CHAPTER 2

REVIEW OF LITERATURE
2.1 PHARMACOLOGICAL PROPERTIES OF ONION

*Allium* vegetables health properties have been supported by numerous *in vitro*, *in vivo*, and *ex-vivo* studies. Particularly, onion has been described to have several health benefits related to its antioxidant, anticarcinogenic, hypolipidemic, hypoglycaemic, or antiaggregatory effects. From a medical and nutritionally point of view, it has to be taken into account that the onion used as a food or a food ingredient in the elaboration of many dishes also exerts a wide variety of medicinal effects which are very interesting for its human health potential benefits. Traditionally, in the folk medicine, it has been described the use of onion as an antimicrobial, cardiovascular-supportive, hypoglycemic, antioxidant, anticancer, and asthma-protective agent. It has been described that a diet rich in *Allium* vegetables, including onion, would lead to several and different health benefits that could be helpful in the prevention of two of the more relevant and prevalent diseases nowadays such as cancer or CVD.

Many epidemiological studies suggest that regular consumption of onions in food is associated with a reduced risk of various degenerative diseases. Their biologically active molecules are effective antioxidants against the lethal effect of oxidative stress [Gülsen et al., 2007; Prakash et al., 2007]. They are also reported to have liver protective effect, immune enhancement potential and anti-infection, anti-stress, anti-cancer and other pharmacological properties [Corzo et al., 2007; Valko et al., 2007].

2.1.1 Antimicrobial activity

*Allium* vegetables have long been known for their antimicrobial activity against various microorganisms, including Gram-positive and Gram-negative bacteria (*Staphilococcus aureus* and *Salmonella enteriditis*) [Zohri et al., 1995] and fungi (*Aspergillus niger*, *Penicillium cyclopium* and *Fusarium oxysporum*) [Benkeblia and Varoquaux, 2003]. There have been few applications of *Allium* vegetables as natural food preservatives, in spite of numerous studies on antimicrobial activity of these vegetables. Different onion extracts and oils have proven antimicrobial and antioxidant properties which are interesting from a technological and nutritional point of view [Irkin and Korukluoglu, 2007; Choi et al., 2008]. Organosulfur compounds
OSCs) and phenolic compounds have been reported to be involved in the onion antimicrobial activity [Takahama and Hirota, 2000; Griffiths et al., 2002; Kim et al., 2004].

2.1.1.1 Antifungal activity

Onions have been shown to possess antibacterial and antifungal properties. Volatile oil of onion has been shown to be highly effective against gram positive bacteria, dermatophytic fungi, growth and aflatoxin production of Aspergillus fungi genera including Aspergillus niger, Brettanomyces anomalus, Candida albicans, C. lipolytica, Cladosporium werneckii, Fusarium oxysporium, Geotrichum candidum and Saccharomyces cerevisiae [Takahama and Hirota, 2000; Griffiths et al., 2002; Corzo et al., 2007]. Aqueous extract or the juice of onion has been reported to inhibit in vitro growth of Escherichia coli, Serratia marcescens, Steptococcus species, Acetobacillus odontolyticus, Pseudomonas aeruginosa and Salmonella typhosa [Bison, 1994].

Onion extracts are also effective against many yeast species and their essential oil inhibits the dermatophytic fungi [Zohri et al., 1995]. The active compounds of onion destroy fungal cells decreasing the oxygen uptake, reducing cellular growth, inhibiting the synthesis of lipids, proteins and nucleic acids, changing the lipid profile of the cell membrane and inhibiting the synthesis of the fungal cell wall [Gupta and Porter, 2001]. The main active antifungal agents from onion are the breakdown products of allicin, including diallyl trisulphide (DATS), DADS and DAS [Tansey and Appleton, 1975]. In addition to sulphur compounds, a great variety of antifungal proteins and peptides have been isolated from several Allium species such as allicepin, a novel isolated antifungal peptide from onion bulbs [Wang and Ng, 2004].

2.1.1.2 Antibacterial activity

The antibacterial effects of onion extracts against oral pathogenic bacteria have been studied by Kim et al. [2004]. A previous study showed that the addition of garlic and onion powders in enhanced meats had an antioxidant activity as effective as that of sodium ascorbate and also an antimicrobial effect to inhibit the growth of total
bacteria and bacteria from the *Enterobacteriaceae* family [Park et al., 2008]. Organosulphur compounds have been reported to be responsible for antibacterial effects of onion extract against oral pathogenic bacteria causing dental caries [Griffiths et al., 2002; Kim et al., 2004]. Onion extract, the activity of which remained stable for 48 h, inhibited *Streptococcus mutans*, a bacterium that causes strep throat, tonsillitis, bacterial pneumonia, as well as other diseases [Ali et al., 2000]. Onion extracts possess an effect on all bacterial strains tested including *Streptococcus mutans* (JC-2), *S. sobrinus* (OMZ176), *Porphyromonas gingivalis* (ATCC 33277) and *Prevotella intermedia* (ATCC 25611). The effects were bactericidal against both cultured and resting bacterial cells. The activity of the onion extracts was stable even after 48 h in the culture medium. The antibacterial effect of onion extracts was not markedly influenced by cysteine (10 mM) treatment, but the activity was significantly decreased with alkali treatment. Activity was lost upon standing of grated onion at 37°C or steam treatment (100°C, 10 min). It is suggested that S-propenylcysteinesulphoxide is the compound that inhibits antibacterial metabolism [Kyung and Lee, 2001].

In addition to organo-sulphur compounds, it has been reported that certain quercetin oxidation products found in onion also present antibacterial activity against *H. pylori* and MRSA (multidrug-resistant *S. aureus*) [Ramos et al., 2006]. In addition to inhibitory effects against pathogenic bacteria, onions have been found to promote beneficial microorganisms. Onions contain FOS, probiotics which are non-digestible ingredients fermented by bifido bacteria in the body that help maintain the health of the gut and colon [Baker, 1980]. Onions contain 2.8% FOS (wet weight) as compared to 1.0% FOS in garlic, 0.7% in rye, and 0.3% in bananas.

### 2.1.1.3 Antiviral activity

It has been reported that quercetin, the major onion flavonoid, also possesses antiviral activity and enhances the bioavailability of some antiviral drugs [Wu et al., 2005]. Lectins are a very heterogeneous group of glycoproteins with the ability to recognize and bind specifically to carbohydrate ligands. Onion lectins have a pronounced anti-HIV activity [Damme et al., 1993].
2.1.2 Anticarcinogenic activity

The association between consumption of Allium vegetables and risk for cancer has been assessed in several epidemiologic studies, mainly case-control [Bianchini and Vainio, 2001; Galeone et al., 2006]. In general, these studies are more consistent in reporting a protective effect of onion in gastric cancer. However, onion consumption has been also consistently related with a decreased colorectal cancer risk. In addition, onion consumption was reported to decrease the risk for the cancer of the lung [Sankaranarayanan et al., 1994] and of the brain [Hu et al., 1999] in case-control studies.

Onion consumption was significantly inversely correlated with the risk of the stomach cancer. Gonzalez et al. [2006] observed a probable protective effect of total vegetables and Allium vegetables intake on the intestinal type of gastric cancer. Most of the case-control studies concerning onion were conducted in China and several of them in Asia and Europe. The chemopreventive effects of onion against stomach and esophageal cancers may be related to their antibacterial properties. Inhibition of bacterial growth in the gastric cavity may result in less conversion of nitrate to nitrite in the stomach, a decreased probability of endogenous formation of carcinogenic N-nitroso compounds, and reduction in Helicobacter pylori infection specifically [You et al., 1989; Tuyns et al., 1992, Hansson et al., 1993, Gao et al., 1999].

Diets rich in fruit and deep-yellow vegetables, dark-green vegetables, and onions and garlic are modestly associated with reduced risk of colorectal adenoma, a precursor of colorectal cancer [Millen et al., 2007]. The effect was particularly significant for consumption of cooked onions and leeks in Belgium [Tuyns et al., 1988], for a combination of garlic, onions, and pepper in Argentina [Iscovich et al., 1992]. It was reported a lower risk for both sexes, with a more pronounced decrease for women and for cancer of the proximal compared with the distal colon [Steinmetz et al., 1993].

Onion exert their anticarcinogenic action of indirect way by different mechanisms: alteration of carcinogen metabolism either increasing the detoxification enzymatic systems activity that increases the carcinogen polarity, facilitating its excretion from
the body; or inhibition of oxidative damage due to their antioxidant action; inhibition of cellular proliferation by induction of apoptosis and inhibition of cell division, prevention of chromosomal damage (anticlastogenic effect); and inhibition of the lipoxygenase and cyclooxygenase activities (anti-inflammatory effect) [Guyonnet et al., 1999; Khanum et al., 2004; Rose et al., 2005; Corzo et al., 2007].

Several investigations have shown that both water- and lipid-soluble sulphur compounds from onion provide their anticarcinogen benefits. Dipropyl sulphide (DPS) and DPDS from onions can inhibit both early and late stages of carcinogenesis [Guyonnet et al., 1999]. Other sulphur compounds, as methiin (abundant in onion), can inhibit the cellular proliferation by inducing apoptosis in human cell cultures, for example, in human leukaemic cells. In addition to organo-sulphur compounds, Selinium (Se) compounds are largely responsible for the anticarcinogenic activity of onion. Se-enriched onion has higher anticarcinogenic activity than the common plants [Corzo et al., 2007]. This increased effect of cancer prevention is achieved at least partly by S substitution with Se. The pure Se compounds have proved to be superior anticancer agents than their corresponding S-analogues. The two major Se compounds possessing anticancer activity in onion are gamma-glutamyl-Se-methyl selenocysteine and Se-methyl selenocysteine, being Se-methyl selenocysteine and Se-allyl selenocysteine the most chemopreventive Se-compounds [Block et al., 1997].

Quercetin and kaempferol, from onion, also possess anticarcinogenic properties. Particularly, they have antineoplastic effects by inhibiting bioactivating enzymes [Lautraite et al., 2002], by inducing detoxifying enzymes, by inducing apoptosis [Brisdelli et al., 2007], and due to their antioxidant and anti-inflammatory activities [Raso et al., 2001]. Several epidemiological studies have found inverse associations between lung cancer risk and onion intake, probably due to its high content of flavonoids [Le Marchand et al., 2000]. Moreover, several studies have reported that quercetin enhances bioavailability of some anticancer drugs, as Tamoxifen, a non-steroidal antiestrogen for treating and preventing breast cancer, by promoting their intestinal absorption and reducing their metabolism; [Wu et al., 2005; Shin et al., 2006].
2.1.3 Use of Onion and Cardiovascular Disease

Cardiovascular disease (CVD) includes coronary heart disease (heart attacks), cerebrovascular disease, raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. If current trends are allowed to continue, by 2015 an estimated 20 million people will die from CVD [WHO, Cardiovascular disease]. Therefore, CVD have a major impact on the mortality and quality of life of human populations across the world, despite improvements in lifestyle and innovations in the prevention and treatment of CVD in previous decades. The study by Galeone et al. [2009], the first from Mediterranean countries, suggests that a diet rich in onions may have a favorable effect on the risk of acute myocardial infarction; therefore these vegetables could be useful in a CVD preventive diet.

Several biomarkers are measured to predict CVD events including blood lipids levels (LDL-cholesterol and triglycerides), fibrinogen (a marker of thrombosis and inflammation), D-dimer (a marker of thrombosis), plasminogen-activator inhibitor type 1 (a marker of fibrinolytic potential and endothelial function), high-sensitivity C reactive protein (CRP) (inflammation marker), homocysteine (a marker of endothelial function and oxidant stress), B-type and N-terminal pro–atrial natriuretic peptides, serum aldosterone, plasma renin (markers of neurohormonal activity), and urinary albumin-to-creatinine ratio (a marker of glomerular endothelial function) [Kannel, 2005; Wang et al., 2006]. Alterations in lipid profiles, diabetes, hypertension, and obesity are risk factors conventionally associated to the early appearance of CVD. Onion has been described to have hypolipidemic, hypoglycaemic, and antithrombotic effects and therefore could be useful in the CVD prevention.

2.1.3.1 Hypolipidemic Effect

Hyperlipidemia is one of the major risk factors for atherosclerosis. Focusing on onion lipid lowering effects, this vegetable has been reported to exert moderately hypolipidemic effects in experimental animal such as healthy pigs fed a high fat diet and consequently potentially reduce risk indices of CVD and obesity [Gabler et al., 2006]. Among bioactive compounds involved in onion hypolipidemic effects,
quercetin has shown to have the ability to reduce serum cholesterol levels and arteriosclerosis severity [Glasser et al., 2002]. A previous study by Kumari and Augusti [2007] also proclaimed for the lipid lowering action of the MCSO isolated from onion.

2.1.3.2 Antithrombotic Effect or Antiplatelet Effect

Thrombosis complications play a major role in CVD. Blood clot formation depends on an intricate series of events involving platelets, other cells, and the activation of specific blood proteins, known as coagulation factors. A thrombus is a blood clot formed when there is an imbalance in the blood coagulation system that can block the flow of blood through a vein or artery, and can detach from the vessel wall to become a life-threatening embolus when it lodges in the lungs or other vital organs. Blood clots in coronary arteries cause acute coronary syndrome and blood clots that form in the heart are the major cause of stroke in people with atrial fibrillation. Onion inhibits platelet aggregation in vitro and in vivo. The mechanism by which onion exerts its antithrombotic effect has been shown to involve the inhibition of thromboxane A2 formation, potent inducer of platelet aggregation [Ali et al., 1999; Ali et al., 2000; Briggs et al., 2001; Jung et al., 2002; Hubbard et al., 2006]. The antiplatelet activity observed in onion is influenced by genotype, environmental factors and genotypically determined sulfur content of the bulb [Goldman et al., 1996; Sance et al., 2008] having onion α-sulfinil-disulfides (cepaenes) a demonstrated antithrombotic activity [Block et al., 1997].

2.1.3.3 Hypoglycemic Effect or Antidiabetic Effect

Onion has also been reported to have hypoglycaemic effects [Srinivasan, 2005]. Several studies have shown that phenolic phytochemicals from onion have blood glucose lowering effect and high antioxidant activity in alloxan-induced diabetic rat [Azuma et al., 2007; EI-Demerdash et al., 2005; Lee et al., 2008]. Administration of onion to diabetic rats was found to improve the diabetic status, including the protection of DNA against oxidatively generated damage and lowering of peroxidized lipids in the circulation and urine and hypoglycemic and hypocholesterolemic effects [Babu and Srinivasan, 1999; Boyle et al., 2000]. Onion intake suppressed oxidative
stress in streptozotocin induced diabetic rats more effectively than the intake of a quercetin-containing diet with an equivalent level of quercetin [Azuma et al., 2007]. A study by Lee et al. [2008] showed that onion skin was effective in controlling hyperglycemia in animal models of type 2 diabetes mellitus, at least in part by inhibiting alpha-glucosidase activity.

Quercetin and sulfur compounds, such as allyl propyl disulfide that have perceived benefits to human health [Griffiths et al., 2002]. Epidemiological studies have also shown that the intake of flavonoids, including quercetin and myricetin is inversely associated with the risk of incident type 2 diabetes [Rigelsky et al., 2002]. Quercetin, Isoquercitrin and rutin have shown inhibitory activities on α-glucosidase from the rat intestine [Jo et al., 2009]. The inhibitory activity of Korean onion extract against rat intestinal α-glucosidases, such as sucrase, maltase, and porcine pancreatic α-amylase were investigated in vitro and in vivo showing that ethyl alcohol extract of onion skin may improve exaggerated postprandial spikes in blood glucose and glucose homeostasis since it inhibits intestinal sucrase and thus delays carbohydrate absorption [Kim et al., 2011].

The bioactive constituents from onion, such as methiin and S-allyl cysteine sulphoxide (SACS), exert their anti-diabetic action by stimulating the insulin production and secretion by pancreas, interfering with dietary glucose absorption, and favouring the insulin saving [Srinivasan, 2005]. Few studies have been directed towards the influence diabetes mellitus and hypoglycemic onion on the activity of GST [Anwar and Meki, 2003]. GST activity might be one of the defense mechanism in these animals to detoxify or neutralize the toxic metabolites, e.g. ketone bodies, generated in liver by the diabetes.

2.1.4 Other Health-Promoting Effects of Onion

2.1.4.1 Bone Health

Bone fractures due to osteoporosis are a health care burden. Dairy and soy have both been proposed as dietary sources of compounds (calcium, phytoestrogens) with potential for improving bone health, but neither has been confirmed as helpful in
clinical trials with humans. Onion consumption has also been reported to be involved in the bone metabolism and in the behaviour as a possible antidepressant agent. Muhlbauer and Li [1999] demonstrated that onion intake by rats was responsible for increasing bone mass, bone thickness, and bone mineral density. Onions inhibited bone resorption by 20% when consumed at a rate of 1g per day per kg of body weight. This was slightly higher than the rate of bone resorption. Matheson et al. [2009] reported that onion consumption seems to have a beneficial effect on bone density in perimenopausal and postmenopausal women. These findings suggest that onion intake may be a useful dietary approach to improving bone health.

Furthermore, older women who consume onions most frequently may decrease their risk of hip fracture by more than 20% versus those who never consume onions. Prevention of low bone mass is important to reduce the incidence of osteoporotic fractures. Onion retains its bone resorption inhibitory activity in the rat when added to a vegetarian diet [Muhlbauer et al., 2002]. Another previous study by Sakakibara et al. [2008] suggests that onion exerted antidepressant-like activity in a behavioural model that acted independently of the hypothalamic-pituitary-adrenal axis.

2.1.4.2 Morphine withdrawal

The in vitro effect of quercetin on morphine withdrawal has been examined. Capasso et al. [1998] found that withdrawal symptoms, measured by naloxone contraction, were reduced on a dose-dependent manner (2.7 x 10^-6 M for IC 50). Previously, Capasso and Sorrentino [1997] showed that arachidonic acid and its metabolites (prostaglandins and leukotrienes) are involved in the development of morphine withdrawal. Onion capaenes and thiosulfimates have been shown to inhibit cyclooxygenase and 5-lipoxygenase activity [Wagner et al., 1990]. The anticholinergic effects of quercetin also were hypothesized to attenuate morphine withdrawal, normally thought to be exacerbated by acetylcholine stimulation [Capasso et al., 1998].
2.2 TECHNOLOGICAL PROPERTIES OF ONIONS

2.2.1 Antioxidant Activity of Onions

Antioxidant activity from a high intake of fruits and vegetables has been reported to prevent alteration of DNA by ROS. Flavonoids, ubiquitous in the plant kingdom, have been widely studied for their antioxidative effects [Rice-Evans et al., 1995]. Onions are known to contain anthocyanins and the flavonoids quercetin and kaempferol [Bilyk et al., 1984; Rhodes and Price, 1996]. However, anthocyanin pigments, concentrated in the outer shell of red onions, are only minor constituents of the edible portion [Rhodes and Price, 1996]. Kaempferol, while detectable in certain onion varieties, is present in much smaller quantities than quercetin [Bilyk et al., 1984; Rice-Evans et al., 1995]. Therefore, quercetin is the major flavonoid of interest in onions. Mechanisms of action include free radical scavenging, chelation of transition metal ions, and inhibition of oxidases such as lipoxygenase [Boots et al., 2008].

The antioxidative effects of consumption of onions have been associated with a reduced risk of neurodegenerative disorders, many forms of cancer, cataract formation, ulcer development and prevention of vascular and heart disease by inhibition of lipid peroxidation and lowering of low density lipoprotein (LDL) cholesterol levels [Frémont et al., 1998; Landis-Piwowar et al., 2008; Orsolic et al., 2004; Zanin et al., 2009]. Inhibition of oxidases, enzymes that liberate free radicals, can be direct or indirect. Protection from arachidonic acid metabolites and lipoxygenase activity is important in prevention of vascular disease [Juurlink et al., 1998]. Quercetin has been shown to not only directly inhibit the lipoxygenase enzyme, but to also suppress consumption of alpha-tocopherol and to preserve human serum paraoxonase (PON 1), both potent antioxidants against lipid peroxidation [Boesch-saadatmandi et al., 2010].

Metal chelation involves formation of a complex with the flavonoid and prevention of catalytic radical production, whereas free radical scavenging activities relate to the flavonoid donating a hydrogen atom and creating a more stable radical [de Groot and Rauen, 1998]. Structural qualities of quercetin and other flavonoids that provide for
effective free radical scavenging are: Presence of ortho-dihydroxyl (catechol) structure in B ring; 2,3-double bond in conjunction with 4-oxo function in C ring; and additional presence of 3- and 5-hydroxyl groups [Boots et al., 2008]. Rice-Evans et al. [1995] found that removal of the 2,3-double bond in the C ring, as in catechin and epicatechin, resulted in a 50% decrease in antioxidant activity [Figure 2.1].

Figure 2.1

![Chemical structure of quercetin](image)

Figure 2.1. Chemical structure of quercetin quercetin and other flavonoids that provide for effective free radical scavenging

Researchers comparing antioxidant activity measured in Trolox equivalents equates 1 glass (150 mL) red wine with 2 cups tea, 5 portions onion (~100g/portion), 7 glasses orange juice, and 20 glasses apple juice [Paganga et al., 1999]. Absorption and bioavailability of flavonoids in onions have been shown to be more effective than from other sources (i.e. tea and apples) [de Vries et al., 1998].

2.2.2 Varietal Differences in Onions

Better quality, high yield, uniformity, and resistance to diseases are major breeding achievements in onion. Important bulb quality traits are bulb size, shape, color, pungency, firmness, dormancy, and amount of soluble solids. Bulb shape, size, and soluble solids show continuous phenotypic variation that suggests quantitative inheritance [McCollum, 1971]. In addition, McCollum [1971] reported significant environmental effects and low heritability of bulb diameter and weight, high heritability of soluble solids (62–82%), and a negative genetic correlation between
bulb size and soluble solids. Havey et al. [2004] evaluated a segregating family from a cross of high and low solids onion parent and reported phenotypic correlations among soluble carbohydrates, pungency and antiplatelet activity. He also emphasized health-enhancing attributes of onion.

Quantities of phytochemicals in onions can vary greatly due to varietal differences [Bilyk et al., 1984]. Geographical location, storage, and genetic factors have all been determined to affect the levels of quercetin found in onions [Patil et al., 1995]. Yellow, red, and pink onions have been shown to contain higher amounts of quercetin than white varieties, but it was determined that color is not the limiting factor [Patil et al., 1995]. Tepe et al. [2005] investigated methanol extracts of five different Allium species other than A. cepa. Prakash et al. [2007] compared phenolic content and antioxidant capacity of four onion scale color, green, white, red and violet, with no variety was specified. They suggested that red onions have the highest quercetin and kaempherol contents among the other onion types. Santas et al. [2008] compared the polyphenol content and antioxidant capacities of white and Calcut onion. Gocke et al. [2010] suggested that the red onions had higher antioxidant activities than yellow and white onions although yellow onions had the richest phenolic contents.

However, accessibility to light (i.e. skin color) has been associated with flavonoid development [Patil and Pike, 1995]. Storage temperature and duration have been shown to have significant effects on quercetin content, but a relative pattern was not elucidated [Patil et al., 1995]. Differences in concentration due to growing location were also found, but identification of exact environmental factors was not determined [Patil et al., 1995]. High bulb sulfur content and percent solids were associated with increased antiplatelet activity [Goldman et al., 1996]. Therefore, highly pungent genotypes may confer more health benefits than mild varieties.

2.2.3 Antibrowning Properties of Onions

The browning reaction is a widespread phenomenon in fruits and vegetables, resulting from mechanical or physiological injury during post-harvest storage or processing. It is a major factor contributing to quality loss in foods and beverages. The polyphenol
oxidase (PPO) enzyme mainly caused the enzymatic oxidation of endogenous phenols into quinones, which then polymerize into brown products, Figure 2.2. Thus, polyphenolic compounds and PPO are directly responsible for the enzymatic browning. The use of sulfiting agents is the most widespread chemical approach for controlling browning. Among the compounds that have been shown to inhibit the PPO activity are sulfites, ascorbic acid and its derivatives, and thiol compounds such as cysteine [Martinez and Whitaker, 1995; Negishi and Ozawa, 2000; Jang et al., 2002].

**Figure 2.2**

![Mechanism of inhibition of enzymatic browning by thiol compounds and protection of active sites of sulfhydryl enzymes [Negishi and Ozawa, 2000]](image)

Onion has been reported to inhibit PPO of fruits such as pear or banana. Kim et al. [2005] reported that thiol compounds in onion might be the active components responsible for the inhibitory effect of onion extract. When the onion extract was dialyzed, the inhibitory effect against pear PPO was completely eliminated, suggesting that the low molecular compounds were responsible of the pear PPO inhibitory effects. Moreover, when onion extracts were heated the pear PPO inhibition
was more efficient. Lee [2007] also found that heat treated onion extracts inhibited banana PPO. Furthermore, it was shown that Maillard reaction products significantly inhibited banana PPO as well as the addition of various antibrowning agents.

2.3 ABSORPTION, BIOAVAILABILITY AND METABOLISM OF FLAVONOIDS IN ONION

Onion, being a source of various biologically active phytomolecules and the role of dietary flavonoids in human health, the concentrations and forms that are present in plasma and tissues after ingestion of these flavonoids present in onion with the diet is an important factor. Therefore, it is essential to study their absorption, metabolism, and bioavailability. Absorption and metabolism of the flavonol quercetin and its glycosides have been described by Aherne and O’Brien [2002]. Quercetin glycosides, the mostly present form in onions, are converted to the respective aglycones in the large intestine by the glycosidase activity of intestinal bacteria and absorbed. Several studies have demonstrated that quercetin glycosides are absorbed more efficiently than quercetin aglycone [Hollman et al., 1995; Moon et al., 2000] irrespective of the position of their glucose moiety [Olthof et al., 1998]. Quercetin glycosides in onion powder were mainly quercetin-3,4′-O-glucoside and 4′-O-glucoside.

A study with ileostomy subjects who lack a colon with bacteria showed unexpectedly high absorption of quercetin-glycosides (quercetin-4O-glucoside and quercetin-3,4′-O-bis-glucoside) from onions (52%) [Hollman et al., 1995]. These data also suggested that absorption of quercetin glucosides takes place in the small intestine. The hydrophilic quercetin glucoside is transported across the small intestine, it was proposed that the intestinal Na⁺-dependent glucose cotransporter was involved [Hollman et al., 1999]. This would imply that intact quercetin glucoside can be transported across the enterocyte and possibly could appear in the plasma.

The bioavailability of quercetin glucosides from onions was superior. After being absorbed, quercetin is metabolized an excreted. Mullen et al. [2006] found five metabolites in quantifiable amounts in human plasma after onion ingestion (quercetin-
3-glucuronide, quercetin-3’-sulfate, isorhamnetin-3-glucuronide, a quercetin diglucuronide and a quercetin glucuronide sulfate). They also reported that total urinary excretion of quercetin metabolites was 12.9 mmol, corresponding to 4.7% of intake. Bioavailability of various quercetin glycosides (b-galactosides and b-xylosides) from apples and of pure quercetin rutinoside was only 30% of that from onions [Hollman et al., 1997]. Thus, the sugar moiety of quercetin glycosides seemed to be an important determinant of their bioavailability, which was confirmed when pure quercetin-b-glucoside or pure quercetin-b-rutinoside was administered to healthy human volunteers [Hollman et al., 1999]. The peak concentration of quercetin in plasma was 20-times higher and reached more than 10-times faster after intake of the glucoside than after the rutinoside. These pharmacokinetic data suggest that quercetin glucoside was absorbed from the small intestine, whereas quercetin rutinoside was absorbed from the colon after deglycosylation. Evidently, the sugar moiety played no role in the elimination of quercetin from plasma: elimination half-life was about 20 h for all glycosides. This is consistent with the observation that quercetin glucosides do not circulate in the blood [Sesink et al., 2003].

One characteristic feature of quercetin bioavailability is that the elimination of quercetin metabolites is quite slow, with reported half-lives ranging from 11 to 28 h. This could favor accumulation in plasma with repeated intakes. A few authors investigated the bioavailability of quercetin after several days or weeks of supplementation. Baseline quercetin concentrations, measured after overnight fasting, were generally 50–80 nmol/L, and values were even lower when a low-polyphenol diet was given to the volunteers before a test meal [Erlund et al., 2002]. The increase was more pronounced in 2 other studies; plasma concentrations reached 1.5 mol/L after 28 days of supplementation with a high dose of quercetin (1 g/day) and 0.63 mol/L after supplementation with 80 mg/d quercetin equivalents for 1 week [Moon et al., 2000; Manach et al., 2005].

In a number of human intervention studies the nature of the conjugates has been identified. Major conjugates of quercetin after onions supplementation were the 30-sulfate, the 30-methoxy-3-glucuronide, and the 3-glucuronide [Day et al., 2003]. The 30-sulfate could not be confirmed with Liquid Chromatography-Mass Spectrometry =

\[ \text{Day et al., 2003} \]
Mass Spectrometry (Tandem Mass Spectrometry) (LC-MS=MS) in another study [Wittig et al., 2001].

Figure 2.3

Figure 2.3. Absorption and bioavailability of quercetin glycosides present in onions

2.4 PROOXIDANT PROPERTIES OF QUERCETIN IN ONIONS

Several studies have shown that catechol-type polyphenols including quercetin besides acting as antioxidants, also acts as prooxidants [Guohua et al., 1997]. The excessive intake of quercetin has been suggested to exert adverse effects on the body by acting as mutagens, prooxidants that generate free radicals, inhibitors of drug-metabolizing enzymes and key enzymes involved in hormone metabolism [Galati and O’Brien, 2004; Bando et al., 2007]. The prooxidant action of quercetin has been shown to induce nuclear DNA damage and lipid peroxidation in the presence of transition metals and is related to mutagenicity [Rahman et al., 1992; Ahmed et al., 1992]. Choi et al. [2003] have found that orally administered quercetin acted as both an antioxidant and prooxidant in the rat liver. Furthermore, the oxidation products of
quercetin are reactive with the protein thiol group resulting in toxic effects. Consequently, the excessive intake of quercetin might show deleterious effects by acting as a prooxidant [Bando et al., 2007]. The excessive intake of quercetin has been suggested to exert adverse effects on the body by acting as mutagens, prooxidants that generate free radicals, inhibitors of drug-metabolizing enzymes and key enzymes involved in hormone metabolism and so on [Galati and O’Brien, 2004; Bando et al., 2007].

Since onions are rich in quercetion, Azuma et al. [2010] assessed the tolerable level of dietary quercetin should be clarified in order to produce the expecting beneficial effect of quercetin and quercetin-rich supplements on the vascular system for providing its antioxidative effect without any adverse actions. The tolerable level of dietary quercetin for isolated compounds and for onion powder, for exerting its antioxidative effect was evaluated in high cholesterol-fed rats, using quercetin-containing diets (31–1260 mg quercetin/kg body weight/day) and onion diets (19–94 mg quercetin aglycone equivalent/kg body weight/day), from the viewpoint of a safety assessment [Azuma et al., 2010]. Results indicated that the tolerable level for dietary quercetin for exerting its antioxidative effect was between 126 and 157 mg/kg/day for the quercetin diet and between 19 and 34 mg/kg/day for the onion diet [Azuma et al., 2010].

Quercetin glucosides in the onion powder diets are absorbed more efficiently compared with quercetin aglycone in the quercetin diets [Azuma et al., 2010]. It is known that a glucoside-hydrolyzing activity and glucose transport system in small intestinal cells, as well as deglycosidation by enterobacteria in large intestine, participate in glucoside absorption [Walgren et al., 2000; Day et al., 2003]. These glucoside-specific absorption pathways might be responsible for the higher absorption of quercetin glucosides compared with that of aglycones. Plasma concentration of quercetin metabolites at which toxicity occurred was found to be more than 2 µM in the case of the quercetin diets. On the other hand, the onion powder diets showed toxic effects at plasma concentrations below 2 µM, indicating that other ingredients are responsible for the toxicity of onion powder [Azuma et al., 2010]. There is a possibility that sulfur-containing compounds in onion may cause some deleterious effects. It has become apparent that these compounds may be transformed in the
human body with the subsequent formation of hydrogen sulfide, which may change the structures of proteins and enzymes resulting in a significant disturbance of their function and activity [Jacob et al., 2008]. Furthermore, lethal, sublethal, and behavioral effects of sulfur-containing products including polysulfide and thiosulfate have been found in bioassays of three species of orchard mites [Beers et al., 2009].

The tolerable level for onion diet was much lower than that for the quercetin diet might be derived from the higher efficiency of the onion diet in the enhancement of plasma quercetin concentration, in addition to the deteriorative effect of ingredients present in onion. The intake of 19 mg quercetin aglycone/kg body weight/ day as an onion powder diet corresponded to that of approximately 3.2 kg of onion with 30 mg quercetin aglycone equivalent/100 g fresh weight per day by a human with a body weight of 50 kg. This level of onion intake is far beyond the daily consumption of onions in normal diets and such an intake level seemed to be achieved by the use of onion powder or quercetin supplement even in the case of humans [Azuma et al., 2010].

2.5 EFFECTS OF OXIDATIVE STRESS

The continuous efflux of ROS from endogenous and exogenous sources results in continuous and accumulative oxidative damage to cellular components and alters many cellular functions. Among the biological targets most vulnerable to oxidative damage are proteinaceous enzymes, lipidic membranes, and DNA [Valko et al., 2007]. The high reactivity of free radicals with biological molecules leads to their extremely short life span, does not permit their distribution within the intracellular environment and limits their ability to cause damage a long distance from their site of formation.

2.5.1 Effect of Oxidative Stress in Erythrocytes

The human erythrocyte, due its role as O₂ and CO₂ transporter, is under constant exposure to ROS and oxidative stress. Oxidative stress occurs in cells or tissues when ROS concentration exceeds antioxidant protection [Al-Omar et al., 2004].
Extracellular antioxidant capacity and reduction of extracellular oxidants allows erythrocytes to respond to stress. The mobility of the erythrocyte makes it an ideal antioxidant not only for its own membrane and local environment, but also as an oxidant scavenger throughout the circulation. In most cells, mitochondria are major source of ROS. Despite their lack of mitochondria, ROS are continuously produced in the red cells due to the high O$_2$ tension in arterial blood and their abundant heme iron content. Various factors lead to generation of oxidizing radicals such as O$_2$$^•$−, H$_2$O$_2$, HO• in erythrocytes [Johnson et al., 2005; Cimen, 2008]. The source of ROS in erythrocytes is the oxygen carrier protein Hgb that undergoes autoxidation to produce O$_2$$^•$−.

As a consequence of their physiologic role, erythrocytes are exposed to continuous oxidant stress. Although the normal red cell reducing capacity is greater than 250 times its oxidizing potential several erythrocyte abnormalities have been identified that circumvent or overwhelm the erythrocyte oxidant defense system. Complex aerobic organisms have assured an adequate and continuous flow of oxygen to their tissues, while simultaneously protecting themselves from the inherent toxicity of oxygen. This occurs by two mechanisms: oxygen-carrying proteins, ie, Hgb, and oxidant defense systems [Scott et al., 1989]. Polyunsaturated fatty acids within the membrane, an oxygen rich environment, and iron-rich Hgb make reds cells susceptible to peroxidative damage. Several reports have documented that in vitro exposure to oxidants increases erythrocyte membrane instability by damaging protein band 4.1 and forming a defective spectrin-band 4.1-actin tertiary complex. Membrane-bound proteinases, the secondary antioxidant defense mechanism, protect erythrocytes by preferentially degrading oxidatively damaged proteins [Dumaswala et al., 1999]. Although many membrane components are possible targets for oxidants, calcium ATPase may be of crucial importance for the survival of red cells. Ca-ATPase contains one or more reactive sulfhydryl groups that are susceptible to oxidation with resultant loss of enzyme activity. Because this enzyme is instrumental to maintaining the very steep gradient between extracellular and intracellular calcium, loss of activity is associated with decreased red cell deformability and premature destruction [Shalev et al., 1981]. Since, blood carries a multitude of substances that are considered oxidative stress markers (e.g., TBARS, protein carbonyls), so changes in the blood concentrations of these markers supposedly reflect corresponding
changes in the tissue of interest. Veskoukis et al. [2009] reported that a combination of markers measured in blood provides a reliable indication about the redox status in skeletal muscle, heart, and liver.

Human erythrocyte membranes exposed to oxidative stress in the circulation undergo various modifications of cellular components. These include formation of oxidatively denatured Hgb, peroxidized lipids, high molecular weight cross-linked membrane proteins, desialylation of glycoproteins. These processes lead to decreased phospholipid symmetry, formation of cross-linked spectrin and Hgb, aggregation of band 3 protein, and increased advanced glycation end products [Muller et al., 2007]. Synder et al. [1985] reported that an irreversible complex between the globin chain of Hgb and spectrin was formed oxidatively during the erythrocyte aging. Erythrocyte lipid peroxidation may be involved in normal cell aging and it has been associated with a variety of pathological events [Ko et al., 1997; Sivilotti, 2004].

Oxidant/antioxidant equilibrium can change in the erythrocyte in several diseases. Erythrocytes are exposed to high oxidant stress may result in accelerated peroxidation reactions and cellular aberration [Claster et al., 1984]. Protective mechanisms exist to scavenge and detoxify ROS, block production, or sequester transition metals. Erythrocytes are well equipped with several biological mechanisms to defend against intracellular oxidative stress comprising antioxidant system consists of enzymatic and nonenzymatic pathways [Rossen van et al., 2000]. Enzymes for preventing oxidative denaturation in erythrocytes include SOD, CAT, GPx, GSH reductase-dependent regeneration of GSH, and NADH–metHgb reductase [Scott et al., 1989]. Endogenous non-enzymatic antioxidants are defined in two phases: lipophylic (vitamin E, carotenoids, ubiquinon, melatonin, etc.) and water soluble (vitamin C, glutathione, uric acid, ceruloplasmin, transferin, haptoglobin, etc.). Several exogen compounds such as inhibitors of NADPH Oxidase, allopurinol, and flavonoids have antioxidant properties. It is known that flavanoids are good exogen antioxidants against free radical initiated lipid peroxidation in human red cells and that the antioxidant activity of flavanoids depends significantly on molecular structure and initiation conditions [Hou et al., 2004; Cimen, 2008].
2.5.2 Oxidative Damage to Lipids

Lipids are the important constituents of membranes and function as steroid hormones, retinoic acids and prostaglandins. Lipid peroxidation was first studied in relation to the deterioration of foods in 1930s, when the study on the chemistry of free radical reactions made remarkable advancements [Walling, 1955; Niki, 2000]. All cellular membranes are especially vulnerable to oxidation due to their high concentrations of unsaturated fatty acid. Unstable carbon radicals from fatty acids can rearrange to short alkanes and conjugated dienes which are exhaled or react with oxygen further to peroxyl radicals and finally by hydrogen abstraction to result in lipid hydroperoxides. Lipid hydroperoxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. Their formation occur in enzymatic or non-enzymatic reactions involving activated chemical species (ROS) which are responsible for toxic effects in the body via various tissue damages.

Oxidative stress-induced peroxidation of membrane lipids can be very damaging. Studies have revealed that lipid peroxidation severely affects biomembranes functioning. It induces disturbance of fine structures, alteration of integrity, fluidity, permeability and functional loss of biomembranes and also modifies LDL to proatherogenic and proinflammatory forms, and generates potentially toxic products [Greenberg et al., 2008]. Moreover, lipid peroxidation may contribute to and amplify cellular damage resulting from generation of oxidized products, some of which are chemically reactive and covalently modify critical macromolecules. Products of lipid peroxidation have therefore commonly been used as biomarkers of oxidative stress/damage [Dalle and Donne, 2006].

The overall process of lipid peroxidation consists of three stages: initiation, propagation and termination [Nyska and Kohen, 2002]. Once formed, peroxyl radicals (ROO•) can be rearranged via a cyclisation reaction to endoperoxides (precursors of malondialdehyde) with the final product of the peroxidation process being malondialdehyde (MDA). Lipid peroxidation generates a variety of relatively stable decomposition end products, mainly α,β-unsaturated reactive aldehydes, such
as malondialdehyde (MDA), 4- hydroxy-2-nonenal (HNE), and 2-propenal (acrolein) [Uchida, 2003], and isoprostanes [Montuschi et al., 2004], which can then be measured in plasma and urine as an indirect index of oxidative stress.

Figure 2.4

Figure 2.4. Overview of Lipid Peroxidation

MDA is mutagenic in bacterial and mammalian cells and carcinogenic in rats. Hydroxynonenal is appears to be the major toxic product of lipid peroxidation. In addition, HNE has powerful effects on signal transduction pathways, which in turn have a major effect on the phenotypic characteristics of cells. Some of these aldehydes have been shown to exhibit facile reactivity with various biomolecules, including proteins, DNA, and phospholipids, generating stable products at the end of
a series of reactions that are thought to contribute to the pathogenesis of many diseases [Dalle and Donne, 2006; Valko et al., 2006].

Lipid peroxidation products have also been shown to be mutagenic and carcinogenic and has been implicated as the underlying mechanisms in numerous disorders and diseases such as cardiovascular diseases, post-ischemic reperfusion injury, neuronal degeneration, cancer, rheumatoid arthritis, neurological disorders, and also in aging [West et al., 2006; Rizvi and Maurya, 2007].

2.5.3 LDL Oxidation

Low-density lipoprotein (LDL) is a major carrier of cholesterol in the circulation. Since cholesterol is insoluble in water, it resides in a large complex composed of various lipids and proteins, which is called lipoprotein. Triglycerides and cholesteryl esters are packed in the core of the lipoprotein particles, and it is surrounded by a phospholipid monolayer sheet. In each LDL particle, one molecule of apolipoprotein B-100 (apoB) is included. Nearly 80% of human LDL is composed of lipids, and more than a half of the lipid molecules are PUFA-containing lipids [Stocker, 1994]. When LDL is exposed to oxidation, the PUFA moieties in those lipids are easily oxidized so as to produce a variety of oxidation products containing oxidized functional groups, such as hydroperoxides, epoxides, endoperoxides, isoprostanes, aldehydes, carboxylic acids and unsaturated ketones. They are chemically active and induce secondary reactions with the side chains of amino acid residues in apolipoproteins.

Oxidized low-density lipoprotein (OxLDL) is characterized by the presence of various oxidized lipids and amino acid residues modified with oxidized lipids, and OxLDL is thus a mixture of those heterogeneously modified LDL particles. Ox-LDL, a recognized oxidative stress marker, has been positively associated with central obesity and metabolic syndrome manifestation [Weinbrenner et al., 2006; Holvoet et al., 2008]. Increased intracellular formation of ROS plays a critical part in OxLDL-induced formation of atherosclerotic plaques [Assinger et al., 2010]. OxLDL presents many deleterious effects such as the transformation of macrophages and smooth muscle cells to foam cells, the production of numerous proinflammatory cytokines
and growth factors by almost all the vascular cells, or changes in the balance between procoagulant and anticoagulant activity of the vascular cell surface. OxLDL initiates an intracellular oxidative stress by means of its lipid peroxidation products, leading to the activation of the tumour suppressor p53 by enhancement of p53 protein synthesis, resulting to cell cycle arrest, necrosis or apoptosis [Maziere et al., 2000].

A positive association between ox-LDL concentrations and lipid biomarkers (total cholesterol, LDL-c and total cholesterol-to-HDL-c ratio), GPx activity and uric acid concentration is also been documented [Barbosa et al., 2011]. A lipid profile characterized by reduced high density lipoprotein-cholesterol (HDL-c) concentrations and increased low density lipoprotein-cholesterol (LDL-c) and triglycerides concentrations as well as increased total cholesterol-to-HDL-c ratio constitutes a high risk for type 2 diabetes and cardiovascular diseases [Hadaegh et al., 2010].

**Figure 2.5**

Figure 2.5. Low Density Lipoprotein

Several studies have pointed out that OxLDL is transferrable between vessel wall tissue and the circulation, so it is a reasonable hypothesis that plasma OxLDL levels
reflect the oxidative status at local sites of atherogenesis. OxLDL measurement has been applied to human gingival crevicular fluids, which can be collected easily and safely, and relatively high levels of OxLDL were shown to be present. These findings, together with recent clinical follow up studies, suggest that oxidized low-density lipoprotein is a predictive biomarker of a variety of diseases related to oxidative stress [Itabe, 2012].

2.5.4 Oxidative Damage to Proteins

Proteins are major targets for ROS because of their high overall abundance in biological systems and because they are primarily responsible for most functional processes within cells. Considerable evidence indicates that the maintenance of protein redox status is of fundamental importance for cell function, therefore structural changes in proteins are considered to be among the molecular mechanisms leading to endothelial dysfunction [Woods et al., 2003; Skvarilova et al., 2005]. They are possibly the most immediate vehicle for inflicting oxidative damage on cells because they are often catalysts rather than stoichiometric mediators; hence, the effect of damage to one molecule is greater than stoichiometric. It has been estimated that proteins can scavenge the majority (50%–75%) of ROS generated [Davies et al., 1999].

Exposure of proteins to ROS may alter every level of protein structure from primary to quaternary, causing major physical changes in protein structure. Oxidative damage to proteins is induced either directly by ROS or indirectly by reaction of secondary byproducts of oxidative stress and can occur via different mechanisms, leading to peptide backbone cleavage, cross-linking, and/or modification of the side chain of virtually every amino acid [Davies et al., 1999; Dalle and Donne, 2006]. Oxidative damage of proteins is one of the modifications leading to severe failure of biological functions and cell death.

Most protein damage is irreparable, and oxidative damage to proteins in vivo may affect the function of receptors, enzymes, transport proteins, and generate new antigens. It also caused oxidative changes of protein structure resulting into various consequences, such as inhibition of enzymatic and binding activities, increased
susceptibility to aggregation and proteolysis, increased or decreased uptake by cells, and altered immunogenicity [Dalle and Donne, 2006]. Products of oxidative protein damage can contribute to secondary damage to other biomolecules, e.g., inactivation of DNA repair enzymes and loss of fidelity of DNA polymerases in replicating DNA [Halliwell and Gutteridge, 1999].

The accumulation of increasing amounts of oxidatively damaged proteins increases with decreased capacity for removal of oxidized proteins, the accumulation of misfolded and damaged proteins is accelerated until the protein aggregates, causing metabolic dysfunctions or the initiation of apoptotic or necrotic events. Protein oxidation is currently considered to be an important player in various diseases including Alzheimer’s and Parkinson’s diseases cardiovascular disease, diabetes, ischemia-reperfusion injury and aging [Olivares-Corichi et al., 2005; Halliwell and Gutteridge, 1999; Dalle and Donne, 2006].

Most amino acid residues in proteins are potential targets for oxidation by various ROS. Phenylalanine and tryptophan, are converted to hydroxyl derivatives, tyrosine is converted to nitrotyrosine, and histidine is converted to 2-oxohistidine. Methionine is unique in that its oxidation to methionine sulfoxide is reversible and can be converted back to methionine by methionine sulfoxide reductase [Levine et al., 2000]. Cysteine can also be oxidized to a series of products, which include reversibly oxidized forms (disulfide, S-thiolated, S-nitrosylated and sulfenic acid) and irreversibly oxidized forms (sulfenic and sulfonic acids). The formation of protein-bound carbonyl groups resulting from the direct oxidation of amino acid side chains, e.g., lysine, arginine, proline and cysteine by free radicals [Dalle and Donne, 2003] or from the modification of proteins by oxidation-derived secondary products, for example, lipid peroxidation products, seems to be a common phenomenon of protein oxidation. It has also been shown that the lipid peroxidation products and advanced glycation end products can react with proteins forming carbonyl derivatives to the side chains of lysine and cysteine by the Michael reaction [Doorn and Petersen, 2002].

2.5.5 Oxidative Damage to Sialic Acids

Sialic acids (SA), a large family of acetylated or glycosylated derivatives of neuraminic acid—is a component of many glycoproteins and glycolipids. They are acidic monosaccharides common in higher animals and some microorganisms. One
branch of the sialic acid family is *N*-acetylated to form *N*-acetylneuraminic acids (Neu5Ac, NANA, Sia), which are the most widespread form of sialic acid and almost the only form found in humans. The other branch is based on *N*-glycolyneuraminic acids (Neu5Gc) which are common in many animal species (best investigated in porcine tissues), but not found in humans except in the case of a particular cancer [Schauer et al., 1995].

SA are found in cellular secretions and on the outer surface of cells, mostly as terminal components of glycoproteins and glycolipids (gangliosides) [Kamerling et al., 2007]. Approximately 80% of SA in serum is *N*-acetylneuraminic acid. It is found in many body tissues and fluids, and in the circulation it is chiefly present covalently bound to glycoproteins as the terminal sugar of oligosaccharide chains and plays a role in the vital function of humans [Varki, 2008]. Many of these glycoproteins are acute phase proteins.

The source of plasma sialic acid is unclear. Some of the sialic acid in the circulation comes from the diet, and, therefore, it has been suggested that concentrations may reflect metabolic status, body tissue levels, and nutritional status [Wang and Brand-Miller, 2003]. Most of the sialic acid in plasma and serum is associated with lipoproteins, although some of the circulating sialic acid may result from cellular cleavage because all tissues have the capacity to synthesize sialic acid, and many cells also express neuraminidases that cause cleavage of sialic acid from the cell surface. Leukocytes from people with type 2 diabetes mellitus and models of diabetes mellitus have been reported to have increased levels or activities of neuraminidases, as well as decreased cellular sialic acid [Murayama et al., 1988].

In the erythrocyte membrane, it is mainly contained in the SA-rich glycophorins comprising three major proteins involved in cytoskeletal interactions: glycophorin A, glycophorin B, and glycophorin C. Red blood cell (RBC) aging is accompanied by desialylation of membrane glycoconjugates caused by cleavage of terminal SA residues or by removal of sialoglycoconjugates [Shinozuka, 1994]. This phenomenon might trigger the clearance of senescent RBCs by unmasking cryptoantigens. SA may
have a variety of functions in vivo, such as stabilization of the conformation of glycoproteins and cellular membranes, cell to cell recognition and interaction, membrane transport, membrane receptor functions [Sillanaukee et al., 1999]. Increased SA concentrations have also been observed during diabetes [Yılmaz et al., 2007], cardiovascular diseases [Serdar et al., 2007], chronic liver disease [Cylwik et al., 2007] and malignancies [Kokoglu et al., 1992]. The mechanisms underlying the elevated SA concentrations in different diseases are not clear [Sillanaukee et al., 1999]. Many studies have reported that SA levels may be increased in biological fluids in alcoholics, and it has been suggested that SA can be valuable as a biomarker for excessive alcohol consumption [Romppanen et al., 2002].

In the erythrocyte membrane, it is mainly contained in the SA-rich glycophorins, mediating or modulating a variety of normal and pathological processes [Varki, 2008]. Due to negative charge and hydrophobicity, sialic acids have many structural and modulatory roles. They affect the action of some hormones, the recognition of different compounds, the cellular adhesiveness, the catalytic properties of enzymes, the transport process and antigenicity [Schauer, 1982].

### 2.5.6 Oxidative Damage to DNA

DNA is a stable, well-protected molecule however, at high concentrations ROS can interact with it and cause several types of damage: modification of DNA bases, single- and double-DNA breaks, loss of purines (apurinic sites), damage to the deoxyribose sugar, DNA-protein cross-linkage, and damage to the DNA repair system. The hydroxyl radical is one of the potential inducers of DNA damage and is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone [Halliwell and Gutteridge, 1999].

The most extensively studied DNA lesion is the formation of 8-hydroxydeoxyguanosine (8-OHdG), when attacked on guanine at its C-8 position by OH• to yield an oxidation product, 8-OHdG. Other positions could be attacked, and other possible products could be formed. Hydroxyl radicals can also attack other
bases like adenine to yield 8 (or 4-, 5)-hydroxyadenine. Other products are the result of interactions between pyrimidines and hydroxyl radicals leading to the formation of thymine peroxide, thymine glycols, 5- (hydroxymethyl) uracyl, and other such products [Kohen and Nyska, 2002]. Permanent modification of genetic material resulting from these “oxidative damage” incidents represents the first step involved in mutagenesis, carcinogenesis, and ageing [Finkel and Holbrook, 2000].

Several studies have shown that ageing cells and organisms accumulate increased levels of oxidant-damaged nuclear DNA [Esposito et al., 1999]. Oxidative stress can induce mitochondrial DNA damage and is generally considered to be even more sensitive than nuclear DNA to oxidative damage because of its proximity to the main source of oxidant generation, or because of a limited DNA repair system. Several studies have shown a significant increase in the level of mitochondrial DNA rearrangements when oxidative stress was genetically engineered by targeted deletions in either Mn-SOD or the adenine nuclear transporter in knockout mice. Increasing damage to mitochondrial DNA inevitably leads to compromised mitochondrial function and integrity. Damaged mitochondria are thought to release more ROS and set in motion a vicious cycle of increasing DNA damage leading to increased ROS production that in turn leads to more DNA damage [Melov et al., 1999; Finkel and Holbrook, 2000].

2.6 OXIDATIVE STRESS AND ENZYME PARAOXONASE 1 ACTIVITY

Serum Paraoxonase 1 (PON1), a calcium-dependent phosphotriesterase synthesized in the liver and closely associated to HDL containing both apolipoprotein A1 and apolipoprotein J (apoA1 and apoJ), is known to be related to the antiatherogenic properties of HDL [Mackness et al., 1996]. The paraoxonase gene family in mammals includes three members: PON1, PON2, and PON3 [Hegele, 1999]. These PON genes seem to have arisen by gene duplication of a common evolutionary precursor, as they share considerable structural homology and are located adjacently on chromosome 7 in humans and on chromosome 6 in mice. Most of the serum PON1 is present in association with HDL [Primo-Parmo et al., 1996].
Figure 2.6

Figure 2.6. General structure of human serum paraoxonase (PON1)

ApoA1: apolipoprotein A1

PON1 has been reported to be an important contributor to the antioxidant and anti-inflammatory activities of HDL, avoiding LDL oxidation. Studies have shown a low PON1 levels also in chylomicrons and VLDL, but not in LDL. The crystal structure of a variant of PON1 obtained by directed evolution was shown to consist of a six-bladed β-propeller with a unique active site and a catabolic mechanism based on a His–His dyad [Harel et al., 2004]. PON3 was shown in plasma, associated with HDL, and PON2 was found only in cells (human endothelial cells and human aortic smooth muscle cells [Ng et al., 2001; Reddy et al., 2001]. PON1 activity is reduced in patients with enhanced atherosclerosis, in disorders of lipoprotein metabolism, in renal insufficiency and after renal transplantation, caused by elevated oxidative stress and disturbances of apolipoprotein metabolism [Varga et al., 2009].
Atherosclerosis is initiated by oxidative modification of LDL in the arterial wall cells including macrophages. HDL can remove excess cholesterol from arteries and its serum levels are inversely related to the risk of developing atherosclerosis [Mackness et al., 2003]. Experimental evidences have shown that PON1 functions as an antioxidative enzyme that inhibits the oxidation of LDL or protecting the endothelium from the pro-oxidant effect of oxidized LDL that prevents the development of atherosclerosis [Mackness et al., 1993; Aviram and Rosenblat, 2004]. In addition, PON1 hydrolyses homocysteine thiolactone and prevents homocysteinemia, which is also involved in atherogenesis [Jakubowski, 2000].

Most of the studies so far suggest that the antiatherogenic role of PON1 is to reduce oxidative stress. PON1 can hydrolyze specific oxidized cholesteryl esters, as well as specific oxidized phospholipids in oxidized lipoproteins [Aviram et al., 1998]. During HDL oxidation by peroxynitrite donor, PON1 hydrolyzes phosphatidylcholine core aldehydes [Ahmed et al., 2001]. Furthermore, PON1 demonstrates high activity toward oxidized eicosanoids and docosanoids [Teiber et al, 2003]. It was shown that covalent linkage of lipid peroxidation products to the LDL protein, as well as the accumulation of lipid peroxides in LDL, is diminished in the presence of PON1 [Sanvanich et al., 2003]. An inverse relationship between serum PON1 activity and lipid peroxidation was demonstrated [Malin et al., 2001]. Reduced serum PON1 activity and increased oxidative stress was also shown in E0 mice and in dyslipidemic obese mice [Mertens et al., 2003]. The direct role of PON1 in reducing oxidative stress was demonstrated in studies using the PON1 knockout mouse model and the human PON1 transgenic mouse model [Shih et al., 2000; Pan et al., 2002]. HDL isolated from PON1 knockout mice was unable to prevent LDL oxidation in cultured arterial cells, in contrast to the HDL isolated from control mice. In PON1 knockout on the background of E0 mice, increased lipoprotein oxidation was shown versus E0 mice [Shih et al., 2000].

Regarding pharmacological regulation, the PON1 gene was shown to be regulated in vitro and in vivo by fenofibrate and statins [Gouedard et al., 2003]. Inflammatory conditions appear to decrease PON1 gene expression in vitro [Van Lenten et al., 2001]. Serum PON1 activity also depends on a number of physiological and pathological conditions: low levels of serum activity have been found in cases of renal
disease, HDL deficiencies, and liver cirrhosis. High-fat diets were also shown to
decrease PON-1 activity, while daily moderate alcohol consumption increased it
[Costa et al., 2003]. Among dietary or lifestyle factors, wine consumption and some
polyphenols present in wine or fruit juice increase serum PON-1 activity in humans
and mice [Fuhrman and Aviram, 2002].

PON1 activity has been shown to be reduced in patients with type 2 diabetes mellitus
in a study where it has been shown that in PON1-knockout or PON1-transgenic mice
demonstrated that PON1 has a protective role against diabetes development,
secondary to the unique antioxidant properties [Rozenberg et al., 2008]. Reduced
PON1 activity is observed in several chronic diseases, including hypercholesterolemia
and during human aging [Ikeda et al., 2009; Mehdí and Rizvi, 2012]. The age-
dependent effect of the PON1 genotype has been investigated, showing the
association of human longevity with PON1 status and its role in delaying the onset of
the major age-related diseases, including cardiovascular diseases [Lescai et al., 2009].
Seres and colleagues investigated the factors influencing PON1 activity and as a
function of age. They reported a significant decrease in PON1 activity with age
without a change in its serum concentration. It was also observed that HDL from
elderly subjects was more susceptible to oxidation than HDL from young subjects
measured in terms of lipid peroxidation rate [Seres et al., 2005].

2.7 OXIDATIVE STRESS AND ENZYME ACETYL
CHOLINESTERASE ACTIVITY

Acetylcholinesterase (AChE) is a key enzyme of the cholinergic transmission in the
central nervous system. It is responsible to promote hydrolysis of the neurotransmitter
“acetylcholine” released at the nerve endings to mediate transmission of the neural
impulse across the synapse. Degradation of acetylcholine is necessary to depolarize
nerves so that it might repolarize in the next conduction event [Suresh et al., 1992]. It
is present in the cholinergic synapses in the central nervous system as well as in
neuromuscular synapses where it rapidly hydrolyzes acetylcholine. It was reported
that AChE varies in different organs in response to environmental stress [Gill et al.,
1990]. The decreased AChE activity could be due to a decrease of the enzyme
REVIEW OF LITERATURE

NUTRITIONAL AND BIOCHEMICAL STUDIES ON ALLIUM VEGETABLES

synthesis by the inhibitory action of the toxicants [El-Demerdash et al., 1999]. Moreover, the decreased enzyme activity may be related to the enzyme inhibitors (competitive inhibitors, non-competitive inhibitors or mixed-type inhibitors) that bind to enzymes or enzyme substrate and receptors inhibition that mediate activity decrease [Hostettmann et al., 2006]. Consequently, its inhibition could decrease cellular metabolism, induce deformities of cell membrane, differential membrane permeability, ionic refluxes, and disturb metabolic and nervous activity [Gill et al., 1990; Tolosa et al., 1996].

Acetylcholinesterase (AchE) in erythrocytes is one of the typical extraneural AchEs. Its activity is high in human erythrocytes. It has been known that the enzyme is present only in the membrane in erythrocytes and that it is localized on the outer side of the membrane. Previous studies have disclosed much of the primary structure of AchE and the membrane anchor structure [Igisu et al., 1994]. AchE is anchored by an attached glycosyl phosphatidylinositol on exterior surface of plasma membrane. However, the physiological functions of erythrocyte AchE are still totally unknown. Nevertheless, the enzyme may be regarded as a model of AchE in the nervous system. In addition, it may be used to examine the status of the erythrocyte membrane. Erythrocyte AchE plays an important role in the preservation of the integrity of the red cell. Markedly reduced erythrocyte AchE activity was demonstrated in cases of paroxysmal nocturnal haemoglobinuria (PNH) [Auditore and Hartmann, 1959]. Previous studies showed a correlation between AchE inhibition in blood and inhibition in target tissues [Kale et al., 1999]. The enzyme activity seems useful as an indicator of the effects of AchE inhibitors, such as pesticides. The determination of AchE activity in blood is a great consideration in the diagnosis of poisonings caused by reversible and irreversible inhibitors of this enzyme including pesticides [Bukowska and Hutnika, 2006].

Acetylcholinesterase (AChE) has an essential role in regulating many vital functions, and responds to various insults including oxidative stress [Silman and Sussman, 2005]. The increased AChE activity of RBCs indirectly reflects the reduced concentration of acetylcholine that, in turn, enhances local and systemic inflammatory events. AChE could serve as a marker of low-grade systemic inflammation and be useful to predict prognosis and response to treatment in Type 2 diabetes mellitus [Rao et al., 2007]. Previous studies have
showed the antioxidant effect of AChE against LDL oxidation as they might be associated with hydrolysis of some specific lipid peroxides. This mode of AChE action resembles that of PON1, since both enzymes were shown to inhibit the production of lipoperoxides and TBARS during the course of LDL oxidation [Fuhrman et al., 2004].

It should be noted, however, that the activity of AchE in erythrocytes is not always a good indicator of intoxication with AchE inhibitors. Non cholinergic function of AChE has been reported in human plasma, lymphocytes and erythrocytes [Bremer et al., 2008]. Despite enzymatic property, acetylcholinesterase has nonenzymatic functions in regulation of several biological functions such as proliferation, differentiation, organization of the cytoskeleton, cell-cell contact, or immune functions [Silman and Sussman, 2005].

2.8 OXIDATIVE STRESS AND PLASMA MEMBRANE REDOX SYSTEM

Eukaryotic cells including erythrocytes display a plasma membrane redox system (PMRS) that transfers electrons from intracellular substrates to extracellular electron acceptors [Hyun et al., 2006a]. There are at least three basic components for the PMRS: membrane associated quinone reductases (e.g. cytochrome b5 reductase (b5R), NADH-quinone oxidoreductase 1 (NQO1), etc.), lipophilic antioxidants (Coenzyme Q (CoQ) and α-tocopherol) and the cytosolic electron donor NAD(P)H [Hyun et al., 2006a,b; Navas et al., 2007]. There are many other components that contribute to the PMRS such as NADH-FeCN reductase, NADH-CoQ reductase and NADH-cytochrome c oxidoreductase.

- NAD(P)H:quinone oxidoreductase 1 (NQO1) is a cytosolic reductase that is able to utilize NADH or NADPH as a reducing cofactor, and which has an equal affinity for both as electron donors. NQO1 catalyzes the reduction of quinones, and in doing so prevents the generation of semiquinone free radicals and reactive oxygen species, which ultimately is believed to limit oxidative stress and perhaps therefore be protective for the cell. NQO1 also acts to generate and maintain the reduced form of
coenzyme Q in the membrane [Beyer et al., 1997; Brunmark et al., 1987; Landi et al., 1997; Duffy et al., 1998].

- Coenzyme Q (CoQ) (also known as ubiquinone) is a lipophilic, redox-active molecule found in the phospholipid bilayer of cell membranes [Battino et al., 1990]. Ubiquinol, the fully reduced form of CoQ, inhibits the peroxidation of membrane lipids by reacting with lipid peroxyl radicals and by reducing tocopheroxyl radicals [Boveris, 1984; Turrens et al., 1985].

- Cytochrome b5 reductase reduces CoQ through a one-electron reaction mechanism, thereby fostering the antioxidant potential of the membrane by maintaining, for example, ascorbate and alpha-tocopherol in their reduced states [Villalba and Navas, 2000].

- Alpha-tocopherol is the only lipid soluble, chain breaking antioxidant present in biological membranes [Burton et al., 1983; Ingold et al., 1987], protecting cell membranes from oxidative damage by neutralizing the effects of peroxide and oxygen free radicals.

The PMRS produces NAD^+ for glycolytic ATP production by transferring electrons from intracellular reducing equivalents to extracellular acceptors, and reduces oxidative stress. Thus, it performs several functions including hormonal signal transduction and protection of cells from oxidative stress. Several findings suggest important roles of the PMRS in aging and neuronal survival [Hyun et al., 2006a, b]. The PMRS has also been observed to protect cells from oxidative stress-induced apoptosis [Rodriguez-Aguilera et al., 2000; Villalba and Navas, 2000]. Multiple enzymes of the PMRS are expressed in neural cells and are up-regulated in response to mitochondrial impairment to preserve energy metabolism and protect the cells against oxidative stress [Hyun, et al., 2007, Rodriguez-Aguilera, et al., 2000, Villalba and Navas, 2000].

The importance of erythrocyte PMRS during oxidative stress has been highlighted [Pandey and Rizvi, 2010a; Rizvi and Jha, 2011]. The activity of erythrocyte PMRS
has found to be elevated in first degree relatives of type 2 diabetic subjects, in patients with diabetic nephropathy and in lymphocytes from insulin dependent diabetes mellitus patients, a disease in which mitochondrial activity is depressed. The increase in PMRS signifies compensatory mechanisms to mitigate increased oxidative stress in diabetic subjects [Lenaz et al., 2002; Rizvi and Srivastava, 2010]. A few studies have shown that the activity of PMRS is modulated during aging in animals, providing an efficient additional cell protection from oxidative stress [Hulbert, 2008; Rizvi et al., 2011]. Previous studies revealed erythrocyte PMRS in maintaining the antioxidant status of the plasma during aging and thereby playing an important role in modulating life span [Rizvi et al., 2009; Rizvi et al., 2011].

Figure 2.7

Figure 2.7. A scheme of the plasma membrane redox system

2.9 ANTIOXIDANT DEFENCE MECHANISMS IN OXIDATIVE STRESS

The harmful effect of oxidative stress is counteracted by the antioxidant action of both antioxidant enzymes and non-enzymatic antioxidants. The most efficient enzymatic antioxidants against ROS-induced damage involve superoxide dismutase, catalase and
glutathione peroxidase [Mates et al., 1999; Valko et al., 2006]. Non-enzymatic antioxidants involve Vitamin C, Vitamin E, carotenoids, thiol antioxidants (glutathione, thioredoxin and lipoic acid), natural flavonoids, a hormonal product of the pineal gland, melatonin and other compounds [McCall and Frei, 1999; Kohen and Nyska, 2002].

2.9.1 Non Enzymatic antioxidants

2.9.1.1 Intracellular Reduced Glutathione

Glutathione (GSH) is a multifunctional intracellular non-enzymatic major thiol antioxidant. It is considered to be the major thiol-disulphide redox buffer of the cell [Masella et al., 2005]. Glutathione is a ubiquitous tripeptide (g-Glu-Cys-Gly), highly abundant in the cytosol (1–11 mM), nuclei (3–15 mM), and mitochondria (5–11 mM) and is the major soluble antioxidant in these cell compartments [Masella et al., 2005]. The reduced form of glutathione is GSH, glutathione, and the oxidised form is GSSG, glutathione disulfide.

Glutathione functions as a direct free-radical scavenger, as a cosubstrate for glutathione peroxidase activity, and as a cofactor for many enzymes, forms conjugates in endo- and xenobiotic reactions and detoxify reactive oxygen metabolites of endogenous or exogenous origins. It plays an important role in the synthesis and repair of DNA, the synthesis of proteins and the activation, maintaining the essential thiol status of protein, immune function, regulate nitric oxide homeostasis, modulate the activity of neurotransmitter receptors and regulation of enzymes [Oja et al., 2000; Hogg, 2002]. The lower level of GSH is related to different physiological and biochemical disturbances.

GSH plays a crucial role in maintaining a normal balance between oxidation and antioxidation. Erythrocyte GSH depletion has been shown to intensify lipid peroxidation and predispose cells to oxidant damage [Pandey and Rizvi, 2010a]. Enhanced oxidant challenge, such as by lipid peroxides, would be expected to result in depletion of the cellular glutathione pool and a corresponding increase in glutathione disulfide.
Cells are rendered more oxidized by the excessively high accumulation of intracellular glutathione disulfide during oxidative stress. This altered thiol-disulfide status coupled with the resultant oxidation of protein sulfhydryls has profound effects on metabolic processes [Ji et al., 1999; Pandey and Rizvi, 2010b]. Depletion of erythrocyte GSH has been documented in many clinical situations such as malnutrition, severe burn injury, human immunodeficiency virus (HIV) infection, and diabetes, both type 1 and type 2. Glutathione depletion may have adverse consequences in diabetic patients, independent of glycemic control, and it may weaken the defense against oxidative stress. This could cause damage to protein, DNA, or membrane lipids and thus potentially lead to cell dysfunction in various tissues [Masella et al., 2005].

GSH has been reported to regulate redox signalling by alterations in both the level of total GSH and in the ratio of its oxidised (GSSG) to reduced (GSH) forms [Jones et al., 2000]. Cellular GSH depletion has been found to be associated with decreased cell proliferation in vascular endothelial cells and increased proliferation of fibroblasts. GSH is involved in regulating the activation of various transcription factors, including nuclear factor NF-κB and activator protein AP-1. GSH protects cells against apoptosis, the protective role originates from multifactorial mechanisms that involve detoxification and modulation of cellular redox state and the subsequent redox-sensitive cell-signalling pathways and interaction with pro- and anti-apoptotic signals [Masella et al., 2005]. GSH in the nucleus maintains the redox state of critical protein sulphhydryls that are necessary for DNA repair and expression. An oxidative environment leads to rapid modification of protein sulphhydryls (protein-SH) [Ji et al., 1999].

The main protective roles of glutathione against oxidative stress are that (i) glutathione is a cofactor of several detoxifying enzymes against oxidative stress, e.g. glutathione peroxidase (GPx), glutathione transferase and others; (ii) GSH participates in amino acid transport through the plasma membrane; (iii) GSH scavenges hydroxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of glutathione peroxidase; (iv) glutathione is able to regenerate the most important antioxidants, vitamins C and E back to their active...
forms; glutathione can reduce the tocopherol radical of Vitamin E directly, or indirectly, via reduction of semi dehydroascorbate to ascorbate [Masella et al., 2005].

2.9.2 Enzymatic antioxidants

2.9.2.1 Superoxide Dismutase

Superoxide dismutase (SOD) is one of the most effective intracellular enzymatic antioxidants (EC 1.15.1.1) discovered by McCord and Fridovich, whose function is protection from ROS which plays an important role in the defense mechanism of biological cells exposed to oxygen [McCord and Fridovich, 1969]. It is the major defence upon superoxide radicals and is the first defence line against oxidative stress. SOD catalyzes the dismutation of superoxide anion radical (\(O_2^-\)) into an oxygen molecule and a hydrogen peroxide. This reaction is recognized as an antioxidant system that protects cells from superoxide toxicity.

Superoxide dismutase exists in several isoforms, differing in the nature of the active metal centre and amino acid constituency, as well as their number of subunits, cofactors and other features. In humans there are three forms of SOD: cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD) [Landis and Tower, 2005]. CuZn-SOD is found in both the cytoplasm and the nucleus. Mn-SOD is confined to the mitochondria, but can be released into extracellular space.

Cu, Zn-SOD (SOD1) is an enzyme with a molecular weight of about 32 kDa and is composed of two identical subunits (homodimer) [Mates et al., 1999]. Cu/Zn - SOD exists in the cytoplasm, lysosomes, and nuclear compartments of mammalian cells. Cu, Zn-SOD specifically catalyzes the dismutation of the superoxide anion to oxygen and water. In humans, the liver has a relatively high amount and activity of SOD1 [Nozik-Grayck et al., 2005]. Each subunit contains as the active site, a dinuclear metal cluster constituted by copper and zinc ions. Enzyme activity is relatively independent of pH in the range of 5–9.5. The copper ion is held by interaction with imidazolate ligands of the histidine residues in SOD1 in the enzymatic active site. The
zinc ion (Zn\(^{2+}\)) contributes to the stabilization of the enzyme [Johnson and Giulivi, 2005].

Mitochondrial Mn-SOD is a homotetramer (96 kDa) containing one manganese atom per subunit [Mates et al., 1999]. This enzyme cycles from Mn(III) to Mn(II) and back to Mn(III) during the two step dismutation of superoxide. Mn-SOD is considered as one of the most effective antioxidant enzymes with anti-tumour activity. Experimental studies indicate that on different cell lines has confirmed that over expression of Mn-SOD leads to tumour growth retardation [Behrend et al., 2003]. The decreased level of Cu, Zn-SOD and Mn-SOD has been found for certain tumour cells [Oberley, 1998]. Extracellular superoxide dismutase (EC-SOD) is a secretory, tetrameric, copper and zinc containing glycoprotein, with a high affinity for certain glycosaminoglycans such as heparin and heparin sulphate [Mates et al., 1999]. Its regulation in mammalian tissues occurs primarily in a manner coordinated by cytokines, rather than as a response of individual cells to oxidants.

Due to their lack of mitochondria, cytoplasmic Cu,Zn–SOD plays a much more important role in erythrocytes. Zinc appears to stabilize the enzyme, while the copper atom and histidine amino acid are required for enzymatic activity [Al-Omar et al., 2004]. Physiologically, erythrocytes are well protected against ROS by abundant Cu,Zn–SOD which scavenges free radicals thus preventing metHgb formation [Dumaswala et al., 1999]. Erythrocyte Cu,Zn–SOD activity tends to be decreased in critical ischemia because production of O\(_2\)\(^{−}\) may be lower than during moderate ischemia. The toxic exposure, ie, smoking, causes no impairment in the enzymatic antioxidant defense systems and does not lead to erythrocyte oxidant stress due to their potent antioxidant defense [Cimen, 2008]. Several researches have reported decreased erythrocyte SOD activity during therapeutic applications and pathophysiologic conditions. SOD scavenges O\(_2\)\(^{−}\) and inhibits the formation of peroxynitrite, thereby suppressing injury and regulating the bioavailability of NO [Durak et al., 2001; Gunduz et al., 2004; Cimen et al., 2008].

2.9.2.2 Catalase

Catalase (CAT) located in a cell organelle called the peroxisomes, is one of the major antioxidant enzymes [Scandalios et al., 1997]. It is one of the first enzymes to be purified and crystallized and has gained a lot of attention because of its link to cancer,
diabetes and aging in humans and animals [Preston et al., 2001]. It is present in every cell and in particular in cell structures that use oxygen in order to detoxify toxic substances and produce \( \text{H}_2\text{O}_2 \). CAT converts \( \text{H}_2\text{O}_2 \) into water and oxygen. CAT can also use \( \text{H}_2\text{O}_2 \) in order to detoxify some toxic substances via a peroxidase reaction [Mayo et al., 2003]. There are many evidences that the changes of catalase activity as well as the mechanisms of its regulation are essential in the response to stress situations which catalyzes the dismutation of \( \text{H}_2\text{O}_2 \), forming \( \text{O}_2 \) and \( \text{H}_2\text{O} \) resulting good protection the cells from the toxic effects of hydrogen peroxide [Brioukhanov and Netrusor, 2004]. The significantly decreased capacity of a variety of tumours for detoxifying hydrogen peroxide is linked to a decreased level of CAT [Valko et al., 2006]. CAT and SOD react synergistically to protect each other was observed earlier in hemolysis studies of erythrocytes. CAT plays an increasingly important role as the erythrocyte is exposed to increased \( \text{H}_2\text{O}_2 \) flux [Cimen, 2008].

### 2.9.2.3 Glutathione Peroxidase and Glutathione Reductase

Glutathione peroxidase and reductase are two enzymes that are found in the cytoplasm, mitochondria, and nucleus. Glutathione peroxidase metabolizes hydrogen peroxide to water by using reduced glutathione as a hydrogen donor. Glutathione disulfide is recycled back to glutathione by glutathione reductase, using the cofactor NADPH generated by glucose 6-phosphate dehydrogenase.

There are two forms of the enzyme glutathione peroxidase, one of which is selenium-independent (glutathione-S-transferase, GST, EC 2.5.1.18) while the other is selenium-dependent (GPx, EC 1.11.1.19) [Mates et al., 1999]. These two enzymes differ in the number of subunits, the bonding nature of the selenium at the active centre and their catalytic mechanisms. Glutathione metabolism is one of the most essential of antioxidative defence mechanisms. Humans have four different Se-dependent glutathione peroxidases [Mates et al., 1999]. GPx decomposes peroxides to water (or alcohol) while simultaneously oxidizing GSH. Significantly, GPx competes with catalase for \( \text{H}_2\text{O}_2 \) as a substrate and is the major source of protection against low levels of oxidative stress. Thus, GPx is generally more efficient with high ROS concentration and CAT has an important action with lower \( \text{H}_2\text{O}_2 \) concentration.
The role of glutathione peroxidase in cellular defense against oxidant attack has been discussed for many years. It has been suggested that GPx has anti-inflammatory activity in the cardiovascular system. An increase in cytosolic GPx is linked to a lower risk of cardiovascular disease [Blankenberg et al., 2003]. The red cell has been a central focus because it is thought to undergo a high endogenous rate of H$_2$O$_2$ production from hemoglobin autoxidation, which can be markedly increased in cells with unstable hemoglobins. In addition, the red cell is probably exposed to H$_2$O$_2$ from stimulated macrophages and, under certain circumstances, from pathogenic bacteria or malarial parasites. It is believed that the glutathione peroxidase enzyme protects the erythrocyte against peroxides that are generated intracellularly or exogenously [Johnson et al., 2000].

Glutathione reductase [EC 1.6.4.2] (GR) plays an important role in protecting hemoglobin, red cell enzymes, and biological cell membranes against oxidative damage by increasing the level of reduced glutathione in the process of aerobic glycolysis. The enzyme deficiency may result in mild to moderately severe hemolytic anemia upon exposure to certain drugs or chemicals. However, hereditary deficiency of the enzyme is extremely rare [Chang et al., 1978].

It is a flavin enzyme involved in the defense of the erythrocyte against hemolysis. This enzyme catalyses reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of NADPH and maintains a high intracellular GSH/GSSG ratio of about 500 in red blood cells [Kondo et al., 1980]. GR is important not only for the maintaining the required GSH level but also for reducing protein thiols to their native state. Disturbances GSH and GR level have been correlated with oxidative stress induced by various factors including toxicity, pollutants, inflammation and different diseases particularly RBC defects [Valko et al., 2006; Cimen, 2008].