Chapter 19

Protective Effect of Silymarin on Liver Damage by Xenobiotics

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http://dx.doi.org/10.5772/51502

1. Introduction

The liver is the vertebrates' largest internal organ. It weighs nearly 1.5 kg, is dark red in color, and is situated in the upper right quadrant of the abdominal cavity. Among the functions that it performs are the following: the metabolism of lipids and carbohydrates, and the synthesis of proteins, coagulation factors, and biliary salts. Eighty percent of the hepatic parenchyma is made up of hepatocytes, which are the cells mainly responsible for maintaining every function that the liver in its entirety requires to sustain the body's normal physiological functions in general. In addition to hepatocytes, the liver possesses other cells, such as the so-called Kupffer cells (hepatic macrophages), Ito cells, endothelial cells. The hepatocytes are disposed in the liver in groups denominated lobules, which have a central orifice comprised of the bile duct and by means of which the biliary salts are excreted. The anatomical loss of the structure of the hepatic lobule is considered a symptom of severe damage to the liver; it can be accompanied by partial or total loss of some physiological function, as in the case of alcohol-related hepatic cirrhosis. [23].

2. Hepatic regeneration

Liver regeneration is a fundamental response of the liver on encountering tissue damage. The complex interaction of factors that determine this response involves a stimulus (experi-

mentally, a hepatectomy), gene expression, and the interaction of other factors that modulate the response. This proliferation depends on the hepatocytes, epithelial bile cells, Kupffer cells, and Ito cells. [24].

The mechanisms of hepatic growth have been studied in detail in experimental models. In the latter, regeneration is induced whether by tissue resection (partial hepatectomy) or by death of the hepatocytes (toxic damage). The principles that govern the growth of this organ in these systems also apply to clinical situations, such as, for example, fulminating liver failure, acute and chronic hepatitis, partial hepatectomy for treating liver cancer, or even in liver transplant donors. Evidence that there is a humoral growth factor of the hepatocyte has been observed in animal models and in patients with liver disease from the 1980s. [1, 13, 34, 10].

3. Ethanol

On being ingested, alcohol (also called ethanol) produces a series of biochemical reactions that lead to the affectation of numerous organs involving economy, having as the endpoint the development of hepatic diseases such as alcoholic hepatitis and cirrhosis. Despite that much is known about the physiopathological mechanisms that trigger ethanol within the organism, it has been observed that a sole mechanism of damage cannot fully explain all of the adverse effects that ethanol produces in the organism or in one organ in particular. [37, 30].

A factor that is referred as playing a central role in the many adverse effects that ethanol exerts on the organism and that has been the focus of attention of many researchers is the excessive generation of molecules called free radicals, which can produce a condition known as oxidative stress, which triggers diverse alterations in the cell's biochemical processes that can finally activate the mechanism of programmed cell death, also known as apoptosis. [28, 26, 17, 19, 25].

Of particular importance for the objective of this chapter is the focus on a particular class of free radicals that are oxygen derivatives, because these are the main chemical entities that are produced within the organism and that affect it in general.

4. Ethanol metabolism

Ethanol is absorbed rapidly in the gastrointestinal tract; the surface of greatest adsorption is the first portion of the small intestine with 70%; 20% is absorbed in the stomach, and the remainder, in the colon. Diverse factors can cause the increase in absorption speed, such as gastric emptying, ingestion without food, ethanol dilution (maximum absorption occurs at a 20% concentration), and carbonation. Under optimal conditions, 80-90% of the ingested dose is completely absorbed within 60 minutes. Similarly, there are factors that can delay ethanol absorption (from 2-6 hours), including high concentrations of the latter, the presence of food, the co-existence of gastrointestinal diseases, the administration of drugs, and individual variations [14, 37].

Once ethanol is absorbed, it is distributed to all of the tissues, being concentrated in greatest proportion in brain, blood, eye, and cerebrospinal fluid, crossing the feto-placentary and hematocephalic barrier [44]. Gender difference is a factor that modifies the distributed ethanol volume; this is due to its hydrosolubility and to that it is not distributed in body fats, which explains why in females this parameter is found diminished compared with males.

Ethanol is eliminated mainly (> 90%) by the liver through the enzymatic oxidation pathway; 5-10% is excreted without changes by the kidneys, lungs, and in sweat [14, 30]. The liver is the primary site of ethanol metabolism through the following three different enzymatic systems: Alcohol dehydrogenases (ADH); Microsomal ethanol oxidation system (MEOS); Catalase system.

5. Liver regeneration and ethanol

Ethanol is a well known hepatotoxic xenobiotic because hepatotoxicity has been well documented in humans as well as in animals. Although aspects concerning the pathogenesis of liver damage have been widely studied, it is known that liver regeneration restores the functional hepatic mass after hepatic damage caused by toxins. Suppression of the regenerating capacity of the liver by ethanol is the major factor of liver damage. [45]. Although the effects of acute or chronic administration of ethanol on the proliferative capacity of the liver to regenerate itself has been studied, the precise mechanism by which ethanol affects hepatocellular function and the regenerative process are poorly explained. [31, 29, 38].

Liver regeneration induced by partial hepatectomy in rats represents an ideal model of controlled hepatocellular growth. This surgical procedure has been sufficiently employed to study the factors than can be implicated in the growth of the liver. Endogenous signals have been described to control hepatic regeneration. The first marker of DNA synthesis in partially hepatectomized rats (70%) occurring normally 24-28 hours postsurgery comprises an enormous action of growth factors and cytokines affecting expression of the gene of the hepatocytes, associated with initiation of the cell cycle. [2]. It has indicated that the hepatocytes enter into a state denominated “priming” to thus begin replication and response to growth factors, that is, which range from the quiescent to the G 1 phase of the cell cycle. The progression of hepatic cells requires the activation of cyclin-dependent kinases that are regulated by cyclins and cyclin-dependent kinase inhibitors. [39]. It has also been demonstrated that a dose of ethanol importantly diminishes the specific activity of two enzymes related with the metabolism of DNA synthesis, which are thymidine kinase and thymidylate synthetase. [47, 11, 32].

6. Free radicals

Free radicals are the result of the organism’s own physiological processes, such as the metabolism of food, respiration, exercise, or even those generated by environmental factors,

such as contamination, tobacco, or by drugs, chemical additives, etc. Free radicals (FR) are atoms or groups of atoms that in their atomic structure present one or more unpaired electrons (odd in number) in the outer orbit. This spatial configuration generates in the molecule distinct physical and chemical properties such as heightened reactivity and diminished lifetime, respectively. [5, 27].

This instability confers on these physical avidity for the uptake of an electron of any other molecule in its ambit (stable molecules), causing the affected structure to remain unstable with the purpose of reaching its electrochemical stability. Once the free radical has achieved trapping the electron that it requires for pairing with its free electron, the stable molecule that cedes the latter to it in turn becomes a free radical, due to its remaining with an unpaired electron, this initiating a true chain reaction that destroys our cells. [4, 7].

In aerobic cells, there are diverse pathways that lead to the production of Oxygen-derived free radicals (OFR). The main sources are enzymes associated with the metabolism of arachidonic acid, such as cycloxygenase, lipoxygenase, and cytochrome P-450. The presence and ubiquity of enzymes (superoxide dismutase, catalase, and peroxidase) that eliminate secondary products in a univalent pathway in aerobic cells suggest that the superoxide anions and hydrogen peroxide are important secondary products of oxidative metabolism. [40, 7]. Reactive oxygen species (ROS) can damage macromolecules such as DNA, carbohydrates, and proteins. These cytotoxic oxygen species can be classified as two types:

1. the free radicals, such as the superoxide radical (O_2) and the hydroxyl radical ($^{\cdot}OH$), and

2. non-radical oxygen species, such as hydrogen peroxide (H_2O_2), the oxygen singlette (O_1), which is a very toxic species, peroxynitrite (ONOO), and Hypochlorous acid (HOCL).

The instable radicals attack cell components, causing damage to the lipids, proteins, and the DNA, which can trigger a chain of events that result in cellular damage. [7, 21]. These reductive processes are accelerated by the presence of trace metals such as iron (Fe) and copper (Cu) and of specific enzymes such as monoxygenases and certain oxidases. [7, 21]

7. Oxidative stress

In 1954, an Argentine researcher, Rebeca Gerschman, suggested for the first time that FR were toxic agents and generators of disease. [12].

Due to the atomic instability of FR, the latter collide with a biomolecule and subtract an electron, oxidating it, losing in this manner its specific function in the cell. If lipids are involved (polyunsaturated fatty acids), the structures rich in these are damaged, such as the cell membranes and the lipoproteins. In the former, the permeability is altered, leading to edema and cell death, and in the latter, to oxygenation of the Low-density lipoproteins (LDL) and genesis of the atheromatous plaque. The characteristics of lipid oxygenation by FR involve a

chain reaction in which the fatty acid, on being oxygenated, becomes a fatty acid radical with the capacity of oxidizing another, neighboring molecule. This process is known as lipid peroxidation and it generates numerous subproducts, many of these, such as Malondialdehyde (MDA), whose determination in tissues, plasma, or urine is one of the methods for evaluating oxidative stress. In the case of proteins, these preferentially oxidize the amino acids (phenylalanine, tyrosine, triptophan, histidine, and methionine), and consequently form peptide chain overlapping, protein fragmentation, and the formation of carbonyl groups, and these impede the normal development of their functions (ionic membrane transporters, receptors, and cellular messengers), enzymes that regulate the cell's metabolism, etc.). [7, 33].

8. Liver regeneration, ethanol, and free radicals

While ROS and FR are generated during ethanol metabolism, causing oxidative stress and lipoperoxidation in the liver, they can also form a significant pathway of damage to the regenerative process of the hepatocyte. In this process, ethanol-induced FR and the generation of ROS involve the mitochondria, the microsomal cytochrome P450 2E1, the iron (FE) ion, and less frequently, peroxisomes, cytosolic xanthines, and aldehyde oxidases, to regulate cellular proliferation, acting as direct or indirect factors. [37, 2].

In general, ROS-derived FR intervene in the persistent bombardment of molecules by reactive oxygen radicals, thus maintaining redox homeostasis, in such a manner that during liver regeneration, these can modify the metabolic response necessary for carrying out cellular mitosis in the hepatocyte. While FR are generated and utilized by the cells as neutrophils, monocytes, macrophages, eosinophils, and fibroblasts for eliminating foreign organisms or toxic substances, the increase of FR due to exposure to ethanol leads to cellular deterioration that in turn produces hepatic alterations, with an unfavorable influence on cell proliferative action. [25].

9. Antioxidants

Halliwell defines an antioxidant as all substances that on being found present at low concentrations with respect to those of an oxidizable substrate (biomolecule), delays or prevents the oxidation of this substrate. The antioxidant, on colliding with FR, is ceded to an electron, in turn oxidizing itself and transforming itself into a non-toxic, weak FR and, in some cases such as with vitamin E, it can regenerate itself into its primitive state due to the action of other antioxidants. Not all antioxidants act in this way: the so-called enzymatic antioxidants catalyze or accelerate chemical reactions that utilize substrates that in turn react with FR. Of the numerous classifications of antioxidants, it is recommended to adopt that which divides these into the following: exogenes or antioxidants that enter through the alimentary chain, and endogenes that are synthesized by the cell. Each antioxidant possesses an affinity for a

determined FR or for several. Vitamin E, beta-carotene, and lycopene act within the liposoluble medium of the cell and their absorption and transport are found to be very much linked with that of the lipids. Vitamin E is considered the most important protector of lipid molecules. [27].

Life in the presence of molecular oxygen requires the possession of a multiple battery of defenses against the diverse oxygen FR, which on the one hand tend to impede their formation and on the other, neutralize them once they are formed. These defenses exert an effect at five levels [7, 21, 33]:

9.1. First level

This consists of editing univalent oxygen reduction through enzymatic systems capable of effecting consecutive tetravalent reduction without releasing the partially reduced intermediaries; this is achieved with great effectiveness by the cytochrome-oxidase system of the mitochondrial respiratory chain, which is responsible for more than 90% of oxygen reduction in the human organism.

9.2. Second level

This is constituted of enzymes specialized in the uptake of the superoxide anion radical (O 2 –). These are Superoxide dismutase (SOD), the methaloenzyme that catalyzes the dismutation of the superoxide anion radical to provide molecular oxygen and hydrogen peroxide, with such great effectiveness that it approaches the theoretical limit of diffusion. In the cells of the eukaryotic organisms, there are two of these: one is cytoplasmatic, and the other is mitochondrial. SOD was described by Fridovich in 1975.

9.3. Third level

This is conferred by a group of specialized enzymes on neutralizing hydrogen peroxide. Among these is catalase, which is found in the peroxisomes and which catalyzes the dismutation reaction.

Also in mammals, glutathione peroxidase (a cytoplasmic enzyme that contains selenium) is the most important.

9.4. Fourth level

Here the hydroxyl radical produced in the Haber-Weiss cycle can neutralized by vitamin E or alpha-tocopherol, which is an effective antioxidant and that due to its hydrophobicity is found in biological membranes in which its protection is particularly important. In addition, vitamin C or ascorbic acid is a reducer agent or electron donor and reacts rapidly with the OH– radical and with the superoxide anion.

9.5. Fifth level

Once the molecular damage is produced, there is a fifth level of defense that consists of repair. It has been demonstrated that FR were capable of causing breaks in the DNA chain and even of inducing mutagenesis, but there are enzymatic repair mechanisms that permit reestablishment of genetic information.

10. Antioxidants and their role in hepatoprotection

The term antioxidant was originally utilized to refer specifically to a chemical product that prevented the consumption of oxygen [6]; thus, antioxidants are defined as molecules whose function is to delay or prevent the oxygenation of other molecules. The importance of antioxidants lies in their mission to end oxidation reactions that are found in the process and to impede their generating new oxidation reactions on acting in a type of sacrifice on oxidating themselves. There are endogenous and exogenous antioxidants in nature. Some of the best-known exogenous antioxidant substances are the following: β-carotene (pro-vitamin A); retinol (vitamin (A); ascorbic acid (vitamin C); α-tocopherol (vitamin E); oligoelements such as selenium; amino acids such as glycine, and flavonoids such as *silymarin*, among other organic compounds [46, 36].

Historically, it is known that the first investigations on the role that antioxidants play in Biology were centered on their intervention in preventing the oxidation of unsaturated fats, which is the main cause of rancidity in food. However, it was the identification of vitamins A, C, and E as antioxidant substances that revolutionized the study area of antioxidants and that led to elucidating the importance of these substances in the defense system of live organisms. [36].

Due to their solubilizing nature, antioxidant compounds have been divided into hydrophilics (phenolic compounds and vitamin C) and lipophilics (carotenoids and vitamin E). The antioxidant capacity of phenolic compounds is due principally to their redox properties, which allow them to act as reducing agents, hydrogen and electron donors, and individual oxygen inhibitors, while vitamin C's antioxidant action is due to its possessing two free electrons that can be taken up by Free radicals (FR), as well as by other Reactive oxygen species (ROS), which lack an electron in their molecular structure. Carotenoids are deactivators of electronically excited sensitizing molecules, which are involved in the generation of radicals and individual oxygen, and the antioxidant activity of vitamin A is characterized by hydrogen donation, avoiding chain reactions. [7, 21, 33].

The antioxidant defense system is composed of a group of substances that, on being present at low concentrations with respect to the oxidizable substrate, delay or significantly prevent oxygenation of the latter. Given that FR such as ROS are inevitably produced constantly during metabolic processes, in general it may be considered as an oxidizable substrate to nearly all organic or inorganic molecules that are found in living cells, such as proteins, lipids, carbohydrates, and DNA molecules. Antioxidants impede other molecules from binding

to oxygen on reacting or interacting more rapidly with FR and ROS than with the remainder of molecules that are present in the microenvironment in which they are found (plasma membrane, cytosol, the nucleus, or Extracellular fluid [ECF]). Antioxidant action is one of the sacrifices of its own molecular integrity in order to avoid alterations in the remainder of vitally functioning or more important molecules. In the case of the exogenic antioxidants, replacement through consumption in the diet is of highest importance, because these act as suicide molecules on encountering FR, as previously mentioned. [7, 21, 33].

This is the reason that, for several years, diverse researchers have been carrying out experimental studies that demonstrate the importance of the role of antioxidants in protection and/or hepatic regeneration in animals. Thus, in this chapter, the principal antioxidants will be described that play an important role in the regeneration of hepatic cells and in the prevention of damage deriving from alcohol.

11. Flavonoids

Flavonoids are compounds that make up part of the polyphenols and are also considered essentials nutrients. Their basic chemical structure consists of two benzene rings bound by means of a three-atom heterocyclic carbon chain. Oxidation of the structure gives rise to several families of flavonoids (flavons, flavonols, flavanons, anthocyanins, flavanols, and isoflavons), and the chemical modifications that each family can undergo give rise to >5,000 compounds identified by their particular properties. [16].

Flavonoid digestion, absorption, and metabolism have common pathways with small differences, such as, for example, unconjugated/non-conjugated flavonoids can be absorbed at the stomach level, while conjugated flavonoids are digested and absorbed at the intestinal level by extracellular enzymes on the enterocyte brush border. After absorption, flavonoids are conjugated by methylation, sukfonation, ands glucoronidation reactions due to their biological activity, such as facilitating their excretion by biliary or urinary route. The conjugation type the site where this occurs determine that metabolite's biological action, together with the protein binding for its circulation and interaction with cellular membranes and lipoproteins. Flavonoid metabolites (conjugated or not) penetrate the tissues in which they possess some function (mainly antioxidant), or are metabolized. [27].

On the other hand, the flavonoids possess implications in health; in recent years, the properties of these compounds have been studied in relation to diverse pathologies. In diabetes, these compounds present regulation of glycemia through diverse mechanisms that include the inhibition of some enzymes such as α-glucosidase, glucose 6 phosphatase, and phosphorylated glycogen. The flavonoids possess other characteristics such as the trapping of molecules of glioxal and methyl-glioxal molecules, which propitiate the formation of advanced final products of glycosylation that are found to be directly related with micro- and macrovascular complications. They also regulate the rise or fall of transporter proteins; the structure of some flavonoids appears to have important participation with regard to the studied benefits. [16].

More research is needed because great majority of the former has been conducted in animals, to determine effects and dosage. Flavonoids in the menopause result in controversial effects due to the population type studied, that is, Asiatic, absorption, metabolism, the binding of isoflavones to estrogen receptors, etc.; however, they appear to possess a beneficial effect in terms of the prevention of certain types of cancer and osteoporosis. [16].

The flavonoids absorb Ultraviolet light (UV) from the sun and possess direct and indirect antioxidant effects (through the induction of cytoprotector proteins). Topical application (on the human skin) of the polyphenolic fraction of green tea protects against immunosuppression and inhibits the erythema and the formation of pyrimidine dimers in DNA caused by UV. On representing one of the most important lifestyle factors, alimentation can importantly affect the incidence and initiation of cardiovascular or neurodegenerative diseases. The cardioprotector effect of flavonoids is based on reducing oxidation and blood concentrations of the binding of cholesterol to Low-density lipoproteins (LDL); flavonoids reduce endothelial dysfunction and blood pressure and increase the HDL-bound cholesterol concentration. Flavonoids possess a neuroprotector effect because they protect the neurons from oxidative stress by means of induction of antioxidant defenses, modulation of signaling cascades, mitochondrial interactions, apoptotic processes, or by synthesis/degradation of the β-amyloid peptide. The potential effect of flavonoids as neuroprotectors is due to three main factors: they prevent neurodegeneration; inhibit neuroinflammation, and reduce the diminution of age-related cognitive functions. [16].

In cancer, the flavonoids have been classified as chemopreventive, as blockers as well as inhibitors, given their functions in carcinogenesis, in which they modulate transduction signaling in cellular proliferation and angiogenesis, modulate enzymes for the metabolic activation of procarcinogens and the detoxification of carcinogens, and modulate enzymes in the biosynthesis of anti-oxidant-pro-oxidant estrogen activity estrogen (promoting oxidative homeostasis, rendering its antioxidative capacity as a contribution to antineoplastic as well as preventive as well as therapeutic activity due to inhibiting the activation of mitogenic kinases and transduction factors, while pro-oxidative activity increases the cell damage that promotes detention of the cell cycle and apoptosis). In obesity, the flavonoids have been identified as reducer factors of fat mass and as inhibitors of fat mass deposition and catabolic activity. [16].

The procyanidins and proanthocyanidins have demonstrated, in human population, to diminish visceral fatty mass (depending on the dose) with an associated increase of adiponectin. This diminution is linked with the malabsorption of carbohydrates and lipids due to enzyme inhibition. It has been observed that the procyanidins increase β-oxidation and inhibit the expression of genes that promote the synthesis of fatty acids. Epigallocatechin gallate can increase energy expenditure and lipid oxidation in humans; it is thought that this is possible because of the increase of thermogenesis and the inhibition of the activity of the lipase, as well as, according to studies *in vitro,* the inhibition of lipogenesis and apoptosis of the adipocytes. Catechins that alter the deposition of adipose tissue related with diminution of the respiratory co-efficient and greater oxygen consumption, and thermogenesis induced by the sympathetic nervous system. Phytoestrogens can improve obesity and its alterations

on diminishing insulin resistance, thus lipogenesis, as well as inhibition of the mechanisms for cell differentiation and proliferation. The study of flavonoids and their effects on the prevention and treatment of obesity is a widespread, yet incomplete research field. [16].

The metabolism of phytoestrogens and their maximum concentration in serum presents great variability, depending on genetic differences and estrogen exposure in early life stages. [16].

12. Silimarina (*silybum marianum*)

Silymarin is a compound of natural origin extracted from the *Silybum marianum* plant, popularly known as St. Mary's thistle, whose active ingredients are flavonoids such as silybin, silydianin, and silycristin. This compound has attracted attention because of its possessing antifibrogenic properties, which have permitted it to be studied for its very promising actions in experimental hepatic damage. In general, it possesses functions such as its antioxidant one, and it can diminish hepatic damage because of its cytoprotection as well as due to its inhibition of Kupffer cell function. [41].

Silymarin, derived from the milk thistle plant named *Silybum marianum*, has been used since time past as a natural remedy for combating liver diseases. *Silymarin* and its active constituents (silybinin, silycristin, and silydianine, among others), have been classified as uptakers of free radicals and inhibitors of lipoperoxidation; some studies also suggest that that they increase the synthesis of hepatocytes, diminish the activity of tumor promoters, stabilize mastocyte cells, and act as iron chelates. [8].

Silybum marianum belongs to the Aster family (Asteraceae or Compositae), which includes daisies and thistles. The milk thistle is distributed widely throughout Europe, was the first plant that appeared in North America to the European colonizers, and is at present established in the South of the U.S., California, and South America. [22].

The name milk thistle is derived from the characteristics of its thorny leaves with white veins, which, according to the legend, were carried by the Virgin Mary. Its name *Cardo lechoso* derives from the same tradition. The mature plant has large flowers, of a brilliant purple color, and abundant thorns of significant appearance. The milk thistle grows in places where exposure to the sun is abundant. [15].

Extracts of the milk thistle have been used as medical remedies from ancestral Greece, when Dioscorides, a Greek herbalist, wrote that the seeds of the milk thistle could cure the bite of a poisonous snake. Pliny noted that the mixture of the juice of the plant and its honey were excellent for bile tract disorders. [9]. In 1596, Gerard mentioned *Silybum marianum* as a major remedy against melancholy or black bile. The milk thistle was sold for treating liver diseases. In the 1960s, observed that milk thistle was an excellent remedy for cleaning obstructions of the liver and spleen, notwithstanding that infusions of the fresh roots and seeds were effective for counteracting jaundice.

The main active agent of the milk thistle is *silymarin*, a mixture of flavonolignans, silydianine, silycristin, and silybinin, the latter the most biologically active extract; the flavonoids appear to be activated as trappers of free radicals and as plasmatic membrane stabilizers. Concentrations of *silymarin* are localized in the fruit of the plant, as well as in the seeds and leaves, from which *silymarin* is extracted with 95%-proof ethanol, achieving a brilliant yellow liquid. The term flavonoid is derived from *flavus*, which denotes yellow. [20, 16].

The standardized extract of *silymarin* contains 70% *silymarin*. Pharmacokinetic studies have shown that there is rapid absorption of silybinin into the bloodstream after an oral dose. Steady-state plasma concentrations are reached after 2 hours and the elimination half-life is 6 hours. [Lorenz et al., 1984, 3]. From 3-8% of an oral dose is excreted in the urine and from 20-40% is recovered in the bile as glucuronide and sulfate. [42].

Silybinin works as an antioxidant, reacting rapidly with oxygen free radicals as demonstrated *in vitro* with hydroxyl anions and hypochlorous acid. Reported activities include the inhibition of hepatocyte lipoperoxidation, the microsomal membrane in rats, and protection against genomic damage through the suppression of hydrogen peroxide, superoxide anions, and lipoxygenase. It is thought that silybinin also increases the synthesis of the proteins of the hepatocyte through stimulation of the activity of the ribosomal RNA (rRNA) polymerase. In addition, silybinin diminishes hepatic and mitochondrial oxidation induced by an iron overcharge and acts as an iron chelate. [16].

13. Antioxidant and hepatoprotector action

Silymarin is an active principle that possesses hepatoprotector and regenerative action; its mechanism of action derives from its capacity to counterarrest the action of FR, which are formed due to the action of toxins that damage the cell membranes (lipid peroxidation), competitive inhibition through external cell membrane modification of hepatocytes; it forms a complex that impedes the entrance of toxins into the interior of liver cells and, on the other hand, metabolically stimulates hepatic cells, in addition to activating RNA biosythesis of the ribosomes, stimulating protein formation. In a study published by [41], the authors observed that *silymarin*'s protector effect on hepatic cells in rats when they employed this as a comparison factor on measuring liver weight/animal weight % (hepatomegaly), their values always being less that those of other groups administered with other possibly antioxidant substances; no significant difference was observed between the *silymarin* group and the *silymarin*-alcohol group, thus demonstrating the protection of *silymarin*. On the other hand, *silymarin* diminishes Kupffer cell activity and the production of glutathione, also inhibiting its oxidation. Participation has also been shown in the increase of protein synthesis in the hepatocyte on stimulating polymerase I RNA activity. *Silymarin* reduces collagen accumulation by 30% in biliary fibrosis induced in rat. An assay in humans reported a slight increase in the survival of persons with cirrhotic alcoholism compared with untreated controls [2].

Silymarin is a flavonoid derived from the *Silybum marianum* plant that has been employed for some 2,000 years for the treatment of liver diseases. At present, its use as an alternative

drug has extended throughout Europe and the U.S. *Silymarin* acts as a hepatoprotector due to its antioxidant effect, which has been observed to inhibit liver damage due to the releasing of the substances of free radicals, such as ethanol, acetaminophen, and Carbon tetrachloride (CCL_4), in addition to increasing the activity of SOD and glutathione. As a uptaker of free radicals, *silymarin* can inhibit the lipid peroxidation cascade in the cell membranes. The hepatoprotector effect of this flavonoid also can be explained by an anti-inflammatory effect, in which it has been observed that *silymarin* acts on the functions of the Kupffer cells. Inhibition also has been reported in the activation of the Nuclear kappa-Beta [NK-B) transcription factor. [2, 16, 7, 21, 33].

14. *Silymarin* and Exercise

During physical activity, oxygen consumption increases, which produces oxidative stress that leads to the generation of free radicals, which are highly toxic for the cell, because these interact with organic molecules susceptible to being oxidized, such as unsaturated fatty acids, which causes lipoperoxidation. To avoid this damage by FR, there are the following antioxidant systems: Superoxide-dismutase (SOD), and Catalase (CAT), in addition to other protector substances such as vitamins A, C, and E and the flavonoids, which trap free radicals. (unpublished data)

In experiments conducted by our research group on groups of rats that were submitted to daily aerobic exercise in a physical-activity cage for 20 minutes during 4 weeks (5 days/week) and on another group of rats submitted to physical activity plus administration of *silymarin* (200 mg/kg of weight) prior to exercise, with daily quantification of physical performance and at the at the end of the experiment, quantification of DNA in serum and of SOD and CAT activity in liver. We found that in the group with physical activity, MDA increased 134% (in serum) and 123% (in liver) vs. control rats. In the group with exercise plus *silymarin*, MDA returned to normality (in serum and in liver). Catalase activity increases during exercise (118%) and with exercise plus *silymarin* (137%). SOD activity exhibited no modifications in any treatment. Finally, we found an increase of physical activity in the group administered *silymarin* (27%) in comparison with the group in which no *silymarin* was administered. (unpublished data)

A protector effect was found of *silymarin* during exercise, because it diminishes MDA levels in serum as well as in liver, which translates into diminution of the production of free radicals, causing as a consequence less cellular damage, which in turn leads to an increase in physical performance.

15. Conclusions

The process of the induction of oxidative stress generated in the liver due to the presence of ethanol implies the conjugation of various factors. The role that these factors play in the de-

velopment of oxidative stress depends in part on whether acute or chronic intoxication is involved. The factors that contribute to the development of oxidative stress imply disequilibrium among pro- and antioxidant factors. It can occur that oxidative stress develops if the xenobiotic increases the pro-oxidant factors (the generation of Oxygen-generated free radicals [OFR]) or decreases intracellular antioxidant factors. In whichever of the two cases, the general result is important damage to the hepatocyte that can lead to general damage to the DNA that, in turn, can comprise a determining factor in the induction of the apoptotic system (programmed cell death) of the cell, thus accelerating its death and destruction.

The study of the factors that determine the increase in the generation of OFR in the liver, originating due to acute or chronic intoxication with a xenobiotic, is of great importance because it will allow diminishing the damage that these reactive species produce within the hepatocyte. On the other hand, despite that at present much is known concerning the physiopathological mechanisms of ethanol ingestion-related liver damage and the role that the production of oxygen-generated free radicals plays in these processes, the exact extent of this damage, as well as how to prevent it, remains unknown with precision. There is evidence obtained from laboratory models that the ingestion of natural antioxidants, such as vitamins A, C, and E, oligoelements (selenium), amino acids (glycine), and principally flavonoids, such as *silymarin*, can in the future be a potential treatment for all persons who present hepatic alterations. However, beyond the remedy, the cooperation of the patient is required to regulate his/her ethanol consumption; as long as this does not take place, taking antioxidant vitamins can be considered within the regular therapy of a patient with alcoholism, taking care above all that this supplement does not reach toxic concentrations, in particular in the case of the vitamin that possess the tendency to accumulate in the liver.

The use of novel experimental procedures that determine the degree of damage caused by xenobiotics, and in particular by free radicals, is of great importance in the management of diseases caused by this type of substance, especially if they damage the liver, because this organ comprises a vital part of our organism on having in its charge the metabolic support of the latter.

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