NOTESKARTS | PREMIUM STUDY NOTES

BP812ET — DIETARY SUPPLEMENTS & NUTRACEUTICALS

UNIT — IV | Free Radicals in Disease, Antioxidants & Functional Foods

B.Pharm 8th Semester · PCI / AKTU Syllabus · Premium Notes ·

UNIT-IV | LEARNING OBJECTIVES

After completing this unit, the student will be able to:

- LO-1: Explain the role of free radicals and oxidative stress in the pathogenesis of major diseases — Diabetes mellitus, Inflammation, Ischaemia-Reperfusion Injury, Cancer, Atherosclerosis.
- LO-2: Describe oxidative stress mechanisms in brain pathology (Alzheimer's, Parkinson's), kidney damage, and muscle damage.
- LO-3: State the Free Radical Theory of Ageing (Harman, 1956) and the Mitochondrial Free Radical Theory of Ageing — evidence and implications.
- LO-4: Classify antioxidants; describe enzymatic endogenous antioxidants — Superoxide Dismutase (SOD), Catalase, and Glutathione Peroxidase — with reactions, types, and clinical significance.
- LO-5: Explain non-enzymatic endogenous antioxidants — Glutathione (GSH), Vitamin C, Vitamin E, α-Lipoic Acid, and Melatonin — with chemical nature, mechanism, and therapeutic applications.
- LO-6: Describe synthetic antioxidants BHA and BHT — structure, mechanism, uses in food, and safety concerns. Explain functional foods for chronic disease prevention.

FREE RADICALS IN DISEASE —

Oxidative stress is the **common pathological thread** linking most major chronic diseases. When **ROS production exceeds antioxidant defence**, the resulting oxidative damage to DNA, proteins, lipids, and carbohydrates initiates and perpetuates disease. Understanding disease-specific ROS pathways is essential for designing targeted antioxidant therapies.

Disease	Primary ROS Source	Molecular Target	Primary Oxidative Damage
Diabetes Mellitus	Glucose autooxidation, NADPH oxidase, AGE-RAGE, mitochondria	Pancreatic β-cells, endothelium, nerves	AGEs, lipid peroxidation, DNA damage, protein carbonylation

Disease	Primary ROS Source	Molecular Target	Primary Oxidative Damage
Inflammation	NADPH oxidase (neutrophils/macrophages), iNOS	Lipids (membranes), arachidonic acid pathway	LPO, NF-κB activation, cytokine amplification
Ischaemia-Reperfusion	Xanthine oxidase (on reperfusion), mitochondria, NADPH oxidase	Cardiomyocytes, endothelium, hepatocytes	Membrane LPO, protein denaturation, DNA strand breaks
Cancer	Mitochondria, NOX, inflammation-associated ROS	DNA (8-OHdG), tumour suppressor genes	Mutagenesis, genomic instability, angiogenesis
Atherosclerosis	LDL oxidation, endothelial NOX, macrophage MPO	LDL, endothelial NO, vascular smooth muscle	OxLDL formation, foam cells, plaque progression
Brain (AD, PD)	Mitochondria, MAO, iron accumulation	Neurons (high PUFA content), mitochondrial DNA	Amyloid aggregation, dopaminergic neuron death, neuroinflammation
Kidney	NOX4 (constitutive renal NOX), ischaemia, nephrotoxic drugs	Podocytes, tubular cells, glomerular membrane	Proteinuria, tubular necrosis, glomerulosclerosis
Muscle	Exercise-induced mitochondrial ROS, inflammation	Sarcolemma, myofilament proteins, dystrophin	Lipid peroxidation, protein carbonylation, impaired contraction

FREE RADICALS IN DIABETES MELLITUS

Diabetes Mellitus — Oxidative Stress Mechanisms	
ROS Mechanisms	(1) GLUCOSE AUTOOXIDATION: Glucose → enediol radicals + $O_2^{\bullet -}$ → H_2O_2; catalysed by trace metals (Fenton → •OH). (2) POLYOL PATHWAY ACTIVATION: Aldose reductase converts glucose → sorbitol; consumes NADPH → GSH ↓ → antioxidant defence depleted. (3) PROTEIN KINASE C (PKC) ACTIVATION: Diacylglycerol (DAG) ↑ in hyperglycaemia → PKC → NADPH oxidase (NOX1/4)

	activation → $O_2^{\bullet-}$ burst. (4) AGE-RAGE SIGNALLING: Glucose glycates proteins → AGEs → RAGE receptor → NF-κB → NOX activation → more ROS (vicious cycle). (5) MITOCHONDRIAL OVERLOAD: Excess glucose → ETC overload → hyperpolarisation → $O_2^{\bullet-}$ burst from Complex I/III.
Cellular Targets	Pancreatic β-cells (islet destruction via ROS — IDDM); Endothelial cells (eNOS uncoupling → NO ↓, $O_2^{\bullet-}$ ↑); Peripheral nerves (demyelination via LPO); Glomerular basement membrane (AGE cross-linking → nephropathy); Retinal pericytes (AGE-VEGF → retinopathy); Lens crystallins (glycation → cataracts).
Oxidative Stress Biomarkers	HbA1c (glycation marker); urinary 8-OHdG (DNA oxidative damage); Isoprostanes (LPO); Protein carbonyls; Nitrotyrosine (peroxynitrite); GSH/GSSG ratio ↓ (antioxidant depletion).
Clinical / Disease Link	Type 2 DM: Insulin resistance perpetuated by ROS (IRS-1 oxidation → impaired insulin signalling); Diabetic triopathy (nephropathy, retinopathy, neuropathy) — ALL caused by AGE-RAGE-ROS axis; β-cell apoptosis in T1DM — excess NO (iNOS) + $O_2^{\bullet-}$ → peroxynitrite → β-cell DNA damage → apoptosis; Diabetic cardiomyopathy — mitochondrial ROS + LPO → contractile dysfunction.

🔬 UNIFYING MECHANISM OF DIABETIC COMPLICATIONS (Brownlee's Unifying Theory, 2001)	HYPERGLYCAEMIA → Mitochondrial ETC overproduction of $O_2^{\bullet-}$

⚠ Exam Trick	Brownlee's Unifying Theory (2001) is the most important concept in diabetic pathophysiology for this exam. ONE molecule — $O_2^{\bullet-}$ from mitochondria — activates FOUR damage pathways simultaneously. This explains why ALL complications of diabetes (nephropathy, retinopathy, neuropathy, CVD) share the same hyperglycaemia → ROS → NF-κB root cause. Always mention this when answering 10-mark questions on DM.

FREE RADICALS IN INFLAMMATION

🔥 Inflammation — Oxidative Stress Mechanisms	
ROS Mechanisms	(1) RESPIRATORY BURST (ACUTE): Neutrophils and macrophages activated → NADPH oxidase (NOX2) → $O_2^{\bullet -}$ → H_2O_2 → HOCl (myeloperoxidase); Deliberate ROS for pathogen killing but also damages surrounding host tissue. (2) iNOS INDUCTION: LPS/cytokines → iNOS expression → large amounts of NO• → NO• + $O_2^{\bullet -}$ → peroxynitrite ($ONOO^-$) → nitrative stress. (3) ARACHIDONIC ACID CASCADE: ROS activates phospholipase A2 → AA release → COX/LOX → prostaglandins/leukotrienes + lipid radicals (LOO•) → more ROS + inflammation. (4) NF-κB ACTIVATION BY ROS: H_2O_2 → IκB kinase activation → IκB degradation → NF-κB nuclear translocation → TNF-α, IL-1β, IL-6, iNOS, COX-2 expression → MORE ROS → vicious inflammatory cycle.
Cellular Targets	Immune cells (neutrophils, macrophages — excessive activation → tissue destruction); Endothelium (HOCl + $ONOO^-$ → endothelial dysfunction, ICAM-1 ↑); Synovial tissue (RA — HOCl cleaves HA → joint viscosity ↓); Gastrointestinal epithelium (IBD); Lung epithelium (ARDS).
Oxidative Stress Biomarkers	C-Reactive Protein (CRP — acute phase); Myeloperoxidase (MPO) activity; HOCl-modified LDL; 3-Nitrotyrosine (peroxynitrite marker); Urinary isoprostanes; 8-OHdG; Nitrite/nitrate (iNOS activity).
Clinical / Disease Link	Rheumatoid Arthritis: Synovial fluid neutrophils → $O_2^{\bullet -}$ + HOCl → HA depolymerisation → cartilage destruction; ARDS (Acute Respiratory Distress Syndrome): Neutrophil-mediated oxidative damage to alveolar epithelium; IBD: Colonic epithelium oxidative damage by activated macrophages; Sepsis: Massive peroxynitrite from iNOS → multiorgan failure; Chronic Inflammation → Cancer (colon cancer from IBD, HCC from hepatitis).

★ Point	ROS and Inflammation form a BIDIRECTIONAL AMPLIFICATION LOOP: Inflammation produces ROS (NADPH oxidase, iNOS, MPO) AND ROS activates inflammation (NF-κB pathway). This 'ROS-NF-κB-ROS' cycle explains why chronic inflammatory diseases (IBD, RA, atherosclerosis) are so difficult to control and why antioxidant therapy can break the cycle.

ISCHAEMIA-REPERFUSION INJURY (IRI)

Ischaemia-Reperfusion Injury (IRI)	The paradoxical phenomenon where restoration of blood flow (reperfusion) to an ischaemic tissue causes ADDITIONAL tissue damage beyond that caused by ischaemia alone — primarily through a massive burst of ROS generated when oxygen is suddenly reintroduced.

🔬 IRI MECHANISM — PHASE BY PHASE	————————————————————

Organ	Clinical Setting	ROS Mechanism	Protective Strategy
Heart	Myocardial infarction (MI) — thrombolysis or PTCA restores flow	XO burst on reperfusion; MPTP opening → cardiomyocyte apoptosis; no-reflow phenomenon	NAC, Allopurinol (XO inhibitor), Cyclosporin A (MPTP blocker), remote ischaemic preconditioning
Brain	Stroke — thrombolysis with tPA restores flow to ischaemic penumbra	•OH from Fenton; iron released from haemoglobin (haem + •OH); excitotoxicity (glutamate → NMDA → Ca^{2+} → NO + $O_2^{\bullet-}$ → $ONOO^-$)	Edaravone (•OH scavenger — approved in Japan/India for stroke); NXY-059 (failed trials)
Kidney	Transplantation; acute tubular necrosis	XO in tubular cells; NADPH oxidase; mitochondrial ROS in high-energy-demanding tubular epithelium	Allopurinol pre-treatment; NAC in contrast nephropathy prevention

Organ	Clinical Setting	ROS Mechanism	Protective Strategy
Liver	Transplantation; shock/resuscitation; hepatic surgery with Pringle manoeuvre	Kupffer cell (resident macrophage) NADPH oxidase burst; XO in hepatocytes	N-Acetylcysteine (GSH precursor); Vitamin E, Allopurinol
Intestine	Mesenteric ischaemia; neonatal NEC; bowel resection	XO most abundant in intestinal epithelium; mucosal barrier breakdown → bacterial translocation → systemic inflammation	Allopurinol; probiotics (gut barrier protection)

⚠ Exam Trick	IRI is the #1 exam scenario for Xanthine Oxidase. Remember: During ISCHAEMIA, hypoxanthine accumulates AND XDH is converted to XO. During REPERFUSION, O_2 arrives → XO uses O_2 as electron acceptor → MASSIVE $O_2^{\bullet-}$ burst (instead of NAD^+ as in normal XDH). Allopurinol (XO inhibitor, used for gout) is a experimental IRI protectant. The 'reperfusion paradox' — restoring blood CAUSES MORE DAMAGE — is frequently tested.

FREE RADICALS IN CANCER

🎗 Cancer — Free Radical Mechanisms	
ROS Mechanisms	(1) INITIATION: •OH + DNA → 8-OHdG → G→T transversion → PROTO-ONCOGENE ACTIVATION (K-RAS, BRAF) or TUMOUR SUPPRESSOR INACTIVATION (p53 — 'guardian of the genome'; BRCA1/2). (2) PROMOTION: ROS → NF-κB → growth factors (EGF, IGF-1) → cell proliferation; COX-2 ↑ → PGE2 → tumour cell immune evasion; AP-1 activation → c-fos, c-jun oncogenes. (3) PROGRESSION: ROS → HIF-1α stabilisation (mimic hypoxia) → VEGF → ANGIOGENESIS → tumour vascularisation; ROS → MMP activation → basement membrane degradation → INVASION + METASTASIS. (4) TUMOUR

	SURVIVAL: Cancer cells produce HIGH ROS (Warburg effect mitochondria) but also upregulate antioxidants (Nrf2 pathway) — ROS paradox: mild ROS = proliferation signal; extreme ROS = cancer cell death (therapeutic opportunity).
Cellular Targets	Nuclear DNA (8-OHdG, strand breaks, crosslinks → mutations); Mitochondrial DNA (10× more sensitive → energy metabolism impairment); Tumour suppressor proteins (p53 carbonylation → inactivation → uncontrolled proliferation); Telomeres (G-rich sequences → ROS → accelerated shortening → genomic instability); Cell membranes (LPO → altered signalling lipid rafts).
Oxidative Stress Biomarkers	Urinary 8-OHdG (↑ in cancer patients); Isoprostanes; Protein carbonyls; OxLDL; Serum Vitamin C/E ↓; Superoxide dismutase activity ↓; Total Antioxidant Capacity (TAC) ↓; Tumour-specific: RAS, p53 mutations in tumour biopsy.
Clinical / Disease Link	SPECIFIC CANCERS — ROS LINK: Lung (cigarette smoke → PAH radicals → p53, K-RAS mutations); Colorectal (IBD → macrophage ROS → mucosal DNA damage → Lynch/sporadic CRC); Prostate (chronic inflammation → NF-κB → androgen-independent growth); Liver (HBV/HCV + iron → Fenton → HCC); Breast (ER + ROS → oestrogen quinone radicals → DNA adducts). ROS PARADOX IN THERAPY: Pro-oxidant cancer therapy (chemo/radiation → generates ROS → kills cancer cells) but cancer cells with high Nrf2 → antioxidant resistance → chemotherapy resistance.

Clinical	THE ROS PARADOX IN CANCER: Low/moderate ROS → tumour promotion (proliferation signal via MAPK, NF-κB). HIGH ROS → tumour cell death (used therapeutically — chemotherapy and radiotherapy work by GENERATING ROS in cancer cells). This means: (1) ANTIOXIDANT SUPPLEMENTS may INTERFERE with chemo/radiotherapy — contraindicated during cancer treatment! (2) Pro-oxidant nutraceuticals (Vitamin C in PHARMACOLOGICAL doses IV > 10g) → H_2O_2 production → cancer cell death. This dual role is the most sophisticated concept in nutraceutical oncology.

FREE RADICALS IN ATHEROSCLEROSIS

🔬 ATHEROSCLEROSIS — STEP-BY-STEP OXIDATIVE MECHANISM	STEP 1 — ENDOTHELIAL DYSFUNCTION (initiated by ROS):

⚠ Exam Trick	OxLDL is taken up by SCAVENGER RECEPTORS (SR-A, CD36) on macrophages — NOT the normal LDL receptor. This is critical because scavenger receptors are NOT downregulated by intracellular cholesterol accumulation — so macrophages keep taking up OxLDL until they become foam cells and die. This lack of feedback inhibition is the reason why OxLDL is so atherogenic. Always mention this in atherosclerosis questions!

Stage	Lesion Type	ROS Involvement	Event
Earliest	Endothelial dysfunction	$O_2^{\bullet-}$ + NO• → $ONOO^-$ → NO ↓; NF-κB → VCAM-1 ↑	Monocyte rolling and adhesion — VCAM-1, ICAM-1 expression
Early	Fatty streak (reversible)	OxLDL formation; foam cell ROS production	Macrophage foam cell formation; T-lymphocyte infiltration
Intermediate	Fibro-fatty plaque	Smooth muscle proliferation (ROS→PDGF); ongoing oxidative damage	Necrotic core forms; fibrous cap develops
Advanced	Complex plaque	MMP activation (ROS→MMP) → fibrous cap erosion	Plaque vulnerability increases; micro-thrombi form
Terminal	Plaque rupture → MI/Stroke	Extreme oxidative/inflammatory stress → cap rupture	Platelet aggregation → occlusive thrombus → MI/Stroke

FREE RADICALS IN BRAIN METABOLISM AND PATHOLOGY

Why is the Brain Uniquely Vulnerable to Oxidative Stress?

Factor	Detail	Consequence
High oxygen consumption	Brain = 2% body weight but uses 20% of O_2	High O_2 → high ROS generation from ETC
High PUFA content	60% of brain dry weight is lipid; DHA (22:6) is dominant	PUFAs = prime LPO targets → neuronal membrane damage
Relatively low antioxidant enzymes	Neurons have LOW catalase, LOW GPx compared to liver	Less ROS detoxification capacity
High iron content (basal ganglia, hippocampus)	Iron catalyses Fenton reaction	Localised •OH production in dopaminergic-rich areas
High dopamine catabolism	MAO → dopamine → H_2O_2 → •OH via Fenton	Ongoing ROS generation in dopaminergic neurons
Post-mitotic neurons	Cannot be replaced; damage accumulates over lifetime	Progressive neurodegeneration with age
Blood-Brain Barrier (BBB)	Limits entry of dietary antioxidants (Vitamin C, E have limited CNS penetration)	Less exogenous antioxidant support

Alzheimer's Disease (AD) — Oxidative Stress Mechanisms

- Amyloid-β (Aβ) aggregation: $A\beta_{1-42}$ → Cu^{2+}/Fe^{2+} complexes → Fenton reaction → H_2O_2 + •OH → neuronal damage; Aβ also inserts into mitochondrial membrane → ETC dysfunction → more ROS (amplification loop).
- Tau hyperphosphorylation: Glycogen Synthase Kinase-3β (GSK-3β) activated by ROS → tau phosphorylation → neurofibrillary tangles (NFTs) → axonal transport blockade → neuronal death.
- HNE modification of proteins: 4-HNE (LPO product) reacts with ERAB (Endoplasmic Reticulum-Associated Amyloid-β Binding Protein), APP processing proteins → increased Aβ production → positive feedback.
- Elevated 8-OHdG, protein carbonyls, isoprostanes found in AD brain tissue — especially in hippocampus and temporal lobe (areas most affected).
- Mitochondrial dysfunction: Complex IV (cytochrome c oxidase) activity ↓↓ in AD brain — 50% reduction in severely affected areas.

Parkinson's Disease (PD) — Oxidative Stress Mechanisms

- Substantia nigra specificity: Rich in dopamine → MAO-B metabolism → H_2O_2; high iron (neuromelanin binds Fe^{2+}) → Fenton reaction → •OH → selective dopaminergic neuron death.
- α-Synuclein aggregation (Lewy bodies): ROS → α-synuclein oxidation → conformational change → oligomers → Lewy body inclusions → mitochondrial dysfunction → more ROS.
- Complex I deficiency: Specifically reduced in substantia nigra of PD patients — most profound ETC defect; primary source of $O_2^{\bullet-}$ in dopaminergic neurons.
- MPTP model of PD: MPP^+ (from MPTP conversion by MAO-B) → Complex I inhibitor → $O_2^{\bullet-}$ burst → selective dopaminergic neuron death; confirms ROS as proximal cause.

- GSH depletion: Substantia nigra GSH levels ↓↓ in EARLIEST stages of PD — before neuronal loss visible; GSH restoration = potential neuroprotective strategy.

> **Clinical** Selegiline (Deprenyl) — a MAO-B inhibitor used in Parkinson's — works partly by REDUCING H_2O_2 production from dopamine metabolism (less MAO-B activity → less H_2O_2 → less Fenton → less •OH → neuroprotection). This is an antioxidant mechanism of a classical anti-PD drug — high-yield pharmacology-nutraceutical integration point!

Other Brain Conditions — Oxidative Involvement

Condition	ROS Mechanism	Oxidative Biomarker
Ischaemic Stroke	IRI on reperfusion; Fenton from haemoglobin degradation (haem iron); excitotoxicity (NMDA → Ca^{2+} → nNOS → NO + $O_2^{•-}$ → $ONOO^-$)	CSF 8-OHdG, isoprostanes; Serum NSE
Amyotrophic Lateral Sclerosis (ALS)	Mutant Cu/Zn-SOD1 (gain of toxic function) → peroxidase activity → •OH + protein damage; mitochondrial dysfunction in motor neurons	Protein carbonyls, 3-nitrotyrosine in spinal cord
Multiple Sclerosis (MS)	Myelin oxidative damage by NO• + $O_2^{•-}$ from activated microglia/macrophages; peroxynitrite → nitration of myelin basic protein	3-Nitrotyrosine in CSF; OxLDL in myelin debris
Depression / Anxiety	Chronic stress → cortisol → mitochondrial dysfunction → ROS; HPA axis → neuro-inflammation; Oxidative damage to serotonergic neurons	Isoprostanes in urine; 8-OHdG; CRP, IL-6 (inflammatory)
Brain Ageing	Accumulation of lipofuscin (oxidised protein-lipid aggregates); mtDNA mutations; telomere shortening → neuronal senescence	Lipofuscin accumulation; mtDNA mutations; 8-OHdG ↑ in aged brain

FREE RADICALS IN KIDNEY AND MUSCLE DAMAGE

Kidney Damage

The kidney is **highly susceptible to oxidative damage** due to its rich blood supply, high metabolic rate, and role in concentrating potentially nephrotoxic substances. NOX4 is the predominant NADPH oxidase isoform in the kidney — it is **constitutively active** (producing H_2O_2 continuously for signalling).

Renal Condition	ROS Mechanism	Structural Target	Clinical Feature
Diabetic Nephropathy	AGE-RAGE → NF-κB → TGF-β → NADPH oxidase; High glucose → NOX4 upregulation; Polyol pathway → NADPH depletion → GSH ↓	Glomerular basement membrane (thickening); Podocyte foot process effacement; Mesangial matrix expansion	Microalbuminuria → macroalbuminuria → proteinuria → GFR decline → ESRD
Acute Ischaemic AKI	IRI on restoration of blood flow; Mitochondrial ROS in tubular epithelium (high O_2 demand); XO in tubular cells	Proximal tubular S3 segment (most vulnerable — high mitochondrial density, low antioxidant)	Oliguria → renal failure; RIFLE/AKIN staging; ATN on biopsy
Contrast-Induced Nephropathy	Iodinated contrast → direct tubular toxicity; vasoconstriction → medullary ischaemia → ROS; contrast → mitochondrial ROS in tubular cells	Renal medullary tubular cells (hypoxic zone — outer medulla)	Creatinine rise 48h post-contrast; prevention: N-Acetylcysteine + IV saline
Cisplatin Nephrotoxicity	Cisplatin → mitochondrial dysfunction → $O_2^{\bullet-}$ + H_2O_2; Fenton from platinum-Fe interaction; TNF-α → NADPH oxidase	Proximal tubular S3 cells (accumulate cisplatin via organic cation transporters)	Dose-dependent nephrotoxicity; tubular necrosis; prevention: NAC, amifostine
Hypertensive Nephropathy	AngII → NOX1/2/4 → $O_2^{\bullet-}$; $O_2^{\bullet-}$ + $NO^\bullet$ → $ONOO^-$ → NO ↓ → afferent arteriole vasoconstriction → glomerular hypertension	Glomerular endothelium, mesangium, tubular epithelium	Glomerulosclerosis → CKD; hypertension both cause and consequence of oxidative renal damage

Muscle Damage

Skeletal muscle produces ROS both during **normal exercise** (physiological/beneficial at low levels) and in **pathological conditions** (rhabdomyolysis, muscular dystrophies, inflammatory myopathies — damaging).

Condition	ROS Source	Mechanism of Muscle Damage	Clinical Feature / Marker
Exercise-induced Muscle Damage (EIMD)	Mitochondrial ETC (during intense exercise); NOX2 (fast-twitch fibres); XO; Phospholipase A2 (PLA2) → LOX	LPO of sarcolemma; protein carbonylation of myofilaments (actin, myosin); Ca^{2+} influx through oxidised ryanodine receptor → uncontrolled contraction	DOMS (Delayed Onset Muscle Soreness); CK elevation; LDH release; MDA ↑; 8-OHdG ↑
Duchenne Muscular Dystrophy (DMD)	Absence of dystrophin → mechanical membrane instability → Ca^{2+} influx → mitochondrial Ca^{2+} overload → $O_2^{\bullet -}$; NOX2 upregulation in dystrophic muscle	Progressive LPO of sarcolemmal membrane; utrophin cannot compensate; necrosis → fibrosis; satellite cell exhaustion	CK markedly ↑ (10-50× normal); EMG myopathic; muscle biopsy: necrosis/regeneration; gene therapy target
Rhabdomyolysis	Crush injury → ischaemia-reperfusion of muscle; Myoglobin release → proximal tubule → Fenton reaction (iron from haem) → tubular ROS → AKI	Myoglobin-catalysed •OH in renal tubules; Direct muscle cell: Ca^{2+} → mitochondrial PTP → necrosis	Myoglobinuria (tea-coloured urine); CK > 5× ULN; AKI — creatinine ↑ rapidly; treatment: aggressive IV saline
Statin-induced Myopathy	Statins inhibit CoQ10 synthesis (same pathway as cholesterol) → mitochondrial dysfunction → $O_2^{\bullet -}$; Reduced CoQ10 → impaired ETC → increased mitochondrial ROS	Mitochondrial LPO; reduced ATP production in type I (oxidative) muscle fibres	Myalgia (5-10% patients); Myositis (0.1%); Rhabdomyolysis (0.01%); CoQ10 supplementation may prevent

Condition	ROS Source	Mechanism of Muscle Damage	Clinical Feature / Marker
Inflammatory Myopathy (Polymyositis)	Autoreactive T-cells and macrophages → respiratory burst at muscle → $O_2^{\bullet -}$ + HOCl; cytokines (TNF-α) → iNOS → $ONOO^-$	Muscle fibre necrosis by HOCl/$ONOO^-$; MHC-I upregulation on muscle → continued immune attack	Proximal muscle weakness; CK ↑; EMG myopathic; muscle biopsy: inflammatory infiltrate; antioxidant (NAC) as adjunct therapy

Remember

Myoglobin-induced AKI (in rhabdomyolysis): Myoglobin per se is not directly nephrotoxic. It is the IRON from haem (Fe^{2+}) within myoglobin that catalyses the Fenton reaction in acidic renal tubule lumen → •OH → tubular epithelial death. This is why ALKALINISATION of urine (IV bicarbonate) protects the kidney in rhabdomyolysis — alkaline urine prevents haem iron release from myoglobin.

FREE RADICALS IN OTHER DISORDERS & FREE RADICAL THEORY OF AGEING

Free Radicals in Other Disorders

Disorder	ROS Mechanism	Oxidative Target	Clinical Relevance
COPD/Emphysema	Cigarette smoke radicals (10^{15}/puff) + macrophage/neutrophil NOX; NF-κB → elastase ↑	Lung elastin (elastase oxidation → loss of elasticity); α_1-Antitrypsin (oxidative inactivation by cigarette oxidants → uninhibited elastase)	Emphysema via 'oxidative enzyme imbalance'; N-Acetylcysteine in COPD treatment
Cataracts	H_2O_2 generated by UV-B in lens; glucose glycation (AGEs) of lens crystallins; mitochondrial ROS in epithelial cells	Lens crystallin proteins (aggregation → clouding); GSH depletion in lens (high GSH normally maintains clarity)	Most common cause of blindness globally; Lutein/Zeaxanthin, Vitamin C, E — preventive; surgical correction

Disorder	ROS Mechanism	Oxidative Target	Clinical Relevance
Rheumatoid Arthritis	Synovial fluid neutrophils → $O_2^{\bullet -}$ + HOCl; ROS → HA depolymerisation; NF-κB → TNF-α → cartilage destruction	Hyaluronic acid (HOCl cleaves → ↓ viscosity); Collagen (cross-linking by MDA); Chondrocytes (apoptosis)	High ROS in synovial fluid; Vitamin E, Fish oil, Boswellia as antioxidant/anti-inflammatory nutraceuticals
Male Infertility	Sperm produce $O_2^{\bullet -}$ from mitochondria + NADPH oxidase; seminal plasma low antioxidant in smokers/varicocele	Sperm membrane PUFAs (high DHA → LPO → membrane fluidity ↓ → motility ↓); Sperm DNA (8-OHdG → DNA fragmentation index ↑)	30-40% of male infertility has oxidative component; Vitamin E, CoQ10, Lycopene, Zinc — antioxidant therapy
Psoriasis	Keratinocyte NADPH oxidase; ROS from mast cells; TNF-α → NF-κB cycle	Keratinocyte DNA (proliferation signal from ROS); Skin LPO (erythema, scaling)	Oxidative biomarkers ↑ in psoriatic skin; Curcumin, Fish oil as adjunct therapy
HIV/AIDS	Viral proteins (gp120, Tat) → NADPH oxidase + mitochondrial ROS; Immune activation → respiratory burst	CD4+ T-cell (oxidative depletion contributes to CD4 decline); Antioxidant enzymes ↓ (GPx, SOD) in HIV patients	GSH depletion in HIV → disease progression; NAC, Selenium, Glutamine supplementation improves immune function in HIV

Free Radical Theory of Ageing (Harman, 1956)

Free Radical Theory of Ageing (Harman, 1956)	Ageing and age-related diseases are caused by the accumulation of oxidative damage to cells and tissues by free radicals produced during normal aerobic metabolism. The degree of ageing is proportional to the rate of free radical production minus the rate of antioxidant defence and damage repair.

FREE RADICAL	PREMISE: Every breath = mitochondrial O_2 consumption → 1-3% electron leak → $O_2^{\bullet -}$

THEORY OF AGEING — CORE ARGUMENT

Theory Version	Proposed by	Core Idea	Molecule	Supporting Evidence
Free Radical Theory	Harman (1956)	Free radical damage accumulates → ageing	•OH, $O_2^{\bullet-}$	Caloric restriction in rodents; antioxidant lifespan extension in invertebrates
Mitochondrial Free Radical Theory	Harman (1972); refined by Miquel (1980)	mtDNA damage is primary driver; mitochondria both generate and are damaged by ROS	$O_2^{\bullet-}$ from Complex I/III	mtDNA mutations 10× nuclear DNA; Complex I activity ↓ with age; mtDNA deletions in aged tissues
Oxidative Stress Theory of Ageing	Sohal & Weindruch (1996)	Quantitative relationship: ROS generation rate vs antioxidant capacity determines rate of ageing	Total ROS output vs total antioxidant capacity	Species-specific antioxidant enzyme levels correlate with lifespan
Inflammaging	Franceschi (2000)	Chronic low-grade inflammation (driven by ROS-NF-κB) is the hallmark of ageing	NF-κB, IL-6, TNF-α (induced by ROS)	IL-6, CRP ↑ in elderly; NF-κB activation ↑ in aged tissues

★ Point

Caloric Restriction (CR) extends lifespan by REDUCING mitochondrial ROS production (lower metabolic rate → lower ETC electron leak → less $O_2^{\bullet-}$). CR activates SIRT1 → mitochondrial biogenesis, efficiency ↑ → ROS ↓. This is also the mechanism behind Resveratrol's anti-ageing effect (SIRT1 activator). CR + SIRT1 + Resveratrol = interconnected anti-ageing pathway — exam-connecting topic across units!

ENDOGENOUS ANTIOXIDANTS — ENZYMATIC

Enzymatic antioxidants are the **PRIMARY LINE of ROS defence** — they catalytically neutralise ROS at rates far exceeding any chemical scavenger. The three enzymatic antioxidants are SOD, Catalase, and Glutathione Peroxidase — often referred to as the **'antioxidant enzyme triad'**.

⚡ Superoxide Dismutase (SOD)

Type / Class	Enzymatic antioxidant — METALLOENZYME (contains metal cofactor)
Source / Location	ALL aerobic organisms; in humans: Cu/Zn-SOD (cytosol, nucleus); Mn-SOD (mitochondria); EC-SOD (extracellular spaces, plasma, lung)
Chemical Nature	Enzyme — NOT a small molecule. Cu/Zn-SOD: MW ~32,000 Da (homodimer with Cu and Zn at active site). Mn-SOD: MW ~80,000 Da (homotetramer with Mn at active site — encoded by mitochondrial genome-associated nuclear gene). EC-SOD: Glycoprotein, homotetramer with Cu/Zn, MW ~135,000 Da.
Mechanism	Catalyses DISMUTATION of superoxide to H_2O_2 — fastest known enzymatic reaction ($k \approx 2\times10^9\ M^{-1}s^{-1}$; near diffusion limit). Cu acts alternately as electron acceptor ($Cu^{2+} + O_2^{\bullet-} \rightarrow Cu^{+} + O_2$) and donor ($Cu^{+} + O_2^{\bullet-} + 2H^{+} \rightarrow Cu^{2+} + H_2O_2$). Net: eliminates $O_2^{\bullet-}$ before it can form •OH or $ONOO^-$.
Reaction / Equation	$2\ O_2^{\bullet-} + 2\ H^{+} \rightarrow H_2O_2 + O_2$ [Dismutation, balanced]
Dose / Normal Levels	Normal serum SOD: 100-250 U/mL. Mn-SOD is INDUCIBLE by ROS, cytokines (NF-κB → Mn-SOD promoter). Cu/Zn-SOD is constitutive.
Clinical Uses / Significance	SOD ↓ in ALS (Cu/Zn-SOD1 mutation — loss of function or gain of toxic peroxidase function); SOD ↑ in Down Syndrome (chromosome 21 has SOD1 gene — 50% more SOD → H_2O_2 accumulation → early Alzheimer's); SOD mimetics (MnTBAP) being

	developed for stroke, IRI; Mn-SOD nitration by peroxynitrite → vicious cycle in inflammation.

⚠ Exam Trick	SOD produces H_2O_2 — which is ALSO a ROS! SOD without Catalase/GPx is dangerous. This is why SOD and Catalase/GPx MUST work together — SOD generates H_2O_2 → Catalase/GPx removes it. This interdependence is a CRITICAL exam concept. Also: Down Syndrome patients have 3 copies of chromosome 21 (SOD1 gene) → 50% more SOD activity → excess H_2O_2 accumulation (catalase can't keep up) → early-onset Alzheimer's. Classic genetics-oxidative stress integration!

Catalase (CAT)

Type / Class	Enzymatic antioxidant — HAEMOPROTEIN (contains Fe^{3+}-haem at active site)
Source / Location	Predominantly in PEROXISOMES (highest activity); also cytosol of erythrocytes (second highest), liver, kidney; LOW activity in brain, heart — explaining their vulnerability to H_2O_2
Chemical Nature	Haemoprotein — four subunits (homotetramer), each with one Fe^{3+}-protoporphyrin IX (haem group) at active site. MW ~240,000 Da. Has NADPH-binding site (protects against H_2O_2-mediated inactivation of catalase itself — NADPH regeneration prevents compound II accumulation). One of the fastest enzymes known (kcat ~6×10^6 s^{-1}).
Mechanism	TWO mechanisms: (1) CATALATIC: Decomposes H_2O_2 to $H_2O + O_2$ (when H_2O_2 concentration HIGH — peroxisome setting). (2) PEROXIDATIC: H_2O_2 + donor-H_2 → 2 H_2O + donor (when H_2O_2 concentration LOW — uses organic substrates like ethanol, formate). Primary function = prevent H_2O_2 accumulation that would lead to •OH via Fenton.
Reaction / Equation	Catalatic: 2 H_2O_2 → 2 H_2O + O_2 [Net reaction, catalytic cycle: $Fe^{3+} + H_2O_2$ → Compound I (Fe^{5+}=O) + H_2O; Compound I + H_2O_2 → $Fe^{3+} + H_2O + O_2$]
Dose / Normal Levels	Plasma catalase: 6.7–24 nmol H_2O_2/min/mL. Inherited deficiency: ACATALASEMIA — increased susceptibility to oral gangrene (Takahara disease), diabetes mellitus. RBC

	catalase is critical — RBCs have NO mitochondria, NO catalase = rely on GPx + GSH for H_2O_2 removal.
Clinical Uses / Significance	15803D

🛡 Glutathione Peroxidase (GPx)

Type / Class	Enzymatic antioxidant — SELENOENZYME (contains selenocysteine at active site — essential selenium cofactor)
Source / Location	Cytosol and mitochondria (GPx1 — major isoform); Plasma (GPx3 — secreted); Gastrointestinal epithelium (GPx2); Sperm/testes (GPx5); Phospholipid-specific (GPx4 — in cell membranes, unique)
Chemical Nature	Selenium-containing enzyme — selenocysteine (SeCys, Sec, the 21st amino acid) at catalytic site. Multiple isoforms (GPx1-8). GPx1 (cytosolic, most abundant): Tetrameric selenoprotein, MW ~84,000 Da. GPx4 (PHGPx — Phospholipid Hydroperoxide GPx): Monomeric, MW ~19,000 Da — ONLY enzyme that reduces phospholipid hydroperoxides in membranes without needing their prior liberation; inhibition → FERROPTOSIS.
Mechanism	Oxidises 2 glutathione (GSH → GSSG) while reducing H_2O_2 or lipid hydroperoxide (LOOH) to water or lipid alcohol. Selenium at active site cycles between Se-H (selenol, reduced) and Se-OH (selenenic acid, oxidised) states. GSSG regenerated to GSH by Glutathione Reductase (GR: GSSG + NADPH → 2 GSH + $NADP^+$).
Reaction / Equation	H_2O_2: $H_2O_2 + 2\,GSH \rightarrow GSSG + 2\,H_2O$ [by GPx1]. LOOH: $LOOH + 2\,GSH \rightarrow LOH + GSSG + H_2O$ [by GPx4]. Regeneration: $GSSG + NADPH + H^+ \rightarrow 2\,GSH + NADP^+$ [by GR]
Dose / Normal Levels	RBC GPx activity: 30–75 U/g Hb. Selenium intake critical — deficiency → GPx ↓ → H_2O_2 + LOOH accumulation → Keshan disease (selenium-deficient cardiomyopathy in China). Optimal selenium intake: 55 mcg/day (RDA); >400 mcg/day = selenium toxicity (selenosis).
Clinical Uses / Significance	3730A3

Enzymatic Antioxidant Triad — Comparison Table

Feature	SOD	Catalase	GPx
Substrate	$O_2^{\bullet-}$ (superoxide)	H_2O_2	H_2O_2 + LOOH (lipid hydroperoxides)
Product	$H_2O_2 + O_2$	$H_2O + O_2$	H_2O + GSSG (oxidised glutathione)
Cofactor / Metal	Cu, Zn (cytosolic); Mn (mitochondrial)	Fe^{3+} (haem)	Selenium (Se — selenocysteine)
Primary Location	Cytosol (Cu/Zn), Mitochondria (Mn), EC (EC-SOD)	Peroxisomes, RBC cytosol	Cytosol, Mitochondria, Membranes (GPx4)
Rate constant	~2×10^9 $M^{-1}s^{-1}$ (fastest known for $O_2^{\bullet-}$)	kcat ~6×10^6 s^{-1} (fastest for H_2O_2)	Rate depends on GSH concentration
deficiency disease	ALS (SOD1 mutation), Down Syndrome (SOD excess)	Acatalasemia (Takahara disease)	Keshan disease (selenium deficiency)
Unique feature	Creates H_2O_2 — needs Catalase/GPx downstream	Cannot reduce LOOH (only H_2O_2)	GPx4 reduces phospholipid LOOH — prevents Ferroptosis

ENDOGENOUS ANTIOXIDANTS — NON-ENZYMATIC

💪 Glutathione (GSH) — Master Antioxidant

Type / Class	Non-enzymatic endogenous antioxidant — thiol tripeptide; MOST ABUNDANT intracellular non-protein thiol
Source / Location	Synthesised in CYTOSOL of ALL mammalian cells (primarily liver — 'glutathione factory'). Intracellular concentration: 1–10 mM (millimolar — extraordinarily high). Exported from liver to blood; then taken up by other tissues. Highest in liver, kidney, lens, RBCs.

Chemical Nature	Tripeptide: γ-Glutamyl-Cysteinyl-Glycine (γ-Glu-Cys-Gly). MW = 307.32 Da. The γ-glutamyl bond (unusual — N-terminus Glu linked via γ-carboxyl, NOT α-carboxyl) makes GSH RESISTANT to ordinary peptidases — only γ-glutamyl transpeptidase (GGT) can cleave it. The -SH group of Cys = functional antioxidant group. GSH/GSSG ratio normally >100:1 (highly reduced intracellular environment); ratio ↓ = oxidative stress marker.
Mechanism	(1) DIRECT SCAVENGER: GSH + •OH → GS• + H_2O; GSH + $^1O_2$ → GSSG; GSH + HOCl → GSSG + H_2O + Cl^-. (2) GPx COFACTOR: 2 GSH + H_2O_2 → GSSG + $2H_2O$ [essential cofactor, not consumed]. (3) PHASE II DETOXIFICATION: Glutathione S-Transferase (GST) conjugates GSH to electrophilic toxins → water-soluble mercapturic acid → excreted in bile/urine. (4) VITAMIN E REGENERATION: GSH regenerates oxidised Vit E (Toc•) indirectly via Vit C → Toc-H. (5) PROTEIN PROTECTION: Glutathionylation (Cys-SH + GSSG → Cys-S-S-G) — reversible, protects critical Cys residues during oxidative stress.
Reaction / Equation	Synthesis: 2 steps — [Step 1] Glu + Cys → γ-Glu-Cys [by γ-Glutamylcysteine Synthetase, GCS — rate-limiting; induced by Nrf2]; [Step 2] γ-Glu-Cys + Gly → GSH [by GSH Synthetase]. Degradation: Only extracellular — by GGT (γ-glutamyl transpeptidase) → dipeptide Cys-Gly → individual amino acids → recycled. Energy cost: 2 ATP per GSH.
Dose / Normal Levels	Intracellular: 1–10 mM (cytosol); 5–10× lower in mitochondria; Plasma: 1–8 μM (oxidised environment — lower). GSH half-life: ~2 days in liver (rapidly recycled). CLINICAL: N-Acetylcysteine (NAC) = GSH precursor drug (provides Cys — rate-limiting precursor); IV GSH infusion; Liposomal GSH; Paracetamol overdose → NAPQI depletes GSH → hepatocyte necrosis — NAC rescues.
Clinical Uses / Significance	Paracetamol overdose antidote (NAC = GSH replenisher); COPD (NAC reduces exacerbations); HIV (GSH depletion → disease progression; NAC prolongs

	CD4+ survival); Cancer prevention (Phase II enzyme induction via GST); Liver disease (NAC in NAFLD, alcoholic hepatitis); Sepsis (GSH depletion → worse outcomes; NAC trial data); Infertility (sperm GSH protects against LPO).

⚠ Exam Trick	N-Acetylcysteine (NAC) is the GSH PRECURSOR drug — it provides N-Acetyl-Cysteine which is deacetylated in cells to Cysteine → the RATE-LIMITING substrate for GSH synthesis. NAC is the ANTIDOTE for paracetamol overdose because paracetamol → NAPQI (toxic radical) → depletes GSH → hepatocyte necrosis. NAC restores GSH → detoxifies NAPQI. This is one of the most important toxicology-antioxidant connections in pharmacy!

Vitamin C (Ascorbic Acid)

Type / Class	Non-enzymatic antioxidant — water-soluble vitamin; PRIMARY water-phase antioxidant
Source / Location	Dietary: Citrus fruits (lemon, orange, amla/Indian gooseberry = RICHEST source: ~600-900 mg/100g), bell peppers, guava, kiwi, broccoli, strawberries. Endogenous synthesis: HUMANS CANNOT synthesise Vitamin C (lack L-gulonolactone oxidase — gene GULO is non-functional pseudogene). Must obtain ENTIRELY from diet — hence it is a VITAMIN (not endogenous per se, but classified as endogenous antioxidant because it is a normal constituent of human blood).
Chemical Nature	L-Ascorbic acid: $C_6H_8O_6$; MW = 176.12 Da. Lactone of 2-oxo-L-gulonic acid. Two ionisable -OH groups ($pKa_1 = 4.17$; $pKa_2 = 11.57$) — both protonated at physiological pH (in REDUCED form). Dehydro-L-ascorbic acid (DHAA) = fully oxidised form — two-electron oxidation product. Intermediate: Ascorbate radical (Asc•) = monodehyroascorbate — relatively stable (one unpaired electron delocalised over enediol system). Water-soluble. Sensitive to oxidation (light, heat, oxygen, alkaline pH).
Mechanism	(1) PRIMARY WATER-PHASE SCAVENGER: Reacts with $O_2^{\bullet-}$, •OH, $^1O_2$, HOCl, peroxynitrite, lipid peroxyl radicals (water phase). Rate constant with •OH: $k \approx 2.5\times10^9\ M^{-1}s^{-1}$. (2)

	VITAMIN E REGENERATION: Asc-H + Toc• → Asc• + Toc-H. CRITICAL: Vit C regenerates Vit E at aqueous-lipid interface — the most important antioxidant synergy in the body! (3) ENZYME COFACTOR: Prolyl hydroxylase (collagen synthesis), Dopamine-β-hydroxylase (noradrenaline synthesis), Carnitine biosynthesis. (4) IRON REDUCTION: Fe^{3+} + Asc → Fe^{2+} + Asc• [PRO-OXIDANT at high dose in iron-overloaded patients — Fenton!]. (5) NITRIC OXIDE SPARING: Reduces $ONOO^-$ → Vit C protects NO from peroxynitrite → sustains vasodilation.
Reaction / Equation	Normal plasma: 50–80 μmol/L. Deficiency (<11 μmol/L) → SCURVY (collagen defects — perifollicular haemorrhage, bleeding gums, impaired wound healing). RDA: 75 mg/day (women), 90 mg/day (men); UL: 2000 mg/day. Smokers need +35 mg/day (cigarette smoke depletes plasma Vit C by ~40%). Pharmacological doses (>1g IV) → H_2O_2 production (pro-oxidant in plasma) → cancer cells killed selectively (cancer cells low catalase).
Dose / Normal Levels	Scurvy prevention/treatment (SPECIFIC indication); Immune support (Vitamin C → neutrophil function ↑, NK cell activity ↑); Collagen synthesis (wound healing, skin health); Prevention of CVD (endothelial function ↑ via NO sparing); Iron absorption enhancement (reduces Fe^{3+} → Fe^{2+} in gut); Pharmacological IV Vitamin C in cancer (emerging — pro-oxidant mechanism); Cataract prevention (high Vit C in lens = 20× plasma concentration).
Clinical Uses / Significance	C2410C

Vitamin E (α-Tocopherol)

Type / Class	Non-enzymatic antioxidant — fat-soluble vitamin; PRIMARY lipid-phase chain-breaking antioxidant
Source / Location	Diet: Wheat germ oil (highest — 150 mg/100g α-Tocopherol), sunflower oil, almonds, hazelnuts, avocado. Endogenous: Found in ALL cell membranes and lipoproteins — incorporated from dietary sources; stored in adipose tissue (fat-soluble). NOT synthesised by humans — must come from diet. Absorbed with

	dietary fat (chylomicrons), transported in LDL/HDL, maintained in membranes by α-TTP (α-Tocopherol Transfer Protein) in liver.
Chemical Nature	α-Tocopherol: $C_{29}H_{50}O_2$; MW = 430.71 Da. Chroman-6-ol ring (fused 6-membered oxygen-containing ring + benzene ring) with phytyl side chain (16-carbon, saturated). Three methyl groups on chromanol ring at positions 5, 7, 8 → HIGHEST biological activity (100%) vs β (50%), γ (10-25%), δ (1-10%). Phenolic -OH group = functional antioxidant group (H-atom donor). HYDROPHOBIC — resides in lipid bilayer and LDL particle.
Mechanism	(1) CHAIN-BREAKING ANTIOXIDANT IN MEMBRANES: LOO• + Toc-H → LOOH + Toc• (terminates lipid peroxidation chain). Toc• is relatively stable (resonance stabilisation, low reactivity). (2) VITAMIN C REGENERATION PARTNER: Toc• + Ascorbate → Toc-H + Asc• (recycled at aqueous-lipid interface). (3) SIGNAL TRANSDUCTION: α-Tocopherol inhibits PKCα (protein kinase C) → reduces platelet aggregation, smooth muscle proliferation (antithrombotic, anti-atherogenic). (4) GENE REGULATION: Inhibits NF-κB → reduces ICAM-1, E-selectin → anti-atherosclerotic. (5) ANTI-INFLAMMATORY: Inhibits phospholipase A2 → reduced AA release → reduced eicosanoid production.
Reaction / Equation	Normal plasma α-Tocopherol: 12–42 μmol/L (closely correlated with LDL/VLDL — fat-soluble). RDA: 15 mg (22.4 IU)/day. UL: 1000 mg/day. CAUTION: High-dose supplementation (>400 IU/day) may be pro-oxidant (Toc• radical at high concentration) and increased ALL-CAUSE mortality in meta-analyses — controversial.
Dose / Normal Levels	Chain-breaking antioxidant in all cell membranes (unique function); LPO prevention → atherosclerosis prevention; Neurological disease (Vit E deficiency → ataxia, peripheral neuropathy — rare); NAFLD (800 IU/day improves histology — guideline-recommended for non-diabetic NAFLD); Male infertility (sperm membrane protection); Haemolytic anaemia prevention in premature neonates; Statin-induced myopathy adjunct (with CoQ10).
Clinical Uses / Significance	15803D

★ Point	The VITAMIN C — VITAMIN E ANTIOXIDANT NETWORK is the most important antioxidant synergy in the human body: Vitamin E (Toc-H) terminates lipid peroxidation (LOO• + Toc-H → LOOH + Toc•) → Vitamin C (Asc-H) regenerates Vitamin E (Toc• + Asc-H → Toc-H + Asc•) → Glutathione (2GSH) regenerates Vitamin C (Asc• → Asc-H via GSH) → NADPH regenerates glutathione (GSSG → 2GSH). This 4-molecule antioxidant cascade is the answer to 'How do antioxidants work together?'

Alpha-Lipoic Acid (α-LA / Thioctic Acid)

Type / Class	Non-enzymatic — UNIVERSAL antioxidant (BOTH water and fat-soluble); Metabolic coenzyme + antioxidant
Source / Location	Endogenous: synthesised in mitochondria (8-carbon fatty acid derivative). Also dietary: Spinach, broccoli, tomato, beef, organ meats (liver, kidney, heart). Available as supplement in RACEMIC (R+S) or pure R-form (R-LA — more bioavailable and effective).
Chemical Nature	1,2-Dithiolane-3-pentanoic acid (cyclic disulfide). MW = 206.32 Da. Unique bicyclic disulfide ring (1,2-dithiane-3-pentanoic acid). Oxidised form = α-Lipoic acid (LA); Reduced form = DIHYDROLIPOIC ACID (DHLA) — the active antioxidant form. DHLA has TWO thiol (-SH) groups. UNIQUE PROPERTY: Both LA and DHLA are amphipathic — soluble in BOTH water AND lipid environments (unlike Vit C = water only; Vit E = lipid only). MW is small enough to easily cross Blood-Brain Barrier.
Mechanism	(1) REGENERATES ALL MAJOR ANTIOXIDANTS: DHLA reduces Vitamin C (DHA → Asc-H); Vitamin E (Toc• → Toc-H); GSH (GSSG → 2GSH); CoQ10 (ubiquinone → ubiquinol). LA is therefore called the 'ANTIOXIDANT OF ANTIOXIDANTS' or 'NETWORK ANTIOXIDANT'. (2) METAL CHELATION: DHLA chelates Fe^{2+}, Cu^{2+}, Cd^{2+}, As^{3+}, Pb^{2+} → prevents Fenton reaction + heavy metal toxicity. (3) DIRECT ROS SCAVENGING: DHLA scavenges •OH, HOCl, $^1O_2$,

	peroxynitrite, peroxyl radicals (in both aqueous and lipid phases). (4) METABOLIC ROLE: Cofactor for pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (KGDH), branched-chain keto acid dehydrogenase → energy metabolism; (5) NRF2 ACTIVATION → upregulates endogenous antioxidant enzymes (SOD, GPx, GCL → GSH synthesis).
Reaction / Equation	R-LA: Endogenous form — more biologically active. S-LA: Not found naturally. Standard supplement dose: 300–600 mg/day (racemic). Blood levels: Both LA and DHLA detectable after supplementation.
Dose / Normal Levels	DIABETIC NEUROPATHY — APPROVED in Germany (Thioctacid® parenteral, Thiogamma® oral) for treatment of symptomatic diabetic polyneuropathy; RCT evidence (ALADIN trial: 600mg IV LA × 3 weeks → symptom improvement); ALZHEIMER'S DISEASE (crosses BBB, regenerates all antioxidants in brain; clinical trials ongoing); LIVER DISEASE (Amanita phalloides poisoning antidote — LA chelates the toxin; chronic hepatitis); HEAVY METAL DETOXIFICATION; METABOLIC SYNDROME (improves insulin sensitivity — Nrf2 → GLUT4 ↑, AMPK activation); WEIGHT MANAGEMENT (appetite suppression via hypothalamic AMPK inhibition).
Clinical Uses / Significance	6D28D9

⚠ Exam Trick	α-Lipoic Acid's MOST UNIQUE property: It is the ONLY antioxidant that is BOTH water-soluble AND fat-soluble AND can regenerate ALL OTHER major antioxidants (Vit C, Vit E, GSH, CoQ10). This makes it the 'Master Antioxidant' or 'Antioxidant of Antioxidants'. Also: R-LA (natural form) is more potent than S-LA (synthetic). Its clinical approval for DIABETIC NEUROPATHY in Germany makes it the most clinically validated nutraceutical antioxidant in the nervous system.

☽ Melatonin (N-acetyl-5-methoxytryptamine)

Type / Class	Non-enzymatic — NEUROHORMONE + potent antioxidant; Pineal gland hormone; Pleiotropic antioxidant cascade initiator
Source / Location	ENDOGENOUS: Synthesised primarily in PINEAL GLAND (brain) — secreted in DARKNESS (circadian rhythm; peaks 2-4 AM). Also produced in: Gut (10× more than pineal), retina, bone marrow, skin, lymphocytes. Synthesis rate: ~25-30 μg/day. Plasma levels: 80-120 pg/mL (peak, night); <10 pg/mL (day). DIETARY: Tart cherries, walnuts, tomatoes, bananas — trace amounts. Supplement: 0.5-10 mg tablets.
Chemical Nature	N-acetyl-5-methoxytryptamine: Indole compound derived from Tryptophan → Serotonin → N-Acetylserotonin → Melatonin. MW = 232.28 Da. groups: Indole nucleus (aromatic; electron donation); N-acetyl group; 5-methoxy group. HIGHLY LIPOPHILIC — crosses all biological membranes, Blood-Brain Barrier, mitochondrial membrane, nuclear membrane with ease. Also water-soluble (amphipathic — like α-LA). Half-life: ~45 minutes.
Mechanism	(1) DIRECT FREE RADICAL SCAVENGER: Melatonin → •OH → 2,3-cyclic-3-hydroxymelatonin (c3OHM); c3OHM → N1-Acetyl-N2-formyl-5-methoxykynuramine (AFMK); AFMK → AMK (both STILL antioxidant!). CASCADE = ONE melatonin molecule scavenges up to 4 ROS/RNS molecules (amplification — unlike Vit C that scavenges one-to-one). (2) STIMULATES ENDOGENOUS ANTIOXIDANT ENZYMES: Melatonin → MT1/MT2 receptor → Nrf2 → upregulates GPx, GR, SOD, Catalase (gene expression ↑). (3) MITOCHONDRIAL PROTECTION: Melatonin accumulates in mitochondria (where it is synthesised locally by some accounts) → prevents MPTP opening → reduces apoptosis; maintains Complex I/IV activity. (4) NF-κB INHIBITION: Reduces pro-inflammatory gene expression; reduces iNOS → less $ONOO^-$. (5) ANTI-APOPTOTIC: Reduces cytochrome c release → caspase inhibition.
Reaction / Equation	Melatonin + •OH → 2,3-c3OHM → AFMK → AMK [ANTIOXIDANT CASCADE — each metabolite still scavenges ROS]. Net: 1 melatonin → scavenges up to 4 free

	radicals (high amplification vs Vitamin C which scavenges 1:1).
Dose / Normal Levels	Endogenous production: ~25-30 µg/day; Peaks 2-4 AM. DECLINES WITH AGE: 20-year-old vs 70-year-old → melatonin production reduced by 70-80% → links melatonin decline to age-related oxidative stress increase. Supplements: 0.5-10 mg/day.
Clinical Uses / Significance	SLEEP DISORDERS (jet lag, shift work, insomnia — primary clinical use via MT1/MT2 receptor, not antioxidant); NEUROPROTECTION (Alzheimer's, Parkinson's — antioxidant + Aβ aggregation inhibition — trials ongoing); CANCER ADJUVANT (antioxidant + cell cycle arrest + apoptosis in cancer cells; reduces chemotherapy side effects — especially carboplatin-induced thrombocytopaenia); IRI PROTECTION (cardiac surgery, organ preservation); AGEING — melatonin supplementation in elderly → improved antioxidant capacity (Pineal pinealectomy = accelerated ageing in animal models).

★ Point	Melatonin's antioxidant cascade: ONE melatonin → c3OHM → AFMK → AMK (each metabolite STILL scavenges ROS). This means melatonin has an 'antioxidant amplification factor' of up to 4 — far superior to Vitamin C (1:1 ratio) or Vitamin E (1:1 ratio). Plus it stimulates endogenous antioxidant ENZYMES (SOD, GPx, Catalase) via Nrf2. This dual action (direct scavenging + enzyme induction) makes melatonin unique among all antioxidants.

SYNTHETIC ANTIOXIDANTS — BHA AND BHT

Synthetic antioxidants are **man-made compounds** added to food, cosmetics, and pharmaceuticals to prevent **lipid peroxidation and rancidity**. They are phenolic compounds that function as **chain-breaking antioxidants** — donating hydrogen atoms to lipid peroxyl radicals, terminating the chain reaction. The two most widely used synthetic food antioxidants are **BHA (Butylated Hydroxyanisole)** and **BHT (Butylated Hydroxytoluene)**.

Butylated Hydroxyanisole (BHA)

🏭 BHA — Butylated Hydroxyanisole (E320)	
Type / Class	SYNTHETIC food antioxidant; Chain-breaking antioxidant; GRAS (Generally Recognised As Safe) — FDA
Source / Location	Does NOT occur in nature. Synthesised industrially by: 4-methoxyphenol + isobutylene (acid-catalysed alkylation) → mixture of 2-BHA and 3-BHA. Commercially available as white waxy solid.
Chemical Nature	Phenolic antioxidant — mixture of two isomers: 2-tert-Butyl-4-hydroxyanisole (2-BHA, minor) + 3-tert-Butyl-4-hydroxyanisole (3-BHA, major ~90%). Chemical formula: $C_{11}H_{16}O_2$. MW = 180.24 Da. Structure: 4-methoxyphenol ring with tert-butyl group at ortho position (2-position or 3-position). Fat-soluble (due to lipophilic tert-butyl group). Melting point: 48-55°C. Boiling point: 264-270°C. STABLE AT HIGH TEMPERATURES — can be used in baked goods.
Mechanism	(1) HYDROGEN ATOM DONATION: BHA-OH + ROO• → BHA-O• + ROOH (terminates lipid peroxidation chain). BHA phenoxyl radical (BHA-O•) is resonance-stabilised and relatively unreactive — does NOT propagate chain. (2) METAL CHELATION: Phenolic OH chelates Fe^{2+}, Cu^{2+} → reduces Fenton reaction (minor contribution). (3) REDUCING AGENT: Converts Fe^{3+} → Fe^{2+} in some systems (pro-oxidant at low O_2 — minor concern).
Reaction / Equation	BHA-OH + LOO• → BHA-O• + LOOH [Chain termination]. BHA-O• + LOO• → BHA-O-OL [Stable non-radical product]
Dose / Normal Levels	Maximum permitted level in food: 200 mg/kg (EU/FSSAI); 0.02% of fat content (FDA/USFDA); Combined BHA+BHT: 0.02% of fat in most foods.
Clinical Uses / Significance	FOOD INDUSTRY: Edible oils, lard, shortenings, potato chips, breakfast cereals, instant noodles, chewing gum, packaged baked goods; COSMETICS: Lipstick, lotions, moisturisers (prevents

	rancidity); PHARMACEUTICALS: Tablet coatings, topical ointments. CONTROVERSY: Animal studies → hyperactivation/behavioural changes at HIGH doses; BHA metabolite = BHA-quinone (potentially carcinogenic — BUT at very high doses far exceeding food levels). IARC: Group 2B (possibly carcinogenic to humans) based on animal studies at supraphysiological doses.

Butylated Hydroxytoluene (BHT)

🏭 BHT — Butylated Hydroxytoluene (E321)	
Type / Class	SYNTHETIC food antioxidant; Chain-breaking antioxidant; GRAS (Generally Recognised As Safe) — FDA
Source / Location	Synthesised by: p-Cresol (4-methylphenol) + isobutylene (acid-catalysed Friedel-Crafts alkylation). Pure compound (unlike BHA which is a mixture). Commercial product: White crystalline solid, characteristic phenolic odour.
Chemical Nature	2,6-Di-tert-Butyl-4-methylphenol. Chemical formula: $C_{15}H_{24}O$. MW = 220.35 Da. Structure: 4-Methylphenol (p-Cresol) ring with TWO tert-butyl groups at positions 2 AND 6 (both ortho positions) — HINDERED PHENOL. The two bulky tert-butyl groups sterically protect the phenolic -OH from easy oxidation (STERIC HINDRANCE), while slowing the antioxidant reaction rate — making BHT a 'retarded' antioxidant with controlled, sustained activity. Fat-soluble. Melting point: 69-71°C. Very stable thermally (higher than BHA).
Mechanism	(1) HINDERED PHENOL HYDROGEN DONOR: BHT-OH + LOO• → BHT-O• + LOOH [steric hindrance makes BHT-O• VERY stable — even less reactive than BHA-O•]. (2) SYNERGIST with BHA: BHT can regenerate BHA from BHA-O•; BHA+BHT combination is synergistic (additive or superadditive antioxidant effect). (3) UV ABSORBER: BHT absorbs UV light → may contribute to antioxidant activity in UV-

	exposed fats. (4) BHT quinone methide: Major metabolite — responsible for both antioxidant AND some potential toxicity.
Reaction / Equation	BHT-OH + 2 LOO• → BHT-O• + 2 LOOH [TWO peroxyl radicals terminated per BHT]. BHT-O• + LOO• → stable non-radical peroxide [Termination]
Dose / Normal Levels	Maximum permitted level: 200 mg/kg in food (EU); 0.02% of fat content (FDA); BHA+BHT combined: 0.02% of fat. Very lipophilic → accumulates in fatty tissues (adipose, liver) — this is the primary safety concern.
Clinical Uses / Significance	FOOD INDUSTRY: Edible vegetable oils, animal fats, margarine, butter, chewing gum, breakfast cereals, snack foods, dehydrated potato products; COSMETICS: Moisturisers, anti-ageing creams, hair care products; RUBBER/POLYMER INDUSTRY: Antioxidant in plastics, rubber, petroleum products; PHARMACEUTICALS: Drug stabilisation in tablet formulations. SAFETY CONCERNS: Accumulates in adipose tissue; BHT quinone methide = reactive metabolite → DNA damage in animal studies at HIGH doses; Potential endocrine disruption (weak oestrogen agonist); IARC Group 3 (not classifiable as to carcinogenicity). Rats: High-dose BHT → lung and liver tumours. However, some paradoxical ANTI-CANCER data (antiviral activity against herpes — BHT disrupts lipid envelope).

BHA vs BHT — Comparison Table

Parameter	BHA (Butylated Hydroxyanisole)	BHT (Butylated Hydroxytoluene)
Full name	Butylated Hydroxyanisole	Butylated Hydroxytoluene
E-number	E320	E321
Chemical formula	$C_{11}H_{16}O_2$	$C_{15}H_{24}O$
MW (g/mol)	180.24	220.35
Structure	2- and 3-tert-Butyl-4-methoxyphenol (mixture of 2 isomers)	2,6-Di-tert-Butyl-4-methylphenol (single compound)
tert-Butyl groups	ONE (at ortho position)	TWO (both ortho positions — fully hindered)

Parameter	BHA (Butylated Hydroxyanisole)	BHT (Butylated Hydroxytoluene)
Methoxy/Methyl	Methoxy ($-OCH_3$) at para	Methyl ($-CH_3$) at para
Phenoxyl radical stability	BHA-O• — stable, non-reactive	BHT-O• — MORE stable (2 tert-butyl steric shield)
Activity mechanism	H-atom donation to LOO•; metal chelation	Hindered phenol H-donation to 2 LOO• per molecule
Thermal stability	Good (stable to 264°C)	Excellent (stable to 320°C) — preferred for frying
Volatility	More volatile — can migrate to food from packaging	Less volatile — better retained in food
Fat solubility	Moderately fat-soluble	Highly fat-soluble (more lipophilic)
Primary food use	Edible fats and oils, breakfast cereals, chewing gum	Edible oils, cereals, snack foods, cosmetics
Maximum permitted	200 mg/kg food; 0.02% of fat	200 mg/kg food; 0.02% of fat
Safety concern	BHA-quinone metabolite — IARC Group 2B (possibly carcinogenic — animal data at high dose)	BHT quinone methide — IARC Group 3 (not classifiable); accumulates in adipose tissue
Synergism	BHA + BHT = SYNERGISTIC combination (most common industrial use)	Same — commonly paired with BHA and/or propyl gallate

⊖ Caution

SAFETY NOTE: Both BHA and BHT are GRAS (FDA) at permitted levels in food. The carcinogenicity data comes from ANIMAL STUDIES at doses 1000× higher than typical human food exposure. At regulated food levels, they are considered safe. However, growing consumer demand for 'clean labels' has led to increasing replacement with natural antioxidants (Rosemary extract = E392, Vitamin E = E306, Vitamin C = E300) in many food products.

Synthetic Antioxidant	Natural Equivalent	E-number	Advantage of Natural
BHA (E320)	Rosemary extract (Rosmarinic acid)	E392	No safety concerns; consumer acceptance; additional health benefits

Synthetic Antioxidant	Natural Equivalent	E-number	Advantage of Natural
BHT (E321)	Mixed Tocopherols (Vit E)	E306	GRAS unlimited; health benefits in human body; no accumulation concerns
BHA+BHT	Vitamin C (Ascorbyl palmitate)	E304	Water-soluble; immune benefits; safe at high levels
Propyl gallate (E310)	Green tea extract (EGCG)	Not approved as additive per se	Antioxidant + anticancer + antimicrobial; clean label

FUNCTIONAL FOODS FOR CHRONIC DISEASE PREVENTION

Functional foods provide health benefits **beyond basic nutrition** and represent the most practical antioxidant delivery strategy for chronic disease prevention. Unlike pharmaceutical antioxidants (which have largely failed in clinical trials), **dietary antioxidants in whole food matrix** are consistently associated with reduced chronic disease risk in epidemiological studies — due to synergistic interactions between multiple phytochemicals.

Functional Foods for Cardiovascular Disease Prevention

Functional Food	Active Compound	Mechanism	Evidence Level
Oat bran	β-Glucan (3g/day)	Bile acid sequestration → LDL ↓ 10-15%; glycemic control	FDA health claim (strongest evidence — Grade A)
Fatty fish (salmon, mackerel)	EPA + DHA (Omega-3, 1-2g/day)	TG ↓ 25-50%; platelet aggregation ↓; arrhythmia ↓; endothelial function ↑	AHA recommendation for CVD prevention; FDA approved drug (Vascepa)
Garlic (2-4 cloves/day)	Allicin, DATS, H_2S	LDL ↓ 5-10%; BP ↓ ~8/5 mmHg; platelet aggregation ↓; HMG-CoA reductase inhibition	Meta-analysis: significant LDL and BP reduction
Red wine/Grapes	Resveratrol, Anthocyanins, Quercetin	SIRT1 → cardioprotection; eNOS → NO → vasodilation; LDL oxidation ↓	'French Paradox' — moderate wine → 25-40% lower CVD; non-alcoholic polyphenols =

Functional Food	Active Compound	Mechanism	Evidence Level
Nuts (Almonds, Walnuts)	Phytosterols, Vitamin E, Omega-3 ALA, Fibre	LDL ↓; HDL ↑; LDL oxidation ↓; endothelial function ↑	PREDIMED trial: 30g/day nuts → 30% CVD risk reduction
Dark chocolate (>70% cocoa)	Flavanols (epicatechin), Theobromine	eNOS → NO → vasodilation; platelet aggregation ↓; LDL oxidation ↓; insulin sensitivity ↑	Cocoa flavanol supplementation → 11% CVD reduction (COSMOS-Heart trial, 2022)
Tomatoes/Lycopene (10mg/day)	Lycopene	LDL oxidation ↓; NO production ↑; antioxidant	Meta-analysis: lycopene supplementation → LDL ↓ 10%; Bp ↓ 5 mmHg

Functional Foods for Diabetes Prevention & Management

Functional Food	Active Compound	Mechanism	Clinical Evidence
Fenugreek (Methi)	4-Hydroxyisoleucine, Galactomannan fiber	Insulin secretion ↑; glucose absorption ↓ (fiber); insulin receptor sensitivity ↑	RCT: 10g/day fenugreek → FBG ↓; HbA1c ↓; widely used in Ayurveda
Bitter gourd (Karela)	Polypeptide-P, Charantin, Momordicin	Insulin-like action (Polypeptide-P → insulin receptor); AMPK activation (Charantin → mimics Metformin)	Multiple RCTs: significant FBG reduction; used in T2DM management in India/Asia
Cinnamon (2-6g/day)	Methylhydroxychalcone polymer (MHCP); Cinnamaldehyde	MHCP: insulin receptor autophosphorylation → GLUT4 translocation ↑; Cinnamaldehyde: amylase/glucosidase inhibition → glucose absorption ↓	Meta-analysis: 6g/day cinnamon → FBG ↓ 18-29 mg/dL; controversial — variable results
Flaxseeds (2 tbsp/day)	SDG (Lignan); ALA; Mucilage fiber	Fiber → glucose absorption ↓; ALA → insulin sensitivity ↑; SDG → phytoestrogen → PPAR-γ → adiponectin ↑ → insulin sensitivity	RCT in T2DM: flaxseed → HbA1c ↓, FBG ↓, insulin resistance ↓

Functional Food	Active Compound	Mechanism	Clinical Evidence
Oats (1 cup/day)	β-Glucan	Viscous gel → slows gastric emptying → postprandial glucose ↓ → insulin demand ↓; GLP-1 release ↑	FDA health claim includes blood glucose reduction; ADA recommends oats for T2DM
Turmeric/Curcumin (500-1000mg/day)	Curcumin	AMPK activation → glucose uptake ↑; NF-κB ↓ (reduces β-cell inflammation); PPARγ activation → insulin sensitivity ↑	RCT: Curcumin prevented T2DM in prediabetes (9-month trial — 16% vs 0% progression)
Berberine (500mg 3×/day)	Berberine (alkaloid)	AMPK pathway activation (identical to Metformin mechanism); incretin (GLP-1) ↑; gut microbiome modulation	RCT: Berberine = Metformin in HbA1c and FBG reduction; being studied for T2DM treatment

Functional Foods for Cancer Prevention

Functional Food	Active Compound	Cancer Target	Mechanism
Broccoli/Cruciferous veg	Sulforaphane, I3C, DIM	Breast, colon, prostate, lung	Nrf2 → Phase II enzymes (GST, NQO1) → carcinogen detox; NF-κB ↓; HER2 ↓
Garlic	Allicin, DATS, DADS	Stomach, colon, prostate	DATS: apoptosis (caspase ↑), G2/M arrest, HIF-1α ↓ (anti-angiogenic)
Green tea	EGCG	Breast, prostate, lung, colon	EGFR inhibition; VEGF ↓ (anti-angiogenic); DHFR inhibition; apoptosis
Soy/Genistein	Genistein	Breast (ER+), prostate	Tyrosine kinase inhibition; antiestrogenic (ER-β selective); phase II enzymes ↑
Turmeric/Curcumin	Curcumin	Multiple (pancreatic, colon, breast)	NF-κB ↓↓; COX-2 ↓; p53 ↑ (tumour suppressor restoration); Bcl-2 ↓ (pro-apoptotic)
Tomatoes/Lycopene	Lycopene	Prostate (strongest evidence)	Singlet O_2 quenching; IGF-1 signalling ↓; connexin (gap junction) ↑; cell cycle arrest

Functional Food	Active Compound	Cancer Target	Mechanism
Flaxseeds/SDG	SDG → Enterolactone	Breast, prostate	Phytoestrogen → antioestrogenic (ER-α antagonist); 5-α-reductase inhibition (DHT ↓)
Raspberries/Ellagic acid	Ellagic acid, Urolithins	Colon, prostate	Phase II enzyme induction; MAPK pathway ↓; urolithins (gut metabolites) → mitophagy → anti-ageing

Functional Foods for Neurodegenerative Disease Prevention

Functional Food	Active Compound	Disease Target	Mechanism
Turmeric/Curcumin	Curcumin	Alzheimer's Disease	Aβ aggregation inhibition (direct binding to amyloid); Tau hyperphosphorylation ↓; NF-κB ↓; GSK-3β ↓; Nrf2 ↑
Ginkgo biloba (EGb761)	Ginkgolides, Bilobalide	Dementia, Alzheimer's	Vasodilation (PAF antagonism); mitochondrial function ↑; antioxidant; Aβ aggregation ↓
Blueberries/Anthocyanins	Anthocyanins (delphinidins, cyanidins)	Cognitive decline, Alzheimer's	BDNF (Brain-Derived Neurotrophic Factor) ↑; neurogenesis ↑ (hippocampus); NF-κB ↓; LPO ↓ in brain
Fatty fish/DHA	DHA (22:6 n-3)	Alzheimer's, Depression, cognitive decline	Neuronal membrane fluidity ↑; neuroprotectin D1 (from DHA) → anti-apoptotic; inflammation resolution; amyloid precursor protein processing ↑
Coffee (3-5 cups/day)	Chlorogenic acids, Caffeine	Parkinson's, Alzheimer's	Caffeine: adenosine receptor antagonism → dopaminergic neuron protection; CGA: Nrf2 → HO-1 → neuroprotection; Parkinson's risk ↓ 30-60%
Green tea	EGCG, L-Theanine	Parkinson's, Alzheimer's	EGCG: iron chelation (Fe^{2+}) → reduces Fenton in substantia nigra; Aβ aggregation ↓; L-Theanine: alpha waves ↑ → stress ↓ → neuroprotection

Functional Food	Active Compound	Disease Target	Mechanism
Resveratrol	Resveratrol	Alzheimer's, general neurodegeneration	SIRT1 → autophagy ↑ → misfolded protein clearance; mitochondrial biogenesis ↑; Aβ clearance ↑; NF-κB ↓

★ Point	PREDIMED Trial (2013, Spain — 7447 high-CV-risk adults): Mediterranean diet supplemented with EVOO (Extra Virgin Olive Oil) or Nuts reduced CVD by 30%; Stroke by 40%; All-cause mortality non-significantly reduced. This is the LARGEST and MOST IMPORTANT functional food RCT ever conducted. Mediterranean diet = olive oil + fish + vegetables + whole grains + nuts + moderate red wine + low red meat = the best-evidenced dietary pattern for chronic disease prevention.

DEFINITIONS GLOSSARY —

Oxidative Stress	Imbalance between ROS production and antioxidant defence mechanisms resulting in net oxidative damage to biomolecules. (Sies, 1985). Root cause of most major chronic diseases.

Ischaemia-Reperfusion Injury (IRI)	Paradoxical additional tissue damage upon restoration of blood flow to ischaemic tissue, caused by a massive ROS burst from Xanthine Oxidase (Hypoxanthine + O_2 → Xanthine + $O_2^{\bullet -}$) and mitochondria.

OxLDL (Oxidised LDL)	LDL modified by free radical attack on its PUFA and apoB100 components. Atherogenic because: recognised by SCAVENGER RECEPTORS (not LDL receptor) → unregulated cholesterol uptake → foam cells → atherosclerotic plaque.

Foam Cell	Lipid-laden macrophage that has engulfed OxLDL via scavenger receptors; hallmark of early atherosclerosis (fatty streak). Does NOT occur with native LDL because the LDL receptor is downregulated by intracellular cholesterol.

Brownlee's Unifying Theory (2001)	Hyperglycaemia → Mitochondrial $O_2^{\bullet-}$ overproduction → simultaneously activates 4 damage pathways (Polyol pathway, AGE formation, PKC, Hexosamine pathway) → ALL diabetic complications.

Free Radical Theory of Ageing (Harman, 1956)	Ageing is caused by cumulative oxidative damage to cells from free radicals produced during normal aerobic metabolism; rate of ageing proportional to ROS generation rate minus antioxidant/repair capacity.

Superoxide Dismutase (SOD)	Primary enzymatic antioxidant; catalyses dismutation of $O_2^{\bullet-}$ to $H_2O_2 + O_2$ at near-diffusion-limited rate ($k \approx 2\times10^9\ M^{-1}s^{-1}$). Isoforms: Cu/Zn-SOD (cytosol), Mn-SOD (mitochondria), EC-SOD (extracellular).

Catalase	Haemoprotein enzyme in peroxisomes that dismutates H_2O_2 to $H_2O + O_2$ at extremely high rate. Deficiency → Acatalasemia (Takahara disease). Low in brain and heart — explains their vulnerability.

Glutathione Peroxidase (GPx)	Selenoenzyme that reduces H_2O_2 and LOOH using GSH as cofactor. GPx4 (unique) reduces phospholipid hydroperoxides in membranes — its inhibition causes Ferroptosis. Selenium deficiency → Keshan disease.

Glutathione (GSH)	Tripeptide (γ-Glu-Cys-Gly); MOST abundant intracellular non-protein thiol (1-10 mM). Master antioxidant — direct scavenger, GPx cofactor, detoxification (Phase II/GST), protein protection (glutathionylation), Vit E regeneration.

N-Acetylcysteine (NAC)	GSH precursor drug — provides N-Acetyl-Cysteine (deacetylated to Cys in cells → GSH synthesis). Antidote for paracetamol overdose (restores GSH to detoxify NAPQI). Also used in COPD, IRI, contrast nephropathy prevention.

Alpha-Lipoic Acid (α-LA)	UNIVERSAL antioxidant — both water and fat-soluble; natural metabolic cofactor (PDH, KGDH); reduced form (DHLA) regenerates Vitamin C, E, GSH, and CoQ10; metal chelator; Nrf2 activator. Approved for diabetic neuropathy in Germany.

Melatonin	Pineal neurohormone (N-acetyl-5-methoxytryptamine); potent antioxidant cascade initiator — 1 melatonin → up to 4 ROS scavenged (metabolites c3OHM → AFMK → AMK all antioxidant); also stimulates SOD, GPx, Catalase via Nrf2.
BHA (Butylated Hydroxyanisole, E320)	Synthetic phenolic food antioxidant (mixture of 2- and 3-BHA isomers); MW 180.24; chain-breaking (H-donor to LOO•); used in edible fats/oils; IARC Group 2B (animal data at high dose); Max 200 mg/kg food.
BHT (Butylated Hydroxytoluene, E321)	Synthetic hindered phenolic food antioxidant; single compound (2,6-di-tert-butyl-4-methylphenol); MW 220.35; TWO tert-butyl groups = steric protection; more thermostable than BHA; IARC Group 3; Max 200 mg/kg food.
Antioxidant Network	Interconnected system of water-soluble (Vit C, GSH) and lipid-soluble (Vit E) antioxidants that regenerate each other: Vit E quenches LOO• → Toc• → Vit C regenerates Toc-H → GSH regenerates Vit C → NADPH regenerates GSH. α-LA and Melatonin regenerate all members.
Ferroptosis	Iron-dependent regulated cell death driven by lipid peroxidation, distinct from apoptosis and necrosis. Caused by GPx4 inhibition → uncontrolled phospholipid hydroperoxide accumulation → membrane rupture. Cancer therapy target.
Inflammaging	Chronic, low-grade, systemic inflammation associated with ageing; driven by ROS → NF-κB → IL-6, TNF-α → chronic inflammation. Proposed by Franceschi (2000). Associated with frailty, CVD, dementia, cancer in the elderly.
PREDIMED Trial (2013)	Landmark RCT: Mediterranean diet with EVOO or nuts → 30% CVD reduction, 40% stroke reduction vs low-fat diet in high-risk adults (n=7447). Most important RCT for functional food/diet-based disease prevention.

Nrf2 (NF-E2-Related Factor 2)	Transcription factor — 'master regulator of antioxidant response'. Activated by: Sulforaphane, Curcumin, α-Lipoic Acid, Resveratrol, Melatonin, EGCG. Induces: GST, NQO1, HO-1, GCL (GSH synthesis), SOD, Catalase, GPx → comprehensive antioxidant defence upregulation.

QUESTION BANK — 2 MARK QUESTIONS

Q. Q1. What is the role of free radicals in the pathogenesis of Atherosclerosis?

Q. Q2. What is the Free Radical Theory of Ageing? Who proposed it?

Q. Q3. Write the reactions catalysed by SOD and Catalase.

Q. Q4. Describe the unique properties of Alpha-Lipoic Acid as an antioxidant.

Q. Q5. Write a short note on BHT — chemical nature, mechanism, and safety.

Q. Q6. What is Ischaemia-Reperfusion Injury? Name the primary enzyme responsible for ROS burst during reperfusion.

Q. Q7. What is the antioxidant cascade of Melatonin? Why is it superior to Vitamin C?

Q. Q8. Explain the role of free radicals in cancer — initiation, promotion, and progression.

QUESTION BANK — 5 MARK QUESTIONS

Q1. Describe the role of free radicals in the pathogenesis of Diabetes Mellitus. (5 marks)

Q2. Classify antioxidants. Describe endogenous enzymatic antioxidants — SOD, Catalase, and GPx with their reactions and clinical significance. (5 marks)

Q3. Write a note on Glutathione (GSH) as a non-enzymatic antioxidant — structure, synthesis, mechanisms, and clinical importance. (5 marks)

Q4. Write a note on Vitamin C and Vitamin E — their antioxidant mechanisms and how they interact. (5 marks)

Q5. Compare BHA and BHT as synthetic antioxidants — structure, mechanism, uses, and safety. (5 marks)

QUESTION BANK — 10 MARK QUESTIONS

Q1. Describe in detail the role of free radicals in Atherosclerosis, Ischaemia-Reperfusion Injury, and Cancer. (10 marks)

Q2. Classify antioxidants and describe the endogenous enzymatic and non-enzymatic antioxidant defence systems in detail. (10 marks)

Q3. Write a comprehensive note on: (a) Free Radical Theory of Ageing (b) Synthetic antioxidants BHA and BHT (c) Functional Foods for Chronic Disease Prevention. (10 marks)

PREVIOUS-YEAR STYLE QUESTIONS

#	Question	Marks
1	Describe the role of free radicals in Diabetes Mellitus and Atherosclerosis with special reference to molecular mechanisms.	10
2	Write a note on Ischaemia-Reperfusion Injury — mechanism, ROS involved, and protective strategies.	5
3	Classify antioxidants. Describe SOD, Catalase, and Glutathione Peroxidase with their reactions and clinical significance.	10
4	Write a note on Alpha-Lipoic Acid and Melatonin as antioxidants.	5
5	Write a detailed note on BHA and BHT — chemical structure, mechanism, food applications, and safety concerns.	10
6	Explain the Free Radical Theory of Ageing. What is the Mitochondrial Free Radical Theory?	5
7	Write short notes on: (a) Functional foods for cancer prevention (b) Functional foods for cardiovascular disease prevention.	5+5

TOP 15 MCQs — WITH ANSWERS & EXPLANATIONS

Q1. The 'Paradox of Reperfusion Injury' is primarily caused by which enzyme producing a burst of superoxide?

(A) NADPH Oxidase (NOX2)

(B) Xanthine Oxidase (XO)

(C) Cyclooxygenase (COX)

(D) Monoamine Oxidase (MAO)

✓ Correct: (B) Xanthine Oxidase (XO)

Explanation: Xanthine Oxidase (XO) is the PRIMARY enzyme responsible for the ROS burst in Ischaemia-Reperfusion Injury. During ischaemia: hypoxanthine accumulates AND Xanthine Dehydrogenase (XDH) is irreversibly converted to XO. When O_2 returns (reperfusion): XO + Hypoxanthine + O_2 → Xanthine + $O_2^{\bullet -}$ + H_2O_2 [MASSIVE BURST]. Allopurinol (XO inhibitor used in gout) prevents this ROS burst and is studied as an IRI protectant.

Q2. OxLDL (Oxidised LDL) is taken up by which receptor on macrophages to form foam cells?

(A) LDL receptor (LDLR)

(B) Scavenger receptor (SR-A / CD36)

(C) VLDL receptor

(D) HDL receptor (SR-B1)

✓ Correct: (B) Scavenger receptor (SR-A / CD36)

Explanation: OxLDL is taken up by SCAVENGER RECEPTORS (SR-A and CD36) on macrophages — NOT the regular LDL receptor. This is atherogenic because: The LDL receptor is downregulated when intracellular cholesterol is high (preventing excess uptake). Scavenger receptors are NOT downregulated — so macrophages continue to take up OxLDL regardless of intracellular cholesterol load → uncontrolled lipid accumulation → foam cells → fatty streak.

Q3. The Free Radical Theory of Ageing was proposed by:

(A) Miquel (1980)

(B) Harman (1956)

(C) Franceschi (2000)

(D) Sohal & Weindruch (1996)

✔ Correct: (B) Harman (1956)

Explanation: The Free Radical Theory of Ageing was proposed by Denham HARMAN in 1956 — stating that free radicals produced during normal aerobic metabolism accumulate oxidative damage to cells over time, causing ageing. Harman extended it in 1972 as the Mitochondrial Free Radical Theory. Miquel refined the mitochondrial aspect (1980). Franceschi proposed 'Inflammaging' (2000). Sohal & Weindruch proposed the Oxidative Stress Theory (1996).

Q4. Which antioxidant is both water-soluble AND fat-soluble, and can regenerate Vitamin C, Vitamin E, Glutathione, and CoQ10?

(A) Melatonin

(B) Vitamin E

(C) Alpha-Lipoic Acid (α-LA)

(D) Glutathione (GSH)

✔ Correct: (C) Alpha-Lipoic Acid (α-LA)

Explanation: Alpha-Lipoic Acid (α-Lipoic acid / Thioctic acid) is UNIQUE as the UNIVERSAL antioxidant — amphipathic (both water and fat soluble). Its reduced form DHLA regenerates ALL major antioxidants: Vitamin C, Vitamin E, GSH, and CoQ10. This makes it the 'Antioxidant of Antioxidants' or 'Network Antioxidant'. It is the only antioxidant approved for clinical use in diabetic neuropathy (Germany). It also chelates metal ions and activates Nrf2.

Q5. The antioxidant enzyme that requires SELENIUM as its essential cofactor is:

(A) Superoxide Dismutase

(B) Catalase

(C) Glutathione Peroxidase (GPx)

(D) Thioredoxin

✔ Correct: (C) Glutathione Peroxidase (GPx)

Explanation: Glutathione Peroxidase (GPx) contains SELENOCYSTEINE (the 21st amino acid, encoded by UGA codon with SECIS element) at its catalytic site. Selenium is essential for GPx activity. Selenium deficiency → GPx ↓ → H_2O_2 and LOOH accumulate → Keshan disease (endemic cardiomyopathy in selenium-deficient areas of China). RDA for selenium = 55 mcg/day. GPx4 is specific for phospholipid hydroperoxides — its inhibition causes Ferroptosis.

Q6. Brownlee's Unifying Theory (2001) states that ALL diabetic complications result from the overproduction of which primary ROS by mitochondria?

(A) Hydrogen Peroxide (H_2O_2)

(B) Hydroxyl Radical (•OH)

(C) Peroxynitrite ($ONOO^-$)

(D) Superoxide Anion Radical ($O_2^{\bullet-}$)

✓ Correct: (D) Superoxide Anion Radical ($O_2^{\bullet-}$)

Explanation: Brownlee's Unifying Theory (2001) — published in Nature — proposes that hyperglycaemia causes a single primary change: MITOCHONDRIAL OVERPRODUCTION OF $O_2^{\bullet-}$ (Superoxide). This $O_2^{\bullet-}$ then SIMULTANEOUSLY activates all four pathways: Polyol pathway, AGE formation, PKC activation, and Hexosamine pathway → ALL diabetic complications. This unifying mechanism explains why all complications (nephropathy, retinopathy, neuropathy, CVD) share the same root cause.

Q7. The melatonin antioxidant cascade can scavenge up to how many ROS molecules per melatonin molecule?

(A) 1

(B) 2

(C) 4

(D) 8

✓ Correct: (C) 4

Explanation: Melatonin undergoes an ANTIOXIDANT CASCADE: Melatonin → 2,3-c3OHM → AFMK → AMK. Each metabolite in this cascade RETAINS antioxidant activity and can scavenge additional ROS. Therefore, ONE melatonin molecule can scavenge UP TO 4 free radicals sequentially through its metabolites — a 4× amplification compared to Vitamin C (which scavenges 1:1 and forms stable Asc• with no further scavenging). This makes melatonin's antioxidant cascade particularly efficient.

Q8. N-Acetylcysteine (NAC) is the antidote for paracetamol (acetaminophen) overdose because it:

(A) Directly inactivates NAPQI

(B) Inhibits cytochrome P450 CYP2E1

(C) Replenishes hepatic Glutathione (GSH) by providing Cysteine

(D) Activates hepatic catalase

✓ Correct: (C) Replenishes hepatic Glutathione (GSH) by providing Cysteine

Explanation: NAC works as a paracetamol antidote by REPLENISHING HEPATIC GSH. Mechanism: Paracetamol → CYP2E1 → NAPQI (N-Acetyl-p-benzoquinoneimine — reactive toxic metabolite). Normal: NAPQI + GSH → mercapturate (detoxified). Overdose: GSH depleted → NAPQI covalently binds hepatocyte proteins → HEPATIC NECROSIS. NAC provides N-Acetyl-Cysteine → deacetylated to Cysteine (rate-limiting GSH precursor) → GSH synthesis restored → NAPQI detoxified. NAC also directly reacts with NAPQI at higher concentrations.

Q9. BHT (Butylated Hydroxytoluene) differs from BHA structurally in having:

(A) One tert-butyl group at ortho position

(B) A methoxy group instead of methyl

(C) Two tert-butyl groups at both ortho positions (fully hindered phenol)

(D) An anisole (methoxyphenol) core

✓ Correct: (C) Two tert-butyl groups at both ortho positions (fully hindered phenol)

Explanation: BHT (2,6-Di-tert-Butyl-4-methylphenol) has TWO tert-butyl groups at BOTH ortho positions (positions 2 AND 6) = FULLY STERICALLY HINDERED PHENOL. BHA has only ONE tert-butyl group. This double steric shielding of the phenolic OH in BHT creates an extremely stable BHT-O• phenoxyl radical (even more stable than BHA-O•) → BHT can terminate 2 peroxyl radicals per molecule. The double steric bulk also makes BHT MORE thermally stable than BHA.

Q10. In Parkinson's disease, free radicals destroy which specific cell type, leading to the cardinal features of the disease?

(A) Hippocampal CA1 neurons

(B) Cerebellar Purkinje cells

(C) Dopaminergic neurons in Substantia Nigra

(D) Cholinergic neurons in Nucleus Basalis of Meynert

✓ Correct: (C) Dopaminergic neurons in Substantia Nigra

Explanation: Parkinson's disease involves selective destruction of DOPAMINERGIC NEURONS in the SUBSTANTIA NIGRA PARS COMPACTA (SNpc). ROS mechanisms in SNpc: (1) High dopamine turnover → MAO-B → H_2O_2 → Fenton (Fe^{2+} from neuromelanin) → •OH; (2) Mitochondrial Complex I deficiency → $O_2^{\bullet -}$ burst; (3) α-Synuclein oxidation → aggregation → Lewy bodies → mitochondrial dysfunction → more ROS. GSH depletion in SNpc is the EARLIEST detectable biochemical change in PD, preceding neuronal loss.

Q11. The PREDIMED trial demonstrated that which dietary pattern reduces cardiovascular events by approximately 30%?

(A) Low-fat diet

(B) Ketogenic diet

(C) Mediterranean diet supplemented with EVOO or nuts

(D) High-protein Atkins diet

✓ Correct: (C) Mediterranean diet supplemented with EVOO or nuts

Explanation: PREDIMED (Prevención con Dieta Mediterránea) trial (2013; Spain; n=7,447 high CV-risk adults) showed that a MEDITERRANEAN DIET supplemented with EXTRA VIRGIN OLIVE OIL (EVOO) or NUTS reduced major cardiovascular events by ~30% and stroke by ~40% compared to a low-fat diet. This is the most important RCT ever conducted for functional food/dietary pattern and cardiovascular disease prevention. Mediterranean diet components: EVOO, fatty fish, vegetables, whole grains, legumes, nuts, moderate red wine, low red meat.

Q12. Which antioxidant enzyme is specifically deficient or mutated in Amyotrophic Lateral Sclerosis (ALS)?

(A) Mn-SOD (SOD2)

(B) Catalase

(C) EC-SOD (SOD3)

(D) Cu/Zn-SOD (SOD1)

✓ Correct: (D) Cu/Zn-SOD (SOD1)

Explanation: Cu/Zn-SOD1 (cytosolic SOD) mutations cause familial ALS (fALS) — approximately 20% of familial ALS cases. IMPORTANTLY, mutant SOD1 does NOT simply LOSE function — it GAINS a TOXIC FUNCTION (becomes a PEROXIDASE, generating •OH from H_2O_2 in the presence of Cu at the active site). This gain-of-toxic-function → motor neuron damage → ALS. Trisomy 21 (Down Syndrome) has EXCESS SOD1 (on chromosome 21) → H_2O_2 accumulates → early Alzheimer's disease at ~40 years — same gene, opposite problem.

Q13. The primary mechanism by which free radicals initiate cancer is:

(A) Protein carbonylation causing enzyme inactivation

(B) 8-OHdG formation in DNA causing G→T transversion mutations in oncogenes

(C) Lipid peroxidation causing membrane damage

(D) Glycation of histones preventing gene transcription

✓ Correct: (B) 8-OHdG formation in DNA causing G→T transversion mutations in oncogenes

Explanation: Free radicals INITIATE cancer primarily through DNA MUTATION. •OH attacks guanine at C-8 → 8-OHdG (8-hydroxy-2'-deoxyguanosine). 8-OHdG adopts a syn conformation → mispairs with ADENINE (instead of Cytosine) → at next replication: A pairs with T → ORIGINAL G:C → MUTANT T:A = G→T TRANSVERSION MUTATION. This is the most common mutation in human cancers — found in CODON 12 of K-RAS in lung cancer (smoking-related), p53 mutations in multiple cancers. Protein carbonylation and LPO cause disease but are not the primary mechanism of cancer INITIATION.

Q14. BHA (Butylated Hydroxyanisole) is classified by IARC as:

(A) Group 1 (Carcinogenic to humans)

(B) Group 2A (Probably carcinogenic)

(C) Group 2B (Possibly carcinogenic — animal data)

(D) Group 3 (Not classifiable)

✓ Correct: (C) Group 2B (Possibly carcinogenic — animal data)

Explanation: BHA is classified as IARC Group 2B — 'Possibly carcinogenic to humans' — based on animal studies showing forestomach tumours in rats and hamsters at very high doses. These doses are orders of magnitude greater than typical human dietary exposure. At regulated food levels (200 mg/kg or 0.02% fat), BHA is considered GRAS (Generally Recognised As Safe) by FDA. BHT is IARC Group 3 (not classifiable as to carcinogenicity). The IARC classification is important for exams — students often confuse BHA and BHT classifications.

Q15. Which functional food is approved in Germany for treatment of symptomatic diabetic peripheral neuropathy?

(A) Curcumin (Turmeric)

(B) N-Acetylcysteine (NAC)

(C) Alpha-Lipoic Acid (Thioctic acid)

(D) Berberine

✓ Correct: (C) Alpha-Lipoic Acid (Thioctic acid)