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Review Article

Relevance and Laboratory Diagnosis of Some Antioxidants

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Abstract

Antioxidants are compounds that are produced in the body and are also present in our foods. They have both natural and artificial sources; their presence in the body protects the body against free radicals or slow down the action of free radicals which are potentially harmful substances that can cause cellular damage to the body. The action of antioxidants prevents oxidative stress which is as a result of the accumulation of free radicals, they are part of the vitamins, minerals and other nutrients relevant to the body. Antioxidants are an essential part for the survival of living things; they often develop when atoms loss or gain charged particles called electrons. In the body, free radicals lack a complete orbit of electrons which makes them highly unstable, they collect electrons from other molecules and end up damaging those molecules. Antioxidants sacrifice their electrons to balance the electron orbit of these free radicals to prevent them from damaging other molecules. But unfortunately these antioxidants are also oxidized in the process, hence they need to be replenished. Their presence in the body puts off the work of free radicals and safeguard the cellular makeup of the body by preserving and protecting it against the possible outcomes of free radicals like cancer and other diseases. This study points us towards the relevance of some antioxidants and the laboratory diagnosis of these antioxidants, this will help us understand how they work and convince us to carry us measures that will further improve the action of antioxidants in our bodies by creating enabling environment where they can thrive in their utmost best and peak performance.

Keywords: Relevance, Laboratory Diagnosis, Antioxidants

INTRODUCTION

The relevance of the contributing efforts and effects of antioxidants cannot be overemphasized; this has overwhelmed a lot of scientists and researchers which has led to a lot of independent and dependent studies on antioxidants.

This work is based on the relevance of some antioxidants to humans and how they can be diagnosed in the laboratory because they form the bedrock of certain diseases. Proper understanding of how they can be assayed in the laboratory will speed-up the process of diagnosis and treatment of health conditions that may have their underlying cause or secondary cause traced to a dysfunction in the activities of antioxidants in the body.

DEFINITION OF ANTIOXIDANTS

Antioxidants are substances that may prevent cell damage. Antioxidants are found in several foods, as well as fruits and vegetables.

There are different complex reactions that take place in the body that may or may not be harmful like oxidation reactions which are crucial for life, but they can also be damaging. Antioxidants are relevant in curtailing the excesses posed by these oxidation reactions [1]

Plants and animals maintain complex systems of multiple types of antioxidants, like glutathione, vitamin C, vitamin A, and tocopherol furthermore as enzymes like enzyme, enzyme and varied peroxides. Before the advent of the several available alternative sources of antioxidants for us; Traditional herbal medicines, dietary foods were the most supply of inhibitor for ancient peoples that protected them from the harm caused by free radicals (Srivastava, 2016)

Antioxidants are our first line of defence against free radical damage, and are critical for maintaining optimum health and prosperity. Regular consumption of anti-oxidative vegetables and fruits has been recognized as reducing the risk of chronic diseases [2].

Antioxidants are also effective in areas outside the human body; they are used in the food industry to protect food from spoilage as a result of the effects of oxidation. Some antioxidants can be employed as dietary supplements to neutralize the adverse effects of oxidative stress.

Many of the natural antioxidants of interest are of plant origin such as carotenoids and antioxidant-vitamins. Their activity and their mechanism of action are dictated by:

- The structural features of the molecules involved,
- The system in which they are present as well as processing and storage conditions [3]

CLASSIFICATION OF ANTIOXIDANTS

Antioxidants can be classified in several ways:

1. Based on their activity: they can be classified as enzymatic and non-enzymatic antioxidants:
   Dangerous oxidative products can be converted to H_2O_2 and then to water by enzymatic antioxidants like superoxide dismutase (SOD) that are able to break down and get rid of free radicals in the presence of cofactors such as copper (Cu), zinc (Zn).

   Vitamin C, vitamin E, plant polyphenol, carotenoids, and glutathione are non-enzymatic antioxidants, which act by interrupting free radicals chain reactions.

2. Based on solubility: Antioxidants can be classified as water-soluble (hydrophilic) or lipid-soluble antioxidants. (Lipophilic).

   Vitamin C is a type of water-soluble vitamin found in cellular fluids such as cytosol and blood plasma.

   Vitamin E is a type of lipid-soluble vitamin that protects the cell membrane against oxidative damage.

3. According to size: Antioxidants can be categorized as small or large-molecule antioxidants. The small molecule antioxidants neutralize the Reactive oxygen species (ROS) in a process named radicals scavenging and carry them away. Vitamin C, vitamin E, carotenoids, and glutathione (GSH) are the main antioxidants in this category [4]

   Large molecule antioxidants include enzymes (SOD) and sacrificial proteins (albumin) that absorb ROS and prevent them from attacking other essential proteins [5]

4. Based on their occurrence: antioxidants are categorized as natural or synthetic
   - Natural antioxidants
     They are classified as chain-breaking antioxidants, which react with radicals and convert them into more stable products.
     Example; Antioxidant minerals like antioxidant enzymes cofactors (selenium, copper, iron) andAntioxidant vitamins such as, vitamin C, E, and B.

   - Synthetic antioxidants
     These are phenolic compounds that carry out the role of capturing free radicals and stopping the chain reaction. These compounds include, metal chelating agent (EDTA) [6]

ENZYMATIC ANTIOXIDANTS

Enzymatic antioxidants are proteins that have the ability to catalyse oxygen species and their by-products into more stable and non-toxic molecules. They are the first line of defence in the body against oxidative stress induced cell damage.

Some few examples of enzymatic antioxidants that help the body in reducing the effect of free radicals during metabolism include but not limited to the following:

1. Superoxide dismutase (SOD) or Superoxide dismutases (SODs) are a group of metalloenzymes that are found in all kingdoms of life. Superoxide dismutases(SODs) constitute a very important antioxidant defence against oxidative stress in the body. The enzyme acts as a good therapeutic agent against reactive oxygen species-mediated diseases. The catalyst will function associate degree anti-inflammatory drug agent and might additionally stop metastatic tumor cell changes. Natural SOD levels in the body drop as the body ages and hence as one age, one becomes more prone to oxidative stress-related diseases. [7]

2. Catalase: It is an antioxidant enzyme that acts as a catalyst for the conversion of hydrogen peroxide to oxygen and water. It nullifies the effect of hydrogen peroxide that is presented intracellular. Oxidative stress is caused

by associate degree imbalance between the reactive atomic number 8 species and inhibitor interaction. Oxidative stress is associate degree etiologic and intensifying consider variety of diseases. [8]

NON- ENZYMATIC ANTIOXIDANTS

1. **VITAMIN E:**
   Most plant-derived foods, especially fruits and vegetables, contain low-to-moderate levels of vitamin E activity; however, due to the abundance of plant-derived foods in our diets, they provide a significant and consistent source of vitamin E. Vitamin E functions as an essential lipid soluble antioxidant, scavenging hydroperoxyl radicals in lipid milieu. Vitamin E, a potent peroxyl radical scavenger, could be a chain-breaking inhibitor that forestalls the propagation of free radicals in membranes and in plasma lipoproteins [9]

2. **CAROTENOIDS:**
   Carotenoids are powerful antioxidants synthesized in plants from a common precursor. Carotenoids are mostly recognized for their vitamin A activity, they have shown to act as antioxidants, with a high potential to quench liposoluble radicals [10]

3. **VITAMIN C:**
   Vitamin C (ascorbic acid) is an essential cofactor. The health-promoting effects of vitamin C can be attributed to its biological functions as a water-soluble antioxidant.
   As an antioxidant, vitamin C provides protection against oxidative stress-induced cellular damage by scavenging of reactive oxygen species [11]

RELEVANCE OF ANTIOXIDANTS

1. They protect the cells against free radicals which are produced when the body breaks down food or when an individual is exposed to tobacco smoke or radiation.
2. A diet high in antioxidants may reduce the risk of many diseases including heart diseases and certain cancers.
3. Antioxidants reduce the damage caused by oxidation
4. They scavenge free radicals from the body and cells
5. They also carry out anti-aging and anti-inflammatory activities
6. In the food processing industry, they are added to foodstuffs to help eliminate problems and enrich the food in the process.

LABORATORY DIAGNOSIS OF ANTIOXIDANTS

There are a number of ways we can assay for antioxidants in the body.

1. **TOTAL ANTIOXIDNT STATUS ASSAY:**
   Reactive oxygen species (ROS) is produced in metabolic and physiological processes, and harmful oxidative reactions may occur. Under certain conditions, the increase in oxidants and decrease in antioxidants cannot be prevented, and the oxidative disorders develop. [12]

   Serum (or plasma) concentrations The ORAC assay measures a fluorescent signal from an exploration that's quenched within the presence of Reactive atomic number 8 Species (ROS) of different antioxidants can be measured in laboratories separately, but the measurements are time-consuming, labour-intensive, costly, and require complicated techniques. Because the measurement of different antioxidant molecules separately is not practical, the total antioxidant capacity of a sample is measured, and this is called total antioxidant capacity (TAC) , total antioxidant activity (TAA) , total antioxidant power (TAOP) , total antioxidant status (TAS) , total antioxidant response , or other synonyms. In the test, antioxidants in the sample reduce dark blue-green coloured ABTS radical to colourless reduced ABTS form. The modification of absorbance at 660 nm is said with total inhibitor level of the sample. The assay is calibrated with a stable antioxidant standard solution which is traditionally named as Trolox Equivalent that is a vitamin E analogue. [13]

2. **Oxygen radical absorbance capacity assay (ORAC Assay):**
   While several methods exist to measure total antioxidant capacity, ORAC has emerged as a low cost method suitable for high throughput automation in a micro plate format. The ORAC assay relies on free radical damage to a fluorescent probe, most commonly fluorescein, caused by an oxidizing reagent resulting in a loss of fluorescent intensity over time. This method measures the antioxidant capacity of a substance. The ORAC assay measures a fluorescent signal from a probe that is quenched in the presence of Reactive Oxygen Species (ROS). Addition of an antioxidant absorbs the generated ROS, allowing the fluorescent signal to persist. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) could be a tocopherol analogue and a glorious inhibitor. It is used as a standard by which all unknown antioxidants are compared. [14]

3. **ABTS Method:** This assay requires 2, 2’-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) which on treatment with sodium / potassium per sulphate or MnO_2 to give a bluish-green radical cation ($ABTS^{+\cdot}$). The radical cation is

reduced in presence of hydrogen donating antioxidants. Trolox can be used as standard antioxidant. The $ABTS^{+\cdot}$ Radical ion is blue in color and absorbs lightweight at 734 nm. The ABTS radical caution is reactive towards most antioxidants including phenolic, thiols and Vitamin C**.** During this reaction, the blue ABTS radical ion is born-again back to its colourless neutral kind. The reaction is also monitored spectrophotometrically. The reaction may complete in four minutes to several hours. This assay is commonly mentioned because the Trolox equivalent inhibitor capability (TEAC-II) assay. The reactivity of the various antioxidants tested is compared to that of Trolox, which is a water-soluble analogue of vitamin E [15]

Conclusion

The relevance of antioxidants cannot be over emphasized because of the vital role they play in our body. More studies continue to reveal their functions and how they significantly decrease the adverse effects of reactive oxygen species on the normal physiological function of human beings.

Due to the studies carried out, researchers like Dr. Richard Cutler (former Director of the National Institute of Aging, Washington) reached the conclusion that “The amount of antioxidants in the human body is directly proportional to how long the individual would live. The laboratory diagnosis carried out to assay for antioxidants will remain relevant and of utmost importance for a long time because studies have proved that anything that causes a decrease in the cellular function of antioxidants or their constituents will result in a deviation from the normal health of the individual.

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