen species (ROS), are very unstable molecules because they have an unpaired (lost) electron. The missing electron is taken up into other molecules in the body, causing damage to the cells (Fig. 1). The substances that neutralize the free radicals and oppose these oxidation reactions are called antioxidants. Many antioxidants can be endogenous in nature and produced by the body, such as vitamins, but these and other compounds can be introduced with the diet as well (1). ROS are associated with liver diseases such as inflammation, steatosis, viral hepatitis, cirrhosis, jaundice, and hepatocellular carcinoma (2-5). Additionally, many chemical compounds such as alcohol and tobacco smoke can cause liver disease (6, 7).

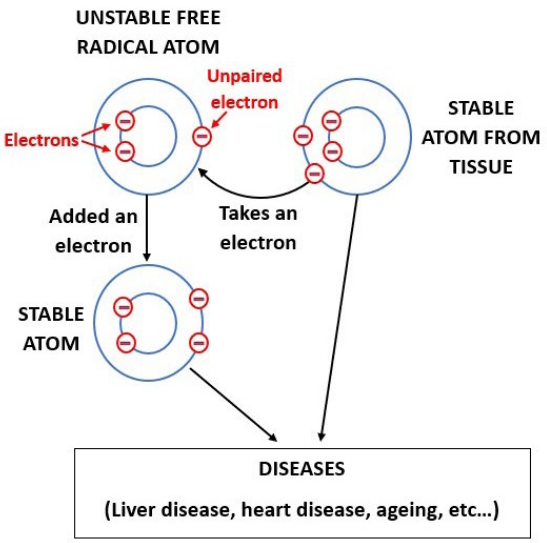

Fig. 1. *Free radicals increase in ageing and in various diseases. This figure shows a free radical with an unpaired electron which takes an electron from a stable atom in the tissue. This reaction can lead to cell death and cause various diseases.*

The correct physiological functioning of the body is regulated by the balance between ROS and antioxidants (8). When the levels of these free radicals increase, they lead to oxidative stress and inflammation, which are present in many human disorders (9, 10) (Table I). Liver inflammation can result in activation of vascular endothelial growth factor (VEGF) and cytokines such as IL-1, IL-6, and tumor necrosis factor (TNF), with the priming of nuclear factor kappa B (NFkB) and nucleotide-binding leucine-rich repeat-containing protein (NLRP12) inflammasome (11, 12).

Many natural products, such as flavonoids, polyphenols, and resveratrol, have been proposed to alleviate the effects of ROS, mediating oxidative stress, metabolic disorders, and inflammation (12-14).

Flavonoids and their derivatives are antioxidant enzymes which modulate the immune response and have anti-inflammatory, anti-apoptotic, and anti-tumor activity (15). They inhibit lipid peroxidation and the oxidation of DNA and proteins, protecting many organs, including the brain and liver (16). Toxic insults to the liver can result in oxidative stress and the synthesis of high levels of free radicals which can lead to apoptosis and hepatocyte damage. Flavonoids exert antioxidant effects on cells by counteracting ROS after organ damage, including that of the liver.

Table I. *Some diseases induced by oxidative stress in humans.*

In humans, oxidative stress induces:		
Ageing	Depression	Liver damage
Alzheimer's disease	Diabetes	Memory loss
Arthritis	Heart failure	Parkinson's disease
Cancer	Infection	Retinal disorders
Cardiomyopathy	Inflammation	Rheumatism
Cataract	Ischemia	Stroke

The use of flavonoids such as silybin can help prevent damage caused by free radicals, such as peroxidation and inflammation (17). In addition, in cancer patients, silybin has a detoxifying power after chemotherapy and is recommended in anti-tumor treatments (18-19). In cases of poisoning, where the liver plays an important role, silybin appears to alleviate liver damage and increases the survival rate (20).

Silybin

Silybin represents about 50% to 70% of the silymarin extract, and it is the active compound of Silybum marianum, which possesses several interesting biological effects (20). Silybin can be distinguished in two forms, A and B, but it can also be found in combination of these two forms as isosilybin. Silybin is an extract of milk thistle, and it has been reported in diverse published articles that it is used against cancer and liver disease. It is poorly soluble in water; however, this drawback can be counteracted when it is used as a prodrug. Silybin is rapidly absorbed into both plasma and tissues when it is administered to mice in free and conjugated forms (20). It is used in acute and chronic liver injury as an antioxidant and anti-inflammatory compound due to its antifibrotic properties (21).

In experiments on rodents *in vivo*, silybin has shown effects on the liver, where there is an increase in glutathione-S-transferase with negligible side effects, such as a mild hyperbilirubinemia, which can regress after discontinuation of the administration (17). Silybin has a strong antioxidant effect, and acts as a scavenger by inhibiting the formation of free radicals and by modulating the permeability of cell membranes. In oxidative stress in the liver of mice, silybin inhibits nitric oxide (NO), glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, and contrarily, it increases ATP levels through the phosphorylation of ADP (22) (Fig. 2). Therefore, in damaged liver tissue, this antioxidant can improve lipid peroxidation, mitochondrial respiration, and cell death. Silybin shows a strong link with some inflammatory target proteins and is used in the prevention of ischemic damage in rats, where it increases the levels of superoxide dismutase and glutathione, while decreasing the levels of TNF and IL-6 both in the hippocampus and in the cortex cerebral (23).

Pro-inflammatory cytokines are produced by hepatocytes via NF-kB after cellular damage which may be physical, chemical, or biological. Silybin interferes with the transduction process controlled by the transcription factor NF-kB that is induced in inflammation (24). Silybin exerts its anti-inflammatory power by inhibiting the activation and translocation of NF-κB into the nucleus, suppressing phosphorylation and increasing IL-10 levels (17). Moreover, it inhibits cyclooxygenase (COX-2) expression, leukotriene formation, and protein kinase activation (20). In the liver, silybin also has antiviral activity, acting on the replication of the HCV virus in infected patients (25).

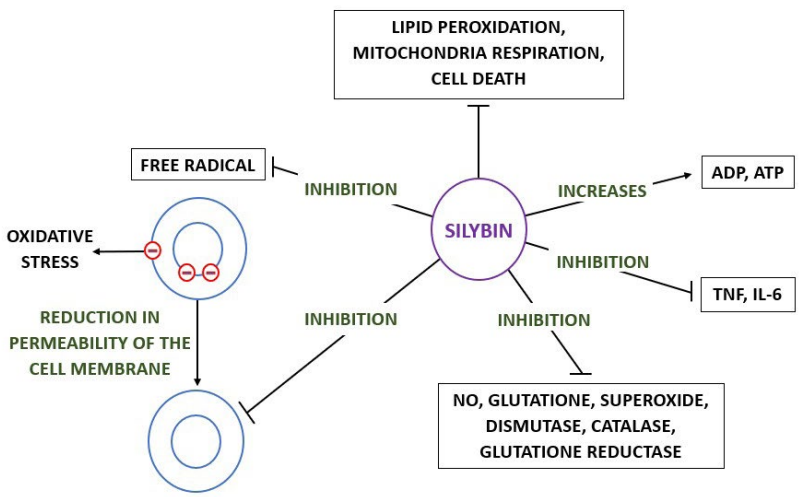

Fig. 2. *Silybin blocks free radicals, which leads to reduced permeability of the cell membrane. Silybin also inhibits lipid peroxidation, mitochondria respiration, cell death, nitric oxide (NO), glutatione, superoxide, dismutase, catalase, glutatione reductase, and tumor necrosis factor (TNF) and IL-6 cytokines. In addition, silybin increases levels of ADP and ATP.*

Silybin has been found to be beneficial in viral diseases such as COVID-19, where oxidative stress, immune system dysfunction, and inflammation are implicated (26). It has been shown to have an anti-inflammatory effect, probably by reducing cytokines such as IL-1, IL-6, and TNF, as well as to have an antiviral action by binding to SARS-COV-2 proteins, including the spike protein, and promoting the elimination of the virus (27) (Fig. 3).

Therefore, silybin proves to be an anti-inflammatory agent by acting as a protector against certain inflammatory liver disorders and diseases of the other organs (23).

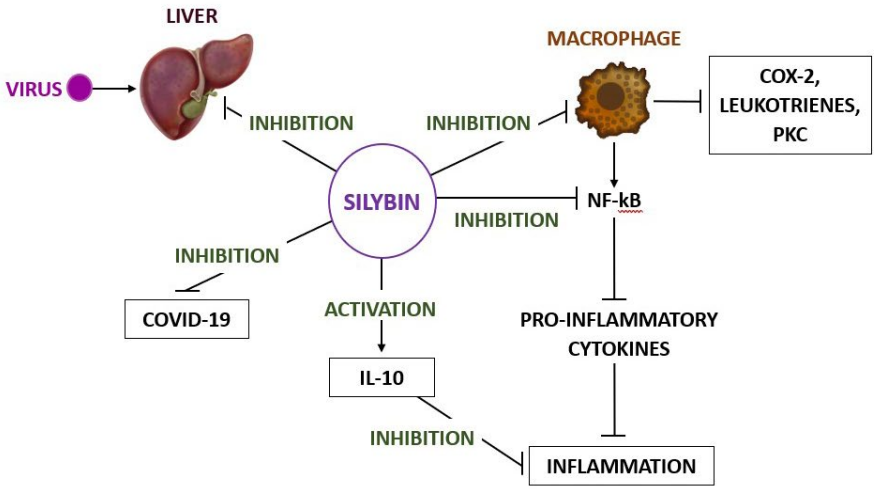

Fig. 3. *Figure showing the antiviral activity of silybin in the liver which inhibits COVID-19. Silybin also inhibits macrophages that produce COX-2, leukotrienes, and PKC. Moreover, silybin inhibits NF-kB, resulting in the decreased release of pro-inflammatory cytokines and reduced inflammation. There is also activation of IL-10, which is an inhibitor of the inflammatory response*.

CONCLUSIONS

In this paper, we report that free radicals are responsible for lipid peroxidation, which is toxic to the body. Oxidative stress can transform DNA, accelerate ageing of the body, and can activate carcinogenesis, neurodegeneration, and autoimmune, cardiovascular, muscular, and liver diseases. On the contrary, antioxidants such as silybin oppose the oxidation process and contrast the effects of free radicals, helping to prevent these disorders, particularly liver damage. The reduction of ROS by silybin and other antioxidants has a therapeutic effect for the entire body and has been shown to prevent and cure liver disease.

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