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The Chemoprevention of Chronic Degenerative Disease Through Dietary Antioxidants: Progress, Promise and Evidences

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Additional information is available at the end of the chapter

1. Introduction

1.1. Epidemiology of chronic degenerative diseases in Mexico and the world

During the last 30 years relevant changes in the public health field have arisen worldwide, among which the most representative are observed in developed countries where a big deal of infectious diseases have been reduced and controlled as a result of the creation and introduction of powerful antibiotics [1].

In countries such as Australia, Austria, Belgium, Canada, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Japan, Luxembourg, Netherlands, New Zealand, Norway, Portugal, Spain, Sweden, Switzerland, the United Kingdom, and the United States, incorporated to the OECD (Organization for Economic Cooperation and Development), mortality due to those diseases has diminished up to 38% in people between 35 and 69 years old. Likewise, the risk of mortality before age 70 has diminished up to the 23%. Those reductions have been the result of social changes and of the improvement of preventive methods of infectious diseases. However, in recent years the prevalence of chronic degenerative diseases has increased [1].

Chronic degenerative diseases (CDDs) represent a problem of public health for they have become the cause of death worldwide both in adolescents and adults. Among the most prevalent CDDs worldwide is obesity, the cardiovascular diseases (such as hypertension, atherosclerosis), heart diseases, diabetes, chronic respiratory diseases, and cancer; which have caused the 60% of the 58 million yearly deaths, which are approximately 35 million people death for these diseases between 2005 and 2007 [2].

Prevalence in chronic degenerative diseases results from different factors, among which the technologic advance and modernization affect life styles where an increase in processed foods consumption with a high level of fat content, a sedentary lifestyle since childhood, alcohol and tobacco, stress and a lack of culture in terms of damage prevention and health risks [1,2].

Mexico does not escape this situation as a result of specific factors to our country such as economic development, concentration of population in urban areas, lack of support to improve the health services and the limitations in preventive programs, particularly in the population under 10 years. Besides, there is a transformation of the population pyramid due to a reduction in mortality and a decrease in birth rate; both phenomena are identified as epidemiologic and demographic transitions [2]. In México, the morbidity data produced by the CDDs are taken from the statistics of the healthcare sector and published by the healthcare ministry. Although in those reports not all the existing cases are included (not all patients request healthcare services), they are a good help to understand the damage behavior along with other indicators of prevalence that estimate the number of cases in the population within a specific period of time. Such indicators are obtained from the national healthcare survey and from the national healthcare and nutrition survey 2006 [2]. On the other hand, the mortality statistics are considered as more reliable due to the permanent job in updating the database. The information is obtained from the records of the national institute of statistics, geography and informatics (INEGI) and the general bureau of health information, in conjunction with the epidemiological AVAD index, which is a measure that combines years of healthy life lost due to premature mortality and years of life lost due to disability [3].

As mentioned above, the epidemiological and demographic transitions are important factors for the prevalence of chronic degenerative diseases and indicate changes in the behavior of population dynamics, as well as damage to health which are the result of the low socioeconomic development and the impact of government policies on public health. The demographic transition shows the change in a steady state population with high fertility and mortality associated with the low socioeconomic development process and/or modernization. This process is irreversible and was constructed from the first countries reaching socioeconomic development in Europe such as France and England. In recent years it has made rapid changes affecting the world population [2].

According to data from INEGI and the national population council (CONAPO), Mexico has experienced an accelerated process of demographic transition, which has influenced the economic development and migration, leading to a reduction in mortality and a parallel high birth rates, as well as the consequent population growth, so it is estimated that between 2010

and 2050 the proportion of elderly people in Mexico will grow from 7% to 28% and with it the possibility of an chronic degenerative disease is greater [2,3].

In the case of the epidemiological transition, this is characterized by a reduction of morbidity and mortality from transmissible diseases and an increase in chronic degenerative diseases. In recent years, this parameter has shown that in both developed and developing countries, the proportion of infectious diseases in individuals over age 15 is stable, but unfortunately the CDDs are increasing, showing that they occupy almost half of value of morbility globally. A relevant fact is observed in developed countries (like France, Germany, Japan, United Kingdom, and United States) where the greatest impact of transmissible diseases remains the HIV/AIDS; but the cerebrovascular diseases and the ischemic heart disease are among the main causes of morbidity and mortality (Table 1) in individuals over age 15, both diseases represent more than 36% of deaths worldwide [2].

In the specific case of Mexico, it is well-known that infectious diseases made up the profile of mortality in the fifties, since half of the deaths were caused by diarrhea and respiratory infections, for reproductive problems and associated malnutrition conditions. Nowadays, these diseases (classified as lag diseases) are concentrated in less than 15% of deaths [2].

In the last 10 years, there has been an overlap between lag diseases and the so-called emerging diseases. Thus, the epidemiological transition has ranked the chronic degenerative diseases among the 10 leading causes of death, highlighting the type 2 diabetes, obesity, cardiovascular diseases, malignant neoplasms and cerebrovascular diseases [4].

Mortality (individuals between 15 and 50 years)			Mortality (over 60 years)		
	Cause	Deaths (thousands)		Cause	Deaths (thousands)
1	HIV / AIDS	2279	1	Ischemic heart disease	5825
2	Ischemic heart disease	1332	2	Cerebrovascular diseases	4689
3	Tuberculosis	1036	3	Chronic obstructive pulmonary disease	2399
4	Injuries from traffic accidents	814	4	Infections of lower respiratory	1396
5	Cerebrovascular diseases	783	5	Trachea and lung cancer	928
6	Self-harm	672	6	Diabetes	754
7	Violence	473	7	Hypertensive heart disease	735
8	Liver cirrhosis	382	8	Stomach Cancer	605
9	Infections of lower respiratory	352	9	Tuberculosis	495
10	Chronic obstructive pulmonary disease	343	10	Colorectal cancer	477

Table 1. Leading causes of death in people over 15 years in the world (as a function of AVAD index)

2. Definition, importance and control of oxidative stress

The term "oxidative stress" was first introduced in the eighties by Helmut Sies (1985), defining it as a disturbance in the prooxidant-oxidant balance in favor of the first. From that time, a great number of researchers have studied this phenomenon; so, the concept has evolved and now, has been defined as "A situation when steady-state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents" [5,6].

However, oxidative stress is a phenomenon not entirely detrimental for the organism; also, free radicals (FR) have an important function in several homeostatic processes. They act as intermediate agents in essential oxidation-reduction (redox) reactions. Some examples are the destruction of microorganisms through phagocytosis, synthesis of inflammatory mediators and detoxification. Therefore, FR in low concentrations are useful and even essential [7].

FR represents any chemical species that exists independently and has one or more unmatched (odd) electrons rotating in its external atomic orbits. This highly unstable configuration causes this chemical species to be very aggressive and to have a short life span. Once generated, FR interact with other molecules through redox reactions to obtain a stable electronic configuration [8-10].

Several authors have classified FR according to the functional group in their molecule, being the most frequent the reactive oxygen species (ROS) and reactive nitrogen species (RNS). Thiol radicals are less important, their reactive group contains sulfur; well as those containing carbon or phosphorus in their reactive center. ROS are constituted by superoxide anion ($O_2\bullet-$), hydroxyl radical ($\bullet OH$), hydrogen peroxide (H_2O_2), and singlet oxygen. While, that the RNS are nitric oxide (NO), nitrogen dioxide ($NO_2\bullet-$) and peroxynitrite ($OONO-$) [11,12].

Due to the constant production of ROS and RNS during metabolic processes, the organism has developed a powerful, complex defense system that limits its exposure to these agents these are the so-called antioxidants (AO). Several antioxidants are enzymes or essential nutrients, or include these in their molecular structure. An essential nutrient is a compound that must be eaten because the organism is unable to synthesize it. Based on this characteristic, some authors classify AO as non-enzymatic and enzymatic [12-14].

2.1. Enzimatic antioxidants

Some researchers state that the AO function performed by enzymes has advantages compared to AO compounds, for this activity is regulated according to cellular requirements: they can be induced, inhibited or activated by endogenous effectors [15]. Ho and colleagues (1998) showed evidence of the importance of AO enzymes in protection against oxidant agents. When using transgenic mice designed to overexpress the activity of some AO enzymes, it was noticed that there is a notorious tolerance of certain tissues when they are exposed to toxics and pathologic conditions that would promote ROS action [9,16].

Enzymatic AO catalyze electron transference from a substrate towards FR. Later, the substrates or reducing agents used in these reactions are regenerated to be used again, they ach-

ieve this by using the NADPH produced in different metabolic pathways [14]. A prolonged exposure to ROS can result in diminished NADPH concentration, which is needed in other important physiologic processes, even though some enzymatic AO do not consume cofactors. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) belong to this group [12,14,17].

2.2. Non-enzymatic antioxidants

Non-enzymatic antioxidants constitute a heterogeneous group of hydrophobic and hydrophilic molecules that trap FR and create chemical species that are less noxious to cell integrity [18]. Essentially, they give an electron to a FR to stabilize it. Hydrophilic non-enzymatic antioxidants are located mainly in the cytosol, mitochondrial and nuclear matrixes and in extracellular fluids. They are vitamin C, glutathione, uric acid, ergothioneine and polyphenolic flavonoids [9,18].

3. Role of oxidative stress in the development and pathogenesis of the chronic degenerative diseases

Currently, studies related to reactive oxygen species (ROS) and reactive nitrogen species (RNS) have become a relevant issue in research with the main purpose of understanding their functions and effects in the organism. Studies developed throughout the 20th century have explained the action mechanisms of ROS and the operation of the systems responsible for their elimination. These evidences have shown the existence of enzyme systems that produce ROS (cytochrome P450, xanthine oxidase, respiratory chain) of the Fenton reaction, catalase, peroxidase, and superoxide dismutase [19].

All researches lead to the same conclusions so far: a) the evidence that the cells have specialized systems to convert ROS into less reactive compounds, and b) if those systems would fail, the ROS could be preexisting compounds for the development of diseases. Thus, several researchers agree in the relevance of "oxidative stress" in medical problems, specifically in pathogenesis and/or complications of chronic degenerative diseases [9,12,20,21].

Different observations suggest that these pathologies could be originated when reactive species are formed and suffer alterations, or when they are eliminated, or both. However, the situation is real and much more complicated, for it is difficult to determine the crucial event that originates this disease due to the diversity of forms of oxidative stress (Figure 1). Different researches indicate that mutations produced in genes are responsible of the metabolic unbalance of ROS, while others suggest that environmental changes and common habits weigh on human metabolic processes. However, doubt remains, if oxidative stress is the primary event that leads to the disease or the oxidative phenomenon is developed throughout the disease [22].

Whatever the means by which oxidative stress is induced and pathology is developed, the majority of evidences coincide in the relevance of alterations or enzyme deficiencies. These

deficiencies are often caused by mutations in genes coding antioxidant or related enzymes, for example, by genetic polymorphism.

This concept is frequently related to large number of pathologies. Enzymes involved in defence against ROS are not an exception. All enzymes contributing to antioxidant defence can be classified to really antioxidant ones, dealing directly with ROS as substrates, and auxiliary ones. The latter enzymes respond for reparation or degradation of oxidatively modified molecules, maturation and posttranslational modification of antioxidant enzymes and metabolism of low molecular mass antioxidants. As a rule, genetic polymorphisms of enzymes may lead to oxidative stress and consequent diseases, among which cancer, neurodegeneration, cardiovascular disorders, and diabetes are most frequently mentioned. Among the most studied enzymes with genetic polymorphism is the glucose-6-phosphate dehydrogenase, catalase, superoxide dismutase, glutathione peroxidase and those involved in reparation of oxidized molecules and the disease progression [22].

3.1. Glucose-6-phosphate dehydrogenase deficiency

The most striking example among polymorphisms of genes coding enzymes related to antioxidant defence is well-known deficiency in glucose-6-phosphate dehydrogenase (G6PDH) which leads to favism; genetic disease characterized by the lysis of erythrocytes when consumed broad beans and other substances which are harmless to the general population [23].

Other pathologies which are related to the same deficiency are diabetes [24,25], vascular diseases [24], and cancer [26]. In these cases, oxidative stress is induced in specific cells; it was shown that GSH may react with superoxide anion radical providing partial defence against this ROS [27]. When decreasing GSH concentration in G6PDH-deficient individuals enhances their sensitivity to redox-active compounds, producing superoxide. Superoxide is able to react also with nitric oxide, leading to the formation of rather harmful oxidant peroxynitrite. However, relation of this reaction to diabetes and vascular diseases is not because of peroxynitrite production and subsequent oxidative damage, but rather because of decrease in nitric oxide level [28].

The latter is an important second messenger in certain signalling pathways particularly related to vasodilation [29]. There is some probability also that individuals with G6PDH-deficiency may fail to regulate properly blood pressure [30]. Despite possible impairment in nitric oxide production, there is also other way to connect G6PDH deficiency with vascular diseases. It is known, that development of vascular diseases depends on the levels of homocysteine and folate, intermediates in metabolism of sulfur-containing amino acids [31]. Production of two these metabolites depends on GSH and NADPH levels in cells [32].

Data regarding association of G6PDH deficiency with cancer are controversial, because some studies demonstrated that G6PDH-deficient patients may additionally suffer from cancer [33], while others state opposite [34]. Nevertheless, both situations are possible. In particular, there is a large data body indicating that different cancer types are developed at increased DNA damage. It often happens under polymorphism in enzymes contributing to DNA repair, what will be discussed below.
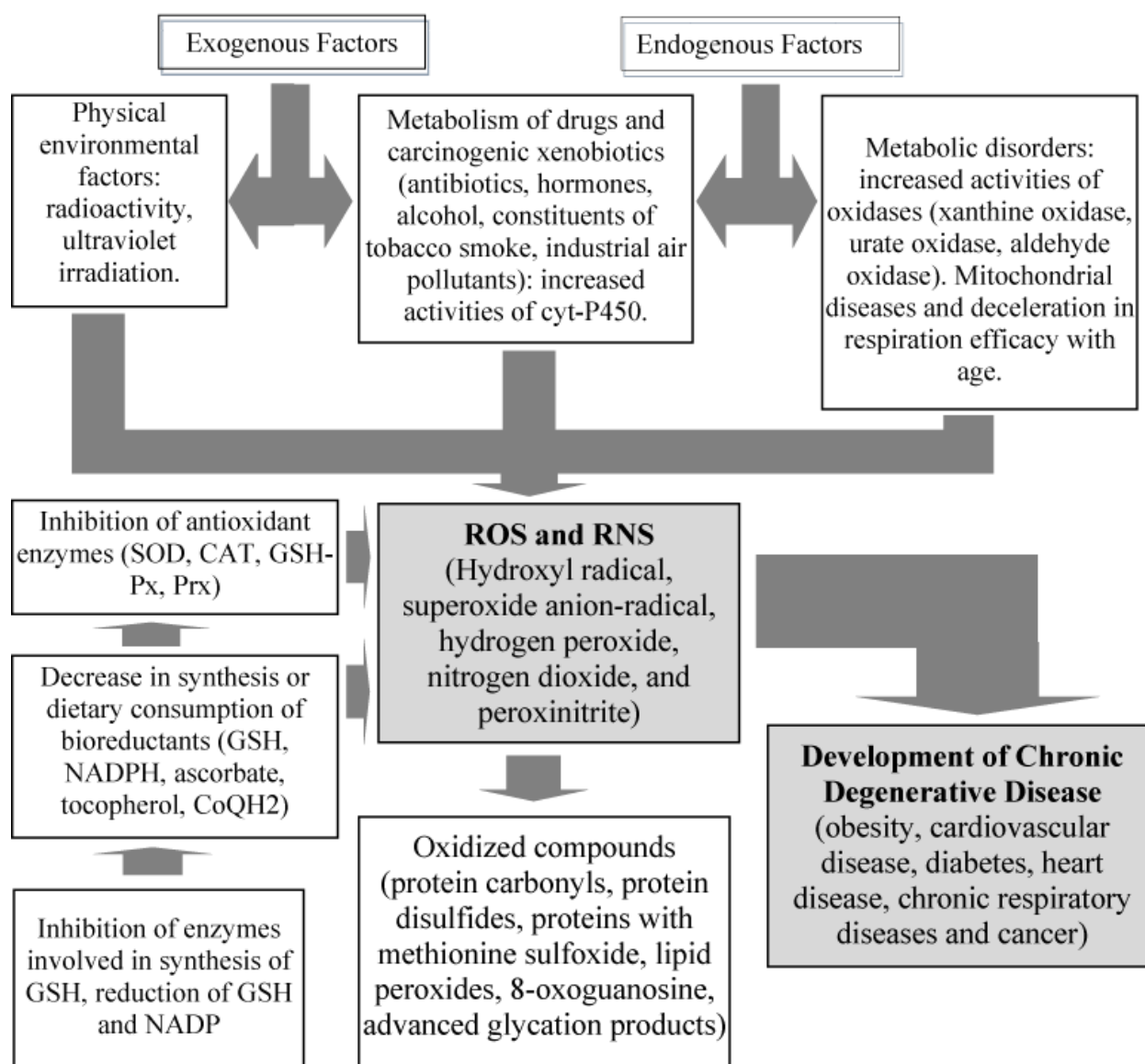
Figure 1. Scheme of the different ways that produce oxidative stress and stimulate the development of chronic degenerative diseases. *Abbreviations*: ROS – reactive oxygen species, RNS – reactive nitrogen species, SOD – superoxide dismutase, CAT - catalase, GSH-Px – glutathione-peroxidase, Prx - peroxyredoxin, GSH – reduced glutathione, CoQH2 – ubiquinol.

On the other hand, NADPH supply at certain conditions may be even harmful leading to enhanced oxidative damage and cancer development. Indeed, it was shown that G6PDH was particularly responsible for cell growth and frequently correlated with cell growth [26]. Tian and colleagues (1998) found that cancer cells possessed several times higher G6PDH activity. The positive correlation between tumour progression and G6PDH activity was found also for humans [35,36].

Increased NADPH supply resulting from G6PDH overexpression can lead to so-called "reductive stress" [37]. Enhanced activity of G6PDH, a lipogenic enzyme, was found at diabetes and obesity [38]. In humans, G6PDH is regulated by many transcription factors, in

particular, SREBP-1a (sterol regulatory element binding protein) [39], AP-1 [40] and Sp1 [41]. It was shown that elevation of G6PDH activity might lead to enhanced lipid synthesis [42] and to possible reductive stress [43].

3.2. Catalase deficiency

The first case of catalase deficiency was described by Shigeo Takahara (1947) in a child with cold sores and called acatalasemia to the patology [44]. The cause of this patology is related to ability of oral Streptococci to produce hydrogen peroxide which may promote death of mouth mucosa cells in acatalasemic patients [45]. Catalase deficiency is also associated with diabetes mellitus [46]. This association is attributed for Hungarian hypocatalasemic patients. They were shown to possess higher levels of homocysteine and lower levels of folate [32]. It hints, on one hand, to abnormalities of sulfur metabolism, but on the other hand, it is commonly known that higher homocysteine levels are related to cardiovascular diseases [47], the fact we mentioned above in the context of G6PDH deficiency.

3.3. Polymorphism of Cu,Zn-SOD and protein aggregation

In recent years, the main attention has focused on the polymorphism of genes coding the enzyme superoxide dismutase. More than 100 nucleotide substitutions for the gene SOD1 coding human cytosolic copper- and zinc containing SOD (Cu,Zn-SOD) were described [48]. It is known that several mutations in SOD1 gene are associated with cases of familial amyotrophic lateral sclerosis (ALS), a neurodegenerative disease which is characterized by paralysis and subsequent death [49]. Mechanisms of the disease development are still unknown, but there are many evidences that oxidative stress, developed in neurons, is rather caused by unexpected pro-oxidative activity of SOD than by the loss of the activity at all [50]. It was found that the aggregates cause harm to the cells not only via oxidative stress, but also via inhibition of glutamate receptors [51] and induction of apoptosis [52].

Irwin Fridovich presented some examples of unusual activities of SOD, such as oxidase-like or reductase-like ones [53]. His works and data of other authors suggest that SOD, being mutated or placed in specific conditions, may produce more harmful ROS tan hydrogen peroxide, i.e. hydroxyl radical [54, 55]. Some studies suggested that SOD aggregation can be triggered by higher susceptibility to oxidation of mutated protein [56,57]. Indeed, Cu,Zn-SOD is considered to be rather stable, resistant to many, deleterious to other proteins, compounds [48]. These evidences suggest that Alzheimer, Huntington, and Parkinson diseases are other pathologies related to this enzymatic alteration [22].

3.4. Polymorphism of Mn-SOD, extracellular SOD and glutathione peroxidase

Unlike Cu,Zn-SOD, less mutations were found in the gene coding human manganese containing superoxide dismutase (SOD2). Substitution of alanine-16 to valine (so called "Ala variant") is the most known mutation [58]. This mutation has recently been associated with cancers of breast, prostate, ovaries and bladder, as well as non-Hodgkin lymphoma, mesothelioma and hepatic carcinoma [58]. Mammals possess also extracellular Cu,Zn-SOD (EC-

SOD) encoded in humans by gene SOD3. The enzyme is a homotetramer presenting in plasma, lymph, and synovial fluid [59]. Extracellular SOD is abundant particularly in the lung, blood vessels, and the heart. Consequently, polymorphism of SOD3 gene is associated with pulmonary and cardiovascular diseases [60].

Polymorphism of glutathione peroxidase (GSH-Px) was found to be associated with some cancers. Four GSH-Px isoforms have been described in humans. It was found that mutations in exon 1 of human GSH-Px-1 gene lead to appearance of polyalanine tract at N-terminus of the protein [59]. These tracts themselves are not connected with diminished enzyme activity. Another polymorphism, substitution of proline-198 to leucine, was found in Japanese diabetic patients and associated with intima-media thickness of carotid arteries [61]. The same substitution for adjacent proline-197 was associated with lung and breast cancers, as well as with cardiovascular diseases [59].

3.5. Polymorphism of enzymes involved in reparation of oxidized molecules

Mutations may also affect enzymes involved in DNA reparation. The enzyme 8-hydroxy-2′-deoxyguanosine glycosylase (hOGG) encoded in human genome by the gene hOGG1 is probably the most known example. Recent studies associate mutations in hOGG1 with different cancer types, such as lung, stomach and bladder cancers [62]. Most of the mutations in this gene affect exon 7 and cause serine-to-cysteine substitution. It was demonstrated that substitution S326C in hOGG1 protein confers susceptibility to oxidation and makes the enzyme prone to form disulfide bond between different polypeptide chains [63].

Hydrolase MTH1 is other important enzyme preventing incorporation of oxidized purine nucleotide triphosphates in DNA [64]. Knockout of this enzyme in mice resulted in increased frequency of lung, stomach and liver tumours with age [65].

Other important antioxidant enzymes are glutathione S-transferases (GSTs). Its main function is to conjugation of different electrophilic compounds with glutathione [66]. Oxidatively modified compounds as well as lipid oxidation products, like 4-hydroxy-2-nonenal, are subjected to conjugation with glutathione. In general, GSTs are belong to xenobiotic-elimitating system. Some of them, namely GSTs of μ class, are known well by their ability to eliminate polycyclic aromatic hydrocarbons, oxidized previously by cytochrome P450 monooxygenases. To date, eight classes of GSTs have been described: α, κ, μ, σ, ξ, π, θ, and ω. Cytosolic enzymes belong to classes α, μ, π and θ [67]. The gene coding GSTM1 (GST of μ class, isoform 1) is appeared to be highly polymorphic and found inactivated in half of human population.

Some studies associate polymorphism of GSTM1 with lung cancer [59,68], although reports are controversial. For example, meta-analysis conducted by [69] found no association of GSTM1 null genotype with lung cancers as well as with smoking. Other authors found such association and reported increased susceptibility to cancerogens among Caucasian and African-American populations [70]. Polymorphism of GSTM1 was also found to be associated with head and neck carcinomas [67]. The need in GSTM1 and its role in prevention of lung cancer are explained by the ability of the enzyme to detoxify constituents of cigarette smoke, such as mentioned above polycyclic aromatic hydrocarbons. Some studies also associate

lung cancer with polymorphism of GSTT1 (GST of θ class) which participates in catabolism of tobacco smoke constituents, such as halomethanes and butadione [70].

3.6. Role of oxidative modifications of antioxidant and related enzymes in disease progression

Many disorders related to the metabolism of transition metals, amino acids or low molecular mass reductants are known to be connected with activities of antioxidant enzymes. Particularly, impairement in selenium uptake or synthesis of selenocysteine needed for glutathione peroxidase may lead to GSH-Px deficiency and subsequent disorders such as cardiovascular ones [47]. Disruption of iron-sulfur clusters by superoxide anion radicals or peroxynitrite leads frequently to impairment of many metabolic pathways. Indeed, aconitase, NADH-ubiquinone-oxidoreductase (complex I of mitochondrial electron transport chain), ubiquinol-cytochrome c oxidoreductase (complex III), ribonucleotide reductase, ferredoxins possess iron-sulfur clusters, susceptible to oxidation. Owing to this, aconitase is used as one of oxidative stress markers [71]. On the other hand, iron is a component of haem, a prosthetic group in catalase holoenzyme. Susceptibility to oxidative modification is described for catalase, glutathione peroxidase, Cu,Zn-SOD, and G6PDH. The latter is believed to be one of the most susceptible to oxidation enzymes [22]. Thus, oxidative stress induced by exogenous factors, like carcinogens, certain drugs, ions of transition metals, etc., or by metabolic disorders, like diabetes, can be exacerbated by oxidative modification of antioxidant enzymes. These assumptions demonstrate the potential of antioxidant therapy in particular cases. At some pathological states, whatever the cause of the disease, oxidative stress is seen to be a powerful exacerbating factor. Type II diabetes, cardiovascular diseases and neurodegenerative diseases, associated with protein aggregation are among such pathologies. Indeed, enhanced level of glucose results in higher probability of protein glycation [72].

4. Impact of chemopreventive agents in the chronic degenerative diseases

The available evidences indicates that individuals with chronic degenerative diseases are more susceptible to oxidative stress and damage because they have elevated levels of oxidants and/or reduced antioxidants. Therefore, it has been posited that antioxidant supplementation in such individuals may be beneficial. Different research has confirmed that many common foods contain nonnutritive components that may provide protection against chronic degenerative diseases, however, the most studies have had impact on the cancer [20,21].

The "chemoprevention" seeks to eliminate precancerous cells in order to avoid the necessity of chemotherapy. It can be further classified as primary, secondary, or tertiary prevention. Primary chemoprevention focuses on preventing the development of precancerous lesions, secondary chemoprevention focuses on preventing the progression of these lesions to cancer, and tertiary chemoprevention aims to prevent the recurrence or spread of a primary cancer [73].

It has been known for some time that dietary factors play a role in the development of some human cancers [73,74] and that some foods contain mutagens and carcinogens [74,75]. Investigations of last decades, has focused on the existence of a number of non nutritional components in our regular diet that possess antimutagenic and anticarcinogenic properties, these compounds have been called as chemopreventers [76,77].

The chemopreventers are classified as food entities that can prevent the appearance of some long-term diseases like cancer or cardiovascular disorders. It has been suggested that chemoprevention should be considered as an inexpensive, easily applicable approach to cancer control and "may become a major weapon in the anticancer arsenal" [76,78,79]. These compounds can be found in all food categories, but mainly in fruits, vegetables, grains and tea [78,79]. Chemopreventers belong to different classes of chemicals but the most recognized are some vitamins, food polyphenols, flavonoids, catechins, and some components in spices [78, 79].

The mechanisms of action of the chemopreventers are complex and can be categorized according to the site of action or by the specific type of action. It appears that most chemopreventers act primarily as antioxidants. As such, they may scavenge free radicals formed during the preparation of food or as a normal biological process in the body. Recall, that the free radicals can react with DNA, lipids, or cell membranes, leading to aging, injuries of the organ, and greater susceptibility to develop the chronic degenerative disease. Therefore, any event that removes free radicals in the human body is considered beneficial for human health. In addition to their antioxidative activities, there are other mechanisms that show in the Table 2 [80-82].

5. Chemopreventive evidence of some fruits and food supplements evaluated by our research group

5.1. Cactus pears

Plants from the genus *Opuntia* are the most abundant of the Cactaceae family, grown throughout the Americas as well as the central area of the Mediterranean, Europe, Asia, Africa, and Australia. *Opuntia* species display flattened stems called "pencas" or cladodes. The cactus pear (also called prickly pears) is the fruit of this plant (*Opuntia* spp.). The fruit is an oval berry with a large number of seeds and a semi-hard rind with thorns, which may be grouped by fruit colors: red, purple, orange-yellow and white. The fruits with white pulp and green rind are preferred for consumption as food, and their domestic production corresponds to almost 95% of the total production. Mexico is the main producer of cactus pears and accounts for more than 45% of the worldwide production; however, only 1.5% of this production is exported [83,84].

Mechanism	Action	Examples
Inhibition of carcinogen formation	Agents that block or inhibit to the enzymes responsible for the biotransformation of procarcinogens to carcinogen form	Dithiocarbamates, isothiocyanates, diallyl sulfide, and ellagic acid
Inducing agents	Agents that induce or enhance enzyme activity (e.g., glutathione S-transferase, GST) for detoxify and reduce the level of mutagenic/carcinogenic species of the body	Isothiocyanates, sulfaraphane, d-limonene, terpinoids, turmeric, and curcurains
Suppressing agents	Agents that may react on different processes (e.g., inhibition of arachidonic acid metabolism, activity of protease or protein kinase C) involved in tumor promotion/progression	isoflavones, phytoestrogens, selenium
Immune activity and modulation	Since the immune system can influence on growth either via effects on the inflammation status or by causing apoptosis. Some chemopreventers can act on the early stages in neoplasia or have effects on frank malignancies	Carotenoid, flavonoids, lactoferrin
Signal transduction pathways and their regulation	Some chemopreventers may alter signaling pathways of receptors for hormones and others factors responsible for cell regulation and can be modified the potential for growth, either by acting to increase mitosis or alter the level of apoptosis.	d-limonene, sulfur compounds, lactoferrin, retinoids

Table 2. Other mechanisms of chemoprevention

A viable strategy to increase the competitiveness of the Mexican cactus pear in national and international markets is the innovation and creation of new high value-added products. This could be achieved by determining the nutritional and functional properties that differentiate the Mexican cactus pear from analogous products. In addition, providing functional products for a market in constant growth would offer a key competitive advantage and would allow the producers to diversify its commercialization, not as fresh fruit only, but also as an ingredient or high-value additive for the food industry. A commercialization of the cactus-pear based on its antioxidant properties could generate competitive advantages that may turn into business opportunities and the development of new products [85].

Different studies with the varieties of European and Asian cactus pears have shown notable antioxidant activities that significantly reduce oxidative stress in patients and may help in preventing chronic pathologies (as diabetes and cancer) [85-87]. For this reason, the cactus pear is considered a functional food; this feature is attributed to its bioactive compounds such as vitamin C and vitamin E, polyphenols, carotenoids, flavonoid compounds (e.g., kaempferol, quercetin, and isorhamnetin), taurine and pigments [88,89].

Betalains are water-soluble pigments. Two betalain derivatives are present in cactus-pears: betacyanin, which gives the red-purple color, and betaxanthin, which gives a yellow-orange

color. These pigments have shown beneficial effects on the redox-regulated pathways involved in cell growth and inflammation, and have not shown toxic effects in humans [90,91].

In addition, a neuroprotector activity against oxidative damage induced in cultures of rat cortical cells has been attributed to the cactus pear flavonoids [92]. Another beneficial effect of the fruit was observed in the prevention of stomach ulcers through the stimulation of prostaglandin production: cactus pear promoted mucous secretion of bicarbonate, involved in the protection of gastric mucosa [93]. On the other hand, their contents of natural antioxidants has raised interest in the use of cactus pears as substitute for synthetic antioxidants, such as butylhydroxytoluene (BHT), butylhydroxyanisole (BHA) [88].

In the Institute of Health Sciences (Autonomous University of Hidalgo State) have been performed studies to demostrate the chemopreventive capacity of the cactus pear. The first studies were developed by Hernández-Ceruelos et al. (2009) with the main objective to evaluate the antioxidant effect of three varieties of prickly pear juice (red-purple, white-green and yellow-orange) in four different concentrations (25, 50, 75 and 100%) by the technique of DPPH (1,1-diphenyl-2-picrylhydrazyl). Their results indicated that the juice of princkly pear variety red-purple (PPRP) had the highest antioxidant capacity in all concentrations in comparison with the positive control (vitamin E). Subsequently, researchers evaluated the anticlastogenic potential of PPRP by micronucleus assay against of methyl methane sulfonate (MMS) in mice. This experiment had a duration of 2 weeks, was included a negative control (animals treated with water), a positive control of MMS (40 mg/kg), a group of mice treated with princkly pear variety red-purple (25mL/Kg), and three groups with PPRP (in doses of 25, 16.5 and 8.3 mL/Kg) plus the mutagen. The PPRP was administered by oral gavage and the mutagen was injected intraperitoneally 5 days before the end of the experiment (single dose). Finally, blood samples were obtained in four times (0, 24, 48 and 72 hours) to determine the frequency of micronucleated polychromatic erythrocytes (MNPE). The result indicated that PPRP is not a genotoxic agent, on the contrary, may reduce the number of micronucleated polychromatic erythrocytes. In this regard, the princkly pear variety red-purple showed an anticlastogenic effect directly proportional to the concentrations. The highest protection was obtained with the concentration of 25 mL/Kg (approximately, 80%) after 48 hours of treatment [94].

In the second study was evaluated the antioxidant activities [with three assays: a)1,1-diphenyl-2-picrylhydrazyl radical-scavenging, b) protection against oxidation of a β-carotene-linoleic acid emulsion, and c) iron(II) chelation], the content of total phenolic compounds, ascorbic acid, betacyanin, betaxanthins and the stability of betacyanin pigments in presence of Cu(II)-dependent hydroxyl radicals (OH$^•$), in 18 cultivars of purple, red, yellow and white cactus pear from six Mexican states (Hidalgo, Puebla, Guanajuato, Jalisco, Zacatecas and the State of Mexico). The results indicated that the antiradical activities from yellow and white cactus pear cultivars were not significantly different and were lower than the average antiradical activities in red and purple cultivars. The red cactus pear from the state of Zacatecas showed the highest antioxidant activity. The free radical scavenging activity for red cactus pears was significantly correlated to the concentration of total phenolic compounds ($R2 = 0.90$) and ascorbic acid ($R2 = 0.86$). All 18 cultivars of cactus pears studied showed sig-

nificant chelating activity of ferrous ions. The red and purple cactus pears showed a great stability when exposed to OH$^\bullet$ [88].

5.2. Cranberries

Among small soft-fleshed colorful fruits, berries make up the largest proportion consumed in our diet. Berry fruits are popularly consumed not only in fresh and frozen forms, but also as processed and derived products including canned fruits, yogurts, beverages, jams, and jellies. In addition, there has been a growing trend in the intake of berry extracts as ingredients in functional foods and dietary supplements, which may or may not be combined with other colorful fruits, vegetables, and herbal extracts [95]. Berry fruits commonly consumed in America include blackberries, black raspberries, red raspberries and strawberries, blueberries, and cranberries.

Other "niche-cultivated" berries and forest/wild berries, for example, bilberries, black currant, lingonberry, and cloudberry, are also popularly consumed in other regions of the World [95]. The North American cranberry (*Vaccinium macrocarpon*) is of a growing public interest as a functional food because of potential health benefits linked to phytochemicals of the fruit. Cranberry juice has long been consumed for the prevention of urinary tract infections, and research linked this property to the ability of cranberry proanthocyanidins to inhibit the adhesion of *Escherichia coli* bacteria responsible for these infections [96]. These studies, which brought to light the unique structural features of cranberry proanthocyanidins [97], have sparked numerous clinical studies probing a cranberry´s role in the prevention of urinary tract infections and targeted the nature of the active metabolites. Further antibacterial adhesion studies demonstrated that cranberry constituents also inhibit the adhesion of *Helicobacter pylori*, a major cause of gastric cancer, to human gastric mucus [98]. The earliest report of potential anti-carcinogenic activity appeared in 1996 in the University of Illinois [99].

Extracts of cranberry and bilberry were observed to inhibit ornithine decarboxylase (ODC) expression and induce the xenobiotic detoxification enzyme quinonereductase *in vitro* [99]. Subsequent studies with cranberry and other berries in cellular models have focused on some cancers such as breast, colon, liver, prostate and lung [100-102]. This biological activity of berries are partially attributed to their high content of a diverse range of phytochemicals such as flavonoids (anthocyanins, flavonols, and flavanols), tannins (proanthocyanidins, ellagitannins, and gallotannins), quercetin, phenolic acids, lignans, and stilbenoids (e.g., resveratrol) [100]. With respect to his genotoxic and/or antigenotoxic potential, there are few reports in the literature that demonstrate this effect and the majority of studies were performed *in vitro* cell culture models [101,103,104]. Boateng *et al*. demonstrated that consumption of some juices of berries (as blueberries, blackberries, and cranberry) can reduce the formation of aberrant crypt foci (ACF) induced by azoxymethane in Fisher male rats [105]. Another study, in which it was administrated a lyophilized extract of *Vaccinium ashei* berries in male Swiss mice during 30 days, showed to have improved the performance on memory tasks and has a protective effect on the DNA damage in brain tissue evaluated with the comet assay [106].

Although the types of berry fruits consumed worldwide are many, the experiment executed in our laboratory is focuses on cranberries that are commonly consumed in Mexico, especially in the states of Tlaxcala, Hidalgo, and Puebla. The purpose of our study was to determine whether cranberry ethanolic extract (CEE) can prevent the DNA damage produced by benzo[a]pyrene (B[a]P) using an in vivo mouse peripheral blood micronucleus assay. The experimental groups were organized as follows: a negative control group (without treatment), a positive group treated with B[a]P (200 mg/kg), a group administered with 800 mg/kg of cranberry ethanolic extract, and three groups treated with B[a]P and cranberry ethanolic extract (200, 400, and 800 mg/kg) respectively. The CEE and benzo[a]pyrene were administered orally for a week, on a daily basis. During this period the body weight, the feed intake, and the determination of antigenotoxic potential were quantified. At the end of this period, we continued with the same determinations for one week more (recovery period) but anymore administration of the substances. The animals treated with B[a]P showed a weight increase after the first week of administration. The same phenomenon was observed in the lots combined with B[a]P and CEE (low and medium doses). The dose of 800 mg/kg of CEE showed similar values to the control group at the end of the treatment period. In the second part of the assay, when the substances were not administered, these experimental groups regained their normal weight. The dose of CEE (800 mg/kg) was not genotoxic nor cytotoxic. On the contrary, the B[a]P increases the frequency of micronucleated normochromatic erythrocytes (MNNE) and reduces the rate of polychromatic erythrocytes (PE) at the end of the treatment period. With respect to the combined lots, a significant decrease in the MN rate was observed from the sixth to the eighth day of treatment with the two high doses applied; the highest protection (60%) was obtained with 800 mg/kg of CEE. The same dose showed an anticytotoxic effect which corresponded to an improvement of 62.5% in relation to the animals administered with the B[a]P. In the second period, all groups reached values that have been seen in the control group animals. Our results suggest that the inhibition of clastogenicity of the cranberry ethanolic extract against B[a]P is related to the antioxidant capacity of the combination of phytochemicals present in its chemical composition [107].

5.3. Grapefruit juice and naringin

The grapefruit is a subtropical citrus tree known for its bitter fruit. These evergreen trees usually grow around 6 meters tall. The leaves are dark green, long and thin. His fruit (called toronja in Spanish) has become popular since the late 19th century, is yellow-orange skinned and largely an oblate spheroid and generally, is consumed in form of juice [108].

The grapefruit juice is an excellent source of many nutrients and phytochemicals that contribute to a healthy diet. Is a good source of vitamin C, contains the fiber pectin, and the varieties pink and red contain the beneficial antioxidant lycopene [108]. But, the main flavonoid, existing in highest concentration in grapefruit juice is naringin, which in humans is metabolized to naringenin [109].

Since grapefruit juice is known to inhibit enzymes necessary for the clearance of some drugs and hormones, some researchers have hypothesized that grapefruit juice and the naringin may play an indirect role in the development of hormone-dependent cancers. A study found

a correlation between eating a quarter of grapefruit daily and a 30% increase in risk for breast cancer in post-menopausal women. The study points to the inhibition of CYP3A4 enzyme by grapefruit, which metabolizes estrogen [110]. However, an investigation conducted in 2008 has shown that grapefruit consumption does not increase breast cancer risk and found a significant decrease in breast cancer risk with greater intake of grapefruit in women who never used hormone therapy [111].

In the case of naringin, this compound exerts a variety of pharmacological effects such as antioxidant activity, blood lipid lowering, anticancer activity, and inhibition of selected drug-metabolizing cytochrome P450 enzymes, including CYP3A4 and CYP1A2, which may result in drug-drug interactions in vivo. Ingestion of naringin and related flavonoids can also affect the intestinal absorption of certain drugs, leading to either an increase or decrease in circulating drug levels [112].

This evidence has motivated to our research group to develop various studies with grapefruit juice (GJ) and the naringin (Nar) to assess his chemoprotective ability.

Our first experience was with naringin in 2001. On that occasion, the study was designed for three main purposes: (1) to determine whether Nar has a genotoxic effect in mouse in vivo. This was evaluated by measuring the rate of micronucleated polychromatic erythrocytes (MNPE); (2) to determine its antigenotoxic and its anticytotoxic potential on the damage produced by ifosfamide. The first study was done by scoring the rate of MNPE, and the second one by establishing the index polychromatic erythrocytes/normochromatic erythrocytes (PE/NE); and (3) to explore whether its antigenotoxic mechanism of action is related to an inhibitory effect of Nar on the expression of the CYP3A enzyme, an effect which could avoid the biotransformation of ifosfamide.

A single oral administration was used for all groups in the experiment: three groups were given different doses of Nar (50, 250, and 500 mg/kg), other groups received the same doses of Nar plus an administration of ifosfamide (60 mg/kg), another group treated with distilled water and another with ifosfamide (60 mg/kg) were used as negative and positive controls, respectively. The micronuclei and the cell scoring were made in blood samples taken from the tail of the animals at 0, 24, 48, 72, and 96 h. The results showed that Nar was neither genotoxic nor cytotoxic with the doses tested, but ifosfamide produced an increase in the rate of MNPE at 24 and 48 h. The highest value was 24+/-1.57 MNPE per thousand cells at 48 h. The index PE/NE was significantly reduced by ifosfamide at 24 and 48 h. Concerning the antigenotoxic capacity of Nar, a significant decrease was observed in the MNPE produced by ifosfamide at the three tested doses. This effect was dose-dependent, showing the highest reduction in MNPE frequency (54.2%) at 48 h with 500 mg/kg of Nar. However, no protection on the cytotoxicity produced by ifosfamide was observed. Immunoblot analysis was used to assess the CYP3A expression in liver and intestinal microsomes from mouse exposed orally to Nar. An induction in the CYP3A protein was observed in both intestinal and hepatic microsomes from treated mice. This induction correlated with an increase in erythromycin N-demethylase activity. These data suggest that other mechanism(s) are involved in the antigenotoxic action of naringin [113].

With regard to grapefruit juice (GJ), we performed two experiments which are summarized below. The first evaluated the capacity of GJ to inhibit the micronucleated polychromatic erythrocytes (MNPE) produced by daunorubicin in an acute assay in mice, as well as to determine its antioxidant potential in mouse hepatic microsomes, and its capacity to trap free radicals in vitro.

The results showed that GJ is not toxic or genotoxic damage; on the contrary, it generated a significant reduction of the MNPE formed by daunorubicin. The effect was found throughout the examined schedule (from 24 to 96 h). The two high doses produced inhibition of about 60% at 48 h, 86% at 72 h and 100% at 96 h after the treatment. With respect to the grapefruit juice antioxidant potential, a 50% decrease in liver microsomal lipid peroxidation produced by daunorubicin was found by quantifying malondialdehyde formation. Finally, a strong GJ scavenging activity evaluated with the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) was observed, giving rise to a concentration-dependent curve with a correlation coefficient of 0.98. Overall, our results established an efficient anticlastogenic potential of grapefruit juice, probably related to its antioxidant capacity, or to alterations of daunorubicin metabolism [114].

Based on this background; recently, we finished another study in which using the comet assay was demonstrated a strong effect by hydrogen peroxide (HP) and no damage by grapefruit juice (GJ) in human lymphocytes. Cells exposed to HP and treated with GJ was shown an increase of DNA damage by HP over the control level, and a decrease of such damage by GJ. With the comet assay plus formamidopyrimidine-DNA-glycosylase we found the strongest increase of DNA damage by HP over the control level, and the strongest reduction of such damage by GJ. By applying the comet/FISH method we determined 98% of the p53 gene signals in the comet head of control cells along the experiment, in contrast with about 90% signals in the comet tail of cells exposed to HP. Cells treated with both agents showed a significant, concentration/time dependent return of p53 signals to the head, suggesting enhancement of the gene repair. Finally, with the annexin V assay we found an increase in apoptosis and necrosis by HP, and no effect by GJ; when GJ was added to HP treated cells no modification was observed in regard to apoptosis, although a decrease of necrosis was observed [115].

5.4. Chamomile

Chamomile (*Matricaria chamomilla* or *Chamomilla recutita*) is an asteraceae plant native to Europe and distributed around the world, except in tropical and polar regions. This plant has been used for its curative properties since ancient Egyptian and Greek times, and at present is frequently used as an antiseptic, antiflogistic, diuretic, expectorant, febrifuge, sedative, anti-inflammatory and anticarcinogen [116]. Pharmacological activities of various components of the plant have been reported, for example, the anti-inflammatory capacity and the modulating effects of the heat shock protein on apigenin and quercetin flavonoids, as well as the anti-inflammatory, antioxidant, and antiseptic activities detected on α-bisabolol, guargazulene, and chamazulene [117, 118]. The essential oil extracted from the chamomile flower var-

ies from 0.42 to 2%, and consists of compounds such as bisabolol, chamazulene, cyclic sesquiterpenes, bisabolol oxides, and other azulenes and terpenes [119].

With respect to his genotoxic and/or antigenotoxic potential, there are few reports in the literature that demonstrate this effect. Therefore, our laboratory performed two investigations with the main purpose to evaluate the chemoprotection capacity of chamomile. Initially, we obtained the chamomile essential oil (CEO) from flowers of *Chamomilla recutita* by steam distillation, and then it was analyzed by gas chromatography to identify the chemical species. Thirteen compounds were determined with this assay, including bisabolol and its oxides, β-farnecene, chamazulene, germacrene, and sesquiterpenes (Table 3).

Compound	RT[a]	Area (%)
(*E*)-β-Farnecene	38.46	28.17
Germacrene-D	39.23	2.19
Unidentified sesquiterpene	40.07	1.40
Unidentified sesquiterpene	41.17	0.78
(Z,E)–α–Farnecene	41.35	1.59
Unidentifiedsesquiterpene	48.52	0.71
α–Bisabolol oxide A	54.46	41.77
α–Bisabolol oxide B	49.28	4.31
α-Bisabolol oxide	50.65	5.30
α–Bisabolol	51.18	2.31
Chamazulene	52.80	2.39
1,6-Dioxaspiro[4,4]non-3-ene,2-(2,4hexadyn-1-ylidene)	60.73	2.19
Hexatriacontane	67.49	0.50

RT[a], Retention time obtained with gas chromatography.

Table 3. Components of the tested chamomile essential oil

The first work was to determine the inhibitory effect of the CEO, on the sister chromatid exchanges (SCEs) produced by daunorubicin and methyl methanesulfonate (MMS) in mouse bone marrow cells.

The authors performed a toxic and genotoxic assay of chamomile essential oil; both showed negative results. To determine whether CEO can inhibit the mutagenic effects induced by daunorubicin, one group of mice was administered corn oil, another group was treated with the mutagen (10 mg/kg), a third group was treated with 500 mg/kg of CEO; three other groups were treated first with CEO (5, 50 and 500 mg/kg) and then with 10 mg/kg of daunorubicin. In the case of MMS, the experimental groups consisted of the following: the negative control group which was administered corn oil, a group treated with 25 mg/kg of MMS,

a group treated with 1000 mg/kg of CEO, and three groups treated first with CEO (250, 500 and 1000 mg/kg) and then with MMS (25 mg/kg). The results indicated a dose-dependent inhibitory effect on the SCEs formed by both mutagens. In the case of daunorubicin, a statistically significant result was observed in the three tested doses: from the lowest to the highest dose, the inhibitory values corresponded to 25.7, 63.1 and 75.5%. No alterations were found with respect to the cellular proliferation kinetics, but a reduction in the mitotic index was detected. As regards MMS, the inhibitory values were 24.8, 45.8 and 60.6%; no alterations were found in either the cellular proliferation kinetics or in the mitotic indices [120]. This results suggested that CEO may be an effective antimutagen and was the reason for develop the second study.

The aim of the second investigation was to determine the inhibitory potential of CEO on the genotoxic damage produced by daunorubicin (DAU) in mice germ cells. We evaluated the effect of 5, 50, and 500 mg/kg of essential oil on the rate of sister chromatid exchange (SCE) induced in spermatogonia by 10 mg/kg of the mutagen. We found no genotoxicity of CEO, but detected an inhibition of SCE after the damage induced by DAU; from the lowest to the highest dose of CEO we found an inhibition of 47.5%, 61.9%, and 93.5%, respectively. As a possible mechanism of action, the antioxidant capacity of CEO was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method and ferric thiocyanate assays. In the first test we observed a moderate scavenging potential of the oil; nevertheless, the second assay showed an antioxidant capacity similar to that observed with vitamin E. In conclusion, we found that CEO is an efficient chemoprotective agent against the damage induced by DAU in the precursor cells of the germinal line of mice, and that its antioxidant capacity may induce this effect [116].

5.5. Silymarin

Silybum marianum is the scientific name of milk thistle or St. Mary's thistle. It is a Mediterranean native plant belonging to the Asteraceae family. It is characterized by thorny branches, a milky sap, with oval leaves that reach up to 30 centimeters, its flowers are bright pink and can measure up to 8 cm to diameter [121].

Milk thistle (Mt) grows of wild form in the southern Europe, the northern Africa and the Middle East but is cultivated in Hungary, China and South American countries as Argentina, Venezuela and Ecuador. In México, is consumed as supplement food for many years ago [122].

In the sixties years, German scientists performed a chemical investigation of his fruits, isolating a crude extract formed by active compounds with hepatoprotective capacity; this group of compounds was called silymarin. In 1975, it was found that the principal components of silymarin are silybin A, silybin B, isosilybin A, isosilybin B, silychristin A, silychristin B and silydianin [123]. Currently it is known that the chemical constituents of silymarin are flavonolignans, ie, a combination conformed by flavonoids and lignins structures [124].

Mt is one of the most investigated plant extracts with known mechanisms of action for oral treatment of toxic liver damage. Silymarin is used as a protective treatment in acute and chronic liver diseases [125]. His protective capacity is related with different mechanisms as suppress toxin penetration into the hepatic cells, increasing superoxide dismutase activity, increasing glutathione tissue level, inhibition of lipid peroxidation and enhancing hepatocyte protein synthesis. The hepatoprotective activity of silymarin can be explained based on antioxidant properties due to the phenolic nature of flavonolignans. It also acts through stimulating liver cells regeneration and cell membrane stabilization to prevent hepatotoxic agents from entering hepatocytes [126].

Silymarin is also beneficial for reducing the chances for developing certain cancers [127]. The molecular targets of silymarin for cancer prevention have been studied. Milk thistle interfere with the expressions of cell cycle regulators and proteins involved in apoptosis to modulate the imbalance between cell survival and apoptosis. Sy-Cordero et al. (2010) isolated four key flavonolign and diastereoisomers (silybin A, silybin B, isosilybin A and isosilybin B) from *S. marianum* in gram scale. These compounds and other two related analogues, present in extremely minute quantities, were evaluated for antiproliferative/cytotoxic activity against human prostate cancer cell lines. Isosilybin B showed the most potent activity [126]. The isolation of six isomers afforded a preliminary analysis of structure-activity relationship toward prostate cancer prevention. The results suggested that an *ortho* relationship for the hydroxyl and methoxy substituents in silybin A, silybin B, isosilybin A and isosilybin B was more favorable than the *meta* relationship for the same substituents in the minor flavonolignans. Silymarin suppressed UVA-induced oxidative stress that can induce skin damage. Therefore, topical application of silymarin can be a useful strategy for protecting against skin cancer [128].

In our laboratory, we evaluated the antigenotoxic effect of two doses of silymarin (200 and 400 mg/Kg) administered by oral gavage against the chronic consumption of ethanol (solution: 92 mL of water/8 mL of ethanol) during a week with alkaline single cell electrophoresis (comet) assay.

Figure 2 shows the comet measurements obtained in our assay. To summarize, at the 24 hours of the schedule we found no significant DNA damage induced in the control group (only water) and the silymarin group (400 mg/kg), both groups had a mean T/N index of 1.1. On the contrary, the mice (strain CD-1) that consumed the solution of ethanol showed a slight comet increase during this same time. But at 48, 72 and 96 hours, this group showed a T/N index increase of about four times as much. During the last times (120, 144, 168 and 192) there is a decrease of DNA damage, suggesting that hepatocytes are in the process of cell regeneration. With respect to the groups treated with the combination of chemicals, a clear antigenotoxic effect was found with the two doses of silymarin; particularly with 400 mg/kg, the prevention of DNA damage was about 70% during the 48, 72, 96 and 120 hours of treatment. At the end of the experiment, these groups reached similar values to the negative control [129].
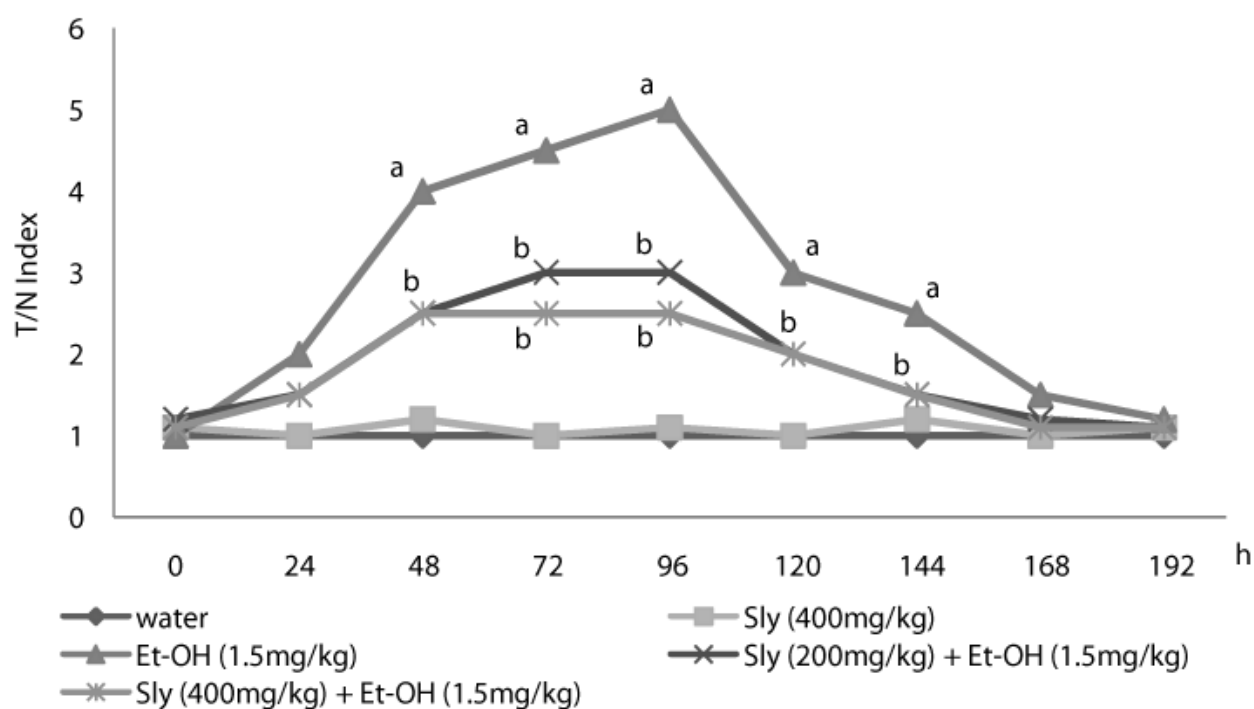
Figure 2. Antigenotoxic effect of silymarin (Sly) against the DNA damage induced by the chronic consumption of etanol (Et-OH). Results are the mean ± SD of 5 mice per group (100 nuclei per doses) [a] statistically significant difference with respect to the value of the control group and, [b] with respect to the value obtained in mice treated with Et-OH only. ANOVA and Student-Newman Keuls tests, $p \leq 0.05$.

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