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OXIDATIVE STRESS AND NATURAL ANTIOXIDANTS

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Oxidative Stress and Natural Antioxidants

(Volume 1)

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FOREWORD

I am pleased to write this foreword for the e-book entitled 'Oxidative Stress and Natural Antioxidants'. This outstanding endeavor by the co-editors represents a multi-disciplinary coverage of all aspects of oxidative stress and the role of anti-oxidants in this fundamental phenomenon. This e-book represents an effective compilation of chapters on the fundamentals of oxidative stress and the role of anti-oxidants in health and disease. The authors deserve credit for their time and effort to contribute excellent chapters relevant to their individual expertise. These chapters include excellent discussions on oxidative stress in human physiology, redox homeostasis, functions of free radicals and intrinsic cellular mechanisms for naturally occurring anti-oxidants. I am confident that this book will be a valuable addition to the bookshelves of teaching faculty, established investigators and young graduate students. I wish all, the success for the launch of this book.

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PREFACE

"*Oxidative Stress and Natural Antioxidants*" presents the one pot solution for the interested readers ranging from an understanding of oxidative stress, recent advances in preparation methods, characterization, and applications of antioxidants. Taken altogether, the gathered information in this volume will cover an array of topics highlighting the importance of natural antioxidants in various oxidative stress associated diseases.

The scientific framework of this e-book contains chapters by eminent experts with in-depth knowledge of antioxidants and oxidative stress. The chapters comprise the role of reactive oxygen species and environmental contaminants in redox homeostasis with cellular mechanisms in oxidative stress that trigger the development and progression of many diseases. The literature includes the extraction, profiling, and characterization of antioxidants *via* different procedures and screening assays. Further, the chapters deliberate the role of antioxidants in human physiology, redox homeostasis, intrinsic cellular mechanisms, and their therapeutic potential with industrial prospects. Authors whose names appear on the chapters have remarkably contributed to the scientific work in this ebook and are responsible and accountable for any scientific queries or questions.

We believe that the chapters published in this volume will enrich the understanding of interdisciplinary domains of natural products as well as offer insights into emerging avenues in drug discovery trends.

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CHAPTER 1

Level of Oxidative Stress: A Fate-Determiner of Carcinogenesis and Anti-Carcinogenesis

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Abstract: Molecular oxygen, a double-edged sword, is both a boon and a curse for the existence of life. Oxidative stress is the disequilibrium between reactive oxygen (ROS)-generation and elimination that inflicts cellular damage. Living cells can adapt to the ever-changing internal or external stresses. However, they gradually lose their radical-scavenging adaptability with persistent stress, which further increases during neoplasia. Cancer cells, well adapted in pro-oxidative milieu, drive metabolic and genomic reprogramming, which further escalates the oxidative load. This vicious cycle promotes further carcinogenic alterations. Contrastingly, the same ROS is essential for the oxidative-burst mediated anticancer host-defense. To sustain this redox pressure, cancer cells hijack the intracellular antioxidants. Therefore, redox reorientation towards enhanced responsiveness may selectively target malignant cells by ROS-enhancement beyond tolerance leading to mortality. Carcinogenesis, a multistep process, requires ROS during initiation, promotion and progression. However, supraphysiological ROS may induce apoptosis in unmanageable malignancies. Interestingly cells possess an evolutionary-conserved nature to get hormetically pre-conditioned by a transient ultra-low exposure of a stressor, which in higher dose may show the opposite effect. Antioxidants are excellent chemopreventives and chemotherapeutics. Here, we have condensed the possible anticancer modulation of oxidative stress by phytochemicals, aiming at an insight for future strategies in cancer management.

Keywords: Anticancer Therapy, Antioxidant, Carcinogenesis, Dietary Phytochemicals, Hormesis, Nuclear Factor (Erythroid-Derived 2)-Like 2 (Nrf2), Oxidative Stressor, Prooxidant, Reactive Oxygen Species, Xenobiotic Metabolism.

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OXIDATIVE STRESS: ORIGIN, DEFINITION AND FEATURES

Oxygen, which is indispensable for existence of all aerobic life forms, becomes lethal when in excess. ROS are oxygen-containing highly reactive species that are produced due to cellular metabolism or environmental stress and can damage nucleic acids, lipids, and proteins structurally and functionally (Jelic *et al.* 2019). ROS are a broad class of chemicals that includes partially oxidized radicals with unpaired electrons, such as superoxide ion ($O_2^{\bullet-}$) and hydroxyl radical ($OH^{\bullet}$), and non-radicals, such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl).

The origin and evolution of aerobic life on Earth was accompanied by ROS and oxidative stress, which has emerged as a concept in redox biology in the past 60-odd years. Oxidative stress was defined by Jones as "an imbalance between oxidants and antioxidants in favour of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage" (Sies 2017). The endogenous sources of oxidants are inflammatory cells, mitochondria, and peroxisomes which produce mostly H_2O_2 and $O_2^{\bullet-}$ as ROS molecules (Jelic *et al.* 2019). Exposomes, which include all the exogenous sources, can be direct environmental oxidants such as pollution, cigarette smoke, microbes, allergens, pesticides and ionizing or solar (UV, visible, infrared-A) radiations. Oxidative stress can be stratified according to intensity ranging from physiological oxidative stress (eustress) essential for redox signaling to supraphysiological oxidative burden (distress), which damages biomolecules (Sies 2017).

Oxidative stress markers can be divided into three categories (Valadez-Vega *et al.* 2013):

1. Modified molecules (nucleotide, protein, lipid) formed by the action of free radicals
2. Antioxidant molecules or enzymes
3. Second messengers and transcription factors

When the ROS production or accumulation exceeds the antioxidant defence, redox imbalance becomes inevitable, which leads to toxic effects on the structural and functional integrity of biological tissues. This imbalance can either arise because of the rise in the ROS production or fall in the antioxidant defence or both. Therefore the main mechanism of antioxidant action is either a) suppression of ROS production b) scavenging free radicals c) upregulation of antioxidative defence or a combination of all these (Valadez-Vega *et al.* 2013). To counteract this inevitable exposure to free radicals from several sources, our physiological system has evolved to develop following mechanisms:

1. Preventive mechanisms
2. Repair mechanisms
3. Physical defences
4. Antioxidant defences

Effect of Oxidative Stress on Life and Disease

Though the average age has increased over the past few decades, simultaneously, the cancer burden has also risen to 19.3 million new cases and 10 million cancer deaths in 2020 (Sung *et al.* 2021). Persistently elevated ROS causes oxidative stress, which plays a vital role in the development of many age-associated diseases, including cancer. Even in the presence of the cell's defence system, oxidative damage acquires throughout the life (Arsova-Sarafinovska and Dimovski 2013). Though the production of ROS enhances during aging, proper ROS signaling is an essential requirement for healthy aging as it can regulate the lifespan directly. Endogenous and exogenous antioxidants can prevent and repair damage caused by ROS. Therefore, they can lower the risk of chronic-ROS driven diseases, including cancer or may even improve its prognosis.

Enzymatic antioxidants, like superoxide dismutase (SOD), glutathione peroxidase (GPx), NADPH quinone dehydrogenase (NQO) and catalase (CAT), act by chelating superoxide and other peroxides. In addition, non-enzymatic antioxidants (flavonoids, alkaloids, thiols, vitamins E and C, coenzyme Q, histidine, carotene, retinoic acid and glutathione) serve as an important biological defence against ROS attack (Sies 2017). In fact, the process of carcinogenesis is intricately linked with the inherited or acquired defects in enzymes responsible for the redox-mediated signaling axis (Tan *et al.* 2018). Therefore, the efficacy of antioxidant molecules that promote chemoprevention or chemotherapy by counteracting oxidative stress is of prior importance. In this chapter, we have highlighted the molecular mechanisms of antioxidants/prooxidants associated with anticancer management.

PHYSIOLOGICAL IMPORTANCE OF ROS

ROS can stimulate pro-inflammatory cytokine secretion from phagocytic cells, fibroblasts, and chondrocytes which can lead to acute disease conditions like, systemic inflammatory response, acute respiratory/renal insufficiencies, ischemia/reperfusion, and acute intestinal/ renal/ arthritic/ cardiac inflammation (Roy *et al.* 2017). However, it has some essential role too for the healthy maintenance of the body. $O_2^{\bullet-}$ due to its highly energized aggressive nature is detrimental and destroys biological macromolecules (protein, nucleotide and lipid). H_2O_2 has a role in regulating protein functioning as a second messenger or as a signaling molecule when its level is within a physiological range (Helfinger

CHAPTER 2

Environmental Contaminants and Redox Homeostasis

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Abstract: Contaminants in the environment, such as oxidant fuels, chemical substances, particulate surfaces, cigarette smoke, toxins, metals, medicines, xenobiotics, or radiation, can trigger the generation of the reactive oxygen species (ROS) or the reactive nitrogen species (RNS), which can lead to oxidative stress. Many ROS-mediated mechanisms shield cells from oxidative damage and help them reclaim their redox homeostasis. The activation of metabolic or bioenergetics reaction processes mediated by thiol redox switches is one of the overt or indirect mechanisms of oxidative stress. Furthermore, toxic agents' oxidative stress can be exacerbated through metabolic processes in cells. Excess ROS is regulated by endogenous antioxidant protection mechanisms (both enzymatic and non-enzymatic), which help remove toxic oxygen molecules or scavenge ROS under normal conditions. To sustain redox homeostasis in the presence of environmental stress, the cells are fitted with several complementing energy-dependent structures. The cytochrome (CYP) enzymes are a monooxygenase superfamily that includes several enzymes involved in xenobiotic detoxification. As a result, it seems that the CYP families are the most prominent members. Heavy metal toxicity, such as zinc, arsenic, and cadmium, is believed to be caused by their interaction with sulfhydryl groups in biological systems. Many sulfhydryl residues in antioxidant proteins, including metallothionein and albumin, serve as a sink for heavy metal ions, saving important protein thiols in the process.

Keywords: Antioxidants, Carotenoid, Drugs and Xenobiotics, Environmental Pollutants, GPx, GSH, Ionizing Radiations, Lipoic Acid, Metals, Pesticides, Reactive Oxygen Species, Redox Homeostasis, SOD, Tobacco Smoke, Vitamins.

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INTRODUCTION

Humans, wildlife, and household animals are all subject to a diverse combination phase of the mitochondrial respiratory chain produce reactive oxygen species (ROS) as a byproduct of normal metabolism in cells (He *et al.* 2017). The exogenous ROS generation can result from the exposure to various environmental contaminants such as oxidant gases, organic compounds, particulate matter, tobacco smoke, pesticides, metal, drugs, xenobiotics or radiation. Homeostasis is the tendency to maintain a reasonably constant internal condition despite changes in the external environment. ROS development and elimination from the body system are also carefully controlled to ensure redox homeostasis (Kong and Chandel 2018). The human body, for example, controls the internal amounts of charged particles, hydrogen, calcium, potassium, and sodium, on which cells depend for normal operation. Water, oxygen, pH, and blood glucose levels are also maintained through homeostatic cycles and are close to core body temperature. Maintaining “redox homeostasis” in the body requires a daily balance of oxidants and antioxidants. This suggests that, in response to a rise in ROS production, the body would increase the activation of endogenous antioxidant systems through the redox signaling mechanism (Valko *et al.* 2007). “Oxidative stress” is characterised as an accumulation of the reactive oxygen species (ROS) as a consequence of an imbalance between their production and the removal (which is controlled by an antioxidant defense system). On a molecular basis, environmental toxins induce various pathways of toxicity and enhance oxidative stress, causing harm to the cell membrane, lipid, DNA, and protein (Valavanidis *et al.* 2006). Metal ion homeostasis disruption may contribute to oxidative stress, a situation in which the enhanced production of reactive oxygen species (ROS) overwhelms the body's antioxidant defences, resulting in DNA injury, lipid peroxidation, protein alteration, and carcinogenesis (Jomova and Valko 2011). The preference for sulfhydryl groups is believed to underpin the toxicity of heavy metals such as arsenic (As), lead (Pb), and cadmium (Cd). Furthermore, environmental air contaminants (a combination of the particles suspended in the liquid and gaseous phase) may cause redox homeostasis to be disrupted.

Many pesticide groups disturb the cellular redox balance (Čermak *et al.* 2018). Multiple complementary energy-dependent mechanisms exist in the cells to sustain redox homeostasis in the presence of oxidative stress from the environment (Samet and Wages 2018). Several means are available for the treatment of free radical production in the cells that includes the non-enzymatic and enzymatic antioxidants.

FREE RADICALS IN REDOX HOMEOSTASIS

In biology, the importance of free radicals as well as other oxidants have become more valuable due to their predominant role in different physiological environments as well as their impact on a very wide variety of diseases. Reactive oxygen species (ROS) generation are initiated by both endogenous and exogenous sources. They are primarily formed as byproducts of natural cellular metabolism during the oxidative reaction phase of the mitochondrial respiratory chain (Balaban *et al.* 2005, Zorov *et al.* 2014) and exogenous sources or environmental sources (exogenous toxicants). ROS are created, during the conversion of xenobiotics from medications like halothane and paracetamol, *via* exposure to UV irradiation or by the metabolism of the toxic compounds including heavy metals, pesticides, tobacco smoke, and pollution, (Jezek and Hlavatá 2005, Phaniendra *et al.* 2015). Some exogenous toxicants' metabolism may result in formation of the ROS, which are more harmful than their parent compounds. As a consequence, exogenous toxicants could be sources of ROS generated by metabolism.

ROS include hydroxyl radicals, singlet oxygen, as well as hydrogen peroxide. They activate signaling pathways resulting in changes in biochemical, physiological, and molecular processes in the cellular metabolism (Xie *et al.* 2019). Moderate amounts of reactive oxygen species have positive effects on invasive pathogen killing, wounds healing, and repair processes (Bhattacharyya *et al.* 2014). ROS also, serve as a signaling molecule for the regulation of biological and physiological processes (Finkel 2011). ROS may be considered as a signal transduction process to allow the adaptation during changes in the nutrients, and oxidizing environment (Wood *et al.* 2003, Xie *et al.* 2019).

Reactive nitrogen species (RNS), like the nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), nitric oxide (NO), peroxynitrite (OONO), and nitrous acid (HNO_2), contribute to the oxidative stress in addition to ROS (Halliwell 2001, Di Meo *et al.* 2016). When cellular NO interacts with ROS, it produces a large number of RNS, which are involved in oxidative and nitrosative destruction.

ROS and RNS could be divided into two classes, namely the radicals and non-radicals. The radicals are species with at least one unpaired electron within shells around the nucleus and may be independent. Examples of the radicals include the superoxide ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), oxygen radicals (O_2), peroxyl radical ($^{\bullet}ROO^-$), nitric monoxide (NO) and nitrogen dioxide (NO_2) (Halliwell 2001). The existence of one unpaired electron around the nucleus, which seeks to donate or receive another electron to achieve equilibrium, results in a higher reaction to certain radicals. The high levels of superoxide anion are more associated with oxidative stress than with cell signaling (Schieber and Chandel 2014). The oxygen

CHAPTER 3

Role of Antioxidants in Redox Homeostasis

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Abstract: Reactive oxygen species are a result of normal oxygen metabolism, which even possess the ability to damage the cells; and thus, it becomes necessary to eliminate them. Redox homeostasis is a natural mechanism that detoxifies these ROS and involves many cellular processes in the detoxification. However, the production of ROS increases dramatically during environmental stress, which can result in the disruption of redox homeostasis. This disruption can lead to several complications that include the generation of tumour cells, ageing, diabetes and neurodegeneration. Antioxidants can prevent this disruption by reducing the propagation of free radicals and thus, they have an important role to play in the process of redox homeostasis. The chapter highlights the role of enzymatic and non-enzymatic antioxidants in redox homeostasis. Non-enzymatic antioxidants have been further divided into two categories namely, metabolic and nutritional antioxidants. The crucial role played by the antioxidants against ROS can be therefore used in therapeutics to treat the major diseases that are caused due to oxidative stress.

Keywords: Ascorbic Acid, Coenzyme Q10, Ebelsen, Haptoglobin, Lutein, MUA2, Quercetin, Rutin, SOD/CAT-Mimetic Drugs, TLK-199, Tocopherol.

INTRODUCTION

Reactive oxygen species (ROS) are free oxygen radicals that are produced as a result of aerobic metabolism in humans as well as in plants by the cell organelles. ROS, when present in normal amounts, can prove to be helpful in many physiological functions. ROS are important for the excitation-contraction coupling of skeletal muscles as it has been found that antioxidants mediated depletion of ROS lead to a decrease in contractile function (Reid *et al.* 1993).

ROS also act as initiators for apoptosis and it has been observed that an increase in ROS level is one of the starting events in apoptosis (Banki *et al.* 1999). Studies

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show that hydrogen peroxide (H_2O_2) acts as a signalling molecule between the sensor and transcriptional activators, indicating an increase in oxygen levels. NADPH-like oxidase acts as a sensor and the modification in the levels of erythropoietin (Epo) indicates the transcriptional activation (Fandrey *et al.* 1994). H_2O_2 increases the production of inflammatory mediators like interleukin-2, interleukin-2R and transcription factor NF-κB, which shows that ROS plays an important part in T-cell activation and it amplifies the immune response (Los *et al.* 1995). It has also been proved that ROS increases the adherence of leukocytes to endothelial cells by 2-2.5 folds compared to that of control (Sellak *et al.* 1994). Lipid peroxidation is thought to be undesirable, however, the non-enzymatic products generated through this process have been found to be useful. For instance, 15-F_{2t}-isoprostanes obtained from the lipid peroxidation of arachidonic acid assist as the biomarkers and mediators for oxidative injury in many diseases such as ischemia-reperfusion injury, cancer and genetic disorders (Milne *et al.* 2015). Along with these, ROS also plays an important role in stem cell differentiation and regulation of aging.

In spite of the useful nature of ROS at physiological levels, it can cause serious repercussions when its level exceeds the control of defence mechanisms (oxidative stress). In order to maintain the physiological levels of ROS, there is an inbuilt mechanism, which is called redox homeostasis. Redox homeostasis involves several responses, which include signalling, adaption, detoxification and apoptosis. The intensity of the response increases with an increase in the levels of ROS (Ayer *et al.* 2014). However, oxidative stress can be induced in response to environmental chemicals or during the biotransformation of certain drugs (Jezek and Hlavata 2005). The failure of defence mechanisms in oxidative stress results in the disruption of redox homeostasis. This disruption can cause inflammatory and cardiac pathologies as well as other disorders like cancer, diabetes, HIV infection, asthma, obstructive sleep apnoea and cataract (Roy *et al.* 2017). It is proven that the amount of ROS is higher in patients suffering from osteoarthritis than that in healthy individuals. This increase may be due to the increase in lipid peroxidation (Maneesh *et al.* 2005). When there is excess ROS production within the mitochondria of cardiac cells, DNA damage occurs and this results in cell injuries. These cell injuries can serve as one of the causes for major cardiac disorders like arrhythmia, atherosclerosis, hypertension, congestive heart failure and cardiac hypertrophy (Kukreja and Hess 1992). In CNS related disorders like Parkinson's and Alzheimer's disease, moderate levels of ROS have been observed too (Roy *et al.* 2017).

Antioxidants are substances that inhibit oxidation and contribute to limit the damage caused due to ROS. Antioxidants can be endogenous as well as exogenous. Endogenous antioxidants include enzymatic and metabolic ones, whereas exogenous antioxidants are obtained from our diet. Antioxidants are useful therapeutically for diseases in which increased ROS levels are observed. In the following chapter, we bring out the role of antioxidants in redox homeostasis with a therapeutic perspective. This chapter covers enzymatic as well as non-enzymatic antioxidants. The various antioxidants detailed in this chapter are superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione reductase (GR), glutathione-S-transferase (GST), urate, bilirubin, coenzyme Q10, transferrin, haptoglobin, ceruloplasmin, albumin, tocopherol, ascorbic acid, flavonoids, carotenoids, selenium and zinc.

ANTIOXIDANTS - SCAVENGERS OF ROS

Enzymatic Antioxidants

Enzymatic antioxidants (Fig. **1**) are the endogenous antioxidants, which at low concentrations inhibit the oxidation and thus neutralize the harmful effects of free radicals generated in our body due to oxidation (Jeeva *et al.* 2015). The enzymatic antioxidants present in our body include SOD, GPx, catalase, glutathione reductase and glutathione-S-transferase. The different classes, location, structure and mechanism of each enzymatic antioxidant are compiled in Table **1**.

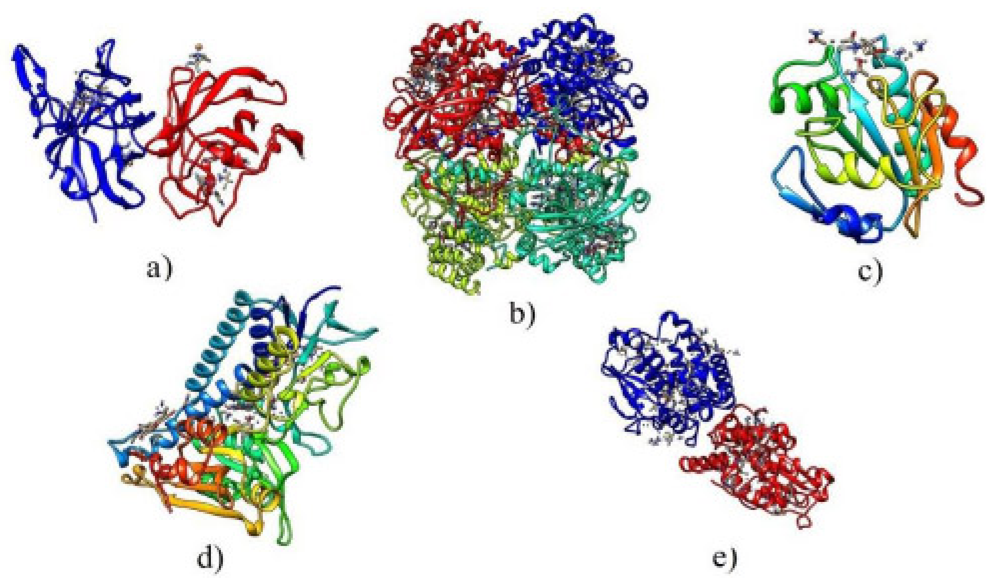

Fig. (1). Enzymatic Antioxidants; a) Superoxide Dismutase (Forest *et al.* 2000); b) Catalase (Foroughi *et al.* 2011); c) Glutathione Peroxidase (Borchert *et al.* 2018); d) Glutathione Reductase (Savvides and Karplus 1996); e) Glutathione-S-Transferase (Meux *et al.* 2011)

CHAPTER 4

Antioxidants (Natural and Synthetic) Screening Assays: An Overview

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Abstract: Antioxidants are used to inhibit the deterioration of a molecule and are used at a low concentration to slow or avoid the degradation of a molecule. They have the ability to chelate transition metals and work through a variety of synthetic processes like hydrogen atom transfer (HAT) and single electron transfer (SET). Understanding the biology of antioxidants, their possible applications, and their synthesis using different biotechnological methods are important aspects of antioxidant mechanisms. Antioxidant molecules can react in one of two ways: through multiple mechanisms or through a single mechanism. Understanding the antioxidant reaction process is possible due to the molecular structure of the antioxidant material. This chapter presents an overview of various antioxidants, their reaction mechanism against free radicals as well as the most utilized techniques to assess their different activities.

Keywords: Antioxidant activity, Antioxidant screening assays, Antioxidant, Free radicals, Hydrogen atom transfer (HAT), Reactive oxygen species, Single electron transfer (SET).

INTRODUCTION

In biological systems, oxidative stress is comprised of multiple and diverse mechanisms, which is considered to be a disproportion amid the creation of the free radicals and capability of our body to remove these species by the utilization of intrinsic and extrinsic antioxidants. Free radicals are extremely reactive species or molecular compound with an odd number of electrons which are considered to be very active with other atoms or molecules in a chemical reaction. Due to their unstable nature within the cell, they can oxidize numerous biomolecules, cause tissue injury and lead to cell death. When our body uses oxygen molecule, free

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radicals are generated due to cell death. When our body uses oxygen molecule, free radicals are generated due to loss or gain of electrons and these reactive molecules damage the internal environment of the cell called oxidative stress. This free radical reaction depends on the existence of oxygen, nitrogen, and sulphur radicals. Superoxide ($\cdot O_2^-$), alkoxyl (RO·), hydroxyl (HO·), peroxyl (ROO·), and nitric oxide (NO·) are examples of O_2 dependent free radicals and reactive oxygen species (ROS). There are other non-radical ROS in the body, including hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) and hypochlorous acid (HOCl) (Pietta 2000).

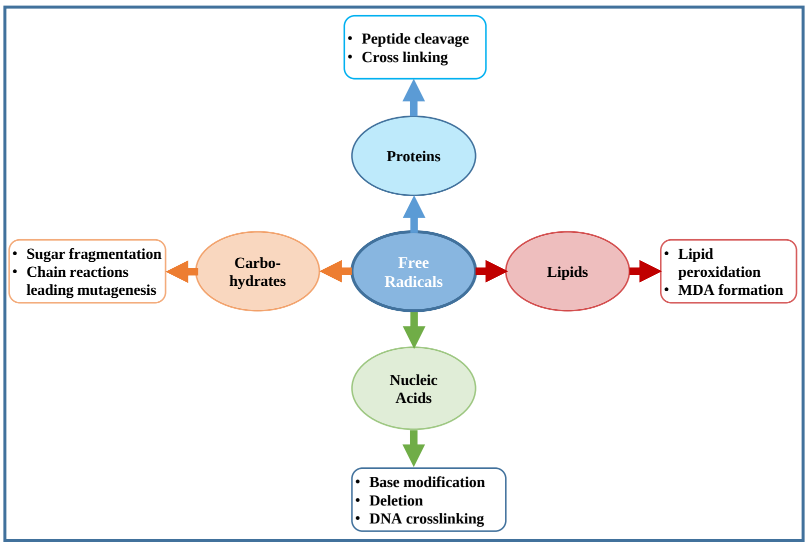

Fig. (1). Biological targets of free radicals.

ROS could be produced throughout the metabolism of biomolecules, respiration, and therefore autoxidation of the xenobiotics as a function of this cause various diseases in the living organisms (Cakmak and Gülçin 2019, Anraku *et al.* 2018). Moreover, there are more reactive nitrogen species (RNS), like, peroxynitrite (ONOO−), nitric oxide (NO•), nitrosoperoxycarbonate ($ONOOCO_2^-$), nitrogen dioxide (NO_2•), and nitronium particles (NO_2^+), dinitrogen trioxide (N_2O_3), and peroxynitrous acid (ONOOH). These reactive species are produced in lesser quantities in the cellular functions including cell flagging, neurotransmission, the unwinding of muscle, peristalsis, accumulation of platelet, pulse adjustment, blood pressure inflection, phagocytosis and cell development (Limón-Pacheco and Gonsebatt 2009). In the biological, environmental conditions, they are generally known as the pro-oxidants or antioxidant agents, and in chemical terms, they are referred to as oxidants and reductants, respectively (Cao and Prior 1998).

The pro-oxidants are agents that cause oxidative harm to the biological targets including sugars, nucleic acids, proteins, and lipids (Fig. **1**).

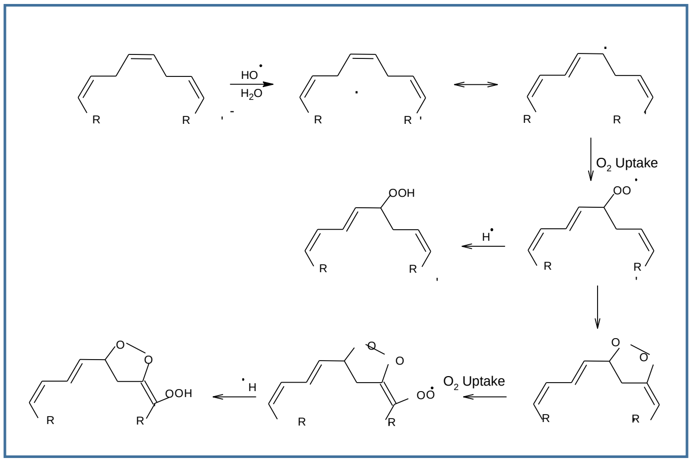

Fig. (2). The hydroxyl (OH) radical reaction with the polyunsaturated fatty acids.

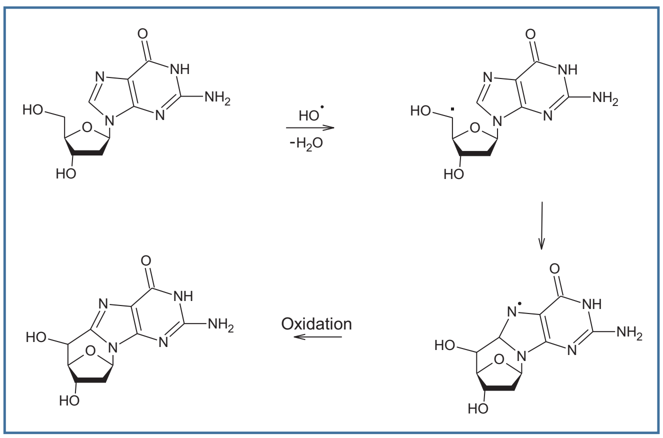

Fig. (3). The hydroxyl radical reaction with the sugar.

Free radicals create assorted activities on the metabolism, which can be the basis of cell injury (Nakamura *et al.* 1997).

1. In the polyunsaturated lipids, lipid peroxidation occurs due to ROS molecules causing cell lysis (Fig. **2**)
2. Altering cellular processes correlated with interleukin involvement and the production of prostaglandins, neurotransmitters, and hormones in the

CHAPTER 5

Oxidative Stress and Biochemical Approaches of Antioxidant Analysis

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Abstract: Abiotic stresses have contributed to the generation of reactive oxygen species called as free radicals which are highly toxic to the organism. Free radicals may be evaluated either explicitly or inadvertently after the production of oxidative by-products of nucleic acids, proteins or lipids, a method also known as fingerprinting. Though the approaches for analyzing such reactive intermediates have been thoroughly studied; we concentrated primarily on recent implementations of these techniques to quantify free radicals and different candidate biomarkers of oxidative stress such as nitrotyrosine, isoprostane, *etc*. Further, the various biochemical approaches along with the conventional methods are also discussed for the evaluation of antioxidant activity of natural products.

Keywords: Antioxidants, Biochemical approaches, Biomarkers, Oxidative stress.

INTRODUCTION

Oxidative stress is associated with a delayed release of free radicals or with a reduction in antioxidant concentration. The disruption in the stability of prooxidants and antioxidants is the result of oxidative stress (Husain and Kumar 2012). Free radicals or prooxidants produce fewer electrons that respond strongly to certain kinds of radicals in an unstable manner. Continuous metabolic pathways in humans generate ROS/free radicals that especially target fats, proteins and DNA. There are few endogenous causes for the production of ROS such as certain organelles (mitochondria, peroxisomes), xanthine oxidase (Sisein 2014), phagocytosis, arachidonic acid pathway (Husain and Kumar 2012), respiratory

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explosion (Takashima *et al.* 2012), whereas exogenous sources include UV radiation, industrial solvents and atmospheric pollutants. In addition, the reactive oxygen species (ROS) are produced as a result of partial reduction by non-reactive dioxygen (Kumar 2014). ROS usually involves nitric oxide (NO), superoxide anion (O_2), H_2O_2, radical hydroxyl (OH), single oxygen *etc*. The importance of free radicals in pathogenesis is increasingly being recognized in past years amongst people for the prevention of various diseases. Oxygen is an important aspect of life however, the regular use of oxygen by the body continually creates free radicals (Shinde *et al.* 2012). The development of chronic and degenerative diseases including cancer, diabetes, aging, cardiovascular and neuropathic disease that has a significant role to play when it comes to oxidative stress (Shinde *et al.* 2012).

The human body offers many mechanisms to combat oxidative stress by providing antioxidants that are produced naturally or delivered externally *via* food and/or supplementation. Antioxidants from external as well as internal sources function as free radical scavengers that can enhance the immune response and reduce the risk of various diseases (Valko *et al.* 2006). The previous reports suggested that disparities in free radicals and saliva antioxidants could be a contributing factor in the development of periodontal diseases, thus it is necessary to evaluate oxidation stress in saliva to provide a more precise account of oral surroundings (Shinde *et al.* 2012).

There are two kinds of biological free radicals: nitrogen-based radicals, also known as RNS and oxygen dependent radicals, also known as ROS. Free radicals may trigger lipid peroxidation (LPO), breakdown in DNA strands and oxidation of proteins and other essential molecules that can cause injury (Phaniendra *et al.* 2015). Some of the RNS and ROS are given in Table **1**.

Table 1. Some of the common reactive oxygen and nitrogen species.

S. NO.	Reactive oxygen species	
1.	Alkoxy radical	$RO^·$
2.	Hydrogen peroxide	H_2O_2
3.	Hydroperoxyl radical	$HOO^·$
4.	Hydroxyl radical	$^·OH$
5.	Hypochlorous acid	HOCl
6.	Ozone	O_3
7.	Perhydroxyl radical	$HO_2^·$
8.	Peroxyl radical	$ROO^·$
9.	Singlet oxygen	O_2

(Table 1) cont.....

S. NO.	Reactive oxygen species	
10.	Superoxide	O_2^-
	Reactive nitrogen species	
1.	Nitric oxide	NO
2.	Nitric dioxide	NO_2
3.	Peroxynitrite	ONO_2^-

BIOMARKERS OF OXIDATIVE STRESS

The cellular ROS rates and ROS mediated protein and membrane lipid products, thiobarbituric acid reactive substances (TBARS), reactive carbonyls and malondialdehyde (MDA) are known to be the main biomarkers of oxidative stress (Anjum *et al.* 2019).

Malondialdehyde (MDA)

MDA is a small, reactive organic molecule omnipresent throughout eukaryotes, which is formed by 3 carbon molecules at C1 and C3 positions containing dual aldehyde groups. Because of its pH-dependent tautomeric chemical activity MDA occurs in aqueous solutions in various types. The dominant form at a pH higher than pKa of 4.46 is the enolic anion which demonstrates weak chemical reactivity. During conditions of oxidative stress, MDA occurs in a balance amongst its protonated enol aldehyde and the dialdehyde form at lower pH (Morales and Munné-Bosch 2019). Numerous approaches for evaluating MDA content have already been introduced through derivatization combined with specific isolation methods, which took advantage of the MDA molecule's electrophilic character. Such approaches include liquid chromatography (LC); gas chromatography (GC) and mass spectrometry (MS) (Morales and Munne-Bosch 2019).

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS is known to be the primary biomarker of oxidative stress (Anjum *et al.* 2019). The technique involves the reaction of lipid peroxidation products, especially thiobarbituric acid (TBA), with MDA which leads to the formation of MDA-TBA2 product, TBARS. TBARS generates a red-pink colour that can be evaluated at 532nm spectrophotometrically. The TBARS test is conducted at 95 °C in acidic conditions (pH = 4). Pure MDA is unstable, but these conditions allow MDA to be released from MDA bis(dimethyl acetal), which is used as an analytical standard. This approach is not very precise, as 2, 4-alkadienals, 4-hydroxyalkenals, and nucleic acids can also react with TBA, resulting in the formation of a chromophore (Miguel 2010).

CHAPTER 6

Advances in Extraction and Profiling of Antioxidants

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Abstract: The natural antioxidants are plant secondary metabolites that play a key role in preventing the development of various oxidative stress-induced degenerative and age-related disorders such as cardiovascular disease, cancer, *etc*. As a result, interest in these antioxidant compounds from natural sources has increased in recent years. For this reason, antioxidant substances in plants are extracted and presented to the market as a standardized solution. The first method of antioxidant extraction from plant sources is classical solvent extraction. Conventional solvent extraction takes place in two ways: liquid-liquid extraction and solid-liquid extraction. However, there are some disadvantages of using the classical extraction method to obtain antioxidants from plant sources. These methods use high amounts of solvents and require more time for extraction. Low selectivity, less efficiency, and environmental effects are some of the disadvantages. Therefore, the trend towards new extraction techniques has increased. Ultrasound-assisted, microwave-assisted, supercritical fluid, and accelerated extraction systems are very effective methods compared to conventional solvent extraction. These extraction procedures can be used in low temperatures and prevent the thermal degradation of antioxidants. In this study, the efficiency of new extraction methods and classical extraction methods are compared and the effect of extraction on antioxidant components has been compiled.

Keywords: Antioxidants, Catalase, Enzymatic antioxidants, Glutathione reductase, Microwave-assisted extraction, Natural antioxidants, Solid-liquid extraction, Soxhlet extraction, Supercritical fluid extraction, Ultrasound-assisted extraction.

INTRODUCTION

Sample preparation is a process that decides the qualitative and quantitative analysis of antioxidants. One of the indispensable steps of analytical processes is extraction. F. Soxhlet developed soxhlet extraction in 1879 with massive

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popularity until mid-1980s and is still most routinely used procedure in laboratories. Demand for advanced extraction techniques has been increasing in recent years. As it is suitable for automation with reduced extraction time and consumption of organic solvents, thereby preventing pollution in analytical laboratories with reduced sample cost (Wan and Wong 1996, Eskilson *et al.* 2000).

The basic understanding of extraction principles has progressed in parallel with the development of new technologies. This progress led to new trends in sample preparation. These are the integration of sampling, separation and quantitation steps used in micro extraction, miniaturization and analytical processes (Pawliszyn *et al.* 2003). The required sample preparation depends on the nature of the sample and the analytical method used. Sample matrices can be classified as organic or inorganic and subdivided into solids, liquids or gases. For example, homogenization and drying are usually the first steps of the process. The next sample pretreatment step is usually extraction. For this purpose, the need for new extraction techniques that shorten the extraction time, reduce organic solvent consumption and prevent environmental pollution is increasing. Ultrasound-assisted, microwave-assisted, supercritical and accelerated extraction systems, which are used in the extraction of antioxidant substances from plants, are very fast and effective. In these techniques, the possibility of working at high pressure and / or high temperatures greatly reduces the extraction time.

ANTIOXIDANTS

Antioxidants are molecules that prevent the formation of free radicals or prevent damage to the cell by sweeping existing radicals and generally carry phenolic groups in its structure (Kahkönen *et al.* 1999). Antioxidants, at lower concentrations than oxidizable substrates severely hamper or delay the oxidation-induced stress. Pro-oxidants (reactive oxygen and nitrogen types, free radicals) are toxic substances that cause oxidative damage in lipids, proteins and nucleic acids, resulting in various pathological events and/or diseases. The presence of these dangerous compounds makes antioxidants important for a healthy life because antioxidants effectively reduce pro-oxidants into low-toxic or non-toxic products (Cao and Prior 1999). The most important factors that determine the place of antioxidants in human health are their chemical structure, solubility, structure/activity relationships and their availability from natural sources (Güçlü *et al.* 2009).

Antioxidants are produced by body cells and can be taken through foods as well. The main natural antioxidants present in foods that protect the human body from harmful free radicals are mainly vitamins (C, E and A), flavonoids, carotenoids and polyphenols. Studies show an inverse relationship between the consumption of fruits/vegetables and the occurrence of certain cancers and heart diseases (Kaur and Kapoor 2001). The most important antioxidants are polyphenols and their derivatives. These compounds can behave in different ways in the oxidative system. For example, they can reduce oxygen concentration by absorbing singlet oxygen, prevent the initiation of chain reactions using their ability to scavenge primary radicals, such as hydroxyl radicals and prevent the catalytic synthesis of pro-oxidants *via* metal ions (Shahidi *et al.* 1996). Antioxidants are oxidizable substances and can protect the biological macromolecules for a limited time only, and after a certain point, the biomolecule continues to oxidize as if there were no antioxidants in the environment. The reduction potential of antioxidants as hydrogen or electron donor is usually expressed as free radical scavenging (Kaur and Kapoor 2001). In an evaluation of chain-breaking antioxidant activity, both the number of electrons that the antioxidant can give per molecule or the number of free radicals it can remove (reaction stoichiometry) and the reaction rate (kinetics) are important (Rice-Evans *et al.* 1997).

Classification of Antioxidants

Cells are protected by antioxidants against oxidative damage caused by free radicals and peroxides under normal physiological conditions (Rice-Evans *et al.* 1997). Antioxidants are divided into two groups, natural and synthetic:

a. Natural Antioxidants: Natural antioxidants are classified as enzymatic and non-enzymatic.
   1. Enzymatic antioxidants are present in all plants, microorganisms and animals. These enzymes are as follows:
      i. Superoxide Dismutase (SOD): Superoxide dismutase (E.C.1.15.1.1) catalyzes single-electron dismutation of superoxide to hydrogen peroxide and oxygen (Chaudiere *et al.* 1999).
      ii. Catalase (CAT): Catalase (E.C.1.11.1.6) is a protein with a tetrameric structure of 240,000 daltons molecular weight, consisting of four subunits, each having a group of [Fe (III) -protoporphirin] in each subunit (Özkan *et al.* 2000). Catalase enzyme neutralizes hydrogen peroxide by converting it into water and oxygen. Although H_2O_2 formed as a result of SOD activity is not a radical, it can cause oxidative damage because it is the precursor of the most reactive species, $^{\bullet}OH$ radical. Therefore, catalase facilitates the reduction of the hydrogen peroxide concentration by catalyzing the dismutation of two electrons of hydrogen peroxide into water and oxygen.
      iii. Glutathione Peroxidase (GPx): Glutathione peroxidase (E.C.1.11.1.4),

CHAPTER 7

Advances in the Profiling and Characterization of Antioxidants

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Abstract: The growing interest in plant foods as a source of phytochemicals in general and antioxidants like polyphenols in particular continues to receive a great deal of attention of nutritionists, food scientists and consumers as well. Food is no more regarded as just a source of energy and nutrition but is gaining importance as a functional or nutraceutical diet ingredient. The functional compounds are the secondary metabolites (PSM), produced by the plants as a natural defense against insect pest damage or adverse environmental conditions and represents a large and diverse group of bioactive compounds. PSMs are strong antioxidants that complement or improve the functions of antioxidant vitamins and enzymes which have a protective role to play in the bodily system against reactive oxygen and nitrogen species, UV light exposure, attack of pathogens, parasites and predators. Antioxidants are prophylactic compounds that can possibly even be used to cure several prevailing human diseases by traditional medicinal and health care system. Antioxidants are very sensitive compounds and their bioavailability in food is subject to their occurrence in food and the food processing conditions. The complexity in structure, function and expression of different antioxidants coupled with their frequent occurrence in different herbals from negligible to significant amounts, extraction, identification and their analysis remain a challenging task as ever for the scientists and technologists, despite the recent advances in the analytical and the instrumentation procedures. Keeping in view the high health potential and the related concerns, the current contribution is focussed on extraction, profiling, characterization, biological activity and implications of antioxidant consumption on human health to diversify food applications.

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Keywords: Antioxidant activity, Biological activity, Characterization, Extraction, Plant secondary metabolites, Profiling.

INTRODUCTION

In the past decades, research conducted and publications dealing with antioxidants, their stability, bioavailability and potential application in human health and disease management reflected tremendous growth. Free radicals can harm DNA, lipids, proteins and negatively affect the aging process and diseases. Antioxidants are substances that neutralize the free radical chain reactions. Plants undergo various environmental stresses like nutrient deficiency, salinity, drought, UV radiation, temperature variations (heat shock, chilling, and frost), heavy metals, pathogen attacks and air pollution, from which they cannot escape. During these oxidative stress, reactive oxygen species (ROS) are formed such as superoxide radicals ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydroxyl radicals ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2). The imbalance in the ROS equilibrium determines its toxic response in a stressed condition. These ROS molecules can attack high molecular mass compounds like DNA. Hence, ROS are capable of causing damage at the cellular levels and antioxidants are essential to scavenge these toxic molecules. Antioxidants act on the ROS and other free radicals to restrict or prevent various cellular damages from free radicals that are responsible for a variety of diseases. The recent research in plant sciences and nutrition has shifted its focus around various practices to protect crucial tissues and organs from damage induced by free these radicals. Mainly, four defence mechanisms of antioxidants suggested by McDowell *et al.* (2007) include (a) quenching active oxygen species, (b) preventive antioxidants, (c) sequestration of elements by chelation, and (d) free radical scavengers. The first mechanism explains the conversion of active oxygen species into a more stable form. For example, vitamin E and carotenoids are helpful in stabilizing singlet oxygen radicals. The second mechanism involves the suppression of free radical generation. The catalase enzymes inhibit hydrogen peroxide and prevent oxygen radical formation. The third mechanism of antioxidant activity suggests their strong bonding with trace minerals like Fe and Cu during protein transportation. These trace elements facilitate the formation of radicals. The fourth mechanism reflects the role of antioxidants in stabilizing free radicals as they donate electrons and oxidize themselves. This process is also referred to as "free radical scavenging." Vitamin E scavenges the peroxyl radical in a similar manner (McDowell *et al.* 2007). The effective action of antioxidants may vary with their activation energy, oxidation-reduction potential, rate constant, their susceptibility towards heat and their stability in various environmental conditions.

Plant tissues are under constant stress (oxidative) and continuously generate the free radicals. As a result, they develop an antioxidant system to protect themselves from free radicals attack. Various drought stressed plants are reported to synthesize low molecular-weight antioxidants, like α-tocopherol. Two types of antioxidants have been reported in the literature, including synthetic and natural antioxidants. Structurally, antioxidants have at least one aromatic ring and their activity greatly depends on a number of –OH groups present on these aromatic rings whereas, the arrangement of this functional group on aromatic rings is helpful in chelating peroxidative metals. The examples of synthetic antioxidants are BHT, BHA and propyl gallate that possess one aromatic ring. Natural antioxidants, ascorbic acid and vitamin E also possess one aromatic ring. However, phenols and other antioxidants possess more than one aromatic ring. Natural antioxidants have great diversity and generally, they include all bioactive compounds (Brewer 2011).

Naturally, each and every cell of the body has a defence mechanism against harmful effects of free radicals which involve various enzymes like superoxide dismutase, glutathione reductase, glutathione peroxidase, thiols and di-sulfide bonding. The theory of free radicals accelerates the broad interest in the bioactivity of antioxidants in preventing chronic disease like stroke, cancer, diabetes, arthritis and neurodegeneration in past few decades. The utilization of dietary antioxidants showed positive evidence especially in preventing fatal disorders like cancer and cardiovascular disease (CV). The traditional medicinal systems like Ayurveda, Siddha, Chinese medicinal system, *etc*., include a wide range of plants for the treatment of many chronic diseases. These plant materials have therapeutic activity and are widely incorporated in the foods through which they enter the body system and interact with the living tissue (Biesalski *et al.* 2009). A vast range of diversity is available in plant compounds, including alcohols, aldehydes, alkyls, benzyl rings, and steroids, and all of them possess different characteristic features (Roessner and Beckles 2009). Further, the concept of nutraceutical and functional foods are trending in developed countries. These functional and nutraceutical foods claim their therapeutic effects due to the presence of bioactive compounds in high concentrations. The bioactive compounds are broadly classified as phenols, terpenes, saponins, alkaloids, vitamins, lipids and carbohydrates. In industries, antioxidants are required for wide spectrum applications *i.e.*, they restrict the deterioration of oxidative products in pharmaceuticals and cosmetics. In plants, the antioxidants are distributed in all parts like leaves, roots, stem/bark, fruits and fruit shells/peels, flowers and seeds. This is the reason behind the extensive study on whole plants for their therapeutic effects in the past few years. In traditional Indian diet, medicinal plants and spices are used which possess a high amount of natural antioxidants. Spices are rich in essential oils that have strong antioxidant

CHAPTER 8

Efficacy of Dietary Antioxidants in Diseases Prevention

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Abstract: Free radicals produced within the body as the inevitable side-effects of standard metabolic procedures of cells, or by exposure to poisons in nature. Excessive levels of free radicals trigger a disorder called oxidative stress, which can destroy cells and contribute to chronic diseases like atherosclerosis, diabetes, rheumatoid arthritis, ocular disease, Alzheimer's disease, deterioration in the immune system, and different kinds of cancer. Antioxidants are materials that counterbalanced free radicals and delay, hinder or remove harm brought about by free radicals. Nutritional antioxidants are commonly distributed in different food forms. Plant foods are major sources of antioxidants. They protect against oxidative stress and reduce the danger of numerous ailments by acting as oxygen and peroxyl radical scavengers. A diet that includes berries, fruits, vegetables, grains, tea, coffee, nuts, and healthy oils has an excellent antioxidant supplement. This combination of multiple detoxifying antioxidants can play a synergistic role in reducing the risk of ailments. Antioxidants including vitamins (A, E, and C), as well as carotenoids and other minerals (zinc, manganese, copper, and selenium) are important for antioxidant enzyme activities. Nutritional polyphenols and flavonoids are also powerful antioxidant compounds. In this chapter, we address the medicinal advantages of various antioxidants in reducing the risk of inflammatory ailments of skin, eye, neurodegenerative, cardiovascular, diabetes and liver diseases.

Keywords: Antioxidants, Cancer, Cardiovascular diseases, Diabetes mellitus, Dietary polyphenols, Eye diseases, Free radicals, Inflammatory diseases, Lipoic acid, Liver diseases, Minerals, Neurodegenerative diseases, Osteoporosis, Vitamins.

INTRODUCTION

Free radicals are produced from both endogenous and exogenous sources. Immune cell activation, irritation, infection, malignant growth, excessive exercise, mental stress, and aging are accountable for endogenous free radical creation for

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the most part during electron transport in mitochondria. While exogenous free radicals are often produced from exposure to ecological stress or toxins (radiations, heavy metals, and cigarette smoke), and xenobiotics (Young and Woodside 2001, Valko *et al.* 2007).

Under normal conditions, a state of equilibrium between the reactive species and endogenous antioxidants was found. When this equilibrium is disrupted, it results in a situation called oxidative stress where the production of these free radicals exceeds the antioxidant potential of the body (Poljsak *et al*. 2013, Pizzino *et al*., 2017). The excess production of reactive oxygen species (ROS) damages unsaturated fatty acids membranes, which cause a loss of membrane fluidity and cell degradation (Nimse and Pal 2015). ROS also leads to the formation of several denatured proteins with deleterious assault on nucleic acids, which ultimately results in mutations that can produce malignancy (Davies *et al*. 1987). ROS attacks on carbohydrates cause severe changes in cell receptors, which significantly alter neurotransmitter and hormonal reactions (Dalle-Donne *et al*. 2003). These radicals damage certain cell organelles, particularly the mitochondria, which can cause energy disturbances and create numerous cytotoxic compounds that harm cells. Most chronic diseases are emerging as a result of these deleterious consequences of oxidative stress. Several investigations have shown that many diseases such as atherosclerosis, cataracts, obesity, diabetes, various types of cancers, Alzheimer's disease (AD), cardiovascular disease (CVD), and arthritis are closely linked to oxidative stress (Labat-Robert and Robert 2014, Liu *et al.* 2018). To overcome these harmful impacts of free radicals for restoring the natural body balance between oxidants and antioxidants, the intake of various kinds of antioxidants are necessary. Dietary natural antioxidants (Fig. **1**) are preferred instead of synthetic antioxidants since the latter has numerous unfavorable impacts. Vegetables and fruits are studied extensively and have been appeared to bring down the occurrence of numerous maladies (Slavin and Lloyd 2012). Numerous edible herbs are rich sources of these antioxidants and have an important role in protection against many diseases (Abdel-Azeem *et al*. 2017). The use of a mixture of antioxidants may potentially be more effective than a single antioxidant, as they can act synergistically (Liu 2003, Sonam and Guleria 2017). Vitamins (vitamin A, E, and C), polyphenols (phenolic acids, anthocyanins, flavonoids, lignans, isoflavones, and stilbenes) and, carotenoids (xanthophylls, carotenes, and lycopene) are common plant-based antioxidants (Manach *et al*. 2004, Baiano and del Nobile 2015). Generally, these natural antioxidants, particularly polyphenols and carotenoids, display beneficial biological actions with anti-inflammatory, antibacterial, antiviral, anti-aging, and anticancer activities (Li *et al*. 2014, Zhang *et al*. 2015, Zhou *et al*. 2016, Xu *et al.* 2017).

DIETARY ANTIOXIDANTS

Vitamins

Natural foods are the main sources of many vitamins, of these, vitamin A is a fat-soluble vitamin. Several carotenoids like lutein, canthaxanthin, astaxanthin, lycopene, and neoxanthin have high antioxidant activity. Vitamin A and carotenoids rich foods include cantaloupe melon, mango, liver, carrot, broccoli, sweet potato, butter, spinach, pumpkin, cheddar, apricot, pear, and egg. Thermal treatment facilitates cell-wall disruption and loosened chemical bonding, which increase the bioaccessibility and absorption of carotenoids (Fernandez-Garcia *et al*. 2012). However, combinations with medications, such as aspirin and sulphonamides, decrease the bioavailability of the β-carotene (Castenmiller and West 1997). The recommended dietary allowance (RDA) for vitamin A is 900 μg/day for men and 700 μg/day for women (Olson 1987). Antioxidant effects of vitamin A and carotenoids are due to the hydrophobic chain of polyene units, which quench or neutralize free radicals (Galano 2007). Nevertheless, a significantly high dose of β-carotene has an adverse effect on the incidence of lung cancer in smokers (Druesne-Pecollo *et al*. 2010).

Vitamin E is a collective term for a group of eight fat soluble compounds, four of which are tocopherols and four are tocotrienols (Wang and Quinn 1999). Alpha-tocopherol is the most abundant type of tocopherol in plasma and possesses the best bioavailability. It shields the cell membrane from oxidative damage by neutralizing lipid radicals created in the lipid peroxidation chain response (Lobo *et al*. 2010). Along these lines, it keeps up the integrity of fatty acids within the cell membranes and improves their bioactivity (Rizvi *et al*. 2014). Tocopherol inhibits chronic oxidative stress-related illnesses (Niki 2015). Nuts, asparagus, wheat germ, avocado, egg, spinach, milk, seeds, and entire grain food are the rich sources of tocopherol. The RDA of vitamin E for both genders is 15 mg/day.

Vitamin C is also referred to as ascorbic acid and ascorbate. It is a crucial nutrient necessary for all our body systems to function properly. Vitamin C plays a powerful role in protecting the various tissues against oxidative stress. It works as a cofactor in numerous enzymatic reactions for collagen synthesis because it is a necessary component of collagen hydroxyproline and hydroxylysine synthesis (Darr *et al.* 1993, Akbari *et al*. 2016). Also, it is a vital component for many enzymatic reactions and the proper functioning of the immune system (Carr and Maggini 2017). There is widespread use of vitamin C in medications against a huge number of disorders. Human diseases, which address the essential effect of vitamin C are common cold, cataracts, malignant growth, atherosclerosis, diabetes, and degenerative neurological disorders (Chambial *et al.* 2013).

CHAPTER 9

Dietary Antioxidants and their Molecular Targets in Oxidative Stress Mediated Cancer Progression

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Abstract: Cancer is a complex disease and is currently the leading cause of mortality and morbidity across the globe. Dysregulated bioenergetics is one of the hallmarks of cancer cells and is characterized by increased activity of several enzymes of metabolic pathways. Consequently, cancer cells produce higher levels of reactive oxygen species (ROS) which contribute to their enhanced proliferation and survival over normal cells. Elevated levels of ROS cause oxidative stress, redox imbalance, DNA damage, activation of oncogenes, chronic inflammation and eventually cancer. Additionally, ROS mediated oxidative stress activates several oncogenic signaling cascades including PI3K/Akt pathway, NF-κB pathway, cyclooxygenase pathway, JAK/STAT pathway, angiogenesis and metastasis. To maintain redox balance and neutralize the detrimental effects of ROS, normal cells exhibit an antioxidant defence system, comprising of both enzymatic and non-enzymatic division. Activation of Nrf2 signaling pathway is the key regulatory pathway that helps in restoring the cellular redox homeostasis. Extensive research in the past decades has witnessed the potential health benefits of dietary antioxidants alone or in combination in the prevention of several chronic diseases, including cancer. A number of antioxidants from dietary backgrounds such as epigallocatechin gallate, resveratrol, curcumin, phloretin, berberine and lycopene have shown appreciable potential as a chemopreventive agent without causing significant toxicity. This chapter presents an extensive analysis of existing knowledge on the protective effects of various dietary antioxidants against cancer with a focus on oxidative stress, redox homeostasis and dysregulated cellular signaling leading to cancer cell proliferation, survival and metastasis.

Keywords: Antioxidants, Cancer, Oxidative stress, Reactive oxygen species, Redox homeostasis.

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INTRODUCTION

Cancer represents one of the major public health problems of the 21st century and is currently the second leading cause of mortality across the globe (Bray *et al.* 2018). In 2018, nearly 9.6 million deaths and 18.1 million new cases of cancer were expected across the globe. There are approximately 6.06 lakh deaths and 1.8 million new cancer cases are expected in 2020 in the United States alone (Siegel *et al.* 2020). Genetic and environmental factors, several of which are associated with socio-economic development have been recognised as risk factors for cancer development (Dean *et al.* 2018, Herceg *et al.* 2018). The striking finding about displacement of cancers caused by infection or poverty with cancers which are largely diagnosed in developed countries (Europe, America, high income countries in Asia), is an indicator of adoption of westernised lifestyle in these countries (Bray *et al.* 2018). This emphasises, albeit indirect, that a large proportion of cancer types can be prevented simply by modifying lifestyle related factors such as diet, physical exercise, smoking and alcohol consumption. These factors play a crucial role in promoting or suppressing carcinogenesis *via* modulation of levels of reactive oxygen species (ROS) and associated redox signaling pathways. Perturbed redox status and subsequently altered redox signaling is a common hallmark of all cancers (Bakalova *et al.* 2013). Free radical generation is a general physiological process, resulting from different biological functions, including metabolism and inflammation. The increased engagement of cancer cells towards metabolic activities generates high levels of cellular ROS and eventually oxidative stress. The enhanced oxidative stress causes genomic instability, genetic mutations in genes whose products keep a check on cell divisions. Additionally, oxidative stress causes aberrant activation of several key signaling cascades such as NF-κB signaling pathway, PI3K/Akt pathway, cyclooxygenase pathway, JAK/STAT pathway aiding cancer cell proliferation and survival, angiogenesis and metastasis. Mitochondria represent the main centre for production of ROS under physiological conditions which subsequently plays a major role in metabolic regulation, cell proliferation and survival mechanisms. To balance ROS, certain defence molecules are present in the cell called "antioxidants". These cellular antioxidants are divided into two major categories: enzymatic and non-enzymatic antioxidants. The enzymatic antioxidant system includes superoxide dismutase (SOD), catalase, glutathione peroxidase (Gpx) and glutathione-S-transferase. The non-enzymatic antioxidant defence system is comprised of vitamin C and E, flavonoids, carotenoids, lipoic acid and others (Watson 2013). In addition to the cellular antioxidant system, regular consumption of foods containing high content of antioxidants also protects against oxidative stress mediated genetic insults and subsequently cancer development.

A series of chemotherapeutic agents have been devised to treat cancers of different origins. However, none of these agents is effective in eradicating cancers of advanced stages. Furthermore, several major adverse effects of chemotherapeutic drugs such as cardiac myopathy, haematological, gastrointestinal, neural, renal and liver damages have been recorded (Pearce *et al.* 2017, Nurgali *et al.* 2018). In this scenario, chemoprevention seems to be a promising window to curb the ever-increasing cancer burden. Moreover, a shift in the perspective of the general public about the origin of medicine has been observed. The acceptance rate of medicines of herbal origin is now gaining more priority over the synthetic ones. The traditional knowledge has indicated the crucial role of diet in promoting or delaying several human diseases including cancer. The idea of preventing most human diseases through certain modifications in diet was suggested by Hippocrates and dates back 2500 years ago . His famous phrase "Let food be thy medicine and medicine be thy food" clearly indicates the critical role of diet in human health and endorses the consumption of food items containing medicinal properties (Langner and Rzeski 2012). Moreover, epidemiological reports have suggested that daily consumption of fruits, vegetables, nuts, flax seeds and fatty fish has an inverse relationship with cancer incidence (Boeing *et al.* 2012, Grosso *et al.* 2013). It has been shown that cancer incidence is half in individuals consuming fruits and vegetables five serves in a day (Surh 2003). Phytochemical analysis of these fruits, vegetables, sea foods revealed the presence of certain kind of bioactive compounds of antioxidant nature including polyphenols, flavonoids, carotenoids and alkaloids. Examples of dietary phytochemicals with chemopreventive activity include epigallocatechin gallate, resveratrol, curcumin, phloretin, berberine and lycopene. The chemopreventive potential of above-mentioned dietary phytochemicals has been supported by numerous pre-clinical and clinical studies (Chikara *et al.* 2018, Choi 2019, Grabowska *et al.* 2019). Additionally, dietary phytochemicals are cost effective, easily administered and are generally recognized as pharmacologically safe. Most of the dietary phytochemicals are suggested to exert their chemopreventive action *via* targeting ROS and associated redox signaling pathways. Daily consumption of dietary compounds enhances cellular antioxidant status, neutralization of free radical species, suppresses expression of proteins regulating cell cycle, inflammation, neovascularization and promotes detoxification of carcinogen and apoptosis of cancer cells. Therefore, targeting ROS signaling pathway and redox homeostasis in cancer cells is a novel approach for the prevention of cancer development. In this chapter, we discuss the basic mechanism of ROS generation, redox signaling mechanism and redox sensitive transcription factors and how dietary phytochemicals target this complex pathway to prevent cancer development. Also, this chapter provides key finding on clinical efficacy of selected dietary phytochemicals in high-risk populations (Table **1**).

CHAPTER 10

Therapeutic Potential of Probiotics on Oxidative Stress and their Role in Human Health

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Abstract: In the industrialized world, functional foods have become part of a diet that provide potential health benefits by curbing various diseases. Currently, the most commonly used functional foods are probiotics which reduce damages caused by oxidative stress and reactive oxygen species (ROS). Probiotics are live microbes used as a therapeutic food with fewer side effects in comparison to other therapeutic agents. The incorporation of probiotics in foods shows many medicinal properties by acting as antioxidant, anti-inflammatory, anti-bacterial and anti-cancer agents. As such probiotic foods (fermented dairy products, drinks, fruits, vegetables, *etc.*) can affect the individual by raising the existing gastrointestinal flora with live microbial nutritional supplements and improve the microbial balance of *Lactobacillus*, *Bifidobacterium* and several other microbial species in the gastrointestinal tract, which causes an alteration in carcinogen metabolism as well as regulation of the immune system. Accumulating evidence highlighted that probiotics have therapeutic effects with a reduction of invasion and metastasis in cancer cells by modulating key signaling pathways. Globally probiotics market extent was valued at $ 48.38 billion in 2018 and expanded at 6.9% annually which indicates the rising demand for probiotics worldwide. Hence, the chapter sheds light on the current state of probiotics and their potential applications for human health and in the development of modern therapeutic drugs for the treatment of diseases.

Keywords: Antioxidant, Immunity, Metastasis, Oxidative stress, Probiotics, ROS.

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INTRODUCTION

Functional foods are supplements or dietary foods usually consumed to get some beneficial results from them. Probiotics are considered as functional food's ideal group with rising large marketable interest and market shares (Mishra *et al.* 2019). Probiotics bacteria have the capacity to colonize the colonic mucosa. These bacteria have the potential to prevent and treat various diseases *viz*. gastrointestinal infections, lactose intolerance, inflammatory bowel disease, urogenital infections, allergies, cancers, cystic fibrosis, reduction of side effects of antibiotics, in oral health like the curing of dental problems and periodontal diseases (Singh *et al.* 2013). However, the alteration in the structure of this defending microbial flora by certain eating and environmental factors makes the host prone to diseases by minimizing its food utilization efficacy (Fuller 1989). Probiotics are used in the treatment of distressed microflora of the intestine and raise gut porousness which are the major features of several intestinal disorders in warm-blooded organisms. The basic action mechanism of probiotics is its ability to compete for the adherence sites on the intestinal epithelium and mucosa and also produce bactericidal substances to neutralize the harmful effects of pathogens and other related toxins (Vanderpool *et al.* 2008).

Probiotics (yogurt and fruits) intake in acceptable quantities has useful health benefits on the host (Fernandez and Marette 2017). The relationship between human beings and live-microbial diet has been well known in history and antedate to the millennium of years ago (Nazir *et al.* 2018). Parker in 1974 used the terms probiotic for the first time and defined as the association of substances and organisms which have a positive influence on their host by maintaining the equilibrium of gastrointestinal flora (Tannock 1999). Metchnikoff and coworkers reported the first study on probiotic and demonstrated the positive impact of the fermented milk on human health. Till now, scientific studies on the valuable outcomes of probiotics on the human have been investigated for treatment and mitigation of gut-related illnesses like indigestion, bowel diseases, stomach swelling and diarrhea (Kim *et al.* 2019). According to the data of PUBMED search, there were 26,207 papers indexed to the term "probiotic" as of March 03, 2020, in comparison to the 714 papers prior to the year 2000. Due to its importance, there is a great increase in the demand for probiotic-based nutrients. In 2017, for probiotic worth, the global market was 42.55 billion US$ and in 2025, it is observed to augment by 74.69 billion US$ (Fortune Business Insights 2019). To date, only scarce information is available about probiotics possessing antioxidative, anti-inflammatory, anticancer and gastroprotective properties. Furthermore, probiotics exhibited the health encouraging efficacy in maintaining hypersensitive, inflammatory and infectious diseases by modifying the functioning of the gut and by enhancing homeostatic immune defenses.

Although probiotics can be found useful in specific clinical applications and human health, the mechanisms behind the modulation of the immune function are understood poorly. Probiotics are usually not necessarily considered as commensal bacteria. They are commonly lactic acid bacteria (LAB), utmost *Bifidobacteria* and *Lactobacilli* species, while *Enterococcus* and *Lactococcus* species of non-pathogenic strains are also identified as probiotics. Though, the available works on the beneficial effects of pro-biotic on these diseases are still controversial and limited. In addition, many studies are not able to sufficiently address the mechanisms through which probiotics treat, reduce and modulate the progression of diseases. Recently, several literature findings showed an upsurge in exploring the beneficial effects of various probiotics in protecting and managing the different human disorders and diseases (Table **1**). Besides the importance of yeasts in the fermentation of beverages and food, it also showed some beneficial effects in promoting human health. Therefore, this chapter will highlight the role of probiotics in averting the incidence of the above-mentioned illnesses besides suggesting its main mechanisms of action.

PROBIOTICS AS ANTIOXIDANT

Oxidative stress (OS) is normally induced due to the formation of ROS. It usually occurs due to disturbance in the equilibrium of antioxidant molecules and pro-oxidant generation (Hussain *et al.* 2012). The main health benefits of probiotics are to improve the antioxidant defense capacity of the human body as these are reported to enhance the total GSH level in the plasma (Mishra *et al.* 2015). Pro-oxidants mostly consist of one or more unpaired electron that is unstable. Mostly, the production of ROS constantly in the cell system can cause damage to the proteins, fats, starches and nucleic acid. Several endogenous sources also generate ROS *i.e.,* xanthine oxidase, mitochondria (Sisein 2014), inflammation, peroxisomes, phagocytosis, exercise (Hussain *et al.* 2012), respiratory burst and free metal ions (Takashima *et al.* 2012). The exogenous sources include industrial solvents, cigarette smoke, UV irradiation and environmental pollutants. The partial reduction of unreactive dioxygen leads to the generation of reactive oxygen species (ROS) (Kumar *et al.* 2014). In OS, cellular mitochondria generate ROS with the reduction in the expression of enzymatic antioxidants and nonenzymatic antioxidants. The elevation in the level of ROS generates oxidative stress and leads to the progression of several chronic diseases, including diabetes, cancer and aging (Valko *et al.* 2007). The antioxidants bind with the free radicals formed in the cells and the chain reaction gets ended before its completion preventing impairment to the vital molecules (Mishra *et al.* 2015). All of these molecules in the body perform diverse physiological roles by suppressing the process of oxidation. All chain reactions are stopped by the antioxidants through the inhibition of free radicals. Therefore, it is crucial to find natural antioxidants that

CHAPTER 11

Expression of miRNA in Regulating Cancer: Role of Phytoconstituents

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Abstract: MicroRNAs (miRNAs) are short, non-coding and functional 18-22 nucleotide sequences, which bind to 3' UTR region of the mRNA and modify mRNA expression by degrading them or modulating their translation process. Besides, miRNAs act as either suppressors or inducers of tumor depending upon binding with the target site. The action of miRNAs is reported for controlling the various important functions like metastasis, angiogenesis, apoptosis and tumor growth. They play an important role in suppressing cancer cell proliferation or invasion by targeting caspases and other factors involved in programmed cell death (apoptosis). So, the application of miRNA is proved to be a novel approach for cancer prevention. According to literature, numerous phytoconstituents isolated from medicinal plants or other botanicals modulate the functioning of different miRNAs which are involved in the pathology and biology of cancer. Therefore, the regulation of miRNA by botanicals or isolated compounds is a new model for researchers to develop/formulate a novel drug to combat this devastating disease. An attempt has been made in this chapter to explore the role of phytoconstituents to control the process of carcinogenesis targeting miRNAs.

Keywords: Apoptosis, Carcinogenesis, Metastasis, miRNA, Oxidative stress, Phytoconstituents, Proliferation, ROS.

INTRODUCTION

The human genome is a set of nucleotides that contains both coding as well as non-coding sequences. The total number of coding and non-coding genes is 19,000-20,000 and 46,831 respectively. Besides this, approximately 1.5% of the genome contains micro-RNA coding sequences. The first microRNA (miRNAs) was discovered by Victor Ambros laboratory in 1993 from *Caenorhabditis*

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elegans (Peng and Croce 2016). miRNA are small group of non-coding sequences consisting of 19-22 nucleotides that regulate the various functions such as differentiation, development, cell proliferation, apoptosis, and stress responses. It may induce or suppress the tumor depending upon its specific binding sites to mRNA by its mature region called seed region (Ryan *et al.* 2010, Reddy 2010).

Mainly 50% miRNA genes are localized in cancer-associated genomic regions or the delicate sites which are prone to mutations (Bandyopadhyay *et al.* 2016). The mutations like amplification, deletion, epigenetic silencing and inhibition of transcription factors in the fragile region of miRNA lead to cancer of prostate, ovary, lungs, pancreas, tongue, colon, liver and diffuse large B-cell lymphoma as well as neurodegenerative disorders, cardiovascular diseases and viral conditions (Pan *et al.* 2010, Kosaka *et al.* 2010). The same kinds of miRNA may act as tumor suppressor genes depending upon their gene expression pattern. It has been reported that miRNA-29 is an oncogene in case of breast cancer whereas the same miRNA-29 acts as tumor suppressor gene in lung cancer. Furthermore, the loss of function of miRNA-23b leads to invasion and migration of bladder cancer cells but if the expression of same miRNA-29 gets knock down then it can reduce the invasion and in turn, promote apoptosis in renal cell carcinoma cell lines (Reddy 2010). The synthesis of miRNA takes place in two compartments *i.e*., nucleus and cytoplasm. It involves various endonuclease enzymes like poly II, poly III and transcriptional factors (c-Myc, p53, MEF 2, PU.1 and REST) (Davis-Dusenbery *et al.* 2010). Any change or mutation in any type of transcriptional factors like c-Myc and p53 can induce different kinds of cancers. Besides this, miRNAs play a vital role in apoptosis. miRNAs modulate the cancer progression or suppression by targeting either extrinsic or intrinsic pathways of apoptosis. In this natural or programmed cell death, various intracellular and extracellular receptors are involved, which receive signal and transmit it to the effector caspases (cleave substrate at aspartic residue) involved in cell death. Due to their significant role in cancer initiation and proliferation, targeting miRNAs has been considered an effective treatment for cancer. According to literature, phytochemicals have a significant role in the intonation of miRNA expression which in turn directly affects tumor inducer, suppressor and cancer-related protein expression. So in this way, phytoconstituents inhibit tumor growth, suppress metastasis and reverse epithelial-mesenchymal transition *via* regulating miRNAs. Moreover, various phytoconstituents isolated from plants either singly or in a mixture may target different types of cancer by inhibiting oncogenes or inducing tumor suppressor genes to modulate cell proliferation. Modulating ROS production and oxidative stress by miRNAs is very crucial for the normal and better functioning of a cell. Due to the significant role of miRNAs in cancer initiation and proliferation coupled with the vital role of phytoconstituents in its inhibition, it is imperative to recognize the modulation of microRNA expression as the potential target for controlling the abnormal signaling pathways of cancer cells.

MICRO-RNA: BIOSYNTHESIS AND FUNCTION

Biogenesis of the human miRNA occurs in the nucleus and cytoplasm. The synthesis involves various enzymes and transcriptional factors that lead to the formation of a complete, mature and functional miRNA. The graphical presentation of the synthesis of miRNA is shown in Fig. (**1**). The transcription of intergenic miRNA containing both exons and introns is catalysed by poly II or poly III form pri-miRNA. The pri-mRNA having a stem-loop structure, single strands overhang at both ends complementary to the target sequence and forms functional miRNA. Then, RNAase and its cofactors *i.e*., DROSHA and DGCR8 lead to the formation of pre-miRNA consisting of 70 nucleotides sequence and stem-loop structure (Hogg *et al.* 2014, Suzuki *et al.* 2012, Hata *et al.* 2015). This precursor product (pre-miRNA) moves to the cytoplasm for further processing by Exportin 5 and RNA-GTP. In the cytoplasm, the stem-loop structure of pre-miRNA is cleaved by RNAase DICER and double-stranded RNA binding protein TRBP which result in the formation of 22 nucleotides containing functional double-stranded RNA. Then this double-stranded RNA is cleaved by enzyme helicase into two single-stranded RNA. One strand is degraded and the other strand is mainly bound to RISC (RNA Inducing Silencing Complex) and performs numerous functions like mRNA cleavage, translational activation and translational repression. The selection and rejection of the miRNA strand is mainly dependent upon the thermal stability of the strand (Iorio and Croce 2012). The mature and functional sequence of the miRNA is highly conserved among species and it regulates various functions such as apoptosis, development, differentiation and cell proliferation (Suzuki *et al.* 2013). Mutation in any step of miRNA synthesis *i.e*. mutations in the promoter region, functional enzyme, transcriptional factors (Drosha, Dicer1, TARBP2 and XPO5), growth factor receptors, chromatin remodelling and any change in apoptosis regulators or signal transducers lead to various types of cancer (Jansson *et al.* 2012, MacFarlane *et al.* 2010).

miRNAs as Oncogene and Tumor Suppressor Gene

Cancer is an uncontrolled division of cells in which normal cells transform into malignant cells due to changes in genetic material. The development of cancer involves multiple biological networks which include: hyper proliferation of tissues, self-sustained growth factors, insensitivity of growth signals, anti-apoptotic activity, induced angiogenesis, replicative immortality, invasion and metastasis. Any mutation/change in miRNAs may function as a tumor suppressor

CHAPTER 12

Antioxidant and Anti-Inflammatory Action of Phytobioactive Compounds in Cardiovascular Disorders

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Abstract: Oxidative stress distorts the mitochondrial function and triggers deleterious effects in the cardiovascular system. Further, oxidative stress-induced overproduction of highly reactive oxygen/nitrogen species (RONS) is amplified in patients exposed to radiation, excessive consumption of alcohol and tobacco, environmental pollutants, exposure to agrochemicals like fertilizers, pesticides or endocrine disrupters. In modern times, oxidative stress-induced cardiovascular diseases (CVDs) have escalated globally. Synthetic medicines prescribed for the amelioration of CVDs are expensive and can cause life-time dependency in some patients, thus escalating the treatment cost. Sometimes, long-term use of synthetic medicines or drug polytherapy for co-morbid conditions can cause undesirable side-effects. Quite often, these therapeutic strategies do not succeed in attenuating the oxidative stress related CVDs. Therefore, researchers are exploring alternative and cost-effective phytobioactive therapies which have strong antioxidant and anti-inflammation properties, and can act as scavengers of RONS. Phytobioactive compounds, nutraceuticals and probiotics prepared from plant/animal origin are potential therapeutic substances for the promotion of health and well-being. Several plant-derived phytotherapies have demonstrated strong antioxidant, anti-inflammatory, cardio-protective effects, inhibition of ischemic injury as well as alleviation in the pathological cardiac biomarkers and cardiac apoptotic proteins. In this review, we have described the therapeutic functions of various phytobioactive compounds and their purported mechanism of action at the genetic, epigenetic, cellular and molecular level with respect to their antioxidant and anti-inflammatory actions for the prevention and treatment of cardiovascular disorders.

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Keywords: Anti-inflammation, Antioxidants, Cardiovascular diseases, Myocardial infarction, Nutraceuticals, Oxidative stress, Phytobioactive compounds, Probiotics.

INTRODUCTION

Cardiovascular diseases (CVDs) comprise collective disorders of the coronary blood vessels, heart, and stroke, which are one of the major causes of deaths in developed and developing countries. CVDs not only pose a major threat to an individual's health but also cause a tremendous economic burden on the healthcare systems globally. The major type of CVDs are caused by oxidative stress that triggers atherosclerosis, coronary artery disease, hypertension, cerebrovascular disease, disorders of the major arteries, and peripheral vascular disorders. Further, congenital heart disease, rheumatic heart disorders, congenital cardiomyopathies, arrhythmias, *etc*., are other types of CVDs (Murabito *et al.* 1993, Riccioni *et al.* 2007). Over 17.3 million deaths are caused annually due to CVDs worldwide (World Health Organization 2011). Deaths due to various types of CVDs in men and women are shown in Fig. (**1**) as below:

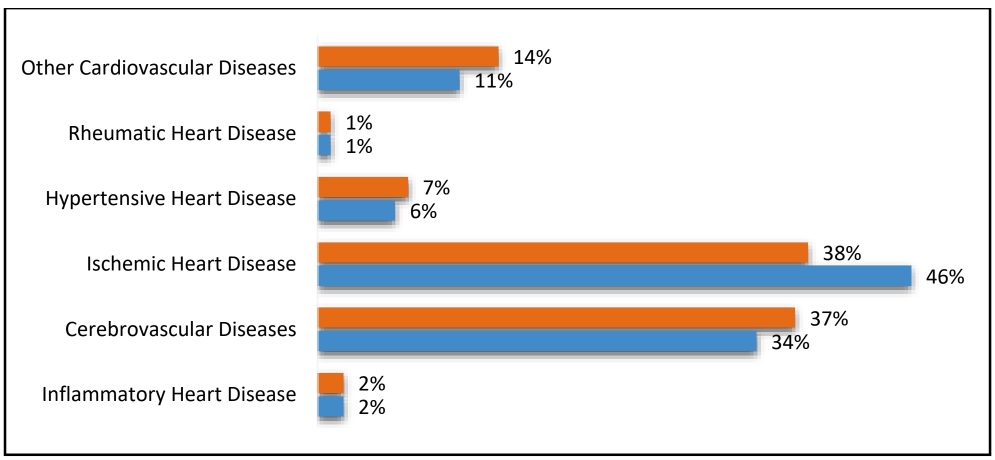

Fig. (1). Distribution of CVD deaths in males and females.

One of the major underlying pathophysiological processes that lead to various types of CVDs is atherosclerosis. Atherosclerosis is caused due to inflammation affecting the lining of blood vessels of the entire cardiovascular system. On exposure to increased levels of LDL cholesterol and further components such as cytokines, hormones and oxidative species on the lining of the blood vessels; the endothelium becomes permeable to certain inflammatory mediators leading to the formation of plaque. A development of cholesterol plaque on the inner side of the artery's walls causes a blockage in the blood flow. The rupture of the plaque may lead to heart attack or stroke because of acute blockage of the artery by the blood clot.

A number of pharmacological and non-pharmacological approaches have been made to combat CVDs. The non-pharmacological approaches include certain lifestyle changes such as smoking cessation, weight control, regular exercising, maintaining a healthy diet and antioxidant therapy (Wilson *et al.* 1998). Our major focus in this chapter is on the role of phytobioactive antioxidants and anti-inflammatory agents to prevent and treat cardiovascular diseases (Nuttall *et al.* 1999).

OXIDATIVE DAMAGE IN CARDIOVASCULAR DISEASES

A large number of reactive oxygen species (ROS) are constantly produced in cells. These reactive oxygen species (ROS) expedite the irreversible oxidation of carbohydrates, proteins, lipids and nucleic acids, which are some of the essential biological macromolecules (Fig. **3**). The development of atherosclerosis is majorly because of the oxidative modification of circulating lipoproteins by free radicals, particularly low-density lipoproteins (LDL). In the early stage of atherogenesis (the first step to the formation of plaque), LDLs are oxidized. Once these Ox-LDLs begin to accumulate, an immune response is stimulated. These immune responses lead to progression of atherosclerosis by releasing reactive oxygen species and pro-inflammatory cytokines which promote the formation and accumulation of oxidized LDLs (Mann 2015). Oxidative stress occurring in the mitochondria in cardiovascular diseases due to increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) lead to free radical formation, which promotes inflammation in the vascular wall and may be the underlying cause of stroke and coronary heart disease. Fig. (**2**) explains the process of oxidative damage in CVDs (Bo *et al.* 2013).

LINK BETWEEN INFLAMMATION AND CVD

Even though inflammation is a part of the natural biological response of body tissues developed by the organisms to get rid of harmful stimuli, persistent increase in certain pro-inflammatory biomarkers leads to chronic low-grade inflammation, which is the key component in the development of cardiovascular disease (Hennekens *et al.* 1996). C-reactive protein (CRP), a predictor of endothelial function and an active mediator in the pathogenesis of vascular disease, is a preliminary example of a reversible atherosclerosis precursor (Gajendragadkar *et al.* 2014). CRP induces vascular remodeling, by producing reactive oxygen species and upregulating angiotensin type 1 enzyme. It also enhances the release of tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β) and interleukin-6 (IL-6), the pro-inflammatory cytokines, through foam cells in

CHAPTER 13

Therapeutic Potential of Phyto-Constituents for the Treatment of Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is acknowledged as one of the most serious and progressive neurodegenerative disorder, and is the leading cause of dementia in late adult life having unknown etiological pathways. AD is characterized by the formation of intracellular neurofibrillary tangles leading to tau phosphorylation and extracellular amyloid deposits that develop into senile plaques. Amyloid beta (Aβ) plaques, the classic hallmarks of AD, in turn, cause the generation of free radical species of different metals (copper, iron) which modulate neuronal growth, differentiation, and progression of cell death through several signalling pathways. The conventional therapies recommended for the amelioration of AD are only restricted to treat the symptoms of AD and do not focus on the underlying causes of the disease. These allopathic medicines are non-economical and also have unwanted side-effects, which further decrease the quality of life (QOL) of the patients. Therefore, it is of utmost importance to explore alternatives to decrease the expression of neurodegeneration. Antioxidant and anti-inflammatory phytoconstituents play a crucial role in preventing the onset of neurodegenerative diseases and exert neuroprotection. Numerous antioxidant phytonutrients, herbal remedies, and food supplements have been reported for the prevention of cognitive decline and management of AD. The neuroprotective potential of phytotherapies has been demonstrated in numerous *in vitro* and *in vivo* studies. The purpose of this review is to describe phytoconstituents based on their therapeutic effects on etiological pathways (microglia, inflammasome, CB2, NLRP3 and NFKβ) of AD and their underlying molecular mechanisms of action involved in neuroprotection and prevention of AD.

Keywords: Alzheimer's disease, Antioxidants, Cognition enhancement, Cognitive decline, Dementia, Dietary phytoconstituents, Microglial cell activation, Neuroinflammation, Neuronal cell injury, Reactive metal ion species.

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INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disease that occurs in old age and worsens with time. According to the Alzheimer's Association report (2020), by 2050, people with age above 65 years suffering from AD and dementia are projected to reach 13.8 million. It is also estimated that by the end of the year 2020, 70% of the world's population above the age of 60 will be suffering from AD, 14.2% from India itself (Mathuranath *et al.* 2010). Between 2000 and 2017, the number of deaths due to AD have increased by 145%, as per Alzheimer's Association report (2019). There are several discoveries made with respect to the pathogenesis of AD, but the major hallmark has been the discovery of the amyloid beta (Aß) of senile plaques. Oligomeric forms of this peptide are the main causative agents in the development of AD.

Oxidative stress is a major cause of various age-related disorders like AD. Major pathophysiology arises due to the occurrence of oxidative stress. It has been proved that an increase in oxidative stress causes a modification in the protein side-chain due to the presence of reactive oxygen species (ROS) or reactive nitrogen species (RNS), thus resulting in tau hyperphosphorylation. Overproduction of ROS results in major oxidative stress, an important mediator of damage to cell structures and leads to lipid peroxidation, mitochondrial dysfunction, protein damage, DNA damage, lysosomal dysfunction causing various age-related disorders including AD (Singh *et al.* 2016, Venkatesan *et al.* 2015). Aß secretion due to the stressed and degenerated neurons leads to the formation of Aß aggregates, in turn causing major degenerative events in the cells like neurons, macrophages, and microglia (Bayer *et al.* 2001). The pathophysiology of AD is mainly related to the neuropathological features *i.e.,* hyperphosphorylated neurofibrillary tangles (NFTs) and amyloid plaques.

Currently, there is no reliable therapy for the treatment of neurodegenerative disorders. However, there has been evidence regarding the use of phytoconstituents for the prevention and treatment of disorders including AD. Phytoconstituents present in dietary supplements help reduce the occurrence and risk associated with several non-communicable diseases such as cardiovascular, cancer and neurodegenerative disorders. People consuming antioxidant and anti-inflammatory dietary supplements are at a lower risk of neuronal dysfunction (Kumar and Khanum 2012). Intake of phytoconstituents containing flavonoids and retinoids have shown beneficial effects on the overall health and well-being as well as helped in improving mental and physical performance by boosting the body's antioxidant systems (Venkatesan *et al.* 2015). The purpose of this review is to highlight how phytoconstituents such as crocin, curcumin, cinnamaldehyde, withaferin, *etc.,* present in dietary supplements like turmeric, cinnamon, ashwagandha, *etc.,* exert useful actions in treating neurodegenerative disorders either as a single entity or in combination with other dietary supplements. Various pathways involved in causing AD, including microglia, NLRP3, NFKB, and CB2, as well as mitochondrial dysfunction pathway and sirtuin SIRT1 are also discussed. It has been reported that various phytoconstituents act on these pathways. Therefore, the overall focus of this review is on the influence of phytoconstituents used for curing AD and other CNS disorders.

LINK BETWEEN OXIDATIVE STRESS, NEURONAL INFLAMMATION AND ALZHEIMER'S DISEASE

AD is characterized by neuronal dysfunction and shrinkage of brain tissue. The two most known hallmarks of AD are amyloid plaques (Aß) and neurofibrillary tangles. These hallmarks are the predisposing factors for causing neuroinflammation. An Alzheimer's associated brain results in atrophy of the cerebral cortex and hippocampus. Also, the gyri are narrowed, the sulci are expanded and cerebrospinal fluid (CSF) in the ventricles is increased. These changes in the brain take place due to neurodegeneration which is associated with the formation of extracellular senile plaques and intracellular neurofibrillary tangles (NFTs). NFTs are intracellular aggregates, in the form of fibrils of the associated tau proteins which show oxidative changes as well as hyperphosphorylation. These tangles and plaques are involved in learning, memory and emotional behavior in regions such as the hippocampus, basal forebrain, and amygdala (Mizuno *et al.* 2012, Morales *et al.* 2014). Oxidative imbalance and neuronal damage play an important role in the progression of AD. The accumulation of Aß increases oxidative stress, elevates mitochondrial dysfunction, increases the phosphorylation of tau protein which induces pathogenesis of AD (Kim *et al.* 2015, Zhao and Zhao 2013). Another important parameter involved in the pathogenesis of AD is the formation of free radicals. Radical species production takes place due to the presence of amyloid beta peptide of 42 residues (Aß42). The amyloid beta peptides bind to metal ions like copper ions (Cu^{2+}) which are present in abundance in the brains of AD patients. Cu^{2+} leads to the formation of H_2O_2, a reactive oxygen species (ROS) leading to oxidative stress and thus induces AD (Rosales Hernández *et al.* 2016). The presence of bound Fe^{3+} to Aß is also observed in AD, which when reduced to Fe^{2+} escalates the production of ROS (Peters *et al.* 2015). Also, there are many genetic and environmental factors that are responsible for AD. As mentioned, the pathophysiology of AD is shown in Fig. (**1**).

CHAPTER 14

Mechanisms of Anti-Glutamate Neurotoxicity of Botanicals and their Chemical Constituents

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Abstract: In many countries, including Asian countries such as Japan, Singapore and Thailand, aging populations have been increasing, thus promoting a high risk for age-associated chronic diseases. One of the devastating chronic diseases in people with old age known to greatly impact the patients' quality of life is a group of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. It has been evident that neurotoxicity is a significant risk of neurodegenerative disorders. One of the crucial contributing factors leading to neurotoxicity in humans is glutamate, the excitatory neurotransmitter. If it is accumulated in the brain, this neurotransmitter can result in neurotoxicity *via* either glutamate-dependent pathway or glutamate-independent pathway. Glutamate neurotoxicity (GNT) is characterized by rising damage of cell components leading to cell death. In the death process due to oxidative stress, reactive oxygen species (ROS) are generated, thus impairing a vast array of cellular functions in many organelles such as mitochondria and endoplasmic reticulum. GNT has been clearly observed in the brain tissue because of the accumulation of glutamate, not only from the endogenous source, but also the exogenous source such as monosodium glutamate. Fortunately, numerous plant extracts and their chemical constituents, particularly the ones with high anti-oxidant activity, have been found to exhibit anti-GNT in both *vitro* and *in vivo* models. Herein, mechanisms of anti-GNT of botanicals and their chemical constituents are presented and discussed in detail. Their anti-GNT mechanisms elucidated could shed light on the discovery and application of neutraceuticals, and the cell defense mechanisms of natural neuroprotectants could certainly be beneficial to improve both healthspan and lifespan in humans.

Keywords: Glutamate neurotoxicity, Natural products, Neuroprotectants, Neutraceuticals, Reactive oxygen species.

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INTRODUCTION

Glutamate is a major excitatory neurotransmitter in the central nervous system which is indispensable in learning and memory formation (Fonnum 1984, Nakanishi 1992). Generally, extracellular glutamate levels are kept low and tightly regulated by glutamate transporters predominantly located on the glial membranes (Auld and Robitaille 2003). The blood-brain barrier functions as a border impermeable to glutamate elsewhere (Hertz *et al.* 1999). The physiological role of glutamatergic transmission is determined by the specific binding between glutamate and various types of glutamate receptors, mainly divided into the ionotropic and metabotropic types (Riedel *et al.* 2003, Willard and Koochekpour 2013b). Dysregulation in the glutamate system can cause an impact on a wide range of neurological disturbances which include psychiatric conditions, neurodevelopmental disorders, neurodegenerative disorders and stroke (Miladinovic *et al.* 2015).

Nowadays, it is evident that consumption of fruits and vegetables exerts numerous health benefits either by boosting the defensive system of the body or assisting in the recovery from diseases (Liu *et al.* 2016, Hu *et al.* 2014a, Hyson 2015). Phytochemicals present in many plants were reported to have strong activity against several diseases and this could be developed for therapeutic purposes. Herbal medicine or phytomedicine which rely on plants or plant products, have been recognized as an effective way to fight against diseases for a long time. There are abundant indigenous plants distributed in several regions of the world that have yet to be studied, which might be useful in terms of health benefits. It is well known, one of the potential characteristics of these plants is that they usually have strong antioxidant activity which contributes to the disease therapy. However, how these dietary antioxidants act in several diseases is elusive. In this regard, Lee and colleagues, did a comprehensive review of how dietary phytochemicals act against adaptive cellular stress responses in the central nervous system *via* several molecular mechanisms (Lee *et al.* 2014b).

Currently, a number of studies have investigated the underlying mechanisms of plants and phytochemicals in the attenuation of glutamate excitotoxicity which includes the over-production of ROS and increase of intracellular Ca^{2+} influx *via* cell surface glutamate receptor activation, leading to neuronal cell death. The proposed neuroprotective mechanisms against glutamate toxicity involve several signaling cascades such as BDNF/TrkB, MAPKs, Nrf2/HO-1 and PI3K/Akt/GSK-3β (Mattson 2008, Wang *et al.* 2007). These molecular mechanisms usually provide protective or survival effects against harmful agents such as toxic levels of glutamate. Interestingly, many plants or plant-derived compounds were found to possess beneficial effects against neurological disorders, including glutamate-related disorders which might be an effective candidate for further investigation and development for therapeutic use. In the present review, we highlight the summary of homeostasis of glutamate in the CNS and how glutamate is relevant to CNS disorders. We also provide a list of natural plants and/or their bioactive compounds with their neuroprotective effect against glutamate neurotoxicity in several glutamate-induced models.

GLUTAMATE METABOLISM AND NEUROTRANSMISSION

Glutamate is a major excitatory neurotransmitter present in the brain (Fonnum 1984). It is a precursor of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter (Petroff 2002). The release of glutamate from presynaptic neurons into the synaptic cleft can be recognized by various types of glutamate receptors in the synapses, predominantly receptors on the postsynaptic membranes which initiate action potentials mediating various physiological functions, including memory and learning (McEntee and Crook 1993, Riedel *et al.* 2003). Glutamate homeostasis in the synaptic cleft is mainly mediated by glial cells *via* glutamate uptake which then converts the glutamate to glutamine and transports it back to the presynaptic neurons as a glutamate precursor (Popoli *et al.* 2011). Undoubtedly, the release and maintenance of synaptic levels of glutamate are well-regulated and the basal concentration level of glutamate is controlled *via* complicated neuronal mechanisms together with the help of glial cells (Auld and Robitaille 2003). However, in pathological conditions, the regulation of glutamate homeostasis and transmission is dysregulated and this can cause a large number of neurological disorders (Miladinovic *et al.* 2015).

Like other amino acids, glutamate is primarily synthesized from glucose which crosses the blood-brain barrier *via* astrocytic endfeet (Pellerin and Magistretti 2004). The α-ketoglutarate from the tricarboxylic acid (TCA) cycle as well as glutamine are the main precursors of glutamate (Tapiero *et al.* 2002). In astrocytes, glutamate is converted to glutamine by glutamine synthetase and is transported out of the cells, which is then taken up by neurons *via* Na^+-dependent uptake proteins (Yudkoff *et al.* 2000). The newly taken up glutamine is then converted back to glutamate *via* glutaminase which is then compartmentalized within the synaptic vesicles *via* vesicular glutamate transporters (vGluTs) (Takamori 2006). This corresponds to evidence suggesting that a high concentration of glutamate is mainly found in synaptic vesicles.

In response to stimuli, vesicular glutamate release is mediated by voltage-dependent calcium channels and soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) to fuse into the presynaptic membrane and release the glutamate into the synaptic cleft (Sudhof and Rothman 2009),

CHAPTER 15

Genistein – A Natural Antioxidant and its Use in Treatment of Various Diseases

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Abstract: Genistein (5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one or 4',5,7-trihydroxyisoflavone) can be found in various plants, though soy is especially rich in this compound. It has multiple biological activities, but one of its major features is its antioxidative function. Either genistein-rich extracts from plants or synthetic genistein have been used in studies on the potential treatment of various conditions and diseases. They are as different as neurodegenerative diseases (including Alzheimer's disease and various genetic diseases), cancer, cardiovascular disorders, liver dysfunctions, and many others. Although for the treatment of various diseases the major mechanisms of genistein action can be based on modulation of specific biochemical pathways, its antioxidative function may contribute significantly to its therapeutic potential. These aspects are discussed in the light of development of genistein-based therapies for a battery of different disorders.

Keywords: Antioxidant, Cancer, Cardiovascular diseases, Genistein, Neurodegenerative diseases.

INTRODUCTION

Naturally occurring compounds and/or their novel derivatives are among the most intensively tested molecules in biomedical and pharmaceutical studies. For instance, from around 1940s to 2014, 49% of approved molecules for cancer treatment were either natural products or derived directly from them (Newman and Cragg 2016). Flavonoids are a class of natural compounds, with anti-oxidative and other actions in various diseases. Therefore, they are used for

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nutraceutical, medical, pharmaceutical or cosmetic applications (Panche *et al.* 2016). These substances are widely distributed in vascular plants, particularly in fruits, vegetables, seeds, nuts, grains, and spices. They contribute to attractive colors of leaves, flowers, and fruits, and play crucial roles in UV protection by rummaging reactive oxygen species (ROS), created by the photosynthetic electron transport system (Pietta 2000). Flavonoids are divided into several subgroups, including flavones, flavonols and isoflavones (Fig. **1**). All the flavonoids have the same base structure of the flavan core, while differing from each other in substituents in the aromatic carbon ring. Among the numerous classes of flavonoids, those specifically noteworthy to this review are isoflavones, in particular one of them – genistein (Fig. **2**).

Isoflavones are bioactive compounds which can be found in the members of the bean family, legumes, including soybeans, fava beans, chickpeas, and peanuts (Setchell *et al.* 2001). They occur in the form of three different types, and each kind being available in four synthetic structures. Soybean contains most of the isoflavones in forms of aglycones (genistein, daidzein, glycitein), β-glucosides, malonyl-β-glucosides, and acetyl-β-glucosides (Wang and Murphy 1994). Aglycones are the most bioavailable isoflavone forms to humans, most likely because their structure does not contain any sugars or other derivatives (Rahman Mazumder and Hongsprabhas 2016). Structures and functions of isoflavones are similar to that of 17-estradiol, the strongest mammalian estrogen, thus, they are also called phytoestrogens, revealing high levels of estrogenic activity (Boué *et al.* 2003). One of the most recognizable aglycone is genistein, having diverse biological activities. This isoflavone interacts with the estrogen receptor, significantly influencing the regulation of expression of many genes. In addition, nonhormonal mechanisms of genistein action consist of antioxidation, anti-inflammatory, and antiproliferative properties (Sarkar and Li 2002).

When different isoflavones were compared according to the level of deoxidation reactions in cell cultures, genistein enhanced inhibition of O_2^- generation more effectively than other compounds, suggesting its high antioxidant potential (Wei *et al.* 1995). Oxidative stress occurs during the generation of ROS, including superoxide (O_2^-), peroxyl (ROO^-), alkoxyl (RO^-), hydroxyl (HO^-) radicals, and nitric oxide (NO^-). Due to their high reactivity, they cause damages in cells, such as destruction of the cell membrane, DNA lesions, and inactivation of proteins.

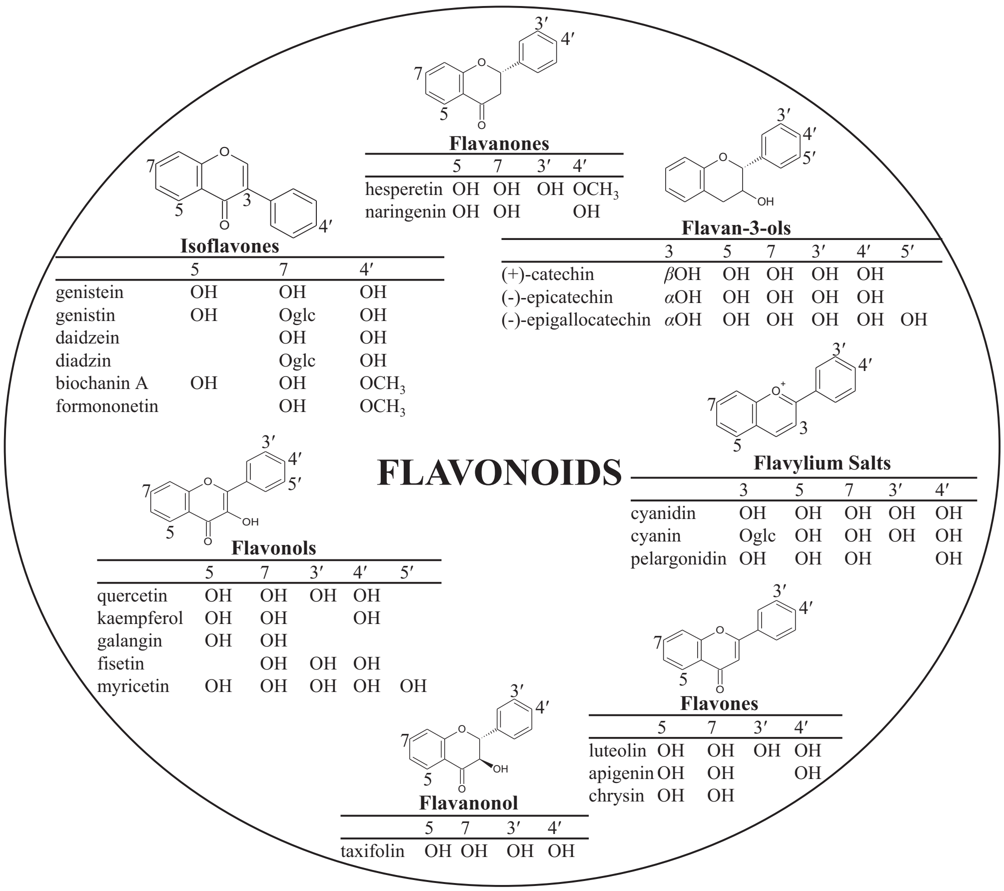

Fig. (1). Structure of flavonoids [based on Pietta, 2000; modified].

Despite the existence of mechanisms controlling ROS, such as actions of antioxidants (tocopherols, ascorbic acid, and glutathione) or enzymes (superoxide dismutase - SOD, catalase or peroxidase), most of them are not sufficient to combat overwhelmed changes in several degenerative diseases caused by ROS (Pietta 2000, Gagné 2014). Genistein is an isoflavone with multiple antioxidant activities, and it is able to decrease levels of lipid peroxidation and ROS-mediated damages. Moreover, genistein is found to activate enzymes involved in deoxidation and to regulate the expression of antioxidation-related genes, like those coding for ERK1/2, and NF-κB (Gaur and Bhatia 2009). This review presents current knowledge on the properties of genistein, focusing on the use of this compound in the treatment of various diseases due to its antioxidative and anti-inflammatory activities.

CHAPTER 16

Industrial Prospects of Antioxidants

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Abstract: The highly reactive free radical species generated through abiotic stress lead to the degradation of essential biomolecules like proteins, carbohydrates, lipids and nucleic acids, thus deregulating a series of cellular functions. Several pathological conditions like wrinkling of skin, ageing, asthma, arthritis, carcinogenesis, cardiovascular diseases, cataract, AIDS, autoimmune disorders, Parkinson's dementia, Alzheimer's disease, *etc*., are the manifestations of free radical toxicity. Apart from these clinical influences, free radicals are associated with spoilage of food resulting through oxidation of fats, oils and lipid content. Antioxidants have enormous potential to neutralize the effect of toxic moieties. Antioxidants can be natural or synthetic with the former taken directly from fruits, vegetables, herbs and spices. Synthetic antioxidants can also inhibit oxidation reactions but their use has been quoted as unsafe for humans. Therefore, expedition on innocuous antioxidants of natural origin has intensified in recent past. The scientific studies have demonstrated the potential of natural antioxidants as: (i) natural preservative for long term storage of ready to eat food products without compromising with their commercial and sensory values; (ii) an anti-ageing, anti-wrinkle agent in the cosmeceutical products; (iii) a medicinal ingredient preventing vesicular calcification and lipid peroxidation responsible for various diseases; (iv) a protective probe against several cardiovascular, neurodegenerative and autoimmune disorders. Owing to such a wide array of industrial applications, natural antioxidants are expected to capture the market in future generating high revenue of billions of dollars. Therefore, through this chapter we focus on bioprospecting diverse sources of natural antioxidant compounds and their industrial prospects.

Keywords: Alzheimer's disease, Anti-carcinogen, Antioxidants, Cardiovascular diseases, Cosmeceutical properties, Flavonoids, Health supplement, Medicinal value, Parkinson's dementia, Reactive oxygen species, Therapeutic aspects.

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INTRODUCTION

Antioxidants are the substances that delay oxidation of carbohydrates, lipids, proteins, and DNA due to oxidative stress at low concentrations (Niki 2004). The oxidative stress is a situation of disparity between reactive oxygen species (ROS) and the protective antioxidant barriers causing several pathological conditions like ageing, cataract, diabetes, asthma, carcinogenesis, arthritis, AIDS, autoimmune diseases, neurodegenerative diseases, Alzheimer's disease, Parkinson's disease and cardiovascular dysfunctions (Bakir *et al.* 2020, Gupta and Sharma 2006, Sindhi *et al.* 2013). ROS possess unpaired electrons in their valence shells and react swiftly with the cell membranes leading to their deterioration and death (Kajarabille and Latunde-Dada 2019). To counter the action of these free radicals, living systems produce antioxidants or these may be incorporated through diet. Broadly antioxidants can be grouped into: a) First line; b) Second line; c) Third line defense antioxidants (Sindhi *et al.* 2013). The first category of antioxidants comprise catalase, glutathione reductase, superoxide dismutase and minerals (Cu, Zn, Se, *etc*.) Second line defense antioxidants cover vitamin C and E, albumins, carotenoids, flavonoids, glutathione, *etc*. The third group of antioxidants include set of enzymes that mediate repair of impaired DNA and proteins, and oxidized lipids and peroxides (Ighodaro and Akinloye 2018, Irshad and Chaudhuri 2002, Sindhi *et al.* 2013). All the above mentioned classes operate either through enzymatic processes or non-enzymatic processes where the former group reduces the amount of antioxidants thus serving the protective function and latter group prevents lipid peroxidation and metal catalyzed radical reactions (Ighodaro and Akinloye 2018, Palozza and Krinsky 1992). Several scientific studies have indicated the significant industrial applications of this elite group of compounds, therefore, through this book chapter we present a cumulative survey of medicinal, pharmacological, therapeutic and food applications of the antioxidants.

SOURCES OF ANTIOXIDANTS

The compounds with antioxidant properties can be derived from natural products and in many cases, these compounds can be semi-synthetic or fully synthetic.

Natural Antioxidants

The natural antioxidants also known as primary antioxidants are present in both plants and animals with plants being the major sources of these compounds. On the other hand, only smaller amounts of these compounds are derived from animals thus making them an insignificant source of natural antioxidants. Vegetables, fruits, cereals, legumes, herbs, spices, tea, coffee, wine and beer are considered the richest sources of antioxidants. Various antioxidants and their sources have been summarized in Table **1**.

Table 1. Antioxidants from natural sources.

Source		**Antioxidants**
Fruits	Blackcurrant	Vitamin C, lutein, β carotene, anthocyanin, m-coumaric acid acid
	Grapes	Gallic acid, catechins, epicatechins, ellagic acid, myricetin, quercetin, kaempferol, anthocyanins, flavonols, trans-resveratrol
	Strawberry	Vitamin C, anthocyanin, ellagic acid, glycosides, ellagitannins
	Bilberry	Vitamin C, anthocyanins, carotenoids, derivatives of hydroxycinnamic acid
	Cranberry	Peonidin, cyanidin, flavanones, procyanidin, quercetin, myricetin, derivatives of hydroxycinnamic acid
	Blackberry	Anthocyanin, flavonols, ellagic acid, procyanidin, epicatechin
	Crowberry	Vitamin C, lutein, β carotene, flavanols, procyanidins, cinnamic acid, trans-resveratrol, p-coumaric acid
	Chokeberry	Anthocyanins, chlorogenic acid, neochlorogenic acid, epicatechins
	Cherry	Anthocyanins, hydroxycinnamic acid
	Plums	Catechins, hydroxycinnamic acid
	Pears	Catechins, hydroxycinnamic acid
	Kiwi	Catechins, hydroxycinnamic acid
	Apple	Epicatechin, procyanidin B2, chlorogenic acid, phlorizin, phloretin---xyloglucoside
	Lemons	Vitamin C, hesperetin, naringenin, eriodictyol
	Oranges	Vitamin C, hesperetin, naringenin, eriodictyol
	Grapefruits	Vitamin C, hesperetin, naringenin, eriodictyol, lycopene
	Papaya	β carotene, tocopherols, lycopene
	Guava	Lycopene, protocatechuic acid, quercetin, ferulic acid, ascorbic acid, gallic acid, caffeic acid

CHAPTER 17

Antioxidants in Cancer Prevention and Combination Therapy

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Abstract: Combination therapy, also known as polytherapy, is a form of treatment that involves the use of several drugs. In fact, the term applies to the use of various treatments to cure a particular illness, with pharmaceutical therapies being the most common. Non-medical treatment, such as the use of a mixture of medications and psychotherapy to relieve depression, may also be used. Polypharmacy, which applies to the usage of multiple medications, is also a related term. When referring to prescription combination treatment, the term polymedicine is also used. The antioxidant protection mechanism, which is responsible for reducing a wide variety of oxidants like reactive oxygen species (ROS), lipid peroxides, and metals, *etc.,* maintains redox homeostasis. Antioxidants are used to guard against the harmful consequences of oxidation and as nutritional additives to counteract the negative effects of stress. Antioxidants are compounds that may prevent or delay cell damage induced by free radicals, which are reactive molecules produced by the body in response to external environmental and other stress. Free-radical scavengers is a term used to describe them. Antioxidants may come from either natural or synthetic sources. Many plant-based foods are thought to have high levels of antioxidants. Plant-based antioxidants are phytonutrients that contribute to disease prevention. These phytonutrients as single entity or in combination have demonstrated beneficial effects in several models and might protect against cancer.

Keywords: Antioxidants, Cancer, Carotenoids, Chemotherapy, Combinational therapy, Free-radicals, Homeostasis, Immunity, Oxidative stress, Reactive oxygen species, Scavengers, Therapeutics, Tumor, Zeaxanthin.

INTRODUCTION

Antioxidants are the constituents that are present abundantly in foods. At small

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concentrations as compared to that of an oxidizable substrate, these compounds substantially delays or averts the oxidation of that substrate. Food manufacturers often use food-grade antioxidants to maintain products' nutritious value while preventing deterioration of their quality. (Senanayake *et al.* 2005, Guo 2013). Biochemists and health practitioners also have an interest in antioxidants because these can help the body defend itself from harm caused by the reactive oxygen (ROS), nitrogen (RNS), and chlorine (RCS) species (Pisoschi and Pop 2015, Winterbourn *et al.* 2016). A number of reactive species including, oxygen radicals, are generated continuously under certain physiological conditions, resulting in severe oxidative damage (Sgherri *et al.* 2018). This free radical generation leads to the development of an efficient defense system in all biological organisms. Therefore, it is assumed that with the evolution of aerobic organisms, there is a development of defense systems with varied functions of antioxidants (Di Meo and Venditti 2020).

Butylated hydroxyanisole (BHA), propyl gallate (PG), butylated hydroxytoluene (BHT), and tert-butyl hydroquinone (TBHQ) are some of the most commonly found antioxidants in foods (Fig. **1 A-D**).

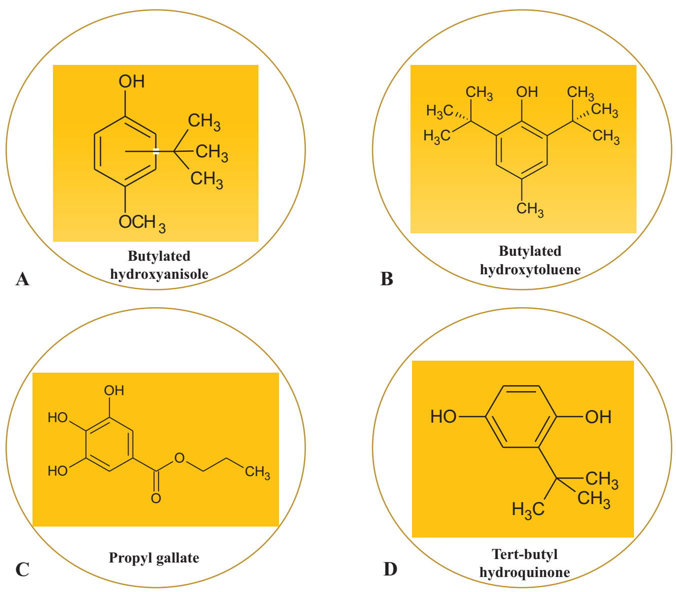

Fig. (1). (A-D) This figure represents the commonly used antioxidants in food like **(A)** Butylated hydroxyanisole (BHA), **(B)** Butylated hydroxytoluene (BHT), **(C)** Propyl gallate (PG) and **(D)** Tert-butylhydroquinone (TBHQ).

The class of phenolic or polyphenolic compounds contains the most potent dietary antioxidants. Phenolic compounds occurring in foods belong to the phenylpropanoids (C6-C3) family and are derivative forms of cinnamic acid. These compounds are formed from the phenylalanine, and to a lesser degree, from tyrosine in certain plants, by way of phenylalanine ammonia lyase's (PAL) mechanism of action, or its corresponding tyrosine lyase (Peter 2012, Cooper and Nicola 2014, Cseke *et al.* 2016) as depicted in the Fig. (**2**).

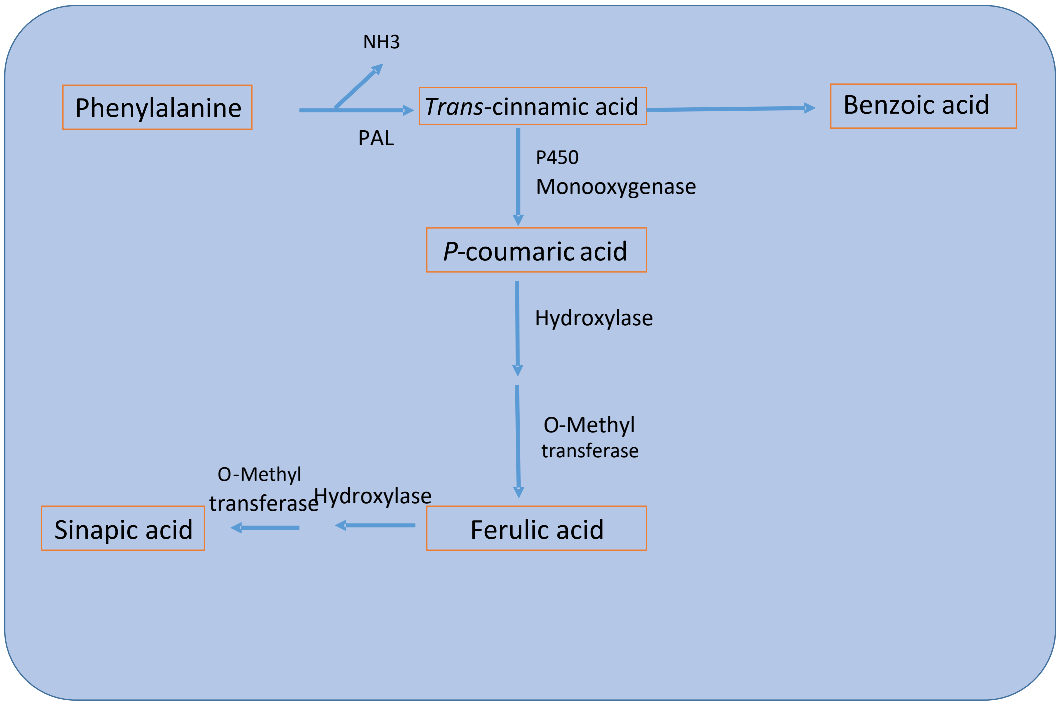

Fig. (2). Biosynthesis of phenylpropanoids and phenolic acids.

Following the loss of a two-carbon moiety, benzoic acid derivatives can be produced from C6–C3 compounds. The participation of malonyl coenzyme A in the condensation of C6–C3 compounds results in the production of chalcones, which can then cyclize under acidic conditions to create flavonoids and isoflavonoids (Fig. **3**) (Vermerris and Nicholson 2007, Hoda *et al.* 2019).

Oxidants and Antioxidants: Basic Concepts

The production and action of ROS acting as oxidants (molecules that have a propensity to donate the oxygen to the other molecules) are accountable for much of oxygen's potentially deleterious outcomes. (Husain *et al.* 2012, Sies and Jones 2020, Saed-Moucheshi *et al.* 2014). ROS are formed in the human body on a continuous basis as a result of natural physiological processes. Free radicals formed as a result of different reactions (Husain *et al.* 2012, Sies and Jones 2020)

SUBJECT INDEX

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Pardeep Kaur

Pardeep Kaur holds M.Sc. (Hons.), M.Phil., and Ph.D. degrees in Botany from Guru Nanak Dev University, Amritsar. Her research interests include medicinal plants, multi-herbal combinations, natural plant products, in-vitro/in-vivo evaluation of plant extracts or isolated compounds for different bioactivities, and determining the molecular mechanisms of action. She has published articles in peer-reviewed international journals with a cumulative impact factor of more than 50. She is also a reviewer of various international scientific journals.

Rajendra G. Mehta

Rajendra G. Mehta is an internationally known researcher in the area of cancer chemoprevention, drug discovery and molecular mechanism of drug action. The major focus of research of his has been in the area of carcinogenesis and chemoprevention for the past 40 years. His group has discovered several products as possible cancer preventive and therapeutic agents. These include Resveratrol (from red wine), 1 alpha hydroxy-Vitamin D5 (vitamin D analogue), Deguelin (from African plant) and Fenretinide (retinoid, vitamin A analogue). In addition, he developed a procedure called mouse mammary gland organ culture model (MMOC) to screen newly identified (or synthesized) chemicals from plants for their cancer preventive properties. The National Cancer Institute (NCI, USA) is using this procedure for screening compounds.