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Research Article

EFFECTS OF ASCORBIC ACID ON BLOOD MALONALDEHYDE (MDA) LEVEL TO FIND OUT ITS ANTI-PEROXIDATIVE POTENTIAL

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ABSTRACT

Anti oxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water soluble antioxidants react with oxidants in the cell cytoplasm and the blood plasma, while lipid soluble antioxidants protect the cell membranes from lipid peroxidation. These compounds may be synthesized in the body r obtained from the diet. The amount of protection provided by any one antioxidant will also depends on its conc., its activity towards the particular reactive oxygen species being considered and the status of the antioxidants with which it interacts [2,3].

Keywords: Anti oxidant metabolites, Cancer, Anti-diabetic etc. & Treatment and Management

INTRODUCTION:

An **Antioxidant** is a molecule capable of slowing or preventing the oxidation of other molecules. The term antioxidant was first coined by scientist **RICHARD PASS WATER[1]**. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reaction can produce free radicals, which start chain reaction that damage cells. Antioxidants terminate these chain reactions by removing free radicals intermediates, & inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as **THIOLS or POLYPHENOLS**.

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants & animals maintain complex systems of multiple types of antioxidants, such as **Glutathione**, **Vitamin C** & **Vitamin E** & as well as enzymes like **Catalase**, **Super oxide dismutase** & various **Peroxidases**. Low levels of antioxidants, or inhibition of antioxidant enzymes, causes oxidative stress & may damage or kill cells[2,3].

CLASSIFICATION OF ANTIOXIDANTS

1. ACCORDING TO THEIR ORIGIN

1. Natural Antioxidants = Ascorbic acid, Citric acid, Glutathione, Carotenes, Alpha Tocopherol.
2. Synthetic Antioxidants = BHA, BHT, EDTA salts, H_3PO_4.

2. ACCORDING TO MECHANISM OF ACTION

1. By preferentially oxidized = Sodium Bisulphate, Thio urea, Vitamin C, Sod. Meta bisulphite.
2. By blocking oxidative chain reaction = BHA, BHT, Alpha tocopherol.
3. By synergistic action = H_3PO_4, Citric acid.
4. By chelating the trace amount of heavy metals = EDTA salts.

3. ACCORDING TO SOLUBILITY

1. Water Soluble Antioxidants = Glutathione, Vitamin C, Lipoic acid, Uric acid.
2. Lipid Soluble Antioxidants = Carotenes, Alpha Tocopherol (Vitamin E), Ubiquinol (coenzyme Q)

HISTORY

The term antioxidant was originally used to prefer specifically to a chemical that prevented the consumption of oxygen.

Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. Antioxidant activity could be measured simply by placing the fat in a closed container with

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O_2 & measuring the rate of O_2 consumption. Vitamins A, C & E acts as antioxidants. The possible mechanisms of action of antioxidants were first explored when it was recognized that a substance with anti-oxidative activity is likely to be one that is itself readily oxidized. Research into how Vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells.

ANTIOXIDANT METABOLITES

Anti oxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water soluble antioxidants react with oxidants in the cell cytoplasm and the blood plasma, while lipid soluble antioxidants protect the cell membranes from lipid peroxidation.

1. ASCORBIC ACID

Ascorbic acid or vitamin c is a monosaccharide antioxidant found in both animals and plants. As it cannot be synthesized in humans and must be obtained from the diet, it is a vitamin.

2. GLUTATHIONE

It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiol group in its Cysteine moiety is a reducing agent and can be reversible oxidized and reduced.

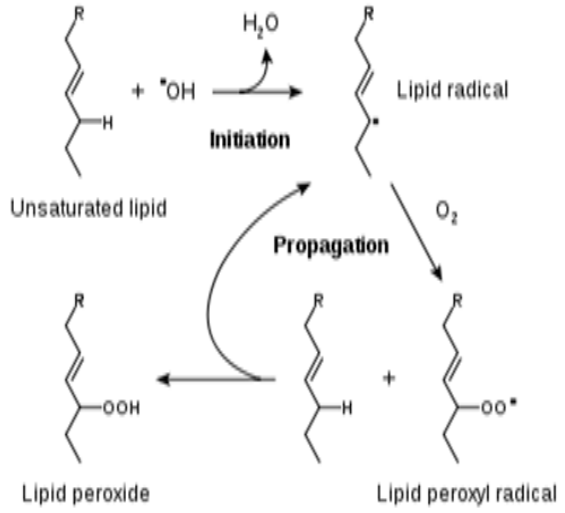


Figure 1:

3. TOCOPHEROLS & TOCOTRIENOLS (VITAMIN E)

Vitamin E is the collective name for a set of 8 related tocopherols & tocotrienols, which are fat-soluble vitamins with antioxidant properties. Of these alpha tocopherols has been most studied as it has the highest bioavailability.

4. MELATONIN

Melatonin is a powerful antioxidant that can easily cross cell membranes & the blood brain barrier. Unlike other antioxidants, melatonin does not undergo redox cycling which is the ability of a molecule to undergo repeated reduction & oxidation.

Pycnogenol is a super-antioxidant

THERAPEUTIC EFFECT OF ANTIOXIDANTS

Antioxidants can cancel out the cell-damaging effects of free radicals. Furthermore, people who eat fruits & vegetables, which are good sources of antioxidants, have a lower risk of heart disease & some Neurological diseases, & there is evidence that some types of vegetables & fruits in general, probably protect against a no. of cancers.

It regulates critical lipid membrane & lipoprotein oxidation events by:

1. Contribution to the formation of more potent secondary oxidants from superoxide (i.e., peroxynitrite).

2. Termination of lipid radicals to possibly less reactive secondary nitrogen containing products, which are in fact organic peroxy nitrites & are expected to be produced in-vitro.

L-ascorbate, Beta-carbonate, Alpha-tocopherol may prevent skin diseases linked to sun exposure like sunburn, photo immuno-supression photo ageing, photo-carcinogenesis. Vitamin C, Methionine & Selenium act as safe & effective medical alternative to surgery for painful chronic pancreatitis. Antioxidants can restore endothelial function & decrease blood pressure in severe models of hypertension. Probucol, a lipid-lowering agent, has antioxidant activity. It can heal HCl + EtOH induced & Acetic acid induced ulcer in rats.

HEART DISEASES

Epidemiologic studies have demonstrated an association between increased intake of antioxidant vitamins such as Vitamin E & Vitamin C & reduced morbidity & mortality from coronary

artery disease. Atherogenesis is initiated by oxidation of the lipids in low density lipoprotein (LDL), also termed as lipid per oxidation. As a corollary to this hypothesis, antioxidants that inhibit lipid per oxidation in LDL should limit atherosclerosis & its clinical manifestations, such as Myocardial Infarction & Stroke.

ANTI-OXIDANTS IN CANCER PREVENTION & TREATMENT

In living cells, reactive oxygen species (ROS) are formed continuously as a consequence of metabolic and other biochemical reactions as well as external factors.

Antioxidants defense systems to counter this can't provide complete protection from the noxious effects of ROS which includes oxidative damage to DNA. Experimental studies in animals and in-vitro have suggested that ROS are an important factor in carcinogenesis. There is a crucial balance between free radical generation and antioxidant defense as a force in disease prevention. An imbalance between protection against free radicals and their generation can be associated with the pathogenesis of a wide variety of diseases. Since, oxidative / electrophillic stress is generally perceived as one of the major causes for the accumulation of mutation in the genome, antioxidants are believed to provide protection against cancer. A no. of natural and synthetic antioxidants is known to retard chemical carcinogenesis. Vitamin E, Selenium and Carotenoids can be recommended diet which is rich in fruit and vegetables.

ANTIOXIDANT SUPPLIMENTS FOR WOMEN

The idea of antioxidants for heart protection sounds great. This is because in the research laboratory oxidation plays a big role in formation of atherosclerotic plaque (the cholesterol formed substance that can eventually rupture to cause a quick heart attack, which is found more in women. So, antioxidant supplements are very much beneficial in women.

Antioxidants are not useful or has no beneficial effects in Acute or chronic ocular diseases.

RENAL DISEASES

Reactive oxygen species (ROS) play a key role in the patho-physiological processes of renal diseases. The cellular damage is mediated by an alteration in the antioxidant status, which increases the concentration of ROS in the stationary state (oxidative stress). Oxidative stress produce renal damage like acute renal failure, Glomerular damage to chronic renal failure. Therefore interventions favoring the scavanging of ROS are done to preventing the oxidative stress.

ANTIOXIDANTS AS ANTI DIABETIC

Hypoglycemia leads to increased oxidative stress & endothelial dysfunction. It has been recognized that oxidation of glucose can generate oxygen free radicals & excess ROS such as super oxides. These molecules can promote lipid peroxidation, leading to excessive oxidation burden in patients with diabetes. Oxidative stress can also influence the expression of multiple genes in vascular cells, including signaling molecules & over expression of these genes may lead to endothelial dysfunction & ultimately to micro & macro vascular diseases. Ages can lead to increased production of oxygen free radicals & may therefore play a role in the development of micro-vascular diseases & atherosclerosis.

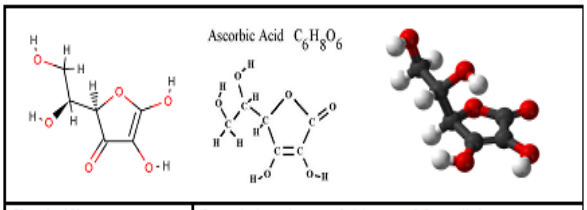
Patients with diabetes appear to have decreased antioxidant defense capability & have lower levels of antioxidants, such as Ascorbic acid (Vitamin C) or Vitamin E, or reduced activities of antioxidant enzymes such as Catalase Super oxide Dismutase or Glutathione Peroxidase.

ANTI AGEING

Free radical affects the skin in 3 ways. They can alter the fatty layers in cellular membranes. These fatty layers provide structure to the cell and control which nutrients can pass or out. They can alter the DNA within the cells, which aside from the potential to develop in to serious illness, can make your skin inclined to wrinkles and sagging before its natural biological time. Antioxidants stop these 3 processes. This is much in use in Cosmetic industry.

ASCORBIC ACID

Table 1.1:



Chemical Name	2-oxo-L-threo-hexano-1,4-lactone-2,3-enediol or (R)-3,4-dihydroxy-5-[(S)-1,2-dihydroxyethyl]furan-2(5H)-one
Chemical Formula	$C_6H_8O_6$
Molecular Mass	176.13 g/mol
Specific Density	1.65
Melting Point	190 °C-192 °C (decomposes)

Ascorbic acid is an organic acid with antioxidant properties. Its appearance is white to yellow crystals or powder. It is water soluble. The L-enantiomer of ascorbic acid is commonly known as Vitamin C.

Uses: The L-enantiomer of ascorbic acid is known as vitamin C

1. The name "ascorbic" comes from its property of preventing & curing scurvy.
2. Ascorbic acid & its sodium, potassium & calcium salts are commonly used as antioxidant food additives. These compounds are water soluble & thus cannot protect fats from oxidation. For this purpose, the fat-soluble esters of ascorbic acid with long-chain fatty acids (Ascorbyl palmitate or Ascorbyl stearate) can be used as food.
3. It can be added to water that has been treated with iodine to make it portable, neutralizing the unpleasant iodine taste.

GLUTATHIONE

Glutathione (g-glutamylcysteinylglycine, GSH) is a sulfahydril (-SH) antioxidant, antitoxin, and enzyme co-factor. Glutathione is ubiquitous in animals, plants, &microorganisms, and being water-soluble is found mainly in the cell cytosol and other aqueous phases of the living system. Glutathione often attains mill molar levels inside cells, which makes it one of the most concentrated intracellular antioxidant.

The term glutathione is typically used as a collective term to refer to the tripeptide L-gamma-glutamyl-L-cysteinylglycine in both its reduced and dimeric forms. Monomeric glutathione is also known as reduced glutathione and its dimmer is also known as oxidized glutathione, glutathione disulfide and glutathione. In this monograph, reduced glutathione will be called as glutathione- this is its common usage by Biochemists.

Glutathione is widely found in all forms of life and plays an essential role in the health of organisms, particularly in aerobic organisms. In animals including humans, & in plants glutathione is a predominant non-protein thiol and functions as a redox buffer, keeping with its own -SH groups those of proteins in a reduced condition, among other antioxidant activities.

Glutathione is present in tissues in concentrations as high as one mill molar. Glutathione plays role in catalysis, metabolism, signal transduction, gene expression and apoptosis. It is also involved in the regeneration of ascorbate from its oxidized form, dehydroascorbate.

Glutathione is present in the diet in amounts less than 100mgs daily, and it does not appear that much of the oral intake is absorbed from the intestine into the blood. Glutathione is not an essential nutrient as it can be synthesized from amino acids L-cysteine, L-glutamate, and glycine .The liver is the principle site of Glutathione synthesis the body. In healthy tissue, more than 90% of the Glutathione pool is in the reduced form & less than 10% exists in the disulfide form.

The consequences of a functional Glutathione deficiency results in the tissue oxidative stress. This condition is characterized by a hemolytic anemia. Oxidative stress caused by glutathione deficiency results in fragile erythrocyte membranes. Chronic functional glutathione deficiency is also associated with immune disorders an increased incidence of malignancies, and in the case of HIV diseases, probably accelerated pathogenesis of the disease. Acute manifestations of functional glutathione deficiency can be seen in those who have taken an over dosage of acetaminophen. This results in depletion of glutathione in the hepatocytes, leading to liver failure and death if not promptly treated.

Glutathione (reduced) is known chemically as N-(N-L-gamma-glutamyl-L-cysteinyl) glycine and is abbreviated as GSH. Molecular formula is $C_{10}H_{17}N_3O_6S$ and its molecular weight is 307.33 Daltons.

FUNCTIONS OF GLUTATHIONE

1. **Essentials co-factors**: It is an essential co-factor for oxidant enzymes, namely the GSH peroxidases.
2. GSH also makes major contributions to the recycling of other antioxidants that have become oxidized.
3. Reducing power of GSH blocks endogenous oxidants.
4. **Exogenous stressors deplete glutathione:-** e.g. Cigarette smoke, Hydrocarbons, X-ray.
5. **Glutathione as a cellular regulator:** GSH has profound importance for cellular homeostasis & for diverse cellular function.

Glutathione, a systemic antitoxin: Normally GSH is abundant in side the cell and relatively lacking out side the cell. One exception is the high concentration of GSH in the lower regions of lungs, where it helps to neutralize inhale toxin and free radicals produced by activated lung phagocytes. GSH may be especially important for those organs most directly exposed to exogenous toxins, such as the lungs, the intestines, the kidneys, and particularly the liver.

THE CHEMISTRY AND OCCURANCE OF LIPIDS

Lipids:

The term lipid includes a variety of naturally occurring compounds, which are insoluble in water, soluble in organic solvents like chloroform, ether, benzene etc., containing long chain hydrocarbon groups & which are present in or derived from living organisms.

A wide range of compounds including long chain hydrocarbons, alcohols, aldehydes, fatty acids & their derivatives fall in the lipid group. The most abundant kinds of lipids are the fats or triacylglycerols, which are the major fuels for most organisms & are most important storage form of chemical energy.

Fatty Acids:

Fatty acids are the building blocks components of most lipids. Long chain carboxylic acids having 4-24 carbon atoms occur in many diverse forms with variations in degree & kind of branching, no. of double bonds (0-6), presence of other functional groups & non-polar hydrocarbon chain as a 'tail'. They are mainly found in esterified form, eg., waxes, glycerides, phosphatides & sphingolipids.

Almost all lipids yield monocarboxylic acids (saturated & unsaturated) on hydrolysis. The acids are straight-chains acids & almost always contain an even no. of carbon atoms.

Saturated Fatty Acids:

These are saturated monocarboxylic acids having general formula $C_nH_{2n}O_2$, but because heir functional group is carboxyl group (-COOH), they are more conveniently expressed as $C_nH_{2n+1}COOH$ or $RCOOH$[3]. The commonest saturated fatty acids are even-numbered acids containing 14-20 carbon atoms, although all the possible odd & even-numbered homologies with 2-30 or more carbon atoms have been found in nature. Some of the important saturated fatty acids include Myristic acid ($C_{14:0}$), Palmitic acid ($C_{16:0}$) & Stearic acid ($C_{18:0}$). These acids are more or less common in plants & animal fats & oils.

Unsaturated Fatty Acids:

These are aliphatic mono carboxylic acids but having one or more than one double bond in their carbon chain. They may be mono saturated (containing only one double bond) or poly saturated (containing more than one double bond in their long carbon chain) fatty acids. They are as abundance as saturated fatty acids in both animal & plant lipids. These unsaturated acids are often referred to as omega-3, omega-6 etc., fatty acids depending on the position of unsaturation.

They can be divided mainly into two different classes:-

Mono unsaturated Fatty Acids (MUFA)
Poly unsaturated Fatty Acids (MUFA)

Monounsaturated Fatty Acids (NIUFA):

These fatty acids are wide spread among plants, animals & microorganisms where they occur mostly as the *cis* isomer, which produces a rigid bond in aliphatic chain. They have the general structure:

$$CH_3(CH_2)_xCH{=}CH_3(CH_2)_YCOOH$$

Where x=0-7, y=0-13

Some examples of monounsaturated fatty acids are Lauroleic acid ($C_{12:1}$), Myristoleic acid ($C_{14:1}$), Palmitoleic acid ($C_{16:1}$), Oleic acid ($C_{18:1}$) etc.

Polyunsaturated Fatty Acids (PUFA):

These acids contain two or more *cis*-double bonds, generally separated by a single methylene group (methylene-interupted unsaturation), & have very low melting point. The more no. of double bonds they posses, the greater their susceptibility to oxidative detoriation (auto oxidation).

Certain PUFAs are essential nutrients as they cannot be synthesized by mammals but has physiological action & so have to be included in the diet. PUFAs play important physiological role i.e., PUFAs present in the lipids act as the precursors of many Eicosanoids (lipid derived autacoids) like Prostaglandins (PGs), Thromboxanes, Leukotrines (LTs) that regulate important body functions & are more prone to lipid peroxidation which may lead to various pathological conditions.

PUFAs like omega-3 fatty acids are involved in maintaining good cardio-vascular health. Examples of PUFAs are Linoleic acid ($C_{18:2,}$ ^{9,12}), Linolenic acid ($C_{18:3,}$ ^{9,12,15}), Arachidonic acid ($C_{20:4,}$ ^{5,8,11,14}), Eicosapentanoic acid ($C_{20:5,}$ ^{5,8,11,14,17}), Docosapentaenoic acid ($C_{22:5,}$ ^{7,10,13,1,19}), Docosahexaenoic acid ($C_{22:6,}$ ^{4,7,10,13,16,19}).

INTRODUCTION TO LIPID PEROXIDATION

Lipid peroxidation can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bonds.

Lipid hydroperoxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. Their formation occurs in **enzymatic** or **non-enzymatic** reactions involving activated chemical species known as "**reactive oxygen species**" (**ROS**) which are responsible for toxic effects in the body via various tissue damages.

In eukaryotic organisms, ROS are mainly generated during the normal respiration process involving oxygen, oxidases and electron transports in mitochondria or endoplasmic reticulum.

FREE RADICAL:

A free radical is any species capable of independent existence & which contains one or more unpaired electrons. It can be anionic, cationic or neutral. Presence of one or more unpaired electrons causes the species to be attracted slightly to a magnetic field (i.e., to be paramagnetic) & sometimes makes the species highly reactive.

TYPES OF FREE RADICALS

Several reactive oxygen species (ROS) and one thiyl radical ($RS^{\cdot}$) are known. Among them, the most frequently studied are given below.

SUPEROXIDE RADICAL ($O_2^{\cdot-}$):

This ROS is formed when oxygen takes up one electron and as leaks in the mitochondrial electron transport but its formation is easily increased when exogenous components (redox cycling compounds) are present. Its first production site is the internal mitochondrial membrane (NADH ubiquinone reductase and ubiquinone cytochrome C reductase). This species is reduced and forms hydrogen peroxide (H_2O_2). The production of superoxide radicals at the membrane level (NADPH oxidase) is initiated in specialized cells (oxidative burst) with phagocytic functions (macrophages) and contributes to their bactericid action. The flavin cytosolic enzyme xanthine oxidase found in quite all tissues and in milk fat globules generates superoxide radicals from hypoxanthine and oxygen and is supposed to be at the origin of vascular pathologies.

HYDROGEN PEROXIDE (H_2O_2):

Hydrogen peroxide is mainly produced by enzymatic reactions. These enzymes are located in microsomes, peroxysomes and mitochondria. Even in normoxia conditions, the hydrogen peroxide production in relatively important and leads to a constant cellular concentration between 10^{-9} and 10^{-7} M. In plant and animal cells, superoxide dismutase is able to produce H_2O_2 by dismutation of $O_2^{\cdot-}$, thus contributing to the lowering of oxidative reactions. The natural combination of dismutase and catalase contributes to remove H_2O_2 and thus has a true cellular antioxidant activity. H_2O_2 is also able to diffuse easily through cellular membranes.

HYDROXYL RADICAL ($^{\cdot}OH$):

In the presence of Fe^{2+}, H_2O_2 produces the very active species $^{\cdot}OH$ by the Fenton reaction (described in 1894):

$Fe^{2+} + H_2O_2$ ----> $Fe^{3+} + {}^{\cdot}OH + OH^-$

This iron-catalyzed decomposition of oxygen peroxide is considered the most prevalent reaction in biological systems and the source of various deleterious lipid peroxidation products. It must be noticed that an important part of hydroxyl radicals is also produced (with $^{\cdot}NO_2$) by the decay of peroxinitrite or peroxynitrous acid.

NITRIC OXIDE ($^{\cdot}NO$):

Nitric oxide is produced in various types of cells of vascular endothelium. It reacts rapidly with oxygen to yield nitrogen dioxide ($^{\cdot}NO_2$) which in turn may react with $^{\cdot}NO$ to yield nitrogen trioxide (N_2O_3). The rapid reaction of $O_2^{\cdot}$-, produced in different pathological states, with $^{\cdot}NO$ gives the extremely reactive peroxinitrite ($ONOO^-$) which mediates oxidation, nitrosation, and nitration reactions[6]. In alkaline solutions, $ONOO^-$ is stable but decays rapidly once protonated into peroxynitrous acid. Its decomposition forms $^{\cdot}OH$ and nitrogen dioxide ($NO_2^{\cdot}$). In acidic conditions, protonation of NO_2^- to HNO_2 can mediate the nitration of polyunsaturated fatty acids and lipid hydroperoxides giving nitrated lipid products whose structure and function are incompletely characterized.

SINGLET OXYGEN ($^1O_2$):

Its toxicity is reinforced when appropriate photoexcitable compounds (sensitizers) are present with molecular oxygen. The presence of metals contributes to increase the production of singlet oxygen, as well as anion superoxide, and thus accelerates the oxidation of unsaturated lipids generating hydroperoxides. It has been suggested that singlet O_2 may be formed during the degradation of lipid peroxides and thus may cause the production of other peroxide molecules.

OZONE (O_3):

This natural compound present in the higher atmosphere and in the lower atmosphere of our polluted cities is a major pollutant formed by photochemical reactions between hydrocarbons and nitrogen oxides. Ozone is not a free radical that stimulates lipid peroxidation and thus induces damages at the lipid and protein levels *in vivo* mainly in airways.

Ozone may add on across a double bond and decomposes to form a free radical. The proposed mechanism is given below.

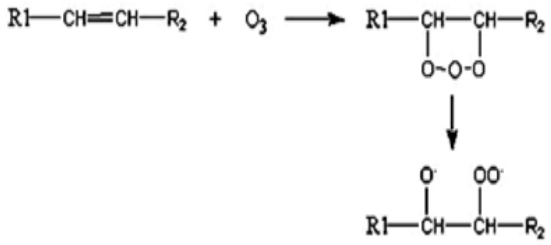


Figure 2:

THIYL RADICALS ($RS^{\cdot}$):

Aliphatic thiols (RSH) are contained in living organisms in high concentrations. It can react as a nucleophile and attack a disulfide bond. In the absence of oxygen, a thiyl radical was shown to induced cis/trans-isomerization of linoleic acid and led to several isomers.

Thiol compounds (RSH) are frequently oxidized in the presence of iron or copper ions:

$RSH + Cu_2^+ \text{----> } RS^{\cdot} + Cu^+ + H^+$

These thiyl radicals have strong reactivity in combining with O_2:

$RS^{\cdot} + O_2 \text{ ---> } RSO_2^{\cdot}$

Furthermore, they are able to oxidize NADH into $NAD^{\cdot}$, ascorbic acid and to generate various free radicals ($^{\cdot}OH$ and $O_2^{\cdot}$-).

GENERATION OF LIPID PEROXIDATION PRODUCTS

Several lipid molecules containing double bond(s) can be peroxidized under appropriate conditions. The mechanisms inducing lipid peroxidation are complex but are similar for the majority of these molecules. Unsaturated fatty acids, free or esterified, are the best studied but cholesterol oxidation appears also to be intensely explored.

PRIMARY PRODUCTS

FATTY ACID OXIDATION:

Fatty acids may be autoxidized either in free form or combined into glycerolipids or glycolipids. Thus, oxidized triacylglycerol monomers include molecules containing different oxygenated groups, mainly hydroxy, keto, and epoxy, as well as short-chain fatty

Oxidation of monoene lipids:- Studies of the autoxidation mechanism of **oleic acid** involved in the formation with near equal probability of a hydroperoxy group at positions 8, 9, 10 and 11 of oleic acid.

Oxidation of diene lipids:- Linoleic acid gives only two autoxidation products in equivalent amount (9-OOH and 13-OOH). These two isomers can change from c,t to t,t structure with exchange of the hydroperoxide group from C9 to C13 or vice versa but keep the conjugated diene structure, thus, four major products are found. They are also able to produce cyclic peroxides by addition of singlet oxygen to their conjugated dienes.

Oxidation of highly unsaturated lipids:- Autoxidation of fatty acids with more than 3 double bonds leads to complex mixtures of products. **Arachidonic**, **pentaenoic** and **hexaenoic acid** oxidation has been largely investigated[6].

CHOLESTEROL PEROXIDATION:

Cholesterol has been exploited with great advantage to detect any oxidation process in cell membranes. In contrast with unsaturated fatty acids, cholesterol exists as a single molecular species, its oxidation products are thus much less complicated to isolate and characterize. Cholesterol may undergo autoxidation and photo-oxidation, both processes give rise to **oxysterols** of various structures depending on the type of oxidation and the physical state of the substrate. Thus, the identification of cholesterol oxidation products may be used as a mechanistic proof in various oxidant systems. When cholesterol esters are oxidized, the structure and the yield of the formed oxysterols depend on the fatty acid species.

SECONDARY PEROXIDATION PRODUCTS

FROM FATTY ACIDS OR MORE COMPLEX LIPIDS

The majority of chain-cleavage products formed from monohydroperoxides is molecules belonging mainly to two groups, simple hydrocarbons and short-chain aldehydes. Other far less characterized molecules were described and bear epoxy, alcohol, or ketone group, either alone or in combination, even with an aldehyde function (hydroxyaldehydes). Dicarboxylic acids may also be formed by a two-step oxidation process. Most of these substances result from cleavage off the carbon bonds adjacent to the hydroperoxy group. Thus, one fragment derives from the methyl end of the fatty chain, the other fragment being free or remaining bound to the parent glycerolipid via the ester linkage. These transformed lipids (with shortened or modified acyl chains) remain poorly studied despite their suggested physiological activity.

MECHANISMS OF FATTY ACID OXIDATION

Three different mechanisms are able to induce lipid peroxidation:

1 - Autoxidation by free radical reaction
2 - Photo-oxidation
3 - Enzyme action

This is a radical-chain process involving 3 sequences:

Initiation, Propagation and Termination.

1.1- Initiation

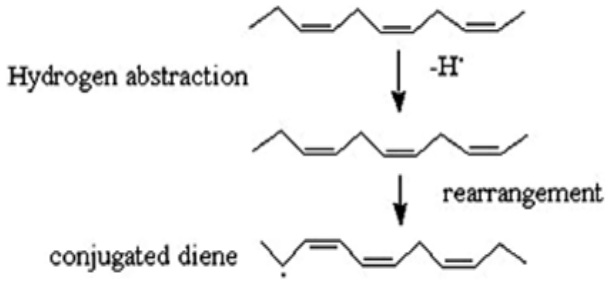
LH + R*⟶ L*+RH

In a peroxide-free lipid system, the initiation of a peroxidation sequence refers to the attack of a ROS (with sufficient reactivity) able to abstract a hydrogen atom from a methylene group (- CH_2-), these hydrogen having very high mobility. This attack generates easily free radicals from polyunsaturated fatty acids. $^{\cdot}OH$ is the most efficient ROS to do that attack, whereas $O_2^{\cdot-}$ is insufficiently reactive.

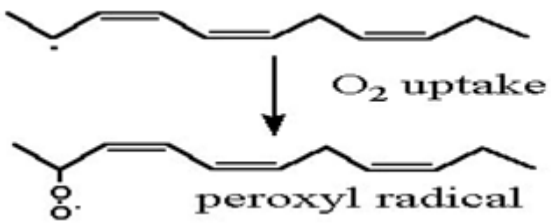
$$-CH_2- + {}^{\cdot}OH \longrightarrow -\dot{C}H- + H_2O$$

This peroxidation process is inhibited by tocopherols, mannitol and formate. The presence of a double bond in the fatty acid weakens the C-H bonds on the carbon atom adjacent to the double bond and so makes H removal easier.

The carbon radical tends to be stabilized by a molecular rearrangement to form a conjugated diene.



Under aerobic conditions conjugated dienes are able to combine with O_2 to give a peroxyl (or peroxy) radical, $ROO^{\cdot}$

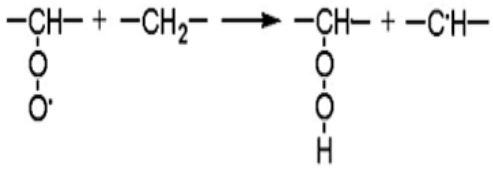


1.2– Propagation

$$L^* + O_2 \longrightarrow LO_2^*$$

$$LH + LO_2 \longrightarrow LOOH + L^*$$

As a peroxyl radical is able to abstract H from another lipid molecule (adjacent fatty acid), especially in the presence of metals such as copper or iron, thus causing an autocatalytic chain reaction. The peroxyl radical combines with H to give a lipid hydroperoxide (or peroxide). This reaction characterizes the propagation stage.



Probable alternative fates of peroxyl radicals are to be transformed into cyclic peroxides or even cyclic endoperoxides (from polyunsaturated fatty acids such as arachidonic or eicosapentaenoic acids).

1.3– Termination

$$L^* + L^* \longrightarrow LL$$

$$LO_2 + LO_2 \longrightarrow LOOL + O_2$$

$$LO_2^* + L^* \longrightarrow LOOL$$

Termination (formation of a hydroperoxide) is most often achieved by reaction of a peroxyl radical with a-tocopherol which is the main lipophilic "chain-breaking molecule" in the cell membranes. Furthermore, any kind of alkyl radicals (lipid free radicals) $L^{\cdot}$ can react with a lipid peroxide $LOO^{\cdot}$ to give non-initiating and non-propagating species such as the relatively stable dimers LOOL or two peroxide molecules combining to form hydroxylated derivatives (LOH). Some bonds between lipid peroxides and membrane proteins are also possible.

2 - Photo-oxidation

As singlet oxygen ($^1O_2$) is highly electrophilic, it can react rapidly with unsaturated lipids but by a different mechanism than free radical autoxidation. In the presence of sensitizers (chlorophyll, porphyrins, myoglobin, riboflavin, bilirubin, erythrosine, rose bengal, methylene blue...), a double bond interacts with singlet oxygen produced from O_2 by light.

Oxygen is added at either end carbon of a double bond which takes the trans configuration. Thus, one possible reaction of singlet O_2 with a double bond between C12 and C13 of one fatty acid is to produce 12- and 13-hydroperoxides.

The lifetime of singlet O_2 in the hydrophobic cell membrane is greater than in aqueous solution.
The inhibition of photosensitized oxidation is efficiently inhibited by carotenoids, the main protective role played by these compounds in green plants. The inhibitory mechanism is thought to be through an interference with the formation of singlet oxygen from the oxygen molecule. In contrast, tocopherols inhibit this oxidation by quenching the previously formed singlet oxygen, this forms stable addition products[4,5].

3. Enzymatic peroxidation

Lipoxygenase enzymes (from plants or animals) catalyze reactions between O_2 and polyunsaturated fatty acids, such as arachidonic acid (20:4 n-6), containing methylene interrupted double bonds.

When 20:4 n-6 is the substrate, these hydroperoxides are known as HpETEs which can be transformed into hydroxy products (HETEs).
These HETEs are also formed directly via cytochrome P450 induced reactions (mono-oxygenases) and sometimes also via cyclooxygenase enzymes.

Six hydroperoxides (5-, 8-, 9-, 11-, 12-, and 15-HpETE) are known to be formed from arachidonic acid in animal cells. Dihydroperoxy compounds (DiHpETEs) may also be formed via the action of 5- and 15-lipoxygenases. These compounds are important metabolic intermediates but are also bioactive.

Cyclooxygenase enzymes (in plants and animals) catalyze the addition of molecular oxygen to various polyunsaturated fatty acids, they are thus converted into biologically active molecules called endoperoxides (PGG, PGH), intermediates in the transformation of fatty acids to prostaglandins[6,7].

MEASUREMENT OF FREE RADICALS IN LIPID PEROXIDATION

Free radicals have a very short half-life, which makes them very hard to measure in the laboratory. Multiple methods of measurements are made today, each with there own benefits and limits. Radicals can be measured using electron spin resonance and spin trapping methods. The methods are both very sophisticated and can trap even the shortest & SHY; lived free radicals (i.e. xenobiotics) are utilized in the spin techniques. The compound and radical together form a stable entity that can be easily measured. This indirect approach has been termed "finger printing". However, this method is not 100% accurate. Spin-trapping collections have very poor sensitivity[8,9].

The methods are:

1. Uptake of oxygen
2. Measurements of peroxides
  a). Iodine liberation
  b). Haem degradation of peroxides
  c). Glutathione peroxides
  d). Cyclooxygenase
3. Diene conjugation
4. Measurement of hydrocarbon gasses
5. Loss of fatty acid
6. Light emission
7. Measurement of fluorescence
8. The thiobarbituric acid (TBA) test
9. Measurements of aldehydes other than MDA

A newly developed technique for measuring free radical production shows promise in producing more valid results. The technique uses monoclonal antibodies and may prove to be the most accurate measurement of free-radicals. However, until further more reliable techniques are established it is generally accepted that 2 or more assays be utilized whenever possible to enhance validity.

In the present study, effects of Ascorbic acid on blood MALONALDEHYDE (MDA) level were evaluated in an attempt to find its anti-peroxidative potential. Plan of the experiment being in an in-vivo system using New Zealand white rabbit (*Oryctolagus cuniculus*) as an animal of choice. Rabbit is chosen for the work as it is a mammal & having similarity with human beings. It is also selected due to its easy availability & ease of experiment.

MATERIALS AND METHOD

Estimation of Oxidative Stress was performed by measuring the level of GLUTATHIONE in the serum of rabbit's blood before and after the administration of Ascorbic Acid.

Estimation was made by using a Standard Curve given below.

STANDARD CURVE PREPARATION

1. Reagents used

- DTNB solution
- Glutathione primary standard.
- Phosphate buffer

2. Equipments & Apparatus used

UV-Visible Spectrophotometer
Volumetric Flask
Graduated Stopper Test Tubes

3. Formula of Standard Curve

A = (0.0058 * C) + 0.00049

where **A**=Absorbance & **C**=Concentration

CONCENTRATION OF GSH

$$\text{Concentration of GSH (nano mole/ml)} = \frac{\text{A- 0.0000085}}{\text{0.0068}}$$

PROCEDURE:

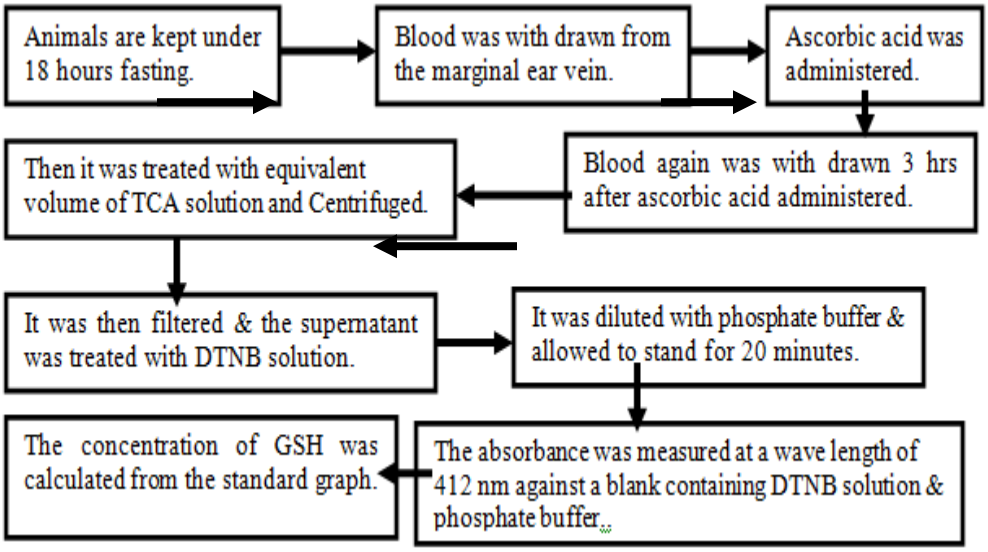


Table 1: Effect of Ascorbic acid on blood MDA level

ANIMAL No	BODY WEIGHT (kg)	ABSORBANCE OF CONTROL	ABSORBANCE OF TEST (Antioxidant)
1	1.8	0.606	0.666
2	1.8	0.406	0.476
3	2.0	0.509	0.553
4	2.0	0.655	0.631

Table 2: Blood MDA content in nano mole/ml in animal models

ANIMAL No	BODY WEIGHT (kg)	CONCENTRATION OF CONTROL	CONCENTRATION OF TEST (Antioxidant)
1	1.8	89.12	97.94
2	1.8	59.70	69.99
3	2.0	74.85	81.32
4	2.0	96.32	92.79

Table 3: Percentage change in blood MDA content of antioxidant treated animals with respect to control

ANIMAL No.	% Change
1	9.90
2	17.24
3	8.64
4	-3.66
AVERAGE	8.03
STANDARD DEVIATION	8.67

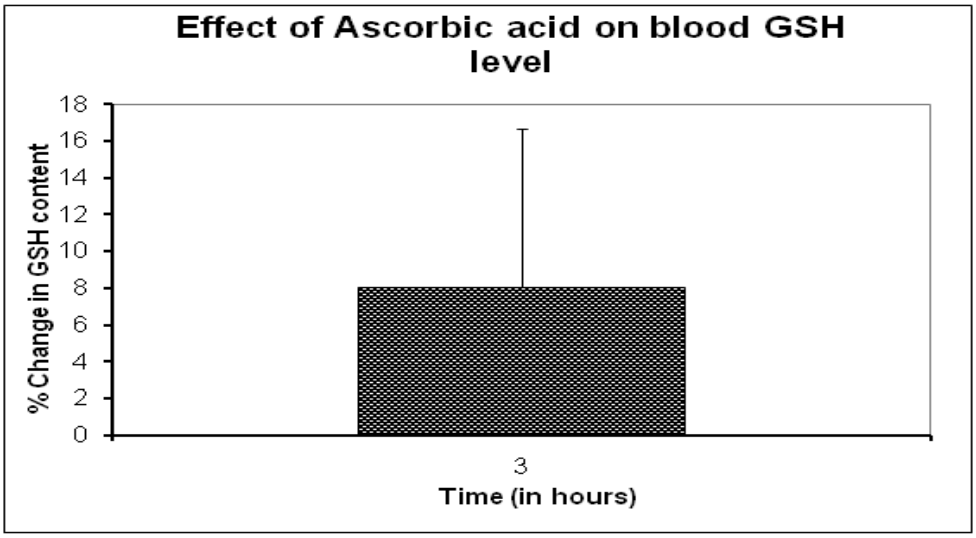


Figure 3:

DISCUSSION & CONCLUSION:

Results of the experiment performed are shown in table 1, 2, & 3 & it was graphically presented in Figure-1. shows absorbance of different samples. Figure 2 shows concentration of GSH content in control animals & antioxidant treated animals. Figure 3 shows % change in GSH content with respect to control.

From figure 3 it is clear that after administration of ascorbic acid GSH level has increased. This supports the antioxidant role of ascorbic acid. Ascorbic acid is a well-known antioxidant & it has good antioxidant potential. The results are also supported by figure 3. Though the results clearly supports the antioxidant capacity of ascorbic acid, but in some experiments it is found that ascorbic acid also has pro-oxidant effect. A final conclusion cannot be drawn considering this little volume of work. We can reach in a final conclusion only after repeated experiments using more animal models.

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