Perspective Aspects for Therapeutic Application of Antioxidants in Veterinary Medicine

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Abstract

Oxidative stress plays a vital role in the pathophysiology of various diseases. It results from imbalance between oxidants and antioxidants in which oxidants activity exceeds the neutralizing capacity of antioxidants. Knowing about antioxidants is essential as most of the diseases are arbitrated through reactive oxygen species (ROS). Corticoid and Eicosanoid synthesis and phagocytic respiratory burst are the main causes of free radicals which challenges the animal antioxidant systems. Antioxidants may act either through single or combination of mechanism and on the basis of their activity they have been categorized into primary, secondary and tertiary antioxidants. Enzymatic and non-enzymatic antioxidants are also the widely accepted another category of antioxidants. This review deals with potential therapeutic value of antioxidant in sick animals.

Keywords: Oxidative stress, ROS, Antioxidants

Introduction

Inside the body, cells maintain the homeostasis with oxidants and antioxidants function. Oxidants are the reactive molecules which are produced inside the body and also in environment. It reacts with certain cellular molecules in the body such as DNA, proteins and lipids (Opera EC, 2006). Certain endogenous sources like mitochondrial leakage, respiratory burst and various enzymatic and auto oxidative reactions results in production of free reactive oxygen species (ROS) within the body. Some environmental sources like tobacco smoke, pollutants, UV light, ionizing radiation and xenobiotics also produce ROS (Young *et al.*, 2001). Free radicals are highly reactive chemical species which are capable of self existence. After free radical production, these ROS undergoes lipid peroxidation, alteration in DNA base pairs and protein damage which ultimately results into tissue damage (Radi *et al.*, 1991). Production of ROS is the structural part of certain normal cellular function like mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, ovulation and fertilization. But, when level of oxidants in animal body exceeds than that of antioxidants, cells undergoes oxidative stress (Liou *et al.,* 1993). This ROS plays an important role in pathogenesis of various diseases. The etiology behind various chronic degenerative diseases like rheumatoid arthritis, cancers, diabetes, obesity, lung and kidney disorders, neurodegenerative disorders, cardiovascular diseases, and other chronic diseases is imbalance between oxidants and antioxidants level. When there is inflammation or certain inflammatory mediators affect cells or tissues then there is production of reactive oxygen species (ROS) which act as antigen or pathogen resulting in oxidative stress (OS). During oxidative stress, body becomes deficient in various antioxidants such as glutathione peroxidase, catalase, superoxide dismutase, cysteine, ascorbic acid and end up with a poor clinical outcome. When cell undergoes damage, ROS release certain biomarker enzymes that lead to infections, tissue inflammation, obesity and even cancers. Biomarkers of clinical importance in companion animals include isoprostane, Nrf-2, microRNA, and others (Liou *et al*., 1993]. By measuring ROS, reaction products and endogenous antioxidants one can assess oxidative stress (Curtin *et al*., 2002)

Primary oxidative stress biomarkers

Isoprostanes

These are series of prostaglandin like compounds produced by peroxidation of arachidonic acid (Morrow and Roberts, 1997). These isoprostanes are considered to be "gold standard" test for quantifying lipid peroxidation *in vivo* in humans and animals.

Glutathione-5-Transferases (GST)

GST belongs to superfamily of certain metabolic isoenzymes which is known for their capacity to catalyze conjugation of the reduced form of glutathione (GSH) to foreign substrates for the purpose of detoxification. GST is normally found in animals, plants, fungi and bacteria. It prevents the cells and tissue from damage induced by ROS, peroxides, lipid peroxides and heavy metals.

Malondialdehyde

It is a reactive aldehyde produced on degradation of polyunsaturated lipids by ROS. It is potentially mutagenic and is found in heated edible oils like sunflower and palm oils (Moore and Roberts, 1998)

Tumor Necrosis Factor-Alpha (TNF-α)

It is also known as cachexin or cachectin that is involved in systemic inflammation. It is produced mainly by activated macrophages CD4+ lymphocytes, natural killer cells. It is an endogenous pyrogen that induces inflammation, fever, cachexia, apoptosis, inhibit tumorigenesis and viral replication, also respond to sepsis via interleukin IL-6 and IL-1 producing cells.

Dysregulation of TNF production results in variety of diseases such as Alzheimer's disease, cancer, depression, psoriasis and inflammatory bowel disease (IBD) in humans.

Cellular biomarkers can be measured in serum and urine of animals (Karihtala *et al.*, 2007). Collection of these samples especially from smaller animal species may cause unnecessary stress (Curtin *et al.*, 2002). Therefore, recent diagnostic techniques have focused on collecting saliva and feces instead of urine and serum samples for assessment of oxidative stress (Halliwell *et al.*, 1999).

Antioxidants

Antioxidant are the substances that scavenges free oxygen radicals by donating a single electron, thus it delays or inhibit oxidation of nucleic acids, lipids, proteins and carbohydrate (Herman *et al.*, 2007) According to Guttering and Halliwell, antioxidants are classified into primary, secondary and tertiary antioxidants. Primary antioxidants are that prevents the oxidant formation, secondary antioxidants scavenges ROS and tertiary one repairs the different oxidized molecules through dietary or consecutive antioxidants.

Antioxidants are also classified as hydrophilic and hydrophobic antioxidants. Antioxidants which react with oxidants in the cell cytoplasm and the blood plasma are termed as hydrophilic antioxidants (ascorbic acid, glutathione, and uric acid) whereas hydrophobic antioxidants are those which protect the cell membrane from lipid peroxidation (ubiquinol, α-tocopherol and carotenes). These are either synthesized in the body or obtained through diet. (Halliwell, 2007)

Antioxidant may be endogenous or exogenous. Endogenous antioxidants are further classified into primary and secondary endogenous antioxidants. Primary endogenous antioxidants enable the conversion of ROS to intermediate. They may be lipid soluble (ubiquinols, tocopherols and carotenoids, etc) or water soluble (uric acid, glutathione, ascorbate, etc). SOD, catalase, and glutathione peroxidase are the examples of primary antioxidant enzymes (Shu, 1998) Secondary antioxidant enzymes act directly upon the ROS and detoxify ROS. They maintain their proper function by reducing the peroxides level and continuously delivering NADPH (nicotinamide adenine dinucleotide phosphate) and glutathione for primary antioxidant enzymes. Glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione-s-transferase, and ubiquinone are the examples of secondary antioxidants. Certain minerals like iron, copper, zinc, manganese, and selenium also increase the antioxidant enzyme activities (Vertuani *et al.*, 2004).

Exogenous antioxidants are obtained through diet and play a crucial role in the defensive mechanism (Rassaf *et al.*, 2002). Many vegetables, spices, herbs, foods, etc., are well-known to be bases of exogenous antioxidants. Different polyphenolic compounds like flavonoids, isoflavones, flavones, anthocyanins, catechins, lignans, epicatechins, and phenolic acids such as hydrocinnamic acid, hydro benzoic acid, ellagic acid, gallic acid, etc act as antioxidant phytochemicals.

Antioxidants are also classified as enzymatic or no-enzymatic antioxidants. The enzymatic antioxidants directly or indirectly provide defense mechanism against ROS (Halliwell, 1999). Examples are superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase etc. Enzymatic antioxidants play an important defense mechanism by three enzymes that control the formation or neutralize free radicals. Glutathione peroxidase act by donating two electrons to decrease peroxides by forming selenols and also removes peroxides. Catalase, converts hydrogen peroxide into water and molecular oxygen (Eaton, 2006) whereas SOD converts superoxide anions into hydrogen peroxide as a substrate for catalase (Rahman et al., 2007). Glutathione reductase act by reducing glutathione from its oxidized form to reduced form, therefore recycling itself by continuing neutralizing more free radicals (Sciuto, 1997). Glucose-6-phosphate creating a reducing environment by regenerating NADPH (a coenzyme used in anabolic reactions). Hence, these two enzymes glucose-6-phosphate and glutathione reductase do not neutralize free radicals directly but shows significant role in helping other endogenous antioxidants. Classification of antioxidants has been presented in Table 1.

Superoxide dismutase (SOD)

SOD requires co-factors such as selenium, iron, copper, zinc, and manganese for their optimum catalytic activity. Reduced dietary intake of these minerals may affect the effectiveness of defense mechanisms. SOD presents in extracellular fluids of all aerobic cells.

Catalase

It is the first antioxidant enzyme that catalyzes two-stage conversion of hydrogen peroxide into water and oxygen by using either an iron or manganese cofactor. Catalase contains 4 protein subunits each carries a heme group and a molecule of NADPH (Kirkman *et al.,* 1987) Catalase is mainly located within cells in peroxisomes. The greatest activity of catalase is found in the liver and erythrocytes.

Glutathione enzymes

Glutathione peroxidase contains four selenium molecules as a cofactor that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. Glutathione peroxidases catalyze the oxidation of glutathione. Hydroperoxides, act as substrates for these enzymes (Takahashi and Cohen, 1986). Cysteine, glycine and glutamate are used in the synthesis of glutathione. The action of the glutathione peroxidase is reliant on the continuous availability of reduced glutathione.(Holben and Smith, 1999).

Non enzymatic antioxidants are mainly vitamin (A, C, E and K) , minerals (Zn, Se) and enzymes cofactors (Q10), organosulfur compounds like allium and allium sulfur, nitrogen compounds (uric acid), peptides (glutathione), and polyphenols (flavonoids and phenolic acid) (Shrestha *et al.,* 2012).

Therapeutic usage of antioxidants

Vitamin A

It is a carotenoid, formed by the breakdown product of β- carotene in the liver. It shows antioxidant activity due to its combining activity with peroxyl radical before they propagate peroxidation to lipids. β carotene enhance bactericidal activity of polymorphonuclear cells in milk and blood against common contagious pathogen, *Staphylococcus aureus* mastitis (Mascio *et al.,* 1991). It may be incorporated or added in the strategic therapeutic intervention against the economically important mastitis condition.

Vitamin C or Ascorbic acid

Ascorbic acid consists of two compounds L-ascorbic acid and L-dehydroascorbic acid. These two compounds interchanged enzymatically in the gastrointestinal tract. Ascorbic acid shows antioxidant property by scavenging the superoxide radical anion, hydrogen peroxide, singlet oxygen, hydroxyl radical, and reactive nitrogen oxide (Barros *et al*., 2011). Vitamin C is the only antioxidant which provides complete protection of endogenous lipids from noticeable oxidative damage produced by ROS (Polidori *et al.,* 2004).Cohort studies indicate that higher vitamin C status minimize the risk of hypertension, coronary heart disease, cataract and stroke. Vitamin C also boosts up the immune function of the body. Vitamin C intake reduces the incidence of stomach cancer, breast cancer and lowers the risk of type II diabetes mellitus (Shrestha *et al.,* 2012). Oxidative damage to the gastric mucosal layer is lessened by ascorbic acid by destroying ROS and diminishing the *H. pylori*-induced inflammatory cascade there by decrease the incidence of gastric carcinoma and bleeding from peptic ulcer disease (Aditi *et al.,* 2012). In young dogs, vitamin C is essential for absorption of antioxidants like N- acetylcysteine, and alpha-tocopherol whereas in geriatric patient, the oral vitamin C gives little antioxidant and immunological effects (Hall *et al.,* 2006; Ogawa *et al.,* 2008; Hesta *et al.,* 2009). If theconcentration of vitamin C is low, then the remaining antioxidants cannot provide complete protection from ROS (Ulutas *et al.,* 2006).

Vitamin E or Tocopherol

This is the major lipid-soluble, chain-breaking antioxidant found in plasma, red cells, and tissues, therefore protecting the integrity of lipid structures, mainly membranes. It prevents lipid peroxidation by donating its phenolic hydrogen to the tocopheroxyl radicals, which is unreactive and unable to continue the oxidative chain reaction. Vitamin E contains eight isoforms. Four are tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol) and four are tocotrienols (α-tocotrienol, β-tocotrienol, γ-tocotrienol, and δ-tocotrienol). The most potent and abundant isoform in biological systems is α- tocopherol.Vitamin E can act as a prooxidant when used in excess. In vitamin E deficit animals, phagocytic activity of neutrophil and macrophage are decreased. Vitamin E stimulates the production of serum antibody, particularly IgG antibodies (Tengerdy, 1980).It was observed by previous workers that level of immunoglobulin in plasma and colostrum was increased in sows following supplementation of α- tocopherol (Mahan, 1994; Mahan *et al.,* 2000). Supplementation of α-tocopherol is effective only when it is being given with selenium for reproductive performance (PinelliSaavedra *et al.,* 2008). Combination of α-tocopherol and selenium stimulates hematopoiesis and protects cell membrane of erythrocytes, resulting in long life span (Mullally *et al.,* 2004; Mohri *et al.,* 2011; Nogueira-Pedro *et al.,* 2011). The decrease in cortisol concentration following supplementation in goat and sows might be due to relief of the severity of oxidative stress (Kobisey 1997; Webel *et al.,* 1998). Vitamin E and Se helps in maintaining the mammary immune function and suggested that 2,000 to 4,000 IU vitamin E/d during the peripartum period may be beneficial in maintaining udder health and milk quality in dairy cows (Seymour, 2001). Intramammary infection was reduced to 42.2 % in vitamin E-Se supplemented versus non supplemented controls (Smith and Conrad, 1987).

Vitamin K

Vitamin K is a fat soluble compound and having two natural isoforms, Vitamin K1 and K2. Vitamin K exhibits antioxidant property due to 4-naphthoquinone structure (Vervoort *et al*., 1997). However, it has not been studied extensively for its antioxidant property in veterinary medicine. It is most widely used to check the bleeding in animals and humans.

Coenzyme Q10

Coenzyme Q10 is a lipid soluble molecule found in all cell and membrane and plays a crucial role in the respiratory chain and other cellular metabolism processes (Turunen *et al*., 2014).It plays a crucial role in revival of vitamin E.The reduced form of CoQ10 i.e ubiquinol is recognized as an antioxidant by scavenging lipid peroxyl free radical, protecting membrane phospholipids, proteins and mitochondrial DNA against oxidative damage (Linnane *et al*., 2007). It is used as an antioxidant in many diseases like cancer, diabetes, cardiac failure and bacterial periodontal disease (Roffe et al. 2004, Adarsh et al. 2008, Prakash et al. 2010, Golbidi *et al.,* 2011). Moreover, authors also observed that supplementation of CoQ10 also reduces the MDA, GST and NO_2 concentrations in pulmonary contusion and cisplatin induced oxidative stress in experimental rat and mice model, respectively (Gokce *et al.,* 2012, Sawicka *et al.,* 2013). Supplementation of CoQ10 would be helpful in *E. coli* associated calf diarrhea for early clinical recovery (Garkhal *et al.,* 2017).

Flavonoid

Flavonoids are the group of compounds that is manly composed of diphenyl propane ($C_6C_3C_6$) skeleton (Rice-Evans *et al*., 1996). These are categorized as flavonols, anthocyanins, isoflavonoids, flavanones, and flavones. Flavanones and flavones are present in the same fruit and are connected by specific enzymes while flavones and flavonols do not share this phenomenon and are rarely found together. Anthocyanins are also absent in flavanone-rich plants. Flavonoids show their antioxidant activity due to presence of phenolic hydroxyl groups attached to ring structures. They act as an antioxidant enzyme, inhibit oxidases, and mitigate nitrosamine stress. Some of the significant flavonoids are quercetin, kaempferol, catechin, and catechin-gallate (Procházková *et al*., 2011). Many plant ingredients contain curcumin, catechin, piperine, quercitin, naigirin and glycurrhizin etc. which possess antioxidant property and helps in regeneration of hepatic insult with proper bioavailability at the target organ.

Phenolic acids

It is made up of hydroxycinnamic and hydroxybenzoic acids. The most promising compound in the hydroxybenzoic group is gallic acid which is the precursor of many kind of tannin, while the cinnamic acid is the precursor of all the hydroxycinnamic acids (Krimmel *et al.*, 2010). They are found in plant material and sometimes present as esters and glycosides. They have antioxidant activity as chelators and also scavenges free radical with special impact over hydroxyl and peroxyl radicals, superoxide anions, and peroxynitrites (Terpinc *et al*.,2019).

Carotenoids

These are the group of natural pigments that are synthesized by plants and microorganisms. They can be classified into two diverse groups: the oxygenated carotenoids also known as xanthophylls, like zeaxanthin and lutein and the carotenoid hydrocarbons also known as the carotenes that contains distinct end groups like lycopene and β-carotene. Carotenoids perform antioxidant activity due to quenching singlet oxygen. The peroxyl is the only free radicals that completely damage these pigments. Carotenoids terminate free radical attacks by binding to these radicals (Paiva *et al.*, 1999)

Minerals

These are present in trace quantities in animals and are a small part of dietary antioxidants, but play significant roles in metabolism. Trace elements, like Cu, Zn and Se are crucial components of the body's defense that play a vital role in preventing free radical mediated damage (Evans and Halliwell, 2001).Selenium exists in both organic (selenocysteine and selenomethionine) and inorganic (selenite and selenate) form in the body. Selenium does not act directly on free radicals but plays a vital role in most of the antioxidant enzymes (metalloenzymes, glutathione peroxidase, and thioredoxin reductase) without which there is no antioxidant effect (Tabassum *et al.*, 2010). Like selenium, zinc also does not directly attack free radicals. Zinc also inhibits NADPH oxidase that catalyzes the production of the single oxygen radical from oxygen by using NADPH as an electron donor. Zinc is present in SOD, an important antioxidant enzyme that converts the singlet oxygen radical into hydrogen peroxide. Zinc helps in the production of metallothionein that scavenges the hydroxyl radical. Finally, zinc competes with copper for binding to the cell wall, thus decreasing the production of hydroxyl radicals [Prasad *et al.*, 2004].Oxidative stress mediated hemolysis in bovine babesiosis could be mitigated by trace minerals administration (Kumar, 2019).

Lipoic acid

It is universal antioxidants, because lipoic acid and its reduced form dihydrolipoic acid, (DHLA), neutralize the free radicals in both lipid and aqueous domains. It is categorized as "biothiol." or "thiol". They are sulfur-containing compounds which catalyze the oxidative decarboxylation of alpha-keto acids, such as pyruvate and alpha-ketoglutarate, in the Krebs cycle.

S- Adenosylmethionine

S- Adenosylmethionine- It is synthesized from l-methionine and ATP. It plays a major role in 3 key pathways i.e, transmethylation, amino propylation, and trans-sulfuration.It alsoincreases the levels of glutathione in the liver in dogs. Glutathione is a tripeptide i.e made up of cysteine, glycine and glutamic acid.

N-acetylcysteine (NAC)

Cysteine is produced by a series of enzymatic steps during SAMe trans-sulfuration. Cysteine synthesis augments glutathione synthesis. Because many of the acute-phase proteins needs cysteine, SAMe and N-acetyl cysteine that may be useful for treatment of animals with inflammatory diseases as well as those with oxidative stress (Eaton, 2006). Like SAMe, N-acetylcysteine is also a glutathione precursor. Glutathione prevents the harmful effect of reactive oxygen and nitrogen species by both direct and indirect scavenging (Dean, Giorlando, & Berk, 2011). It is used as an antidote for paracetamol toxicity. Efficacy of NAC as an antioxidant has been evaluated against hepatitis and cholangiohepatitis of dogs and other noninfectious diseases of rat (Dean *et al.,* 2011; Ribeiro *et al.,* 2011).N-acetylcysteine treatment protects against endotoxin challenge, radiation-induced injury, and pulmonary injury from toxic gases (Bernard *et al*., 1984).N-Acetyl cysteine as an adjunct antioxidant is less effective than vitamin E in reducing mineralo-oxidative pathology of bovine pediculosis (Madhesh *et al.,* 2019)

Failure of antioxidant therapy

Oxidative stress is a major contributor towards morbidity for various diseases. Many factors affect the relationship between oxidative stress and disease progression. Knowledge about the assessment of damage caused by oxidative stress and response to treatment is an essential step for evaluating the success of clinical trials. No single agent has been reported with complete amelioration of oxidative stress due to *low bioavailability,* poor target specificity, and inappropriate time and duration of therapy. Oxidative stress is not detected clinically therefore it is difficult to monitor treatment. Combination therapy that targets the various steps in oxidative stress pathway would help in ameliorating the oxidative stress efficiently than individual therapy.

Antioxidants			
Enzymatic (Endogenous)		Non-Enzymatic (Exogenous)	
Primary	Secondary	Hydrophilic	Hydrophobic
Super oxide dismutase (SOD) Catalase Glutathione peroxidase (GPx)	Glutathione reductase Glucose s transferase Glucose 6 phosphatae dehydrogenase Ubiquinone	Ascorbic acid Ubiquinol Glutathione	Vitamin E Uric acid Vitamin A

Table 1. Classification of Antioxidants

Conclusion

Free radical formation plays a vital role in developing oxidative stress. Oxidative stress develops when free radicals formation exceeds the capacity of the antioxidant system of body cells. Excessive generation of ROS may be due to mitochondria dysfunction or aberrant accumulation of transition metals, resulting in oxidative stress. Antioxidants perform a very good control over the oxidative stress. Antioxidants covers vitamin C and E, carotenoids, andenzymes containing Se, Cu, Mn, Fe and Zn. Free radical production can be categorized as environmental (e.g., temperature and humidity), nutritional (e.g., high PUFA and or excesses of certain minerals and vitamins), and infectious origin (e.g., bacterial, viral and fungal diseases). Nutritional antioxidants play a dynamic role in animal health by deactivating harmful free radicals formed by normal cellular activity or from various stressors. Therefore, dietary antioxidants should be supplemented in adequate amount.

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