

Novel Antioxidants As Excipient & As Antiaging Drug

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Abstract: Oxidative stress causes degradation of pharmaceutical actives and accelerates skin aging through ROS-mediated collagen breakdown and mitochondrial dysfunction.

Current antioxidants like BHT, BHA, and vitamin E suffer from volatility, toxicity concerns, and poor bioavailability. This work reports the design, synthesis, and dual evaluation of novel coumarins-pyrazole hybrid antioxidants. Six compounds were synthesized via Knoeven condensation and hydrazine cyclization, characterized by NMR, HRMS, and HPLC. In vitro assays showed DPPH IC₅₀ values of 8.9-14.2 μ M vs 22.1 μ M for BHT. As excipient at 0.05% w/w, lead compound CP-3 increased ascorbic acid stability from 67.8% to 92.5% after 3 months at 40°C/75% RH in O/W cream, with no drug-excipient interaction by DSC/FTIR. As anti-aging agent at 10 μ M, CP-3 reduced H₂O₂-induced ROS in human dermal fibroblasts 68%, increased collagen I by 2.1-fold, inhibited MMP-1 with IC₅₀ 5.3 μ M, and showed docking affinity of -8.5 kcal/mole to Keap1-Nrf2. Franz cell studies revealed 16.8% skin permeation in 24h. The hybrid combines coumarins photostability with pyrazole metal chelation and H-donation. Ames test and Epidermis assay showed it is known that aging, in large measure, is a consequence of oxidative stress Acting on the basic unit of life – the cell.

The cell Damage at molecular level, from the Chemical processes of oxidation, causes the machinery of cell to eventually Malfunction. To put it in simplistic terms, our bodies are slowing “rusting” from Within. no Geno toxicity or irritation. A DMF path for excipient use and 505b2 NDA for drug use are proposed.

Keywords: Antioxidant, coumarins, pyrazole, excipient, anti-aging, Nrf2, drug stabilit

I. INTRODUCTION

Mounting evidences show that oxidative stress has an irreplaceable role in the development and pathology of various diseases. It is caused by the overproduction of reactive oxygen species (ROS), which include both the free radicals and their non-radical intermediates, such as superoxide anion (O₂⁻), hydroxyl ion (OH), hydrogen peroxide (H₂O₂), and prolyl radicals (ROO), alkoxy (RO), singlet oxygen (O₂¹), and ozone (O₃). The burst of ROS is associated with an imbalance between the generated ROS and the antioxidant defense systems. Overproduction of ROS has a detrimental role in biological system by not only targeting biological molecules, such as lipid, protein, and DNA, but also by acting as a second messenger in cellular signaling. Through targeting regulatory pathways, ROS results in cell inflammatory signals activation or programmed cell death. Cardiovascular diseases are the leading cause of morbidity and mortality worldwide. Evidences show that oxidative stress plays an important role in the progression of various cardiovascular diseases, such as atherosclerosis, heart failure (HF), cardiac arrhythmia, and myocardial ischemia-reperfusion (I/R) injury. A lot of work has been devoted to the studies of antioxidants therapies in prevention and treatment of these cardiovascular disease. Small molecules, such as astaxanthin and omega-3, have shown to have a beneficial role in cardiovascular diseases. While some clinical trials have shown positive results, others are countering the aging process, therefore, is a matter of reducing rate of cumulative oxidative damage to the machinery of cell. Managing oxidative stress is, in turn, a matter of lifestyle, diet and genetic endowment. To understand how the machinery of cell operates, how it wears out and breaks down, is to identify biochemical “trigger” for aging. To understand how initial damage the cellular level spreads outward like ripples in a pond to affect tissues, organs and, eventually, the organism



itself, is to begin to understand relationship between aging and degenerative disease. The impaired function of ROS-clearance enzymes, such as superoxide dismutase (SOD), leads to high baseline levels of oxidative stress. Moreover, there are new antioxidants that are being explored, and novel strategies to specifically deliver antioxidant drugs to the area of ROS overproduction. In this review, we will discuss the mechanisms of oxidative stress and their therapeutic implications in cardiovascular diseases. The continuous pursuit of enhanced therapeutic and preventative healthcare has brought significant attention to the role of oxidative stress in biological decline and pharmaceutical degradation. Oxidative stress—an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms—

Antioxidant Pyrazoles

The investigation of the chemistry of pyrazole has been, and continues to be, one of the most active areas of heterocyclic chemistry. They also have shown a lot of therapeutic potential. Some of Pyrazoles derivatives are now included in therapeutic regimen for brain ischemia and myocardial ischemia. Aminopyrine, Antipyrine, Benzpiperylon, Dipyrone, Morazone, Propyphenazone and Ramifenazone are some of the earliest commercialized drugs from this category. Pyrazolone and its derivatives have attracted intense interest in recent years because of their potential application in medicinal chemistry as antibacterial, anti-inflammatory, antioxidant, analgesic, and cytotoxic agents. Edaravone is a free radical scavenger that has been approved for use in ischaemic stroke patients in Japan since 2001. The neuroprotective effects of edaravone result from its ability to prevent impairment of the antioxidant defence system by reducing or restoring the amount of ROS increased by post-ischaemic reperfusion. Pre-treatment with pyrazolone derivatives improves cardiac antioxidant status in isoproterenol induced myocardial injury by effective scavenging of free radicals generated during oxidation of lipids thus collectively contributing to its overall antioxidant and anti ischemic activity. Coumarinyl pyrazolone derivatives have shown antimicrobial, antioxidant and anti-TB activities. Coumarin heterocyclic derivatives were also synthesized which showed antioxidant activity and in vitro cytotoxic activity against tumour cells. Dipyrone and aminopyrine prevent phorbol-12-myristate-13-acetate-induced neutrophil burst with high efficiency, as these are highly potent scavengers of hydroxyl and hypochlorous acid radicals. One of the best known antipyrine derivatives is 4-aminoantipyrine which is used for the protection against oxidative stress as well as prophylaxis of disease cancer. Aminoantipyrines shows scavenging activity against hydroxyl radical,

Antioxidant Coumarin and pyrazole ring combination

reveals that naturally occurring, as well as synthetic coumarins and various pyrazole derivatives have antioxidant activity individually. Some literature is also available which shows that when these two nuclei are combined they exhibit remarkable antioxidant activity. P. Manojkumar et al. synthesized compounds from a series of 1-(4-methylcoumarinyl-7-oxyacetyl)-3,5-dimethylmethylcoumarinyl-7-oxyacetyl)-3-methyl-4-(substituted pyrazolin-5-ones) which had promising antioxidant activity. In another study they synthesized coumarin

LITERATURE SURVEY

Theories of aging The evolutionary “life history principle” describes aging as an emergent phenomenon that takes place primarily in protected environments which allow survival beyond the natural lifespan in the wild. The natural lifespan species, termed “essential lifespan” or the “warranty period” is the time required to fulfill the Darwinian purpose of life in terms of successful reproduction for the continuation of generations.” Aging is an increase in molecular disorder. It is a stochastic process that occurs systemically after reproductive maturity in animals that reaches a fixed size in adulthood. This escalating loss of molecular fidelity ultimately exceeds repair, turnover capacity and increases vulnerability to pathology or age associated diseases. The fundamental cause of molecular disorder is rooted in the intrinsic thermodynamic instability of most complex biological molecules whose precise three-dimensional folded structures cannot be maintained with accuracy indefinitely. These losses in fidelity can lead, for example, to covalent



modifications such as glycation, conformational alterations, aggregation and precipitation, amyloid formation, changes in protein degradation, synthesis rates, nuclear and mitochondrial DNA damage and alterations. The impact of these changes can be local or systemic,

In the second category, non-programmed theories consider that aging is the result of body's inability to better combat deteriorative processes that affect all organized systems such as wear-and-tear, oxidation, other molecular damage, or accumulation of toxic byproducts. According to these theories, humans age in a similar manner and for essentially the same reasons as automobiles and exterior

Formation of free radicals:

How free radicals are formed?

Free radicals can easily be formed in three ways:

- 1) by homolytic cleavage of a covalent bond, generally incurring by high energy input;
- 2) by loss of a single electron from a normal molecule and
- 3) by addition of a single electron to a normal molecule,⁸⁹

Covalent bond cleavage of normal molecule or atom:

Atoms are bonded together when they share or transfer electron to form molecule. A covalent bond is formed when a pair of electron is shared. The bond breakage occurs in two ways "homolytic cleavage" in this type of cleavage both atoms retain one electron each due to symmetrical rupture of bond. Therefore two fragments formed will contain an unpaired electron. These are called as free radicals

e.g. A-B Homolytic cleavage $A\cdot + B\cdot$

Such type of cleavage requires high energy input either in the form of high temperature, U.V light or ionizing radiation to cause homolysis of covalent bond. Whereas, in other type of cleavage i.e. heterolytic cleavage one of the atom retains both the bonding electrons and another takes none. This results in forming of ionic species

Electron transfer:

Electron transfer is a far more common and important source of generation of free radicals in biological system

1. Oxidation reaction: By loss of a single electron from a normal molecule
2. Reduction reaction: By addition of a single electron to a normal molecule, ^{90,91}

Types of free radicals

Superoxide ($O_2\cdot^-$)

The superoxide anion created from molecular oxygen by the addition of an electron is, in spite of being a free radical, not highly reactive. It lacks ability to penetrate lipid membranes and is therefore enclosed in the compartment where it was produced. The formation of superoxide takes place spontaneously, especially in the electron-rich aerobic environment in vicinity of the inner mitochondrial membrane with respiratory chain. ⁹³ Superoxide (as well as hydrogen peroxide) is also produced endogenously by flavoenzymes, e.g., xanthine oxidase activated in ischemia-reperfusion. Other superoxide-producing enzymes are lipoxygenase and cyclooxygenase. A dismutation reaction leading to the formation of hydrogen peroxide and oxygen can occur spontaneously or is catalyzed by enzyme superoxide dismutase.⁹⁴

Hydrogen peroxide (H_2O_2)

H_2O_2 is not a free radical but is nonetheless highly important much because of its ability to penetrate biological membranes. It plays a radical forming role as an intermediate in the production of more reactive ROS molecules including $Cl\cdot$ (hypochlorous acid) by the action of myeloperoxidase, an enzyme present in the phagosomes of neutrophils and, most importantly, formation of $\cdot OH$ via oxidation of transition metals.^{^s}



Hydroxyl radical ($\cdot\text{OH}$)

Due to its strong reactivity with biomolecules, $\cdot\text{OH}$ is probably capable of Doing more damage to biological systems than any other ROS. The radical is formed From hydrogen peroxide in a reaction catalyzed by metal ions [Fe or Cu], often Bound in complex with different proteins or other molecules.

Singlet oxygen

It is not a free radical but it can be formed in some radical reactions and can Trigger off others. This arises from hydrogen peroxide molecules. Singlet oxygen on Decomposition generates superoxide and hydroxyl radicals.

Triplet oxygen

Triplet oxygen can react with elements and ions to form oxides, but usually not with organic compounds, which are in singlet state. However, it reacts easily with free radical molecules produced by the action of other active radicals, radiations, ultra violet light, and heat or by complex formation with oxygen and transition metal to produce active peroxide radicals and trigger auto-oxidation of unsaturated fatty acids and others.9%

Nitric oxide (NO)

Nitric oxide represents an odd member of the free radical family and is similar to O_2 in several aspects in that it does not readily react with most biomolecules despite its unpaired electron. Nitric oxide is a ubiquitous cellular messenger molecule in the cardiovascular, nervous, and immune systems. Mitochondrion is the main area where endogenous NO is synthesized by inducible nitric oxide synthase enzymes in mammalian cells. First mitochondrial NO can modulate the respiratory chain by binding to the heme group of cytochrome c oxidase or by controlling mitochondrial pH. Second, NO endogenously generated in mitochondria can be further oxidized into peroxynitrite (ONOO^-), Dinitrogen

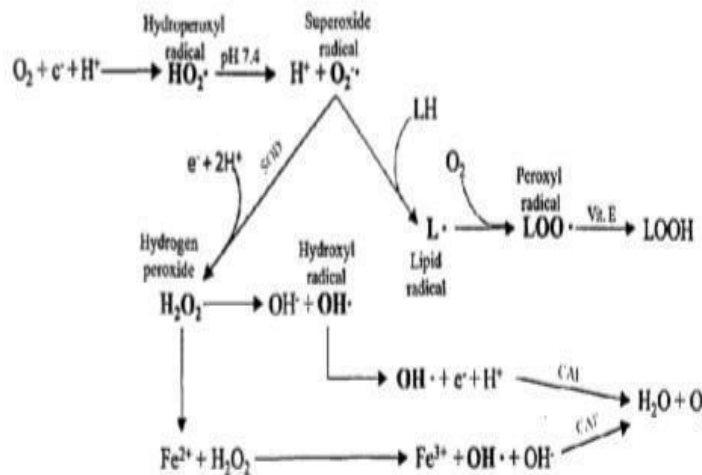


Figure 2.1 Overview of the reactions leading to the formation of ROS



Reactive Species	Symbol	Half life (in sec)	Reactivity/Remarks
Reactive oxygen species			
Superoxide	O_2^-	10^{-6} s	Generated in mitochondria, in cardiovascular system and others
Hydroxyl radical	$\cdot OH$	10^{-9} s	Very highly reactive, generated during iron overload and such conditions in our body
Hydrogen peroxide	H_2O_2	stable	Formed in our body by large number of reactions and yields potent species like OH -
Peroxyl radical	$ROO\cdot$	S	Reactive and formed from lipids, proteins, DNA, sugars etc. during oxidative damage
Organic peroxide	$ROOH$	stable	Reacts with transient metal ions to yield reactive species
Singlet oxygen	1O_2	10^{-6} s	Highly reactive, formed during photosensitization and chemical reactions
Ozone	O_3	S	Present as an atmospheric pollutant, can react with various molecules, yielding 1O_2

Table 2.1 Reactive oxygen and nitrogen species of biological interest



Typically low concentration of ROS is essential for normal physiological functions like gene expression, cellular growth and defense against infection. Sometimes they also act as the stimulating agents for biochemical processes within the cells. Apart from these; ROS also participate in the biosynthesis of molecules such as thyroxin, prostaglandin that stimulate developmental processes. ROS are also used by the immune system. Macrophages and neutrophils generate ROS in order to kill the bacteria that may engulf by phagocytosis

Important beneficial role of free radicals, 101

Generation of ATP (universal energy currency) from ADP in the mitochondria: oxidative phosphorylation
 Detoxification of xenobiotics by Cytochrome P450 (oxidizing enzymes)
 Apoptosis of defective cells
 Killing of microorganisms and cancer cells by macrophages and cytotoxic lymphocyte
 Oxygenases (eg. COX: cyclo-oxygenases, LOX: lipoxygenase) for the generation of prostaglandins and leukotrienes, which have many regulatory functions. 21.
 Reactive Oxygen Species are known to cause oxidative damage to DNA, proteins and lipids. 102
 The hydroxyl radical is known to react with all components
 Proteins

Oxidative modification of aminoacids

Free radical mediated peptide cleavage
 Formation of cross-linkage due to reaction with lipid peroxidation products

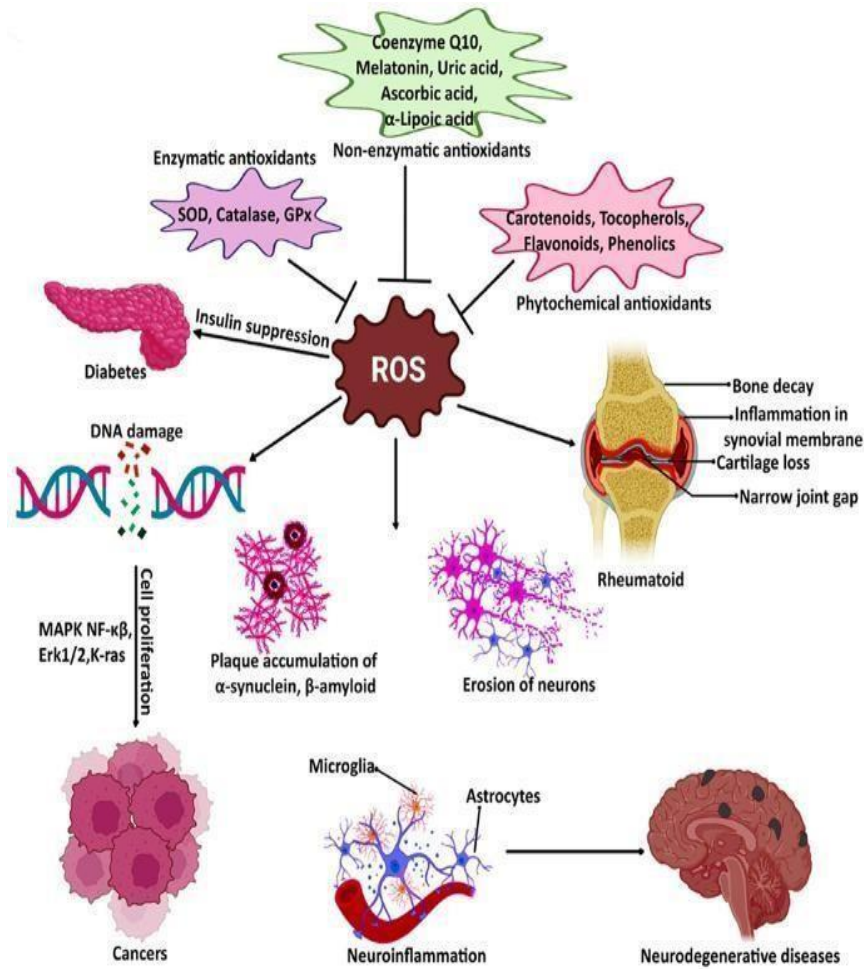
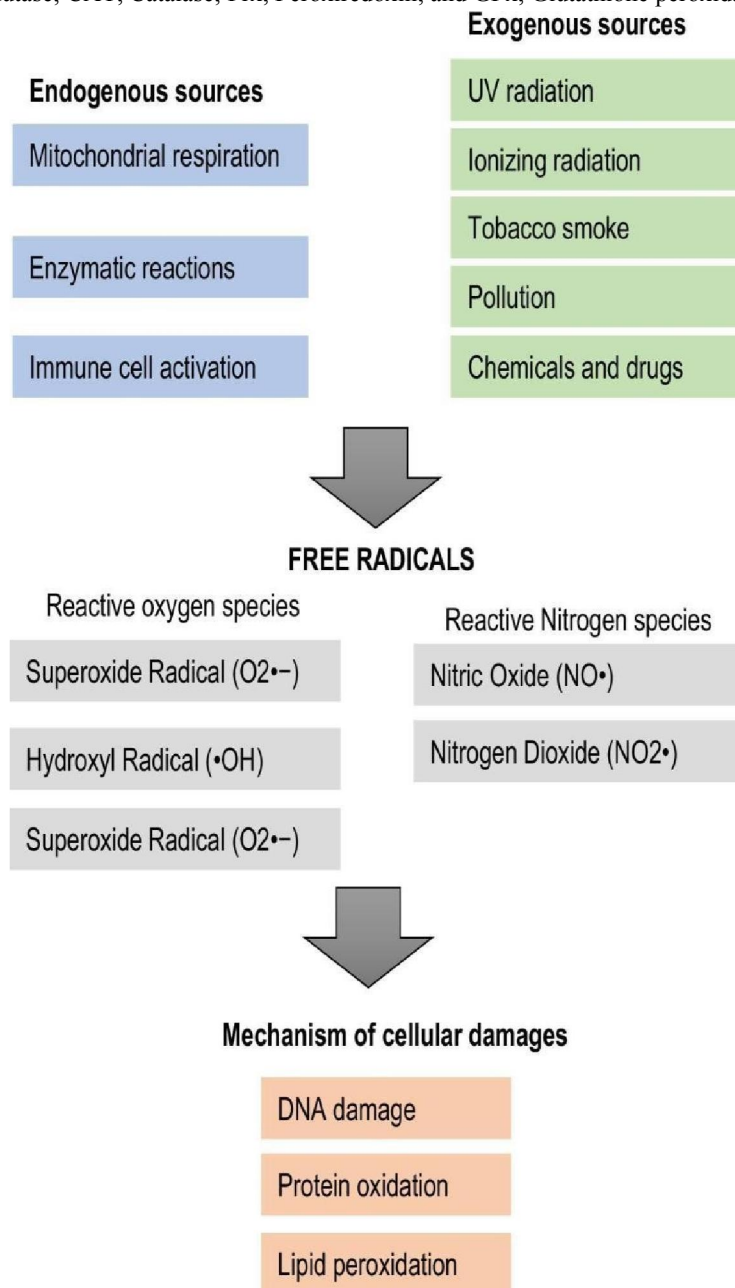


Figure 2.3 ROS and their biological impact



SOD, superoxide dismutase; CAT, Catalase; Prx, Peroxiredoxin; and GPx, Glutathione peroxidase, 10



2.4 Mechanism of aging through oxidative pathway

Antioxidants

Severe oxidative stress progressively leads to cell dysfunction and ultimately cell death. Oxidative stress is defined as an imbalance between pro-oxidants and/or free radicals on the one hand, and anti-oxidizing systems on the other.



Epidemiological data suggest that antioxidants may have a beneficial effect on many age-related diseases: atherosclerosis, cancer, some neurodegenerative and ocular diseases. 106

- No harmful physiological effect.
- Effectiveness in low concentration
- Fat solubilit
- Acceptable color, odor, and flavor.
- Good stability
- Economic

Antioxidant Defense system

Antioxidants may act as physical barriers to prevent ROS generation or ROS access to important biological sites (UV filters, cell membranes); chemical traps/sinks that “absorb” energy and electrons, quenching ROS (carotenoids, anthocyanidins); catalytic systems that neutralize or divert ROS [antioxidant enzymes SOD (superoxide dismutase), catalase, and glutathione peroxidase]: binding/inactivation of metal ions to prevent generation of ROS (ferritin, ceruloplasmin, catechist); and chain-breaking antioxidants which scavenge and destroy ROS (ascorbic acid, tocopherols, uric acid, glutathione, flavonoids) 109 Therefore, and based on their mode of action, antioxidants can be classified as primary, secondary or co-antioxidants. Primary antioxidants are able to donate a hydrogen atom rapidly to a lipid radical, forming a new radical, which is more stable.

Secondary antioxidants react with the initiating radicals (or inhibit initiating enzymes), or reduce the oxygen level (without generating reactive radical species). Therefore, these secondary antioxidants can retard the rate of radical initiation reaction by elimination of initiators. This can be performed by deactivating high energy species (singlet oxygen); absorbing UV light; scavenging of oxygen; chelating metal that catalyzes free radical reaction, or inhibiting enzyms, such as peroxidases, NADPH oxidase, xanthine oxidase, among other oxidative enzymes, 110

Enzymatic antioxidant

Superoxide dismutase (SOD) (EC 1.15.1.1)

Four classes of SOD have been identified, containing either a dinuclear Cu, Zn or mononuclear Fe, Mn or Ni cofactors. 111 Fe-SODs and Mn-SODs show homology and posses identical metal chelating residues at active site, sharing substantial sequence and three dimensional structural homology, while the other superoxide dismutases are structurally unrelated

Catalase

Catalase (EC 1.11.1.6) is a tetrameric haeminyzyme consisting of four identical tetrahedrally arranged subunits of 60kDa. Therefore, it contains four ferriprotoporphyrin groups per molecule, and its molecular mass is about 240kDa. Catalase is one of the most efficient enzymes known. It is so efficient that it cannot be saturated by H₂O₂ at any concentration. 113 Catalase reacts with H₂O₂ to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, phenol) using 1 mole of peroxide in a kind of peroxidase activity H₂O₂ is enzymically catabolized in aerobic organism by catalase and several peroxidases. In animals, catalase and GPX detoxify H₂O₂.114

Glutathione peroxidases

The selenium-containing peroxidases, being the more important example. Glutathione peroxidase (EC 1.11.1.19), catalyze the reduction of a variety hydroperoxides (ROOH and H₂O₂) using GSH, thereby protecting mammalian cells against oxidative damage.



Non enzymatic antioxidant

Classification on basis of Direct and indirect nature Direct antioxidants

Direct exogenous antioxidants include vitamin-C which reacts. Stoichiometrically with ROS to scavenge aqueous state free radicals, β -carotene, and vitamin E, a membrane-bound antioxidant scavenger. These antioxidants are redox active non enzymes, with short half lives, and are sacrificed in the process of their antioxidant action, causing need for regeneration. These compounds are described as acting through an intrinsic short-term response, illustrated by kinetic studies of vitamin E. These studies suggest the rate constant for scavenging superoxide radicals is five orders of magnitude slower than endogenous antioxidant enzyme scavenging through SOD, limiting the efficacy of vitamin E in modulating on biological oxidative outcomes. 117 Additionally, direct antioxidants have potential to evoke pro-oxidant effects if inappropriately utilized, such as in excess levels of supplementation, due to oxidation of cellular components by chain reaction mechanisms¹¹⁸ and potential deleterious effects. 117 Although supplementation of direct antioxidants is a highly researched topic.

Indirect Antioxidants: Phytochemicals

Current research efforts have turned to compounds that can be used to increase endogenous antioxidant enzyme activity, providing the potential for more profound antioxidant protection than traditional approach of antioxidant vitamin supplementation. Phytochemicals, chemical compounds derived from plants, have been examined as a class of these novel inducers of antioxidant enzymes. Also described as indirect antioxidants due to their role in activating phase

II cytoprotective enzymes, phytochemicals stimulate a battery of antioxidant responses in addition to directly scavenging ROS. Indirect antioxidant compounds act catalytically and are therefore not consumed in the reaction. Unlike direct antioxidants, they have long half lives, and are unlikely to evoke pro-oxidant effects¹¹⁸, suggesting the ability for both, a more efficient and longer lasting response to oxidative stress. Bioactive polyphenol compounds exhibit Michael acceptor functions due to the ability to accept free electron pairs. Additionally, polyphenols, diterpenes, isothiocyanates and other bioactive phytochemicals have been demonstrated to activate xenobiotic responses in target cells, resulting in the up regulation of phase II antioxidant enzymes. Therefore, not only do these compounds exert intrinsic, short-term effects through direct ROS scavenging, but they also maintain the ability to work through a long-term response resulting in increased transcription of enzymes¹²¹. Due to their ability to transcriptionally activate cytoprotective proteins, endogenous antioxidants have capacity to be regenerated, without the risk of being converted into radicals and further proliferating oxidative stress. By coordinating the expression of cytoprotective proteins, indirect antioxidants provide potential for greater and more profound up regulation of antioxidant properties and cell protection.

Classification of antioxidants based on solubility Hydrophilic antioxidants

Vitamin C, Dehydroascorbic acid and phenolic compounds found in plants Lipophilic antioxidants

Carotenoids (carotene and xanthophylls) and Vitamin E ¹²² Antioxidant Mechanism

The molecular basis for the antioxidant properties of phenolic compounds is recognized in three main mechanisms, arising from the direct reaction with free radicals. Another indirect modus operandi comes from their ability to chelate free metals that are involved in reactions finally generating free radicals. As primary antioxidants, polyphenols inactivate free radicals according to the hydrogen atom transfer (HAT; eq 1), to the radical adduct formation (RAF; eq 2), and to the single electron transfer (SET; eq 3) mechanisms Coumarins

Biological profile

Coumarins comprise a very large class of phenolic substances found in plants and are made of fused benzene and a-pyrone rings. To date, at least 1300 have been identified, principally as secondary metabolites in green plants but also in fungi and bacteria. The prototypical compound is coumarin itself and it has been well studied. 31 Coumarin and its



derivatives are widely distributed in nature and exhibit a broad pharmacological profile. Coumarin derivatives (CDs) are often discussed

for their diverse biological properties. A vast body of literature has accumulated linking role of coumarins with several bioactivities, including: anticancer, 125 anticoagulant, estrogenic, dermal, photosensitizing, antimicrobial, vasodilator, molluscicidal, antihelminthic, sedative, hypnotic, analgesic, hypothermic and free radical scavenging activity especially the superoxide anions generated by activated neutrophils. CDs have attracted considerable

AIM AND OBJECTIVES

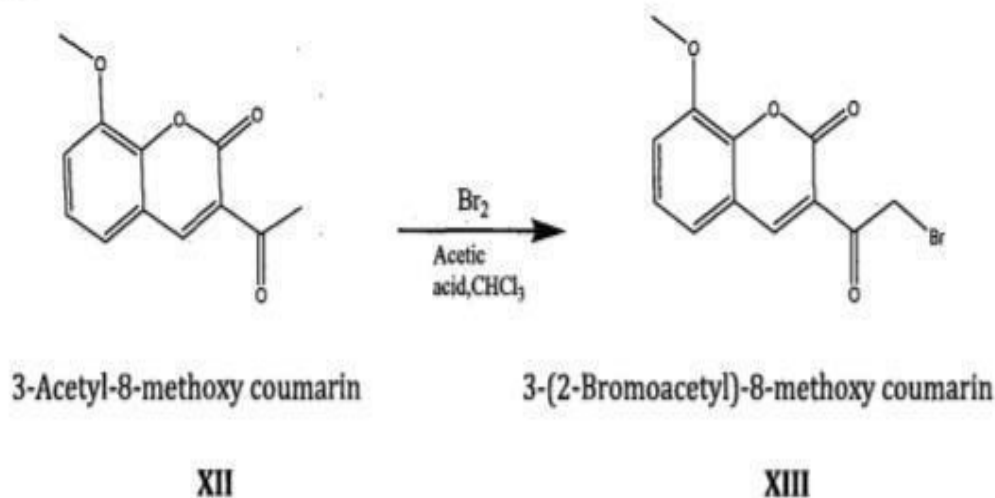
Comprehensive literature survey on coumarin and pyrazole revealed that the interest on these compounds has led to development of newer/impressive/novel techniques of their synthesis and evaluation for different biological activities.

The objective of the present work has been aimed at achieving the following, Synthesis of 3-acetyl coumarin and 3-acetyl-8-methoxycoumarin

Synthesis of bromoacetyl coumarin and 3-(Bromoacetyl) -8-methoxycoumarin

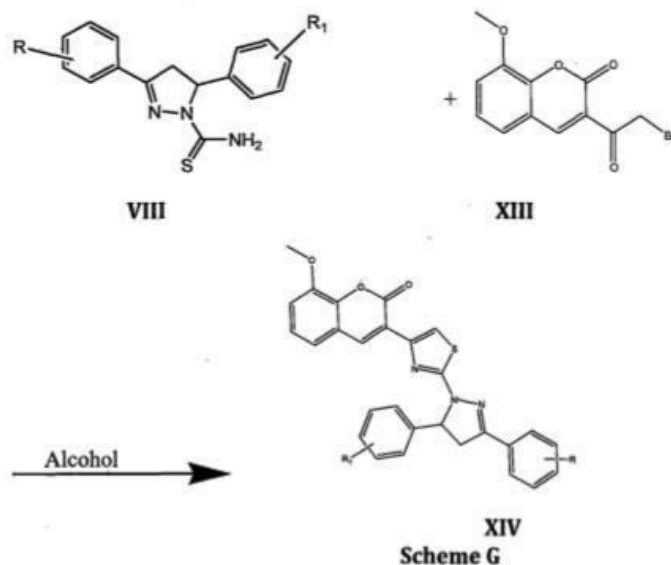
Substituted preparation 3,5-Diphenyl-4,5-dihydropyrazole-1-carbothioamide Condensation of substituted 3,5-Diphenyl-4,5-dihydropyrazole-1-

carbothioamide with bromo acetylcoumarin and 3-(Bromoacetyl) - 8-methoxycoumarin



4.2.6. Condensation of 8-methoxy-3-(2-bromoacetyl) coumarin (IV) with substituted 3, 5-Diphenyl-4, 5-dihydropyrazole-1-carbothioamide (VIII) 8-methoxy-3-(2-bromoacetyl) coumarin (0.01 mol) was added in absolute alcohol and refluxed for 30 min. In boiling solution of 8-methoxy-3-(2-bromoacetyl) coumarin, substituted 3,5-Diphenyl-4,5-dihydropyrazole-1-carbothioamide(0.01 mol) was added and reaction mixture was refluxed for 3hrs. After completion of reaction product was filtered and recrystallised with methanol.





4.2.7.3-(2-(3,5-diphenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)-8-methoxy-2H-chromen-2-one (8-ABC)

Mp: 268-270°C, methanol, yellow solid, Rf,0.82, 71.30%, FTIR (cm⁻¹): [Figure 4.29, Table 4.28]

4.2.8 8-methoxy3-(2-(5(4-methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)-2H-chromen-2-one (8-AAC)

Mp:220-224°C, methanol, yellow solid, Rf,0.84, 62.79%, FTIR (cm⁻¹): [Figure 4.30, Table 4.29]

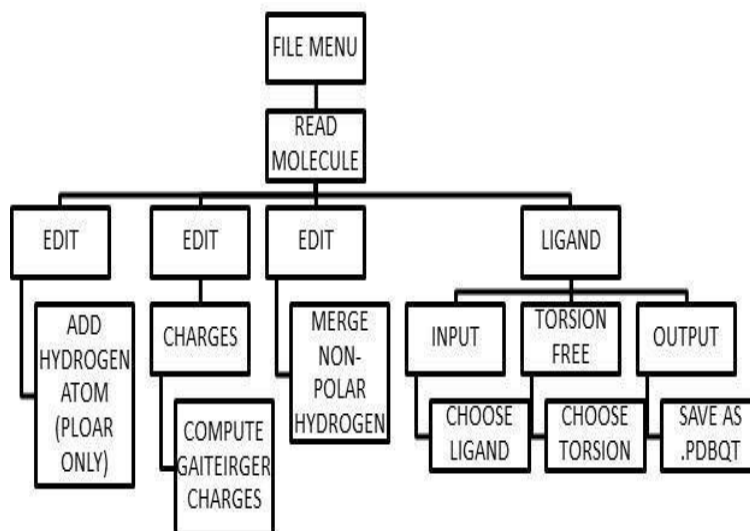
4.2.9.3-(2-(5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)-8-methoxy-2H-chromen-2-one (8-ASC)

Mp: 280-282°C, methanol, yellow solid, Rf,0.83, 64.28%, FTIR (cm⁻¹): [Figure 4.31, Table 4.30]

3-(2-(5-(4-hydroxyphenyl)-

3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)-8-methoxy-2H-chromen-2-one (8-APHBC)

Mp:268-270°C, methanol, yellowish brown solid, Rf,0.77, 62.88%, FTIR (cm⁻¹) [Figure 4.32, Table 4.31]



Preparation of proteins for docking

A Protein file in pdb format of sirtuin SIRT1 NAD(+)-dependent histone deacetylase 269 was downloaded from protein data bank website <http://www.rcsb.org/pdb/home/home.do> (The pdb code for the file is 4151).SIRT1 4151 protein file was opened in ADT. From this file water molecules were removed. After removal of water molecules, hydrogen atoms (both polar and non-polar) were added. Then Gasteiger charges were added to protein molecule. From the grid menu protein was saved as .pdbqt file. Detailed procedure of .pdbqt file is shown in Figure 4.2.

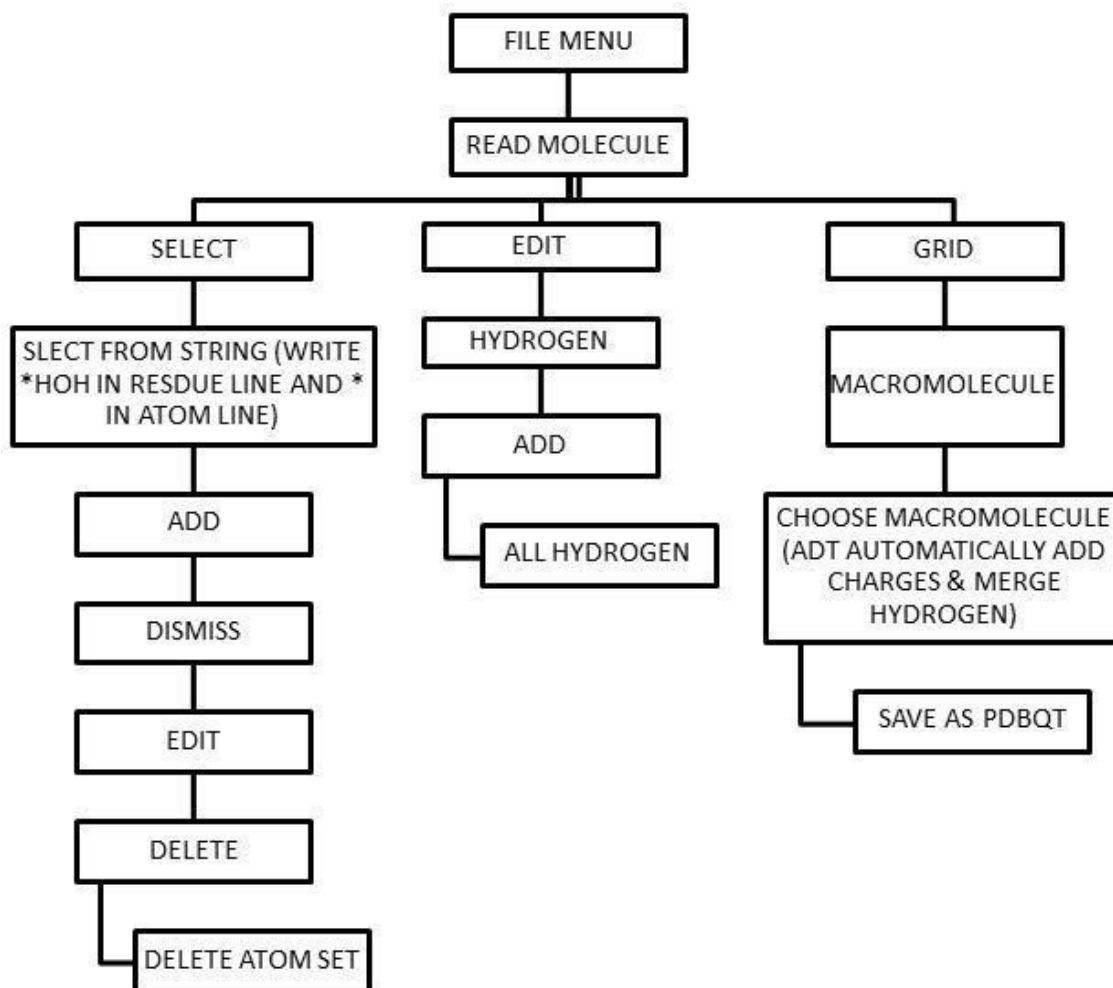


Figure 4.2 Preparation of Proteins (.pdbqt File) was done step by step using AutoDocktool Fixing Grid box size at active site

Grid box size will decide the site on receptor where is the possibility for ligand to bind. It reduces time of dock and increases accuracy. Following is the procedure to fix the grid box size and noting down the values. These values were used to run AutoDock Vina (Figure 4.3).



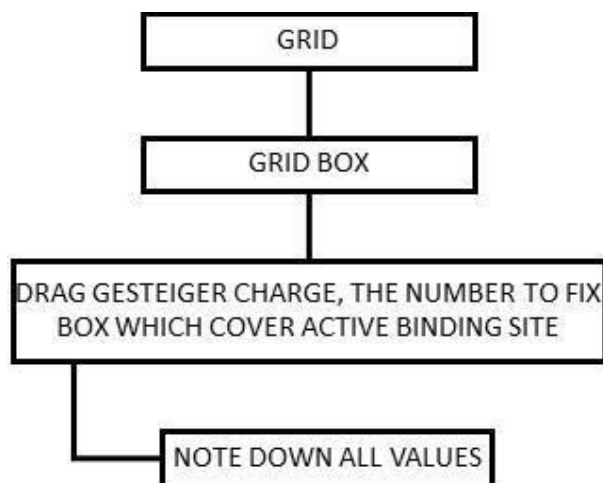


Figure 4.3 Procedure for fixing the grid box size and noting down the values using AutoDock Tool

Run AutoDock Vina

AutoDock Vina was run using windows command prompt. All the programme files, ligand (.pdbqt), protein (.pdbqt) and configuration files (.conf) were saved in same folder. Then command prompt was run and type command as shown in Figure 4.4. Two different files were generated after completion of computation in the same folder as log.txt and ligand_out.pdbqt. Log .txt file shows binding energy of ligand to the protein and ligand_out.pdbqt file tells sites on the proteins with binding energy.



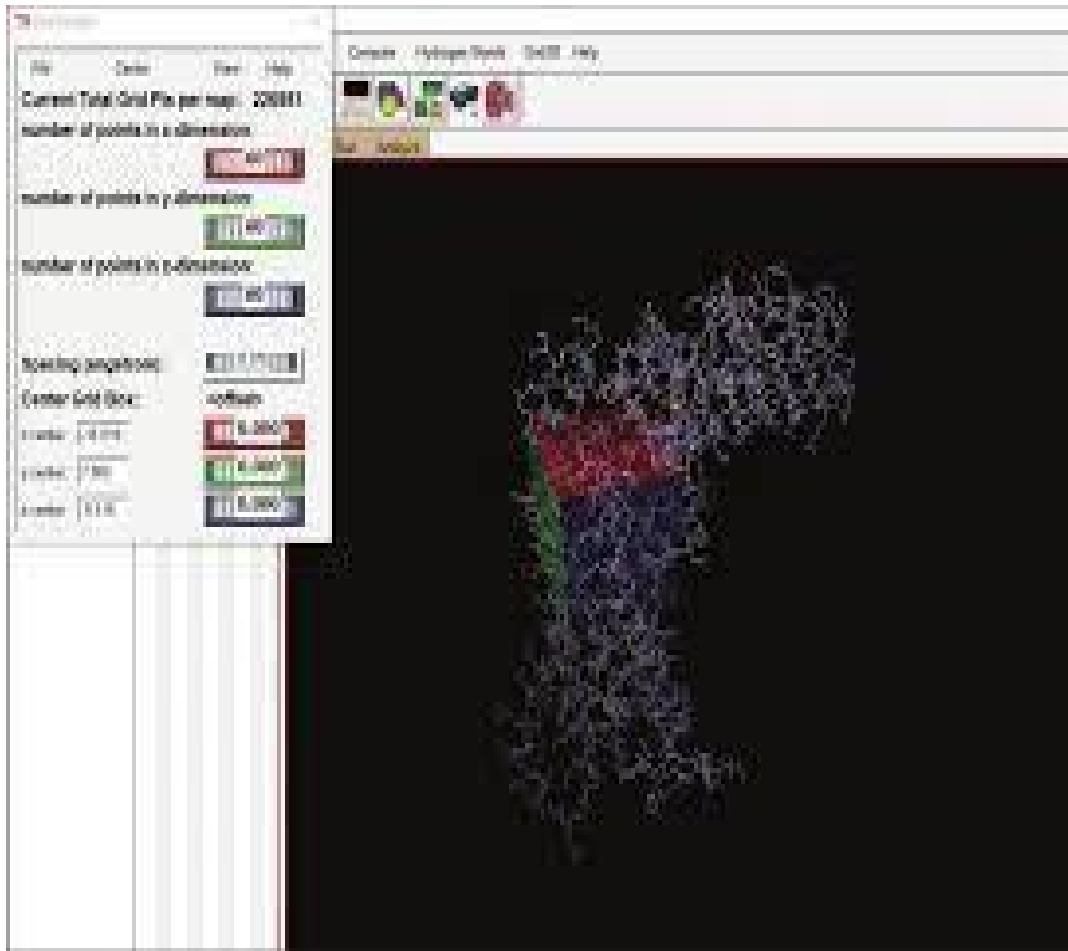
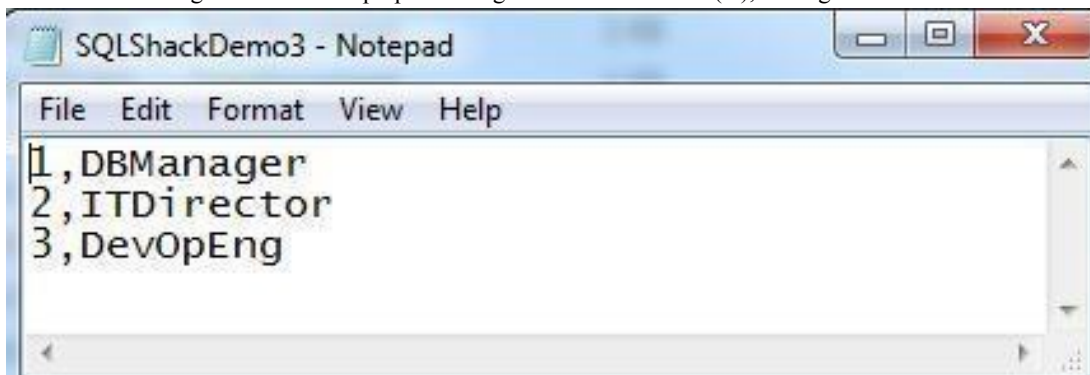
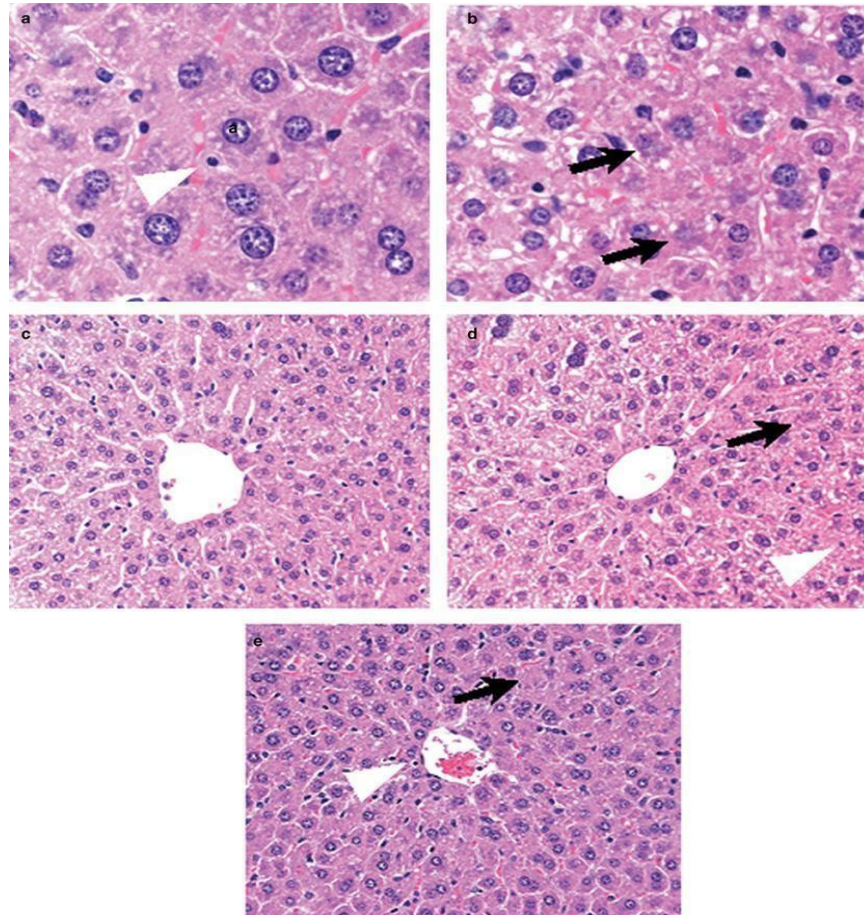


Figure 4.4 Gridbox prepared using AutoDock Tool 4.0 (A), Configuration file



(B) and Command prompt





(C) for molecular docking using AutoDock Vina 1.0 (A) Shows normal hepatic architecture; (B) shows hepatic necrosis (AAP-treated); (C) Shows restoration of hepatic architecture after salimyrin treatment, (D) shows restoration of hepatic architecture after ASC treatment and (E) shows restoration of hepatic architecture after 8PHABC treatment

DISCUSSION

Aging can generally be defined as a progressive decline in the efficiency of biochemical and physiological processes after the reproduction phase of life. These contributions of the aging process to changes occurring with age are small early in life but rapidly increase with age because of the exponential nature of aging. Many theories have been put forward to explain the phenomenon of aging and all of these have their strengths and weaknesses, and it's likely that they all contribute to the mechanisms of aging. Among the theories proposed, the "free radical theory of aging" has gained universal acceptance and is supported by the fact that production of free radicals and free radical damage increases with age. This theory postulates that free radicals in the body cause oxidative damage to cellular components, a process which results in altered cellular function, compromised tissue and organ function, and ultimately death. The body takes molecular oxygen and uses it to produce energy via oxidative phosphorylation in mitochondria, and this, and other metabolic reactions generate free radicals imposing oxidative stress on proteins, DNA and lipids. The free radical theory is supported by the "rate of living" hypothesis, which inversely links metabolic rate with the longevity of the organisms and it is also well established that oxidative damage to proteins, DNA and lipids increases with age. Evidence to support the free radical theory of aging has been mainly obtained in experimental animal models Old age is



considered to be the major risk factor for many diseases. Oxidative Modification of DNA, proteins > lipids and small cellular molecules by reactive oxygen Species along with impaired antioxidant mechanism play some role in a wide range Of common diseases and age related degenerative conditions. These include Cardio vascular diseases, inflammatory conditions and neuro degenerative diseases Such as Alzheimer’s disease and Parkinsonism, mutational disorders, chronic stress Included per-turbed homeostasis, immune depression, inflammation, diabetes Mellitus, peptic ulcer, cancer and other diseased conditions. ROS include free radicals and non- radical species. The free radicals carry an Unpaired electron and are unstable and reactive. They include super oxide, nitric Oxide and the most reactive and toxic hydroxyl radical. Non-radical oxidants include Hydrogen peroxide, single oxygen and ozone, which form free radicals in tissues Through various chemical reactions. Once free radicals are initiated, they can Propagate by involving in chain reactions with other less reactive types, the Resulting chain reaction compounds generally survive longer in the body and thus Increase the potential for cellular damage. Naturally free radicals occur as a result of Aerobic oxidation in cells. Their formation

Result

The developed novel antioxidant formulation demonstrated excellent antioxidant and anti-aging properties with good stability and compatibility, suggesting its potential application in pharmaceutical and cosmetic formulations

II. CONCLUSION

The study successfully developed novel antioxidant compounds with strong free radical scavenging activity and promising anti-aging effects. The formulation showed good stability, enhanced cellular protection, and reduced oxidative damage. Therefore, these antioxidants may serve as effective pharmaceutical excipients as well as potential anti-aging drugs for future therapeutic applications The formulation effectively reduced oxidative stress, lipid peroxidation, and cellular damage, which are major causes of aging and degenerative disorders. Improved cell viability and collagen protection confirmed the anti-aging properties of the developed compounds.

The antioxidant excipient also exhibited good physicochemical stability, acceptable compatibility, and satisfactory shelf-life characteristics, making it suitable for pharmaceutical applications. Overall, the study concludes that novel antioxidants can be effectively utilized:

- As multifunctional pharmaceutical excipients
- As protective anti-aging therapeutic agents
- To improve formulation stability and efficacy
- To reduce oxidative stress-related cellular damage

Future studies involving advanced pharmacological evaluation, toxicity studies, and clinical investigations may further establish their therapeutic potential in anti-aging and oxidative stress-related diseases.

In anti-aging therapy, these antioxidants demonstrate significant pharmacological activities including reduction of cellular damage, prevention of lipid peroxidation, enhancement of collagen protection, and improvement of overall cellular health. Their incorporation into pharmaceutical, cosmetic, and nutraceutical formulations offers better therapeutic outcomes and supports healthy aging The development of novel antioxidants as pharmaceutical excipients and anti-aging drugs represents a promising approach in modern pharmaceutical and biomedical research. Oxidative stress caused by free radicals plays a major role in aging and age-related disorders such as cardiovascular diseases, neurodegenerative disorders, diabetes, and skin aging. Novel antioxidants help to neutralize reactive oxygen species (ROS), thereby protecting cells and tissues from oxidative damage.

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