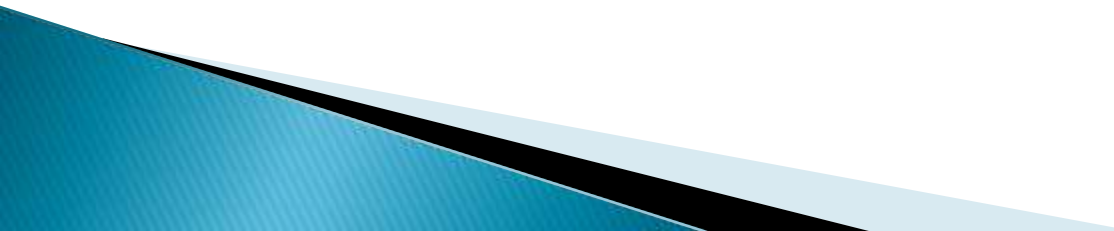


# ANTIOXIDANTS

# CONTENTS

- ▶ INTRODUCTION
  - ▶ HISTORY
  - ▶ FREE RADICALS
  - ▶ ANTIOXIDANTS
  - ▶ CLASSIFICATION
  - ▶ MECHANISM OF TISSUE DAMAGE
  - ▶ ROLE OF ANTIOXIDANTS IN PERIODONTICS
  - ▶ CONCLUSION
  - ▶ REFERENCES
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# INTRODUCTION

- ▶ Oxygen is synonym for life
- ▶ Few oxygen atoms are **destructive** too – free radicals or **reactive oxygen species**
- ▶ Unpaired electron in its structure
  - Hydroxyl radical ( $\text{OH}$ )
  - Superoxide anion ( $\text{O}_2^-$ )
  - Hydrogen peroxide ( $\text{H}_2\text{O}_2$ )
  - Hypochlorous acid ( $\text{HClO}$ )

## Free radicals are produced during:

### Normal cellular activities

- ETC reactions
- Liver detoxification reaction
- Immune reactions

### Pathological events

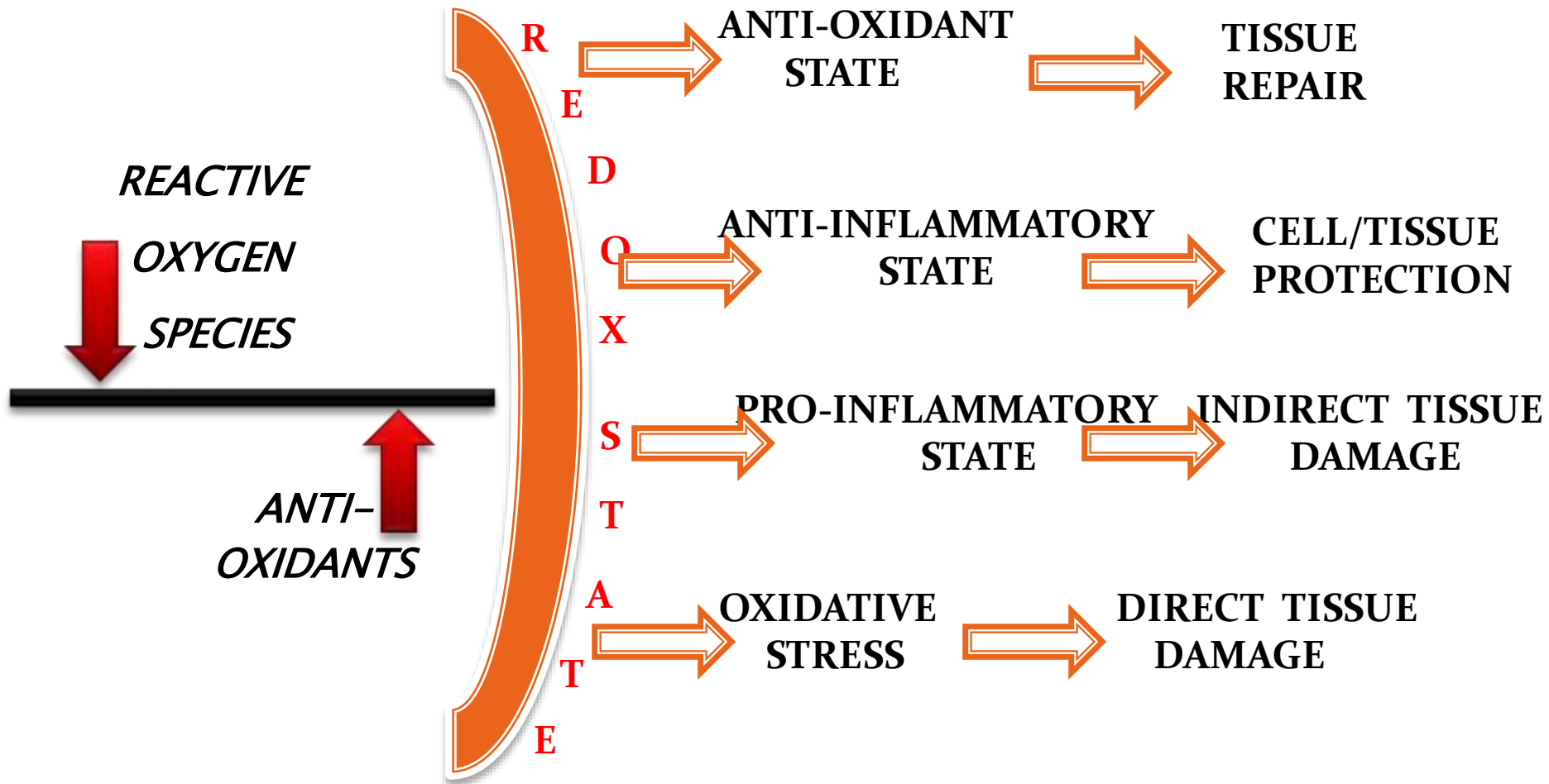
- Ionizing radiation
- Toxic chemicals
- Tissue ischemia

### Disease states

- Inflammatory disease
- Degenerative disease
- carcinogenesis

- ▶ **Antioxidants:**
- ▶ protect against harmful oxidative reactions
- ▶ Any substance that when present **in low concentrations** compared to that of an oxidisable substrate significantly **delays or or inhibits** the oxidation of that substrate.

Intoduction .....

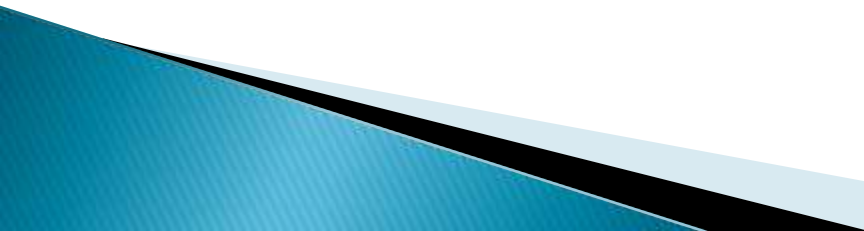


In health a balance exists between pro oxidant and the antioxidant mechanisms but in disease this balance is tipped in favour of the former.

# HISTORY

- ▶ Oxygen

*Joseph priesley, 1775*

- ▶ In 19<sup>th</sup> century – Engineers discovered anti- metal corrosion substance
  - ▶ In mid 20<sup>th</sup> century – From metal to **food** to **cells**
  - ▶ Prevents oxidative damage to cellular components like DNA & Proteins.
- 

# FREE RADICALS

- ▶ Any species capable of **independent existence** that contains one or more **unpaired electrons**.
- ▶ Highly unstable & reactive
- ▶ Toxic – react with DNA & cell membrane

## What are **Free radicals** ?

- Free radicals are like robbers which are deficient in energy.
- Free radicals attack and snatch energy from the other cells to satisfy themselves.



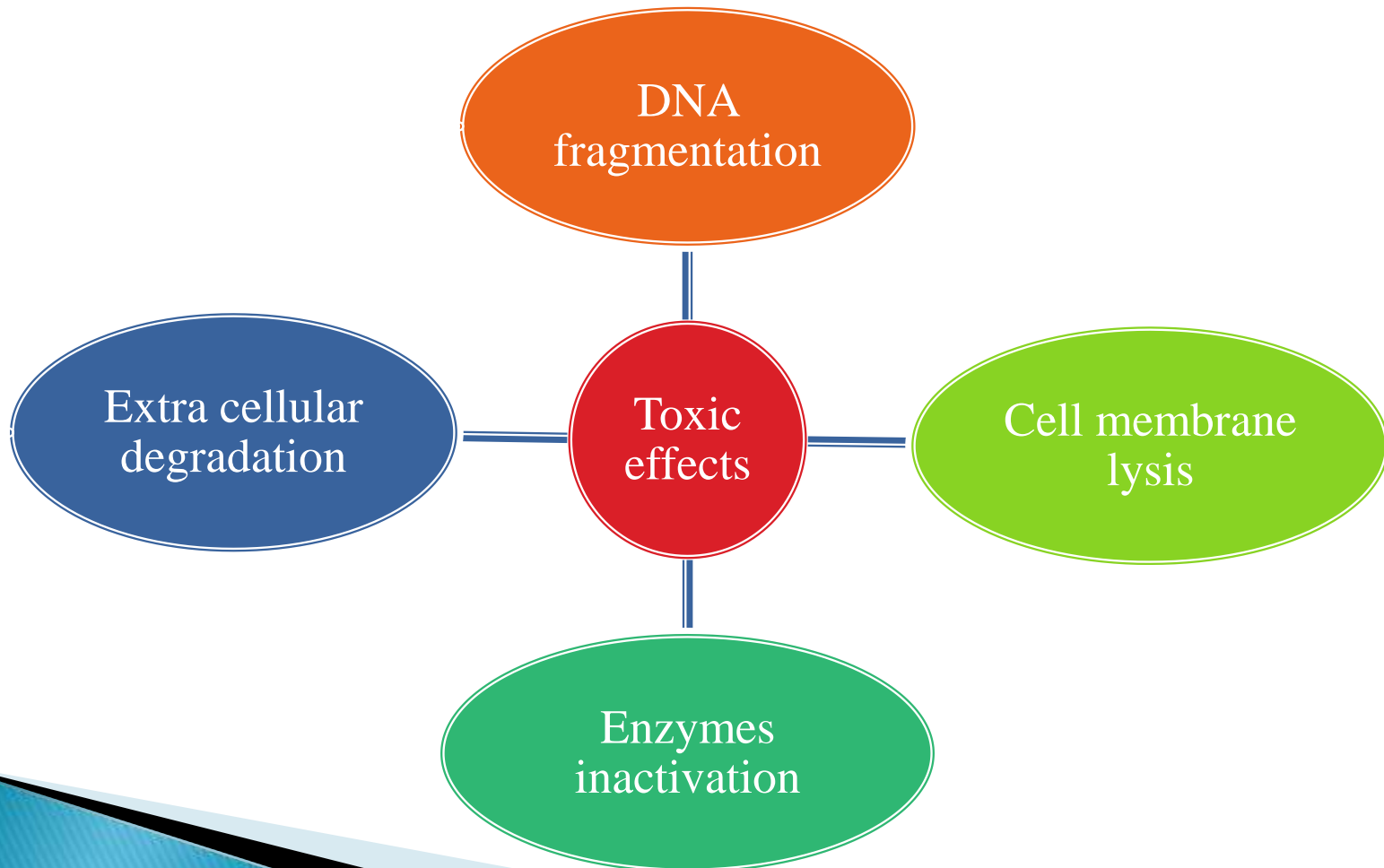


## Free radicals .....

INTERNAL SOURCES	EXTERNAL SOURCES
Mitochondria	Cigarette smoke
Phagocytes	Environment pollution
Arachidonate pathways	Radiation
Peroxisomes	UV light
Xanthine oxidase	Certain drugs & anaesthetics
Exercise	Organic compounds like pesticides
Inflammation & ischemia	Ozone

## Free radical .....

- ▶ Phagocytes ,fibroblast, vascular endothelial cells & osteoclasts.



## Free radicals.....

- ▶ Free radical species have been implicated in the pathogenesis of over 100 conditions
- Rheumatoid arthritis
- Type 2 diabetes mellitus
- Adult respiratory distress syndrome
- AIDS
- Atherosclerosis
- Myocardial infarction
- Cataracts
- Periodontal disease

Free radicals.....

# Superoxide

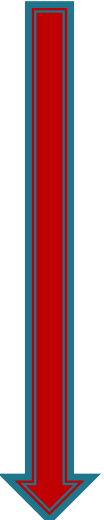
Respiratory burst



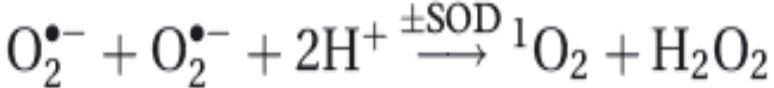
Superoxide



H<sub>2</sub>O<sub>2</sub>




superoxide  
dismutase 2  
enzyme

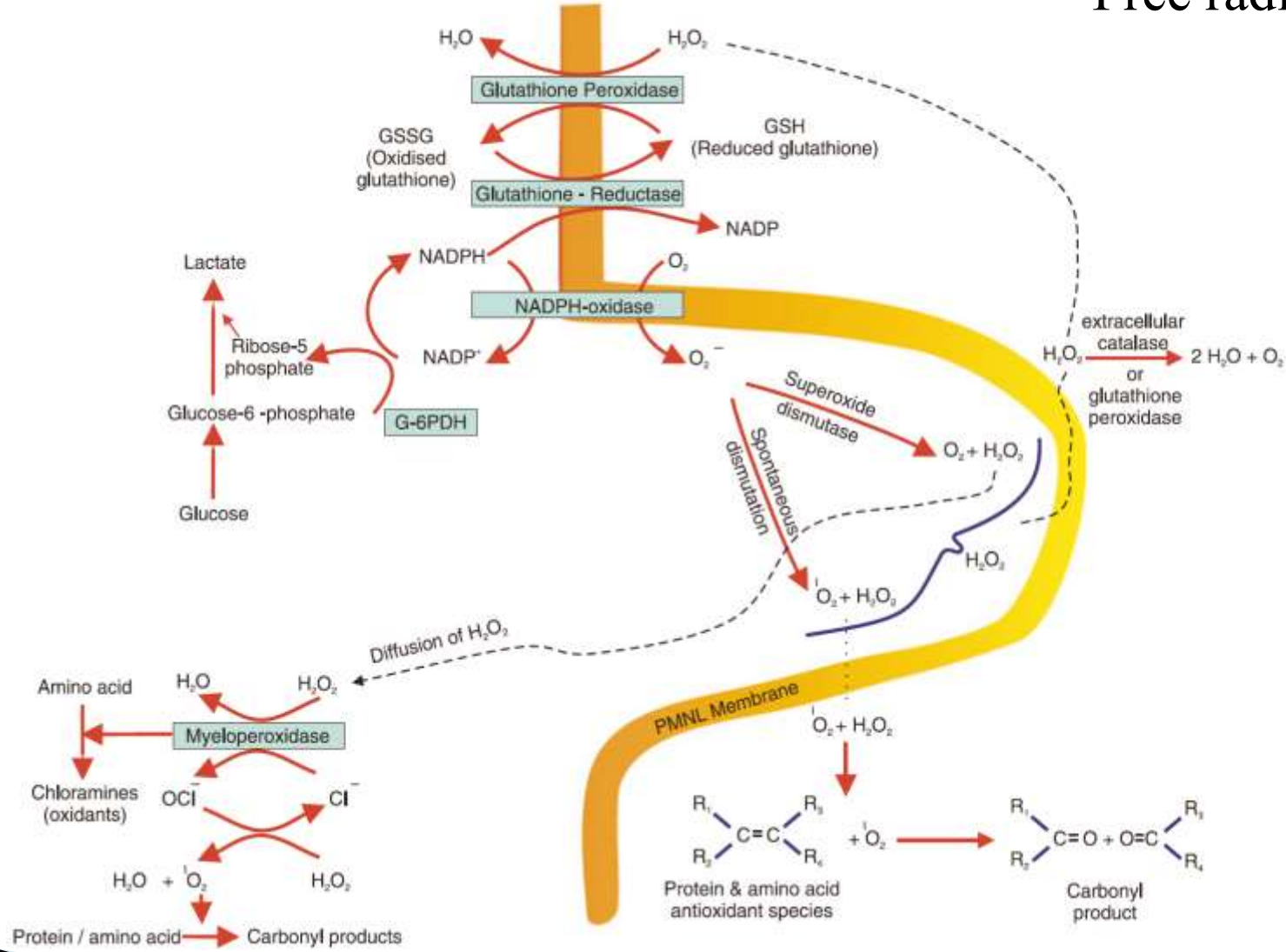


H<sub>2</sub>O<sub>2</sub>

Superoxide establishes a pro-inflammatory state

- Triggering nuclear factor- $\kappa$ B transcription
  - Endothelial cell damage
  - Increased vascular permeability
  - Neutrophil chemotaxis via leukotriene B4 formation
  - Lipid peroxidation
  - DNA strand breaks
- 

# Free radicals.....



## Nitric oxide

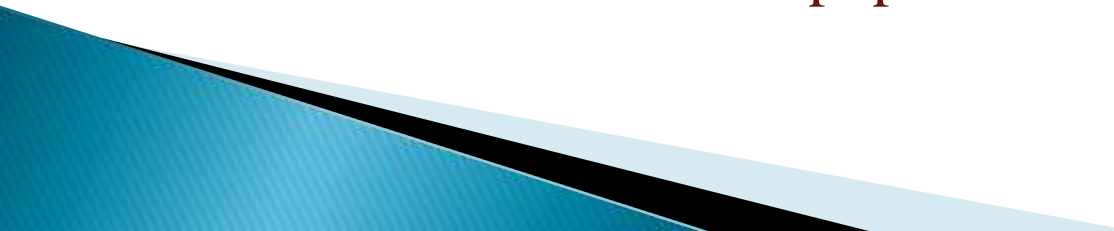
- Nitric oxide synthases.
- Macrophage-derived nitric oxide synthase 2



- When released simultaneously with superoxide it forms the reactive nitrogen species **peroxynitrite anion** .

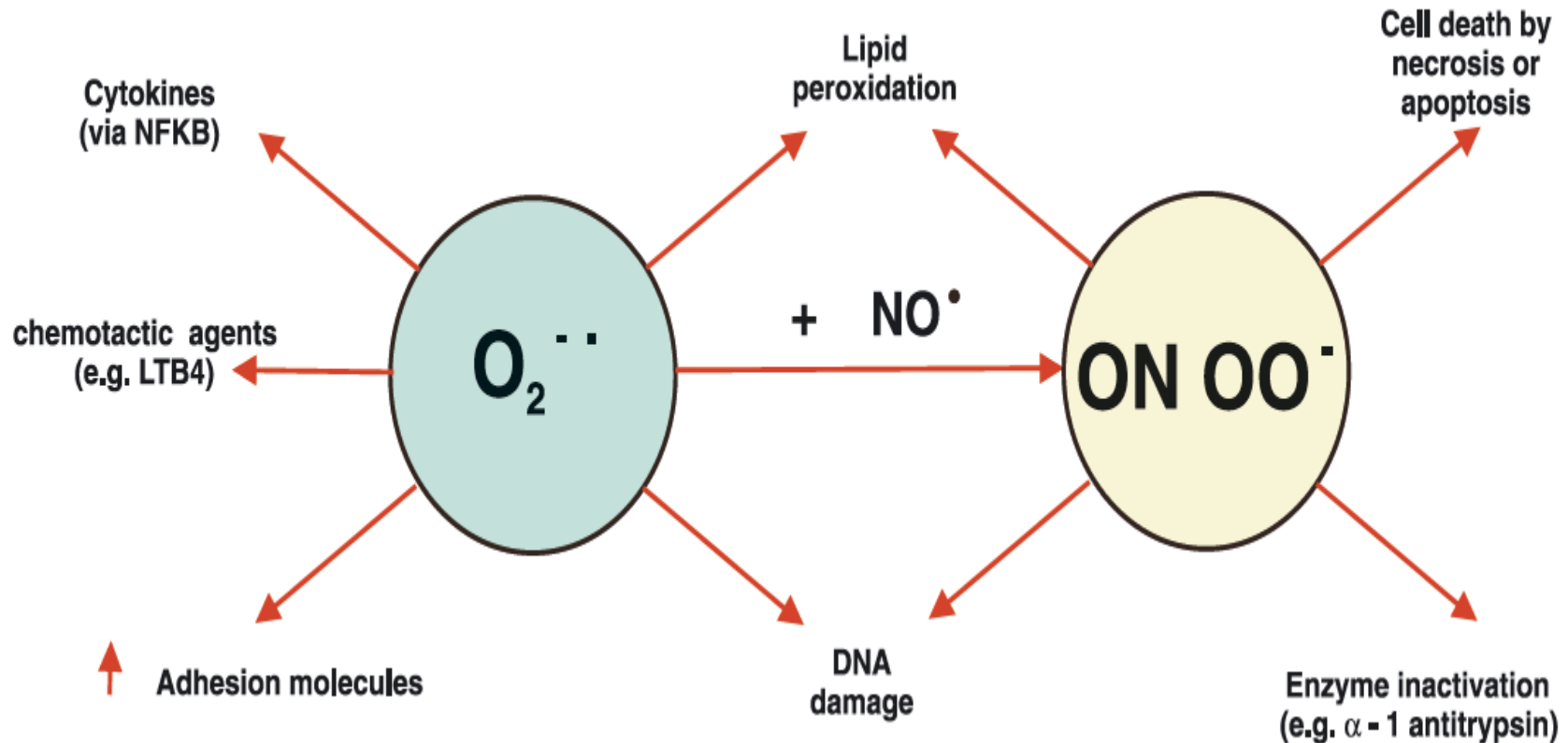
## Peroxynitrite

Responsible for many of the cytotoxic effects:

- lipid peroxidation;
  - glutathione depletion by oxidation;
  - inhibition of superoxide dismutase activity;
  - DNA damage by nitrosilation, deamination and oxidation;
  - high concentrations cause rapid **cellular necrosis**
  - low concentrations cause **apoptosis**.
- 



# Interaction between superoxide & nitric oxide to form peroxynitrite

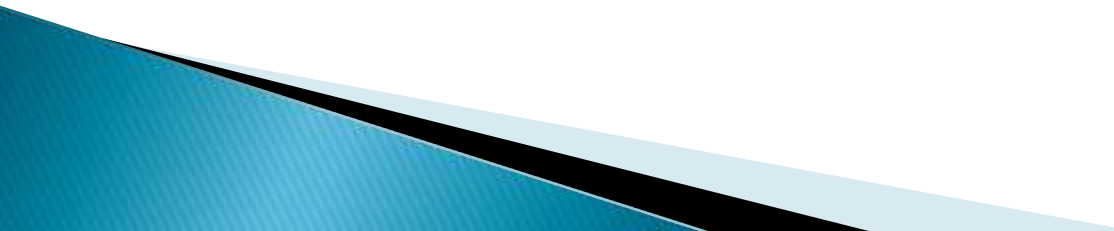


# Hydrogen Peroxide

- A weak ROS
- $< 50 \mu\text{m}$  – limited cytotoxicity
  - more as a cell signaling molecule.
- Hydrogen peroxide – as a second messenger in nuclear factor- $\kappa\text{B}$  activation.

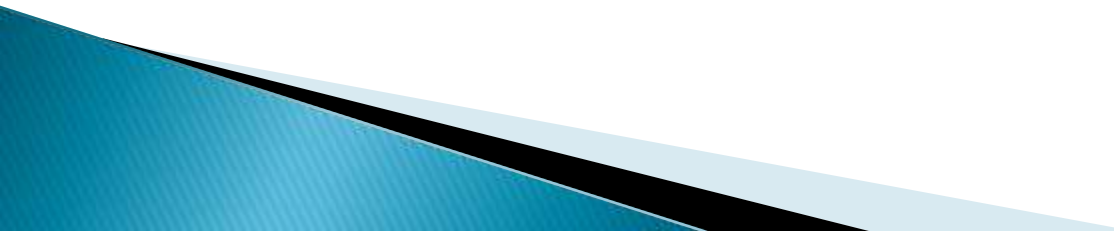
## Free radicals.....

Where inflammation is present it may:

- increase adhesion molecule expression;
  - cell proliferation;
  - induce apoptosis;
  - modulate platelet aggregation.
- 

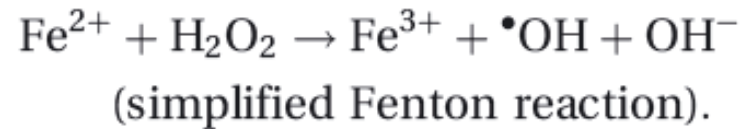
## Free radicals.....

The principal enzymes charged with removal of  $\text{H}_2\text{O}_2$  are :

- **Catalase** - predominantly acts intracellularly,
  - **Glutathione peroxidase** - within mitochondria and extracellularly
  - **Thioredoxin linked peroxidases**
- 

## Hydroxyl Radical

- Most potent species.



- Cellular targets include:

- Lipids - lipid peroxidation

- Carbohydrates - forming carbohydrate radicals or depolymerizing mucopolysaccharides

- Protein - most potent in oxidizing aliphatic amino acids
  - hydroxylation of tyrosine, tryptophan, phenylalanine etc

- DNA - most significant damage.

Free radicals.....

Extracellular targets include:

- **Extracellular matrix components** – proteoglycan

-

glycosaminoglycan

- **Collagens and structural proteins** – proline sites

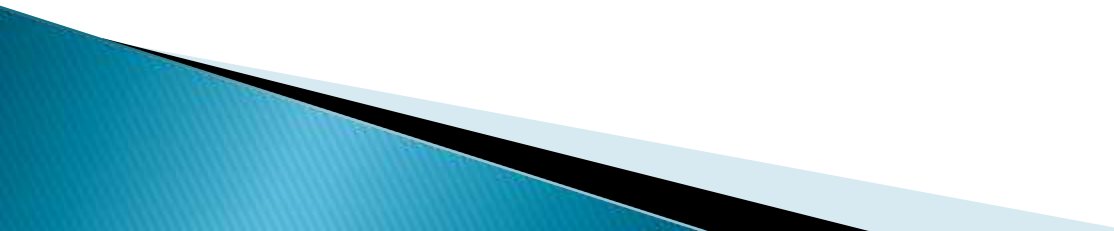
The type 1 collagen of periodontal ligament - particularly sensitive.

▶ **Counteracting free radical damage:**

- 1.) **Enzymes** like glutathione peroxidase, superoxide dismutases & catalase.
- 2.) **Antioxidants** –stable molecule to donate electron like glutathione, ubiquinol & uric acid.  
Vit A ,vit C, vit E.

# ANTIOXIDANTS

## **Ideal antioxidants:**

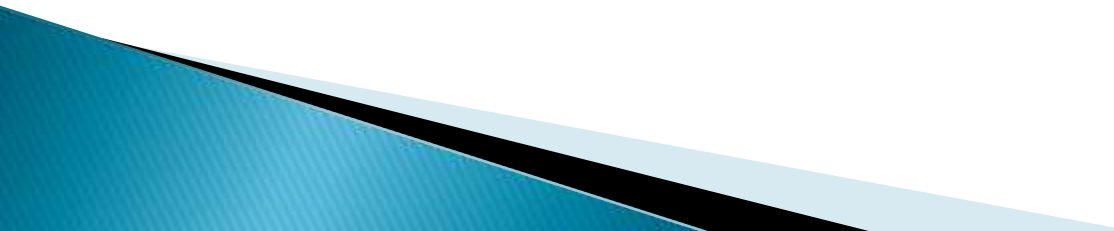
- ▶ No harmful effects
  - ▶ Effective in low concentration
  - ▶ Fat soluble
  - ▶ Readily available
  - ▶ Not contribute to objectionable flavor ,odor or colour to the fat.
- 



▶ Different kinds of antioxidants:

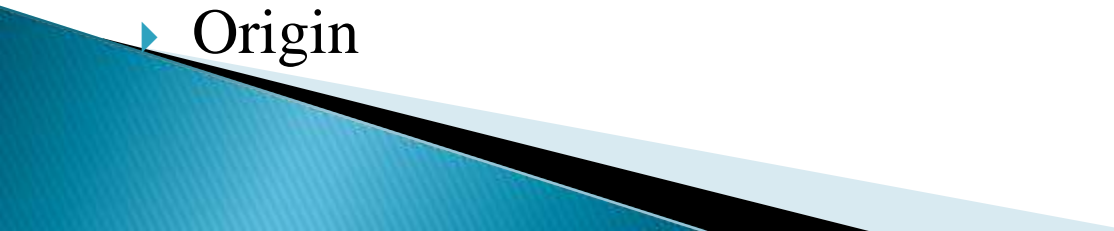
Natural	Synthetics
Tocopherols (delta > gamma > beta > alpha)	Butylated hydroxy anisole (BHA)
Nordihydroguaretic acid (NDGA)	Butylated hydroxy toluene (BHT)
Sesamol	Propyl gallate (PG)
Gossypol	Tertiary butyl hydroquinone (TBHQ)

## **Mechanisms by which antioxidants may offer protection**

- ▶ prevention of formation of free radicals
  - ▶ interception of free radicals
  - ▶ facilitating the repair
  - ▶ providing a favourable environment
- 

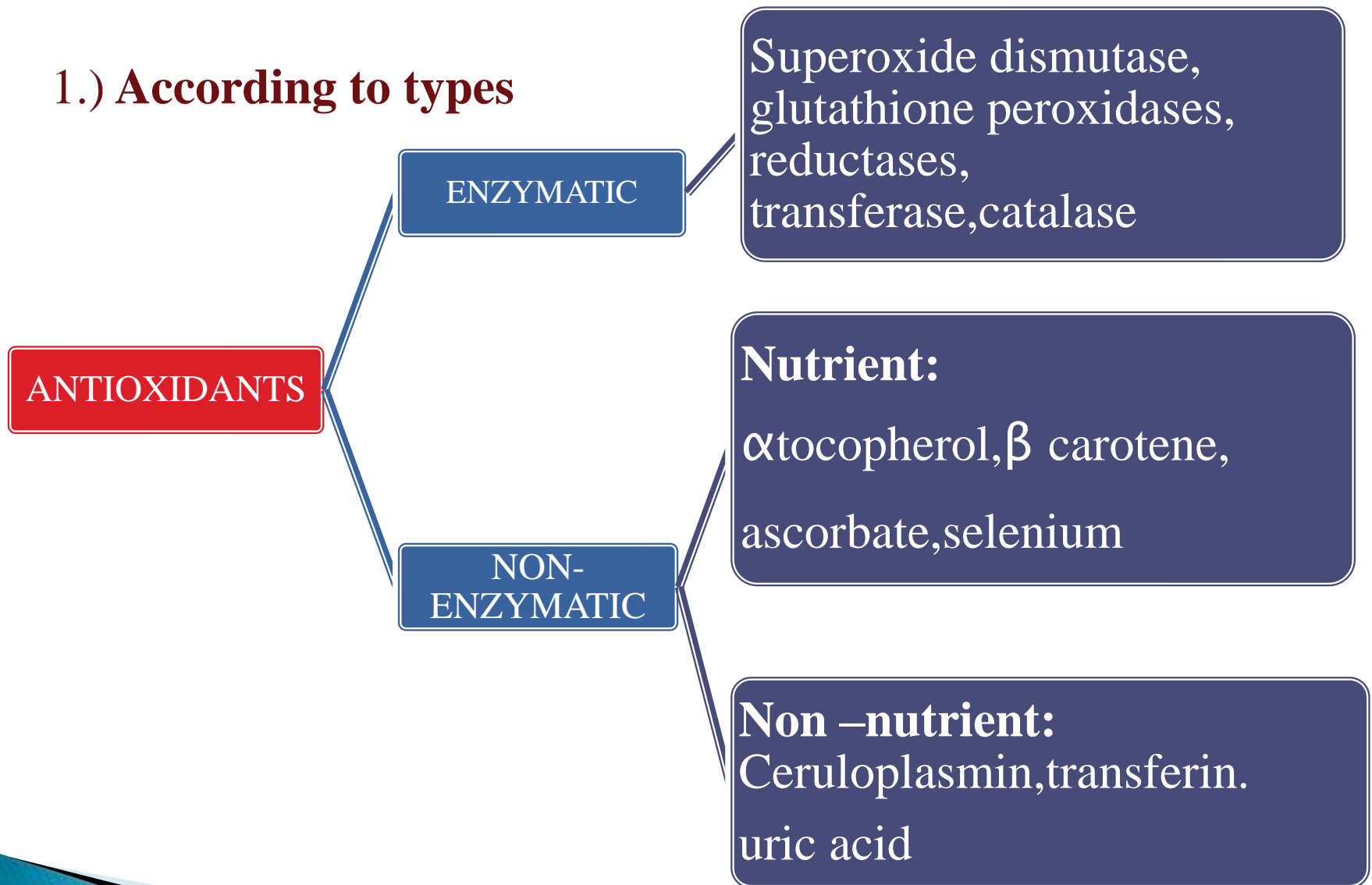
# CLASSIFICATION

**Antioxidants can be categorized by several methods:**

- ▶ Types
    - Mode of action
    - Location
    - Solubility
  - ▶ Structural dependents
  - ▶ Origin
- 

# Classification.....

## 1.) According to types



## 2.) Mode of Action

### PREVENTIVE

- Enzymes: superoxide dismutase , catalase, glutathione
- Metal ion sequestrators: carotenoids, superoxide dismutase, catalase, glutathione, uric acid, flavenoids

### SCAVENGING

- Ascorbate, carotenoids, uric acid,  $\alpha$ -tocopherol. flavenoids, ubiquinone, thiols

### 3.) Location

#### INTRACELLULAR

- SOD 1 and 2, catalase, glutathione peroxidase, DNA repair enzymes

#### EXTRACELLULAR

- SOD 3, reduced glutathione, ascorbate, carotenoids, uric acid

#### MEMBRANE ASSOCIATED

- $\alpha$ -Tocopherol

## 4.) Solubility

### WATER SOLUBLE

- Ascorbate, Uric acid, Flavenoids, thiols, Cysteine, Transferins

### LIPID SOLUBLE

- $\alpha$ -Tocopherol, Carotenoids, bilirubin

## 5.) Structures they Protect

### DNA

- SOD1 and 2, glutathione peroxidase, DNA repair enzymes, reduced glutathione, cysteine

### PROTEIN

- Sequestration of transition metals by preventive antioxidants

### LIPID

- $\alpha$ -Tocopherol, ascorbate, carotenoids, glutathione



## 6.) Origin

### EXOGENOUS

- Carotenoids, ascorbic acid, tocopherols, polyphenols

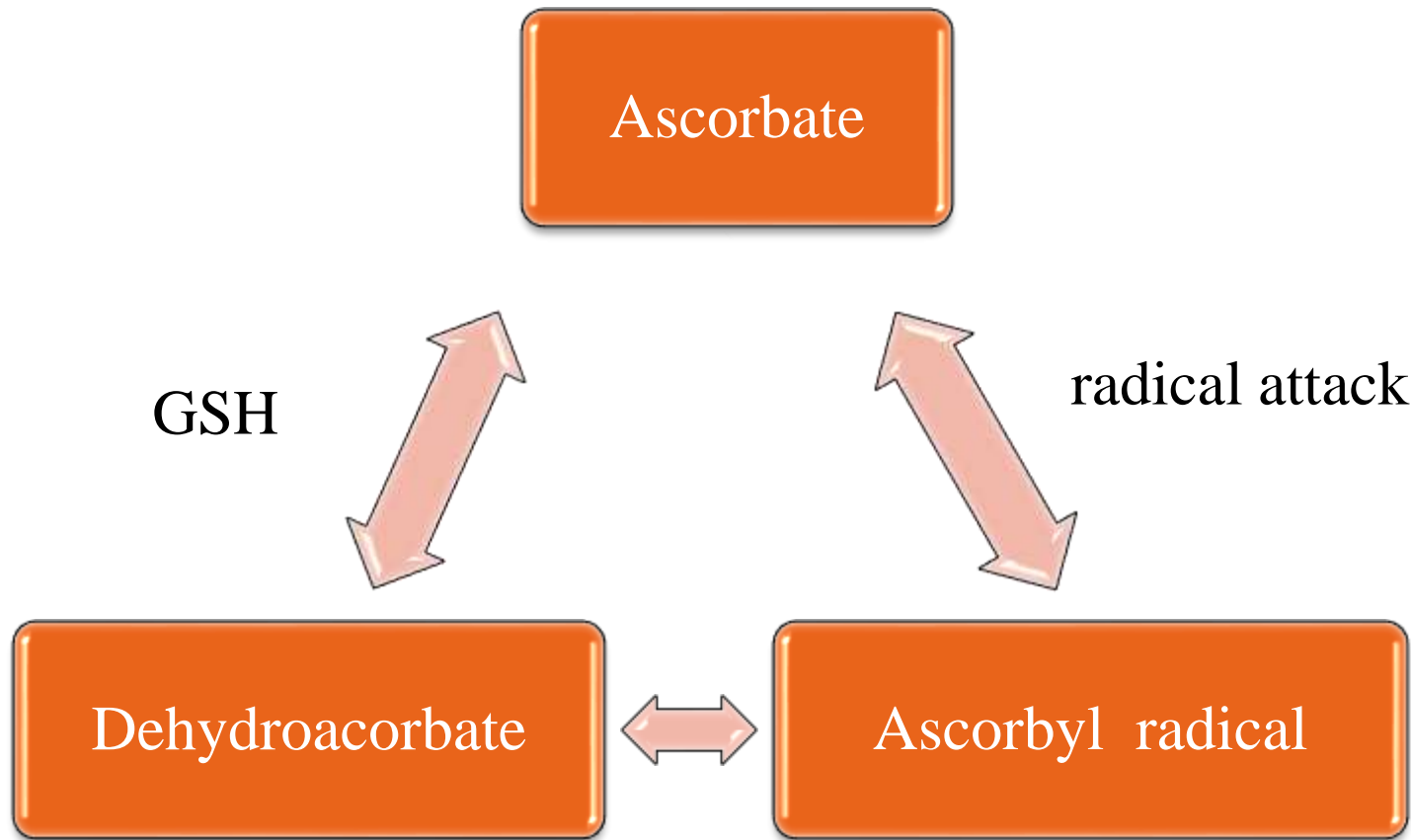
### ENDOGENOUS

- Catalase, superoxide dismutase, glutathione

# Ascorbic acid (Vitamin C)

**SOURCES : Citrus fruits, oranges, pineapple, grapes, green peppers, cabbage, watermelon, papaya, spinach, & strawberries**

- Scavenging water-soluble peroxy, perhydroxyl, superoxide, hypochlorous acid, & singlet oxygen
- Decreases heme breakdown by preventing fenton reactions
- Re-forms  $\alpha$ -tocopherol from its radical;
- Protects against ROS-release from cigarette smoke.



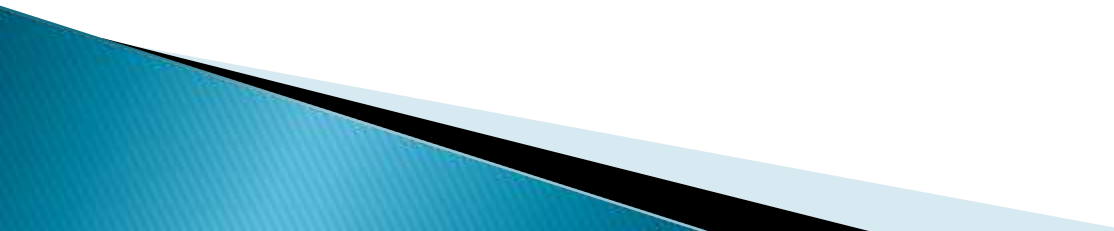
These systems are intracellular

# $\alpha$ -Tocopherol (vitamin E)

**SOURCES** : Unsaturated fats like sunflower, safflower, olive, and wheat germ oils, whole-wheat flour

- Most important and effective **lipid-soluble** antioxidant
- Vital to maintaining cell membrane integrity
- Requires other antioxidant species to be re-constituted  
(co-enzyme Q10 and ascorbic acid)
- Levels in plasma - significantly compromised in smokers.

## Vitamin E possesses anti-inflammatory & antioxidant properties

- Inhibition of protein kinase C and subsequent platelet aggregation
  - Inhibition of nitric oxide production by vascular endothelium
  - Inhibition of superoxide production by macrophages and neutrophils
- 

The limitations as an antioxidant are the result of :

- Its limited mobility within cell membranes
- Its lack of water solubility (many ROS are generated in the aqueous phase).

# Carotenoids

**SOURCES** : Deep orange, red, yellow fruits & vegetables like carrots, pumpkin, sweet potatoes, red grapes, watermelon, tomatoes

Carotenoids are tetraterpenes with over 600 variants.

- lycopene
- $\alpha$ -carotene
- $\beta$ -carotene
- cryptoxanthine
- retinol (vitamin A1)
- dehydroretinol (vitamin A2)

# Lycopene

**SOURCES:** Tomatoes, apricots, guava, watermelon, papaya

- 2 times as great as carotenes
- Cooked tomatoes are better than raw ones
- Protective against cancer of lung, stomach, & prostate


Vitamin A is controversial as an antioxidant

Behavior depends upon the oxygen tension of the immediate environment.



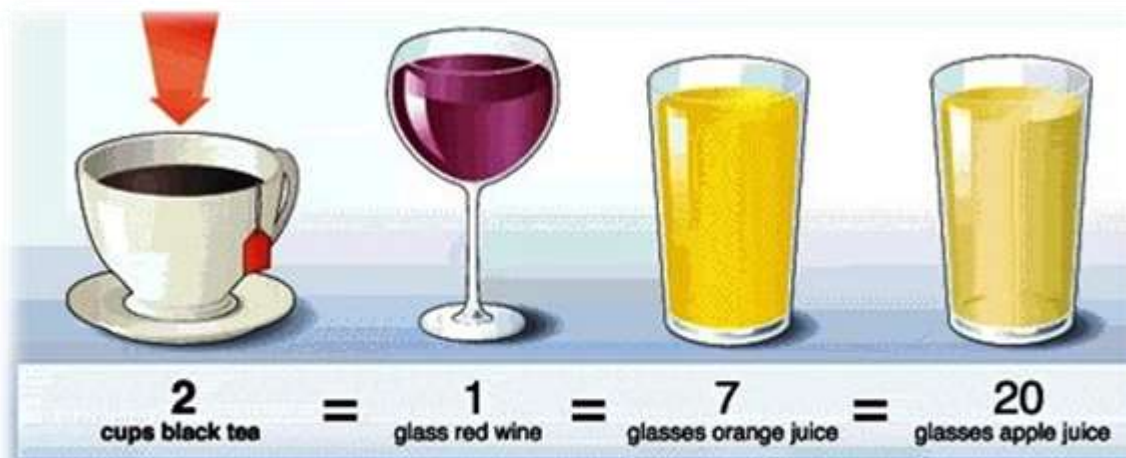
- At low partial oxygen pressures found in most tissues
  - as an **antioxidant**
- At higher oxygen tensions
  - **Pro oxidant behaviour**

# Polyphenols (Flavenoids)

- Absorbed following dietary intake of vegetables, red wine, tea.
  - There are over 4,000 known flavenoids
  - Most researched - catechin, epigallocatechin gallate, polyphenol,
  - radical scavenging
  - terminating lipid peroxidation
  - iron chelation
  - vitamin E & vitamin C
- 

## Other sources of Antioxidants are:


Honey	Green tea
Caffeic acid phenethyl ester(CAPE)	Epigallocatechin 3-gallate
Anti cancer	Effective in oral leukoplakia



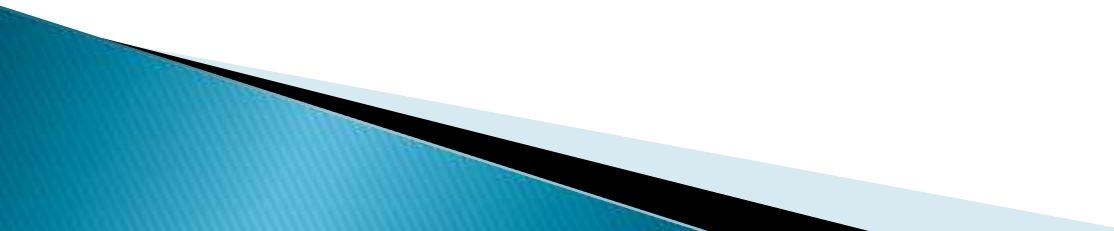
Source: The Polyphenolic Content of Fruit and Vegetables and their Antioxidant Activities: What Does a Serving Constitute?, Paganga et al., Free Radical Research, Volume 30, February 1999

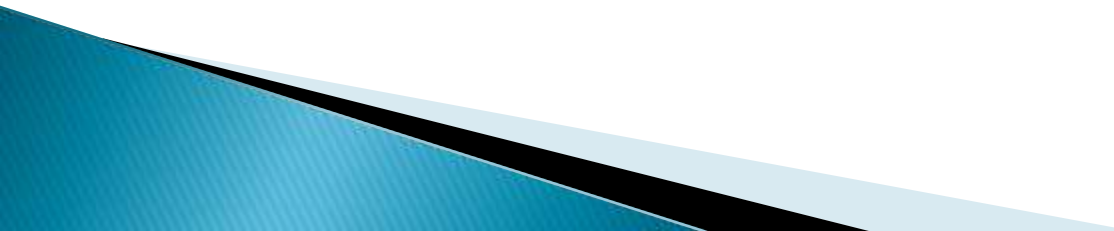
# Uric acid

Major radical scavengers within plasma, urine, and saliva.

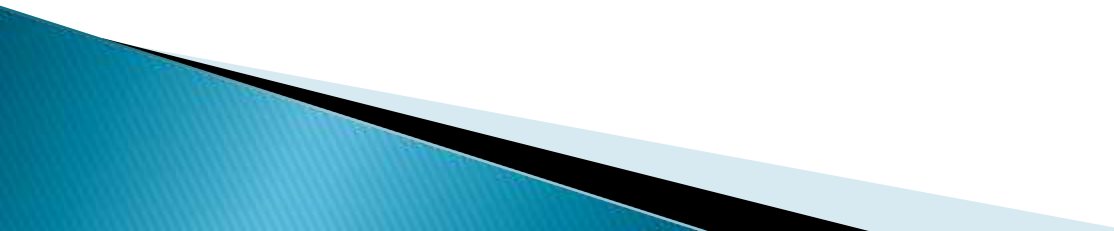
- Scavenger of singlet oxygen
  - Scavenger of hydroxyl radicals
  - Scavenger of hypochlorous acid
  - Protection of  $\alpha$ 1-antitrypsin
  - Preventing fenton chemistry by binding of divalent metal ions .
- 

# Reduced Glutathione

- A non essential tripeptide
  - Reduced form (GSH)- an antioxidant (radical scavenger).
  - Regulation of IL-2 dependent T-lymphocyte proliferation.
  - Maintains intracellular redox balance - signaling pathways
  - A neurotransmitter
  - Preservation and restoration of other antioxidant species
- 

- Innate and fundamental defense strategy at exposed epithelial surfaces.
  - Smoking of a single cigarette – significantly reduces salivary & plasma glutathione
  - Protects against the cytotoxic actions of nicotine on fibroblasts.
  - Some periodontal pathogens convert it to the cytotoxic  $H_2S$
- 

# Superoxide Dismutase


- localized within human PDL & important defense mechanism within gingival fibroblasts
  - SD 1 – a  $\text{Cu}^{2+}/\text{Zn}^{2+}$ -dependent enzyme found within the cytosol
  - SD 2 – the  $\text{Mn}^{2+}$ -dependent enzyme located within the mitochondria;
  - SD 3 – at low levels extracellularly
- 

# **MECHANISMS OF TISSUE DAMAGE**

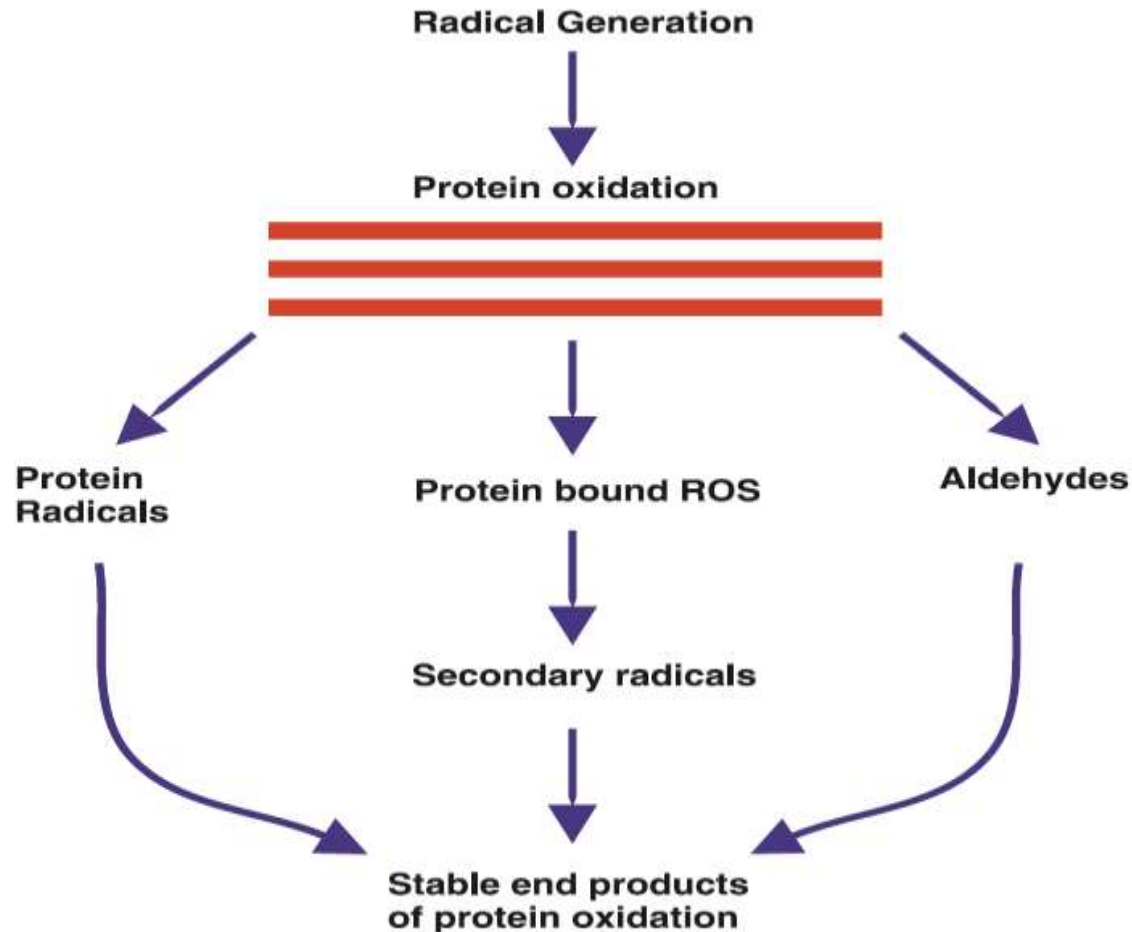




## **Protein damage:**

- protein folding or unfolding
  - protein fragmentation and polymerization reactions
  - protease degradation of the modified protein
  - formation of protein radicals
  - formation of protein-bound ROS
  - formation of stable end products e.g. carbonyl compounds
- 

# A schematic view of the effects of ROS on proteins and amino acids (Dean et al.)



## **Lipid peroxidation.**

- Most common
- Most effective - hydroxyl radical, peroxynitrite anion
- Halliwell describes the reaction in three major stages:

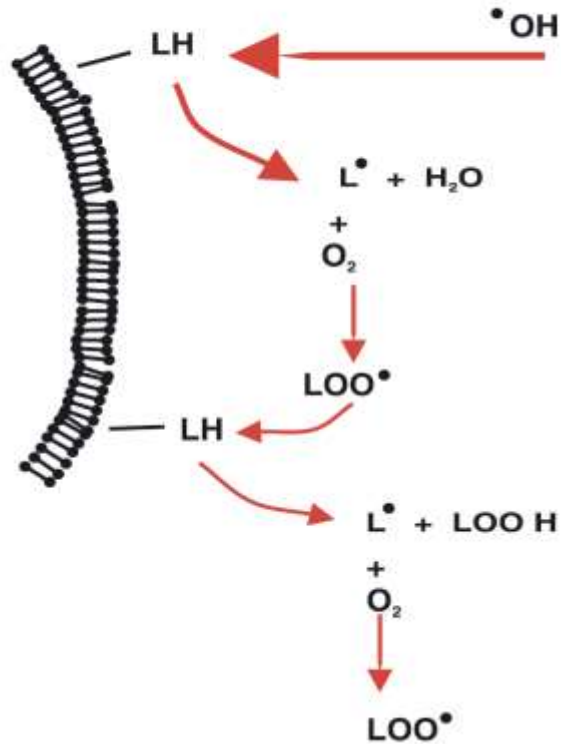
initiation;

propagation;

termination.

# The lipid peroxidation chain reaction initiated by hydroxyl radicals

PUFAs  
e.g. arachadonic acid



$\text{L}\cdot$  = carbon - centred radical

$\text{LOO}\cdot$  = lipid peroxy radical

$\text{LOOH}$  = lipid hydroperoxide

## **DNA damage**

### Mechanisms of DNA damage :

- strand breaks
- base pair mutations (purine and pyrimidine bases)
- deletions;
- insertions;
- nicking;
- sequence amplification.

**Hydroxyl radicals -cause damage to all four bases**



# ROLE OF ANTIOXIDANTS IN PERIODONTICS

- ROS in pathogenesis of periodontal disease

*Halliwell, 2000*

- PMNs (respiratory burst) are primary source of ROS
- Hydroxyl radical production by PMN

*Tauber et al, 1997*

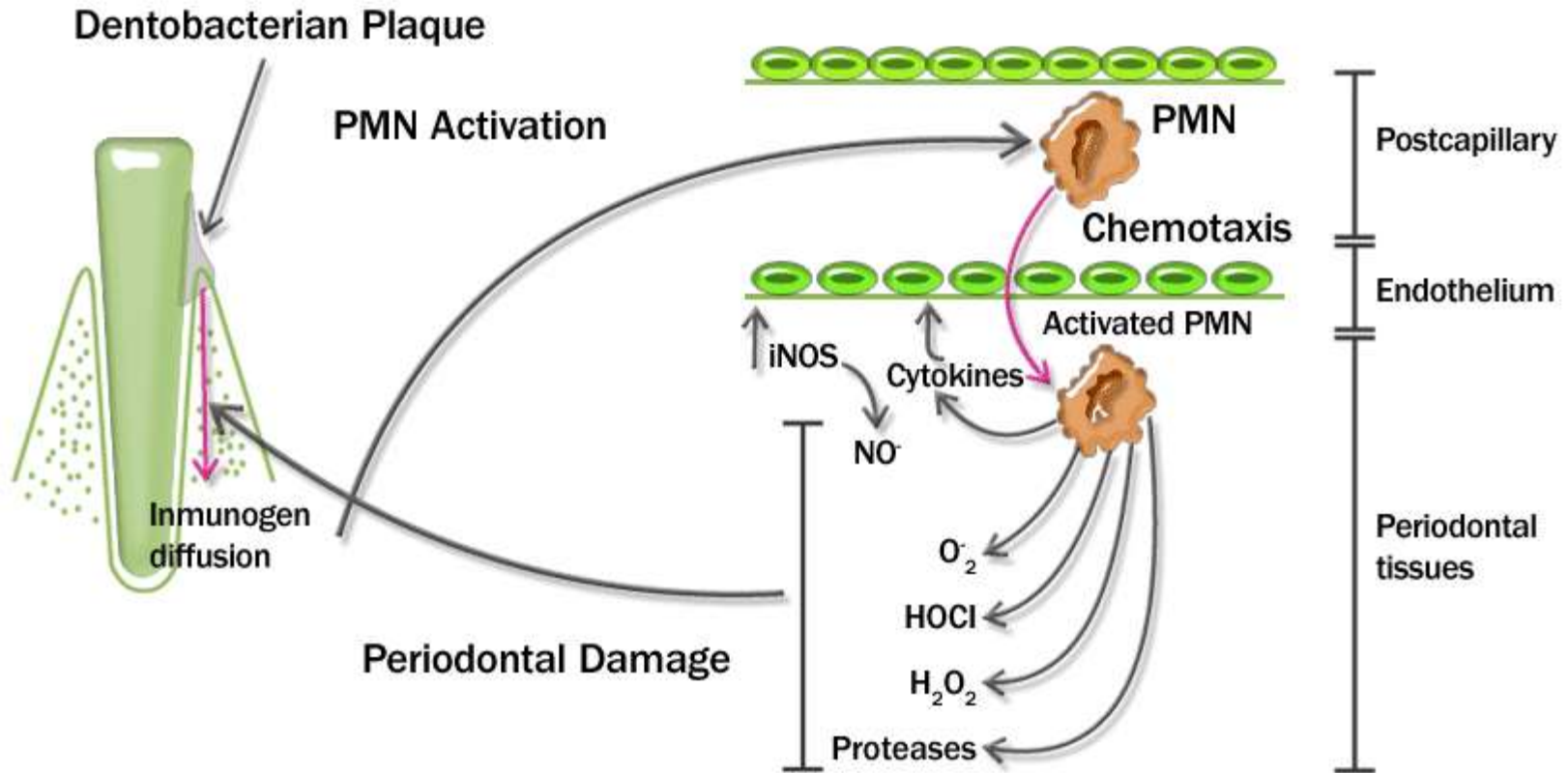
- Free radical in collagen destruction

*Asman B et al, 1994*

- Decrease in total antioxidant activity of saliva & serum in periodontitis

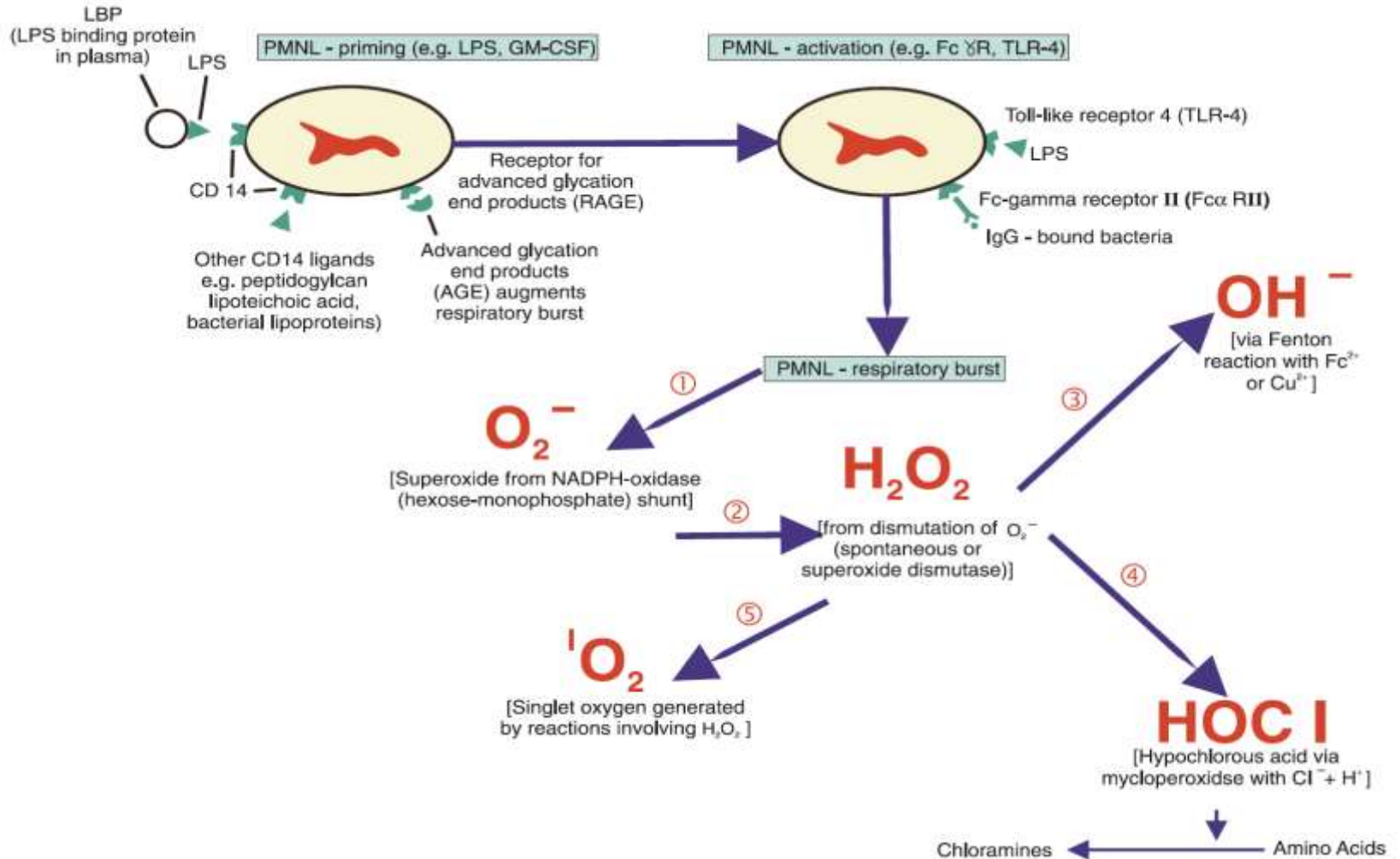
*Diab LR et al 2003, Pendyala G, 2009*

## ROLE OF ANTIOXIDANTS.....



The protective or destructive effect of PMN could be associated to the antioxidant capacity of tissues in an oxidative stress condition.

# ROLE OF ANTIOXIDANTS.....





## ROLE OF ANTIOXIDANTS.....

- ▶ Within the pocket a low redox potential - essential for growth of subgingival anaerobes ,also protective against oxidative stress.
- ▶ ROS production : mothwashes(listerine)  
toothpastes( Na ascorbyl PO<sub>4</sub> )

*Battino et al ,2002 & 2005*

- Poor periodontal health is associated with increased carbonyls in saliva

*DV Scully,2003*

## ROLE OF ANTIOXIDANTS.....

- ROS generation in periodontal disease causes bone resorption, increases matrix metallo proteinases activity

*Chapple IL,2006*

- Progressive reduction in SOD levels from healthy non-smokers to light smokers to heavy smokers

*Agnihotri R,2010*

- Antioxidant supplementation can reduce the incidence & recurrence of periodontal disease

# Antioxidants for oral lesions

- ▶ Lycopene protect against cell damage & progression of dysplasia.

Mohitpal etal ,2004

- ▶ Superoxide dismutase, vit E &  $\beta$ -carotene in OSMF

Kumar A etal ,2006

- ▶ Tr tretinoin topically for OLP

Sloberg etal,1979

- ▶ Tazarotene for OLP

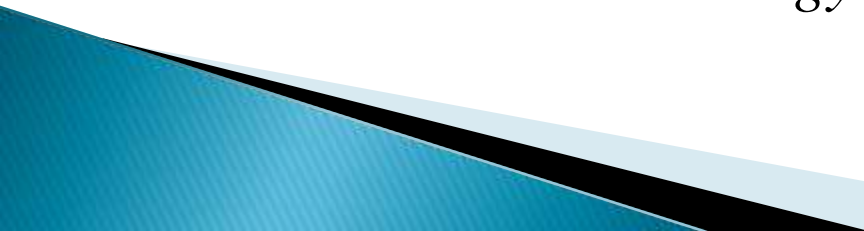
Petruzzi etal ,2002

**Noni** (anti oxidant vitamins)for reducing severity of viral infections like **herpes**

# Conclusion

# REFERENCES

1. **Haffajee AD, Socransky SS.** Microbial etiological agents of destructive periodontal diseases. *Periodontology* 2000. 1994;5:78–111.
2. **Jain I. C. Chapple & John B. Matthews** The role of reactive oxygen and antioxidant species in periodontal tissue destruction *Perio 2000*, Vol. 43, 2007, 160–232
3. **Liwell B.** Oral inflammation and reactive species: a missed opportunity? *Oral Dis* 2000: 6: 136–137.
- 4 **Battino M, Ferreiro MS, Armeni T, Politi A, Bompadre S, Massoli A, Bullon P.** In-vitro antioxidant activities of antioxidant-enriched toothpastes. *Free Radic Res* 2005: 39:343–350.

- ▶ **5. Royack GA, Nguyen MP, Tong DC, Poot M, Oda D.** Response of human oral epithelial cells to oxidative damage and the effect of vitamin *E*. *Oral Oncol* 2000; 36: 37–41.
  
  - ▶ **6. Battino M, Ferreiro MS, Galalrdo I, Newman HN, Bullon P.** The antioxidant capacity of saliva. *J Clin Periodontol* 2002;29: 189–94.
  
  - ▶ **7. Chapple IL, Matthews JB.** The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontology* 2000. 2007;43:160–232.
- 

# Thank you



Anti-oxidants kicks out the free radicals