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Neuroprotective Role of Thymoquinone in Alzheimer's Disease Models: Mechanisms and Drug Design Perspectives

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects millions of people worldwide and is one of the leading causes of dementia in the elderly. It is mainly characterized by memory loss,behavioral changes, and a steady decline in cognitive functions. The underlying causes of AD are complex and involve multiple biological pathways such as oxidative stress,chronic inflammation, accumulation of amyloid-beta (Aß) plaques, tau protein tangles, mitochondrial dysfunction, and neuronal apoptosis. Because current treatments only provide symptomatic relief without effectively halting disease progression, there is an urgent need for novel, multi-targeted therapeutic agents.Thymoquinone (TQ), the main bioactive compound derived from the seeds of Nigella sativa (commonly known as black cumin),has emerged as a promising natural molecule with strong neuroprotective potential. TQ possesses potent antioxidant,anti-inflammatory, and anti-apoptotic properties that make it highly valuable in neurodegenerative research. Its antioxidant effect comes from its ability to neutralize reactive oxygen species (ROS) and enhance the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Through these mechanisms, TQ helps to maintain redox balance and prevent brain cells.In addition, TQ exhibits significant anti-inflammatory effects by reducing pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-a), interleukin-1ß (IL-1ß), and cyclooxygenase-2 (COX-2). By suppressing these inflammatory pathways,TQ protects neurons from damage caused by chronic neuroinflammation - one of the key contributors to AD pathology. Experimental studies have also shown that TQ inhibits the aggregation of amyloid-beta peptides,reduces acetylcholinesterase activity, and improves cholinergic transmission, leading to better memory and learning performance inanimal models of Alzheimer's disease.

Keywords

Nigella sativa (black cumin), Thymoquinone (TQ),Neurodegenerative disorder, Alzheimer's disease (AD), Computer Aided Drug Design, Molecular Docking .Chronic inflammation, Amyloid-beta (Aß) plaques, Alzheimer's disease models.

1. Introduction

The severity of cognitive impairment in patients with AD varies. The earliest manifestations can be a subjective decline in mental abilities in the absence of impaired performance on objective cognitive

testing. Mild cognitive impairment (MCI) refers to the earliest symptomatic stage of cognitive impairment in which a single cognitive domain or, possibly, multiple cognitive domains are impaired to at least a mild extent whilst functional capacities are relatively preserved. By contrast, dementia is defined as cognitive impairment of sufficient magnitude to impair independence and affect daily life. Dementia of gradual onset and ongoing progression with prominent amnestic symptoms and signs is the prototypical clinical phenotype of AD3. AD was originally considered a clinicopathological entity, meaning that, if the patient fulfilled the clinical syndrome of an amnestic dementia and other conditions were ruled out, one could assume that AD pathology was the cause. However, increased clinical sophistication together with biomarkers of AD, namely cerebrospinal fluid (CSF) and PET markers for Aβ and tau, has transformed the concept of AD to a neurobiological condition that affects different aspects of cognition. Of note, there is a greater appreciation of the relationship between AD and other aetiologies of cognitive impairment. Although multiaetiology dementia (which is the preferred term over 'mixed dementia') is not the focus of this Primer, it is important to remember that AD pathology rarely occurs in isolation in patients >65 years of age. [1]

Thymoquinone (TQ) is a natural compound found mainly in black cumin seeds (Nigella sativa), which has been used traditionally as a remedy for many ailments. Here's a simple explanation of what thymoquinone can do for the body:Thymoquinone is a powerful antioxidant, meaning it helps protect our cells from damage caused by harmful molecules called free radicals[2]. It reduces inflammation in the body,which is important because chronic inflammation can lead to diseases like arthritis, heart disease, and more.Studies show that TQ may help reduce joint pain and swelling, similar to some over-the-counter anti-inflammatory drugs but with fewer side effects. It supports the immune system by boosting the activity of important immune cells that fight infections andviruses.TQ has shown promise in protecting organs like the liver, kidneys, heart, and brain from damage.It has been studied for its potential benefits in fighting certain cancers by slowing tumor growth and aiding the body's natural cancer-fighting processes. Researchers are exploring TQ for its ability to help in managing conditions like asthma, diabetes, and even viral infections such as COVID-19. Because it is natural and gentle, black seed oil containing TQ is sometimes used as a supplement for general health and wellness.[3]Although the knowledge of the exact pathophysiological mechanisms remains an unresolved issue, an emerging evidence underlines the role of the oxidative damage and microglia-mediated neuro-inflammatory responses in the initiation of neurodegenerative disorders including Alzheimer's disease (AD) (Mosher and Wyss-Coray, 2014). Experimental findings demonstrated that in AD pathogenesis, the 4.2-kD amyloid β peptide (Aβ)-dependent microglial activation leads to neuronal injury through a complex cascade by involving the secretion of various pro-inflammatory molecules such as tumor necrosis factor-α (TNF-α), interleukin (IL) IL-6, IL-1β, reactive oxygen species (ROS), and reactive nitrogen species (NOS) (Agostinho et al., 2010). In turn, the neuroinflammation and oxidative stress processes are responsible for the impairment of the neurovascular working leading to an axonal demyelination, local hypoxia–ischemia, and to restoring white matter damages (Iadecola, 2010).[4]

Fig 1 :-Drug Discovery of Tymoquinon

- **Alzheimer's**

Alzheimer's disease is a slowly progressive brain disorder that affects a person's memory, thinking, and ability to carry out simple daily activities. It is the most common cause of dementia among older adults. The condition develops gradually and worsensover time, eventually leading to severe memory loss and personality changes.Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disorder that primarily affects memory, learning, and behavior. It is the most common cause of dementia, accounting for around 60-70% of all dementia cases globally. The disease was first described by Dr. Alois Alzheimer in 1906, when he identified abnormal protein deposits and tangled nerve fibers in the brain of a woman who had suffered from memory loss and confusion.Over time, Alzheimer's causes shrinkage of brain tissue (especially in the hippocampus and cerebral cortex), leading to irreversible loss of cognitive and physical abilities.

Inside the brain, two abnormal proteins play a major role:

. Amyloid plaques - clumps that form between nerve cells and block communication.

. Tau tangles - twisted fibers that form inside brain cells and working properly.

Natural and Herbal Remedies Nigella sativa (Black Seed): Acts as an antioxidant and protects nerve cells.

Modern Research and Drug Discovery New approaches use computer-aided drug design (CADD) to find better treatments faster:

. Molecular Docking: Finds molecules that can block harmful proteins.

. QSAR Modeling: Predicts how chemical structures affect activity.

. Virtual Screening: Tests thousands of compounds quickly using Al tools.

These computational techniques save time, money, and reduce the need for animal testing.

1.Pathophysiology

1. Formation of Amyloid Plaques(Aß): Abnormal buildup of ß-amyloid peptides between neurons disrupts communication and triggers immune response.

2. Neurofibrillary Tangles: Tau proteins inside neurons become twisted and tangled, preventing nutrients from moving through the cell.

3. Neuroinflammation: Activated microglia and astrocytes release inflammatory cytokines that worsen cell damage.

4. Neuronal Death and Brain Atrophy: Over time, neurons die, causing brain shrinkage-especially in memory-related areas like the hippocampus.

The neuro-pathology of AD can be described by the accumulation of hyper-phosphorylated tau proteins containing neurofibrillary tangles (NFTs) and amyloid beta (Aβ) containing plaques with degenerating and frequently swollen axons, neuritis and glial cells . The pathophysi ology of AD is complex involving disturbances and imbalances occurring in a variety of mechanisms including amyloid beta plaque formation, hyper-phosphorylated tau protein aggregation, disturbance in the ho meostasis of calcium and other metal ions, lowered expression of BDNF, insulin resistance, low choline uptake (Fig.2). Apart from this, there are some other pathways in which AD mediates its severity by neuronal loss and memory decline.[27]

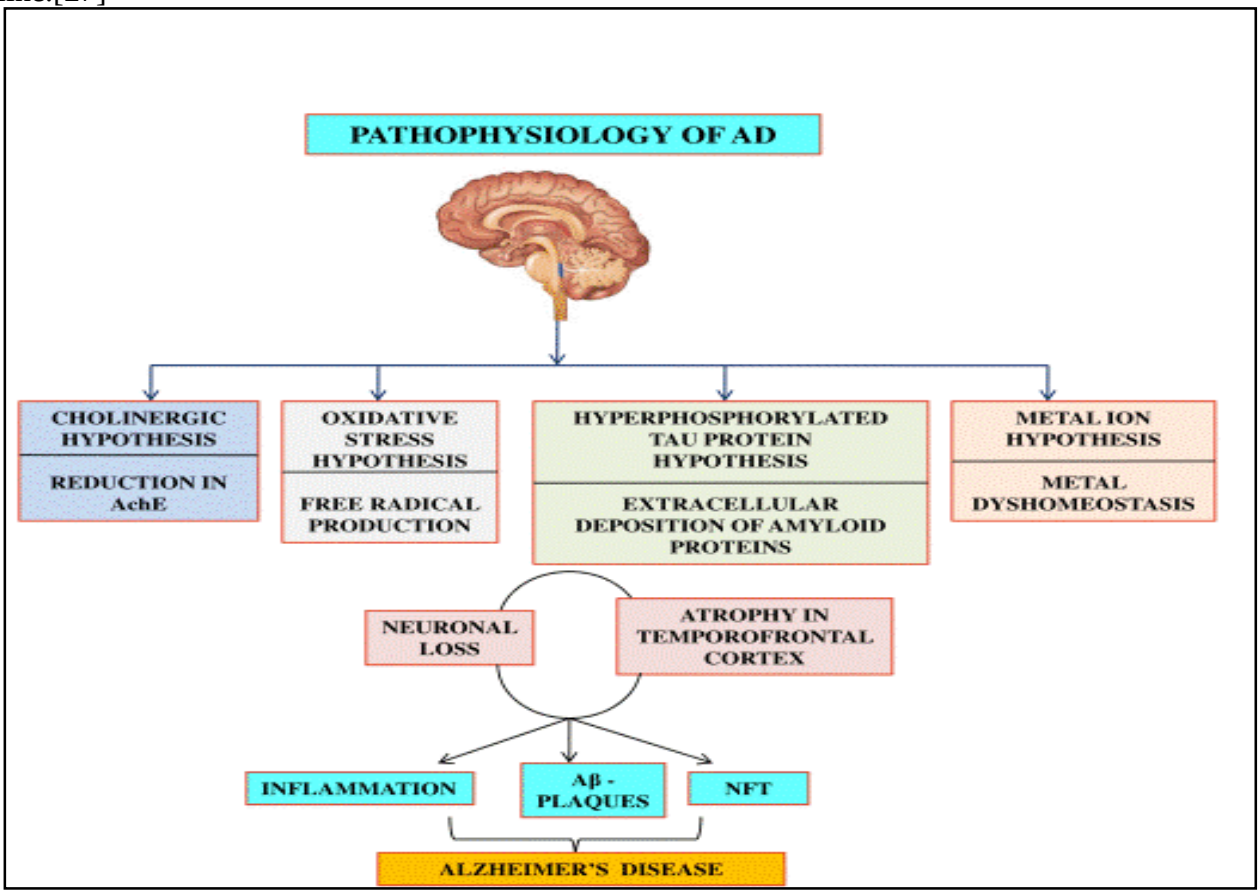

Fig 2:-Pathophysiology Of Alzhemer Disease

1.1. Amyloid β pathway of protein aggregation

AD is characterized by malfunction of amyloid precursor protein (APP) processing pathway which leads to the formation of amyloid proteins generating plaques. The proteolytic processing for amyloid peptides occurs in a chronological manner through two pathways-the α pathway and the β pathway [28]. The α pathway is conducted by cleavage with alpha secretase and produces sAPP, soluble fragment which functions in regulation of enhancing synaptic plasticity, neuronal excitations, protection of neurons from oxidative and metabolic stresses and improvement of learning and memory. This is the non-amyloid genic pathway as it occurs under normal physiological state. On the contrary, in the β path way in the neuropathological state, an additional 99-amino acid fragment is generated which is further cleaved by gamma secretase to form soluble Aβ(1–40) or insoluble Aβ(1–42) peptides leading to senile plaque production . Comparative study between ADpatients and normal individuals reported higher levels of Aβ (1–40) (>60–70%) than Aβ(1–42) (~15%) in normal individual's brain [29]. This insoluble Aβ (1–42) peptides exert an opposite effect to that of the sAPP causing synaptic degradation, reduced neuronal plasticity, alternation in energy metabolism pathway and oxidative stress with mitochondrial dys functions . This Aβ overproduction is also accompanied with insulin degrading enzyme down-regulation (IDE), in the common non-autosomal dominant forms of AD, which has the function of degrading Aβ [30]

1.2. Phosphorylation of the tau proteins and destabilization of microtubules

Along with APPs, tau proteins play a role in AD pathogenesis by bringing alternations of neurofibrillary degeneration . These tau proteins are robustly expressed disorganized proteins encoded by microtubule associated protein tau (MAPT) gene on the 17q21 human chromosome and are engaged in the activity of facilitating intra cellular transport, preservation of the integrity of cellular structures as well as neurite outgrowth and their formation processes . Insoluble forms of fibrillary tau may exert its neurotoxic effect as a result of hyperphosphorylation which makes it resistant to calcium activated proteases, calpains and the ubiquitin-proteasome pathway . After hyperphosphorylation of these proteins, they segregate from the micro tubules and keep burgeoning up in the somatodendritic neuronal regions and eventually forms NF. These NFTs remain organized in paired helical filaments showingtheir emergence in thelaterstagesof AD. Moreover, studies demonstrated that hyperphosphorylated tau are capable of disrupting neuronal and synaptic functions as well as sup pressing pre-synaptic protein expressions even before their deposition in the form of NFTs . Besides these, a number of studies depicted the phosphorylated forms of these proteins to be responsible for amyloid induced neurotoxicity and cognitive deterioration [31, 32].

Drug :- Thymoquinone

2.Chemistry of thymoquinone

TQ (IUPAC name of 2-isopropyl-A 5-methylbenzo-1, 4-quinone) (Fig.9) is a plant-derived, potential bioactive monomer compound (about 30–48%)inthevolatileoil ofNS seedswiththemolecularformula C10H12O2 and molecular mass of 164.20 g mol-1. Chemically, NS contains 30% fixed oils (mainly fatty acids), 0.40–0.45% volatile oils, vitamins, amino acids, proteins, carbohydrates, alkaloids, saponins, crude fiber, and minerals. The oil part is enriched in polyunsaturated fatty acids (PUFA), phytosterols, TQ, carvacrol, t-anethole, sesquiterpene longifolene and terpinen-4-ol [33]. The concentration of TQ in the oil has been reported to be 18–25 μg/mL . Due to the presence of a basic quinone ring moiety, TQ exhibits keto enrol tautomer's in which the keno-version is the major form showing pharmacological capabilities

such as antitussive, gastro protective, anti-inflammatory, ant nociceptive, antihistaminic, antibac terial, anthelmintic, antioxidant, immunomodulatory, anticancer, hepatoprotective, cardioprotective, antidiabetic, ototoxicity protective, and nephroprotective effects. It also provides neuroprotective ef fects by reversing memory impairments and enhance cognitive func tioning as well as attenuate drug tolerance and dependence. It exhibits low stability in aqueous solutions particularly in alkaline pH and possess a solubility range of 549–669 µg/mL in all aqueous solutions [34] (Fig.3)

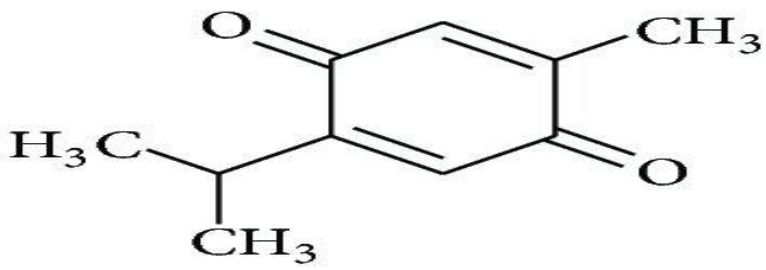

Fig 3 :- Chemical structure of thymoquinone

3. Effects of Thymoquinone in Alzheimer's Diseas

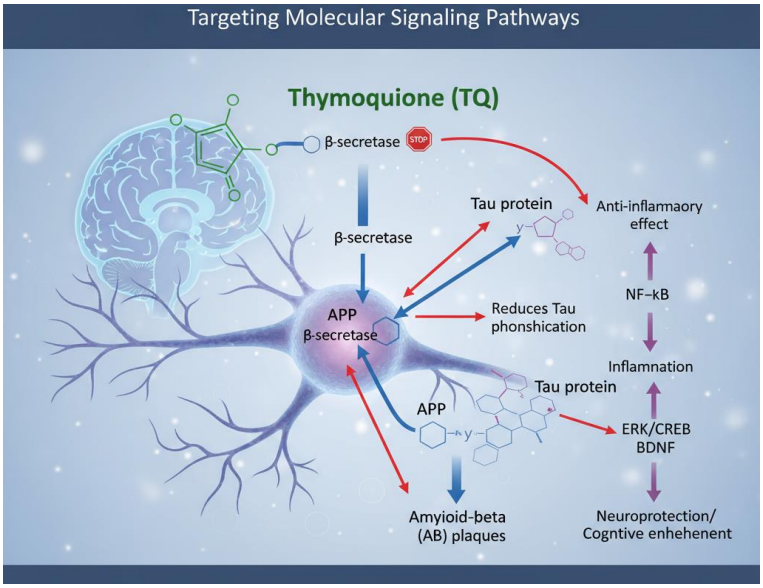

Fig 4 :-TQ Targeting Molecular Signaling Pathaway

3.1. Anti-inflammatory effect

The anti-inflammatory activity of TQ is mediated through the Toll like receptors (TLRs) which are transmembrane proteins possessing an extracellular leucine-rich domain followed by an intracellular toll interleukin-1-receptor (TLR) domain . The activation of these re ceptors trigger two separate downstream

signaling pathways one of which includes the Myeloid differential factor 88 (MyD88), where TLRs dimerization stimulates NF-kB in order to generate pro-inflammatory cytokines such as TNF- α and IL-1,6,7 . NF-kB is primarily associ ated with the CNS microglia and its activation causes nuclear trans location of p65 subunit followed by its phosphorylation, acetylation and methylation, as well as DNA binding and gene transcription. Afterwards, the activated NF-kB binds to particular DNA sequences of these target genes for neuro inflammatory cytokine production [35]. TQ in hibits nuclear translocation of NF-kB which subsequently blocks the production of NF-kB mediated neuroinflammatory cytokines . In the other pathway, there occurs the activation of interferon regulatory factor-3 (IRF-3) which causes multiple Type-1 interferon inducible genes formation by utilizing the TIR-domain-containing adapter-inducing interferon-β (TRIF) pathway independent of MyD88 [36] . Exhibiting the anti-inflammatory effect, TQ administration at different doses (10, 20, 40 mg/kg) significantly down-regulated the mRNA expression of TLR-2, TLR-4, MyD88, TRIF and their downstream effectors Interferon regulatory factor 3 (IRF-3) and NF-kB which conse quently caused a decline in protein levels of the pro-inflammatory cy tokines such as IL-1B and TNF-α [19]. Similarly, TQ also inhibits LPS induced pro-inflammatory cytokine release like IL-1B, IL-6 and IL-12 p40/70 via its interaction with NF-kB [37].(Fig.4)

3.2. Antioxidative effect

Oxidative stress plays a prominent role in AD pathogenesis of cognitive impairments. Any means of chronic cerebral hypoperfusion generates the formation of free radicals which wanes the antioxidant defense mechanism, primarily superoxide dismutase (SOD), thereby leading to neuronal degeneration and death. In a study conducted on male Wistar rats with cerebral hypoperfusion leading to learning and memory impairments, TQ prominently mitigated hippocampal lipid peroxidation and improved SOD activity . Moreover, the NS oil caused an improvement in the spatial working performance on a radial arm maze model and subsequently developed the spatial cognition ac tivity with global cerebrovascular hypoperfusion [38]. TQ is a strong hydrogen peroxide, hydroxyl scavenger and lipid peroxidation inhibitor with a percent inhibition value of 79.5 2.12%, whereas it possesses a low antioxidant activity against 2,20-diphenyl-p picrylhydrazyl radical (DPPH) and 2,20-azino-bis(3-ethylbenzothiazo line-6-sulfonic acid) (ABTS) radicals which is attributed to its chemical structure providing the site for the free delocalization of electrons as well as Hþ donation for the radical scavenging action. It exhibits a potential neuroprotective activity against Aβ1-40 mediated neurotoxicity in rat hippocampal cells by refining the oxidative stress conditions [38]. A study by Ismail and colleagues showed that pretreatment of primary cultured cerebellar granule neurons (CGNs) with varying therapeutic levels of TQ (0.1 and 1 μM) ensured the inhibition of free radical gen eration, lowering of the release of lactate dehydrogenase (LDH) along with inhibition of both extrinsic and intrinsic caspase pathways, thereby, improving conditions of oxidative damage after subsequent exposure to Aβ1-40 peptide . Similar phenomenon was also observed in case of differentiated pheochromocytoma (PC-12) cells of rats . TQ can also act as a therapeutic agent against ethanol-induced neuronal apoptosis in the cortical neurons [38]. (Fig.10)

3.3. Anticholinesterase (AChEI) activity

ACh is an essential neurotransmitter for memory and learning pro cessing, serving as the primary media to carry nerve impulses between nerve cells where enzyme AChE acts to rapidly degrade ACh into choline and acetate [39]. Researchers demonstrated that elevated levels of AChE activity is strongly co-related with cognitive deficiencies due to the impact on the cerebral blood flow . Therefore, suppression of this

enzyme may serve as a potential approach for the treatment of AD which elevates the synaptic concentration of ACh, allowing a higher occupancy rate and longer duration at its receptor . The NS oil seed has a prominent dose dependent impact on AChE inhibitory activity (r2 ¼ 0.989) compared to essential oils from other medicinal and aromatic plants and edible oils such as olive oil used in the Mediterranean diet. A study with methanolic extract of NS demonstrated almost identical levels of AChEI activity compared to the standard drug donepezil [40]. TQ exhibited the highest AChEI activity of 53.7 g/mL in which NS extract overall exhibited 84.7 g/mL, which suggests a signifi cant AChE inhibition. In another study, L-Cys was administered to mimic the biochemical changes induced by ACh decline and found that administration of TQ (5 and 10 mg/kg; p. o.) lowered the L-Cys induced stimulation of AChE enzyme function at the same dose level of donepezil (10 mg/kg; p. o), the reference control for the study . This done pezil effect was mimicked by improving cognitive capacities by sup pressing AChE, TNF- α levels, inhibiting lipid peroxidation, and increasing glutathione levels [41]. (Fig.10)

3.4. Prevention of neuro-degeneration and neurotoxicity

Neuroprotection can be defined as the strategic steps to protect the central nervous system (CNS) in the face of neuronal injury due to both acute (e.g., stroke or trauma) and chronic neurodegenerative disorders (e.g. Alzheimer's disease and Parkinson's disease) . Studies on cultured hippocampal and cortical neurons with TQ (100 nM) efficiently suppressed Aβ1-42 induced neurotoxicity by improving the cellular activity, inhibiting mitochondrial membrane depolarization and suppressing ROS[43] . In a similar experiment, Al-Majed et al showed that TQ (5 mg/kg/day, orally) has a defensive effect for these neurons against transient forebrain ischemia-mediated injury by means of diminishing the content of dead hippocampal neuronal cells . TQ also provided protection against Aβ1-40-induced neurotoxicity in vitro and induced cell death in neuronal cultured cells . TQ has been shown to provide some neuronal and morphology-based improvements in the degenerated hippocampus of mice due to long-term toluene exposure [42]. TQ was also seen to prevent neurotoxicity by lowering the Bax, caspase-3,-8 and-9 and flourishing the B-cell lymphoma 2 (Bcl-2) levels. Neuroprotective properties in nanomolecular con centrations are mediated by TQ based antioxidants which accumulates in the mitochondria as TQ inhibits Aβ induced hyperproduction of ROS by the mitochondria through a cascade of reactions leading to neuronal death. In fact, TQ based antioxidants can serve as the new type of neu roprotectors . This can be termed as a potential neuroprotective agent by elevating antioxidant genes such as SOD1, SOD2, CAT expres sion as well as signaling related genes such as Jun N-terminal kinase (JNK), P38 and protein kinase B and diminishing the ROS levels [44]. (Fig.4)

5. Molecular Docking:

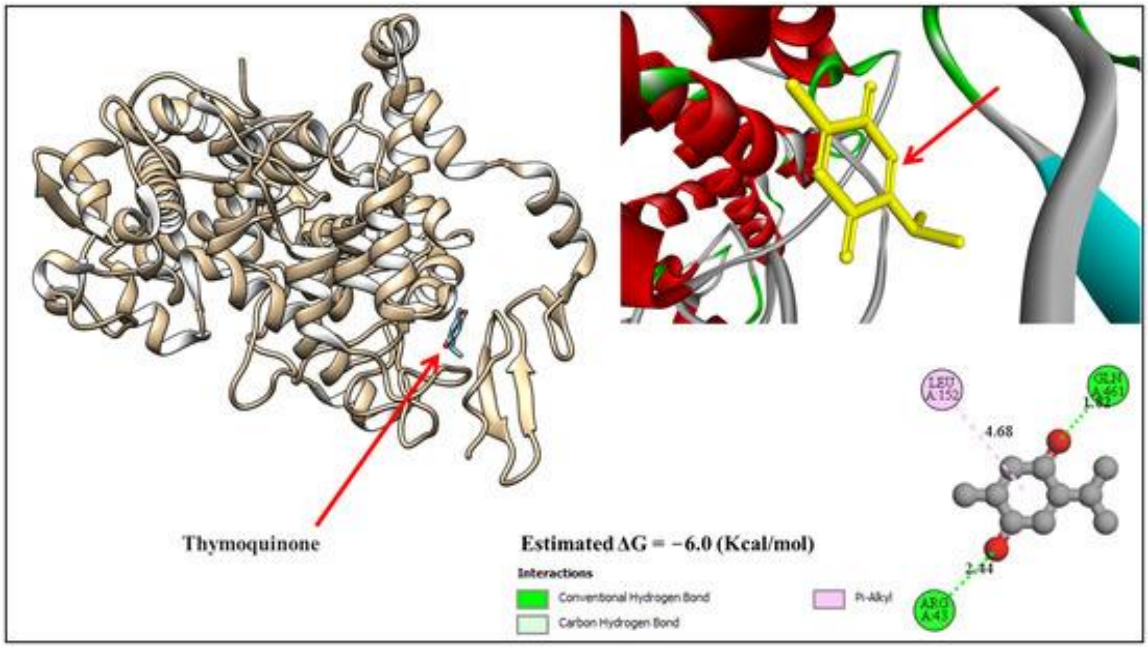

Fig 5 :- Molecular Docking of Thymoquinone

The image helps us understand how Thymoquinone interacts with a specific target protein at the molecular level. The large ribbon-like structure in the image represents the 3D model of the protein. The small molecule (highlighted by a red arrow)is Thymoquinone, which binds at a specific site on the protein's surface. This interaction site is known as the binding pocket.

A closer view (shown in the zoomed section) illustrates how Thymoquinone fits into the active site of the protein. The yellow structure represents Thymoquinone, surrounded by red and green protein regions, indicating the amino acids that interact with it. These interactions can include hydrogen bonds, hydrophobic forces, and van der Waals interactions.At the bottom, the 2D diagram shows the interaction map between Thymoquinone and the amino acid residues of the protein. Each small circle or line represents the type of bond formed and its strength[45].(Fig.5)

The estimated binding energy (AG) is shown as -4.6 kcal/mol, which indicates a stable and favorable binding. A negative value means that Thymoquinone can attach well to the protein, suggesting it may have a biological or therapeutic effect.

In Simple Terms:

. Thymoquinone can effectively bind to the target protein.

. The binding energy shows the interaction is stable and meaningful.

. Such interactions may explain its anti-inflammatory, antioxidant, or neuroprotective effects[46].

- **Drug Design and Discovery**

Drug design and discovery is a critical aspect of pharmaceutical sciences, involving the identification and development of new therapeutic agents to treat various diseases. For pharmacy professionals,

understanding this process is essential, as it forms the foundation of modern pharmacotherapy. The journey begins with target identification, where a biological molecule (such as a receptor, enzyme, or gene) linked to a disease is selected. This is followed by target validation, confirming that modulating the target can produce a therapeutic effect.Once the target is validated, the next step is lead compound identification[5]. This involves screening chemical libraries (using techniques like high-throughput screening) or employing computer-aided drug design (CADD) to find compounds that interact effectively with the target. Pharmacy professionals should also be familiar with structure-based drug design (SBDD) and ligand-based drug design (LBDD), which use knowledge of the target's 3D structure or known ligands to design new drugs.(Fig.6)

After a lead is identified, it undergoes lead optimization to enhance properties such as potency, selectivity, bioavailability, and safety. The optimized compounds are then tested in preclinical studies, using in vitro and in vivo models to assess pharmacokinetics (ADME) and toxicityModern drug discovery is increasingly influenced by advances in technology.[4] Artificial intelligence and machine learning are now used to predict drug-target interactions, toxicity, and to aid in drug repurposing. Personalized medicine, which tailors therapy based on an individual's genetic makeup, is becoming a major focus, especially in oncology and rare diseases. Other emerging areas include RNA-based therapies (such as mRNA vaccines), biologics, biosimilars, and the use of organoids and 3D cell cultures for more accurate preclinical testing. Despite these innovations, the process remains costly and time-consuming, with only a small percentage of candidate drugs reaching the market. Understanding the science and strategy behind drug design and discovery is crucial for pharmacy professionals, as it enhances their ability to contribute meaningfully to research, clinical care, and the safe use of new medications in practice.[6]

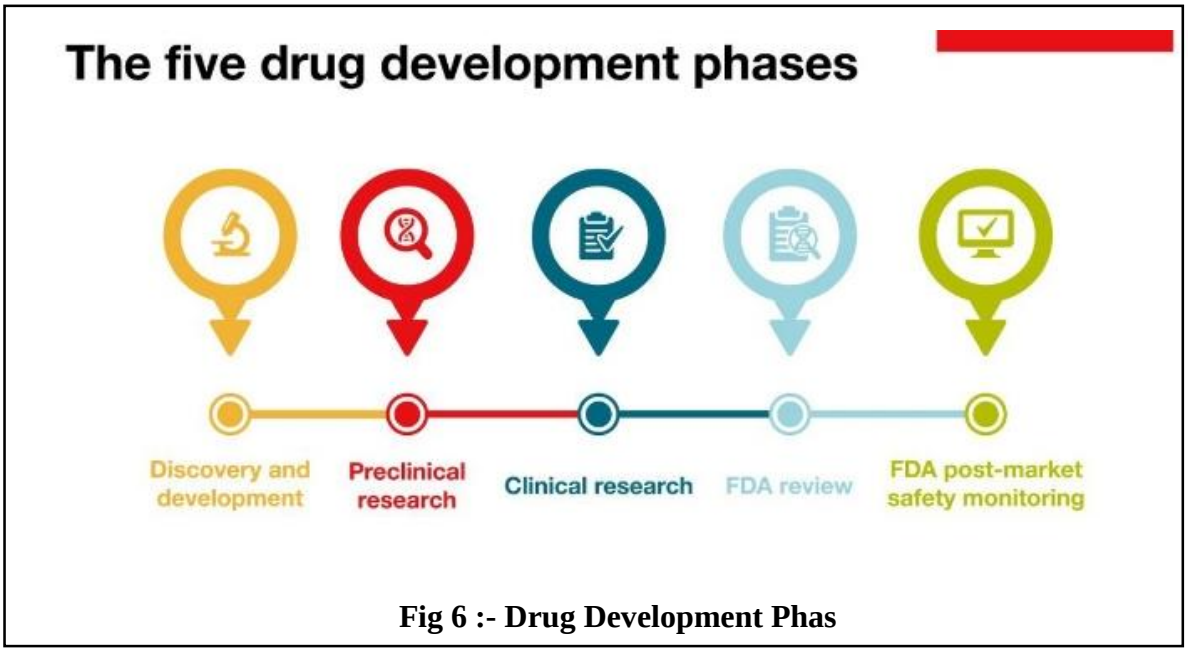

Fig 6 :- Drug Development Phas

Stages of Drug Discovery

1. Drug Discovery (2-5 years)

Involves identifying and validating drug targets, and finding lead compounds.About 100 projects may start, but only a few promising moleculesmove forward.

2. Preclinical Development (1-2 years)

Laboratory and animal studies to evaluate safety,efficacy,pharmacokinetics toxicity.

Only the safest and most effective molecules enter human trials.

3. Clinical Development (5-7 years)

Human testing in three phases:

Phase I: Safety and dosage in healthy volunteers.

Phase II: Efficacy and side effectsin patients.

Phase III: Large-scale testing for Effectiveness and monitoring of adverse reactions.

4. Regulatory Approval (1-2 years)

Data is submitted to authorities (e.g.,FDA, EMA, CDSCO).(Fig.2)[7]

Principle of Drug Design

1. Understanding the Disease and the Target

The first step in drug design is to clearly understand the disease at a molecular level. Scientists study how the disease developsand identify the biological molecules involved in its progression. These molecules, often enzymes, receptors, or nucleic acids,become the potential targets for drug action[8].

For example, in Alzheimer's disease, the enzyme acetylcholinesterase breaks down the neurotransmitter acetylcholine. Drugs that inhibit this enzyme can help increase acetylcholine levels and improve memory function.

2.Target Identification and Validation

After finding a possible target, researchers must confirm that it truly plays a critical role in the disease. This process is called target validation.

3. Discovering a Lead Compound

Once a valid target is known, the next step is to find a chemical compound that can interact with it. This initial compound is called a lead compound.

Leads can come from:

. Natural sources such as plants or microorganisms

. Synthetic chemical libraries

. Computer-based screening (virtual screening)[9]

4. Studying the Structure-Activity Relationship (SAR)

The structure-activity relationship (SAR) helps researchers understand how the structure of a molecule affects its biological activity.

5. Lead Optimization

After understanding which parts of the molecule are important, the lead compound is optimized to make it safer and more effective.

Optimization focuses on improving:

. Target binding strength

. Selectivity (so it acts only where needed)

. Pharmacokinetic properties (absorption, distribution, metabolism, and excretion)

. Toxicity (to reduce side effects)

. Stability and solubility

6. Drug-Target Interactions

Drugs work by binding to their targets through various forces such as:

. Hydrogen bonding

. lonic interactions

. Hydrophobic forces

. Van der Waals interactions

7. Pharmacophore & Ligand-Based Design

Sometimes, we don't have the 3D structure of the target protein. In those cases, scientists use information from other molecules that are known to work. They create a kind of "feature blueprint" of what the molecule needs to have—called a **pharmacophore**—and use that to design or search for new drugs.[10]

8. ADME and Safety

Even the best-designed molecule is useless if it doesn't behave properly inside the body[11]. That's why scientists test for **ADME**—which stands for:

- Absorption: Can the drug get into the bloodstream?
- Distribution: Can it reach the right parts of the body?
- Metabolism: Is it broken down safely by the body?
- Excretion: Can it leave the body without causing harm?

1. Drug Design Development

Drug design and discovery is a critical aspect of pharmaceutical sciences, involving the identification and development of new therapeutic agents to treat various diseases. For pharmacy professionals, understanding this process is essential, as it forms the foundation of modern pharmacotherapy. The journey begins with target identification, where a biological molecule (such as a receptor, enzyme, or gene) linked to a disease is selected. This is followed by target validation, confirming that modulating the target can produce a therapeutic effect.Once the target is validated, the next step is lead compound identification. This involves screening chemical libraries (using techniques like high-throughput screening) or employing computer-aided drug design (CADD) to find compounds that interact effectively with the target. Pharmacy professionals should also be familiar with structure-based drug design (SBDD) and ligand-based drug dsesign (LBDD), which use knowledge of the target's 3D structure or known ligands to design new drugs.[12]

After a lead is identified, it undergoes lead optimization to enhance properties such as potency, selectivity, bioavailability, and safety. The optimized compounds are then tested in preclinical studies, using in vitro and in vivo models to assess pharmacokinetics (ADME) and toxicity. Successful candidates move into clinical trials, which are conducted in four phases: Phase I (safety), Phase II (efficacy), Phase III (comparison with existing therapies), and Phase IV (post-marketing surveillance).Pharmacy professionals play a significant role throughout this pipeline—not just in understanding the pharmacology of new drugs, but also in regulatory affairs, clinical trials, and ensuring safe and effective use of medications in practice. Compounds that pass these stages proceed to clinical trials, which are conducted in four phases: Phase I tests safety in healthy volunteers, Phase II assesses efficacy in patients, Phase III involves large-scale trials for regulatory approval, and Phase IV monitors long-term safety after the drug is marketed. Throughout this process, pharmacy professionals play a key role—not only in understanding and applying pharmacological principles but also in clinical trial management, regulatory compliance, and ensuring patient safety[13]. Their knowledge of drug mechanisms, adverse effects, interactions, and therapeutic monitoring is essential for the development and appropriate use of new medications.(Fig.7)

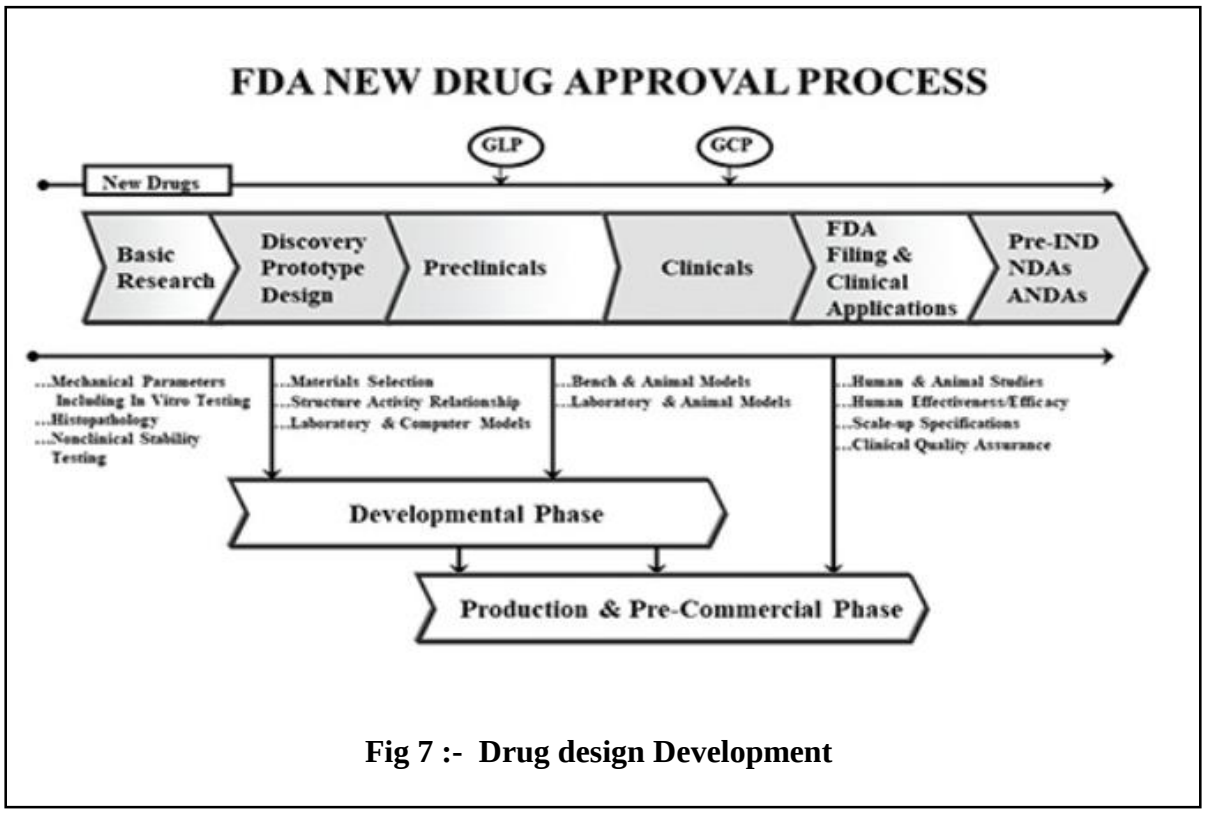

Fig 7 :- Drug design Development

2.QSAR (Quantitative Structure Activity Relationship):

Quantitative structure activity relationship (QSAR)studies represent a non experimental part of drug design encompassing the study of both structure activity and structure property relations in broad sense. The most commonly used mathematical techniques in elassical quantitative structure activity relationahips (QSAR) work is multiple regression analysis. QSAR is an intellectual exercise of assemhling , manipulating . and examining data obtained from physical, chemical. and hiological experiments, and correlating them to biological activity . Biological activity of a drug depends on the types and magnitude of interactions between the receptor and the drug molecule[14].

The main properties of a drug that seem to influence its activity measure its lipophilicity, the electronic effects within the molecule and the size and shape of the molecule. Lipophilicity is a measure of a drug's solubility in lipid membeanes. This is usually an important factor in determining how easily adrug passes through lipid membranes. It is used as a live of the convenience of distribution of a drug to its target website. The parameters commonly used to represent these properties are partition coefficients and lipophilic substitution constants for lipophilicity, Hammetts constants for electronic effeets and tatts Es steric constants for steric effects.

QSAR derived equations take the general form

Biological activity = Function (parameter)

In which the activity is normally expressed as log [1/(concentration term)]where the concentration term is usually C the minimum concentration required to cause a defined biological response.[15]QSAR studies square mensures ordinarily applied on tcams of connected compounds. However, QSAR studies on structurally diverse sets of compounds are becoming more common.In both instances it is important to consider as wide range of paramelers as possible,The different parmeters are:

2.1 QSAR Parameters

When scientists design drugs using QSAR, they study how the features of a molecule like its size, shape, charge, and water-likingnature - affect how well it works as a medicine.

These features are called parameters descriptors.

1.Physicochemical Parameters - Basic Nature of the Molecule these describe the physical and chemical characteristics of the compound.They help us know how the molecule behaves in the body. Think of it like: whether the molecule is heavy or light, solid or soft, and how easily it dissolves.[16]

Examples:

- Molecular weight - tells how big or small the molecule is
- Solubility - whether it dissolves in water or fat
- Melting or boiling point - shows how stable it is

2.Electronic Parameters – Charge and Reactivity

These explain how electrons are arranged in the molecule.

They affect how the molecule interacts with receptors or enzymes (the target in the body).

Think of it like: how attractive or reactive the molecule is toward its target.

Examples:

- Dipole moment - shows how polar(charged) the molecule is
- Hammett constant - shows how different parts of the molecule pull or push electrons Hammett Sigma Constant (o)

3.Steric Parameter–Size and Shape These tell us about the 3D structure of the molecule its bulkiness, volume, and how it fits in the receptor site.Think of it like: whether the molecule's shape fits perfectly into the lock (receptor) or not.

Examples:

- Molar refractivity - gives an idea of molecular volume
- Taft's steric factor - shows how bulky a substituent is

4.Hydrophobic Parameters – Water or Fat-Loving Nature

These describe whether the molecule likes water (hydrophilic) or fat (hydrophobic) environments.This affects how the drug passes through cell membranes and spreads in the body.

Think of it like: Does the molecule prefer to stay in water or in oil?

Examples:

- logP - tells how much the molecule prefers oil over water
- Tt constant - measures how hydrophobic a chemical group is

5.Topological & Geometrical Parameters - Structural Arrangement

These are mathematical representations of a molecule's structure - like its connectivity, surface area, and shape - but don't need full 3D data.

Think of it like: creating a digital blueprint of the molecule.

Examples:

- Molecular surface area
- Connectivity indices

6. Hansch Analysis

QSAR based on Hammett' s relationship utilize electronic properties as the descriptorsof structures. Difficulties were encountered when investigators attempted to applyHammett-type relationships to biological systems, indicating that other structuraldescriptors were necessary. In 1962, Hansch et al entered the scenario with the numerical information on lipophilicity,electronic, and steric effect on the model development. The general form of Hansch equation is as follows:

$$\text{Log BA} = a \log p + b\, o + c\, Es + \text{constant (linear)}$$

$$\text{Log BA} = a \log p + b (\log p)2 + c\, o + d\, Es + \text{constant (nonlinear)}$$

Partition coefficient; log P

Hammett constant; o

Taft's steric parameter; Es

Hansch model correlates biological activity with physicochemical properties. The coefficients (a, b, c, d, and constant) are determined by multiple regression analysis[17].

- Selection of Relevant Parameters

Not all calculated descriptors contribute to biological activity.Thus, feature selection methods like principal component analysis (PCA) or genetic algorithms are applied to identify the most influential parameters.[18]

- Proper parameter selection ensures:
  - Better model accuracy
  - Avoidance of overfitting
  - Improved interpretability

2.2 Types of QSAR

Imagine you are a security guard checking IDs. A 0D QSAR model is like only being allowed to see a person's name and weight. It uses simple, basic molecular properties that don't depend on the molecule's shape or arrangement in space.

1] 1D-QSAR –TheBasic Level Think of this like the simplest form of QSAR. Here we look at basic numbers that describe a molecule –

Example: The more oily the molecule, the better it might pass through cell membranes.

. How oily or water-loving it is (logP)
. How acidic or basic it is (pKa)
. Its molecular weight
  - Easy and quick

2] 2D-QSAR – A Better Picture Now we look at the molecule's 2D structure - how atoms are connecte and what bonds exist.

We use mathematical models (like regression or computer algorithms) to find patterns.

We can study:

Example: Drugs with more positive charge near a certain receptor.

. Electronic properties (charges, dipoles)

. Size and shape (steric factors)

. Hydrophobic properties (how it interacts with water or fat

- Gives more information than 1D

3] 3D-QSAR – Seeing in Three Dimensions Here we go one step further - we study the3D shape of the molecule.We imagine how the molecule fits into itstarget site, like a key fitting into a lock

Famous methods:

Example: We can see that a bulky group on one side increases activity, but on the other side, it blocks binding.

. CoMFA (Comparative Molecular Field Analysis)

. CoMSIA (Comparative Molecular Similarity Indices Analysis)

- Very accurate and realistic
- Needs computer Heling and 3D alignment.

4] 4D-QSAR – Adding Movement Molecules are not frozen - they move and change shape in real life!So, 4D-QSAR includes different shapes (conformations) a molecule can take.It studies how these shapes affect biological activity.

Example: A flexible molecule may bend perfectly to fit inside the enzyme pocket.

- More realistic than 3D

5] 5D-QSAR –The Full Picture Now we also include the surroundings – like the effect of the solvent (water, lipids) and receptor environment.It's like seeing the molecule inside the human body, not just on paper.[19]

Example: How the molecule interacts inside the receptor pocket in real-life biological conditions.

- Most accurate and detailed

2.3 Methodology of the QSAR

QSAR methodologies have the potential of decreasing substantially the time and effort required for the discovery of new medicines A major step in constructing the QSAR models is to find a set of molecular deseriptors that represents variations of the structural properties of the molecule. The QSAR analysis employs statistical methods to derive quantitative mathematical relationship between chemical structure und biological activity.

The process of QSAR modelling cun be divided into three stages: development, model validation and application.(Fig.8)

QSAR Methodology Steps

1.Collect data - Take several compounds and note their biological effects (like how strongly they block an enzyme).

2.Study their structures - Look at properties such as size, shape, charge, and how oily or water-loving they are.

3.Find patterns - Use math and computer programs to find a relationship between structure and activity.

4. Build a model - The computer creates an equation that can predict the activity of new compounds.

5. Use the model - Predict which new compounds might work best before even making them in the lab![20]

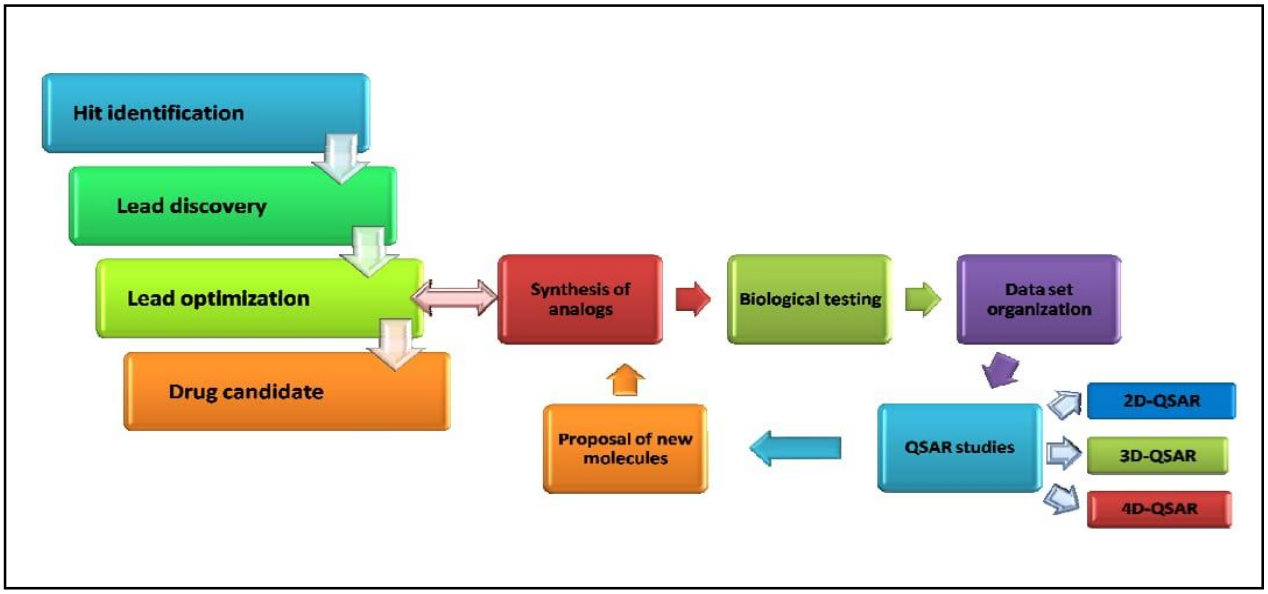

Fig 8 :- methodology of QSAR

3.Introdaction to CADD:

Drug discovery is a multistep process that begins with the Identification of suitable drug target, validation of drug target, hit to Lead discovery, optimization of lead molecules, and preclinical and Clinical studies. The application of rational drug design as an Integral part of CADD provides useful insights into the Understanding of the binding affinity and molecular interaction Between target protein and ligand. Additionally, lead identification In pharmaceutical research has been facilitated by the availability of Supercomputing facility, parallel processing, and advanced Programs, algorithms, and tools. Furthermore, recent Advancements in artificial intelligence (AI) and machine learning Methods have greatly aided in analyzing, learning, and explaining The pharmaceutical-related big data in the drug discovery process. Different methods employed in the identification of new Inhibitors from chemical databases include pharmacophore Modelling, quantitative structure-activity relationship (QSAR), Molecular docking, quantum mechanics, and statistical learning Methods. (Fig.9)

CADD Overview;The main categories of CADD tectiniques a Computer-aided design is the process of creating and documenting a product utilising software.Engineering drawings need the use of visual symbols such point lines, curves, planes, and shapes. In essence, it gives a detailed graphical depiction of each component's explanation.

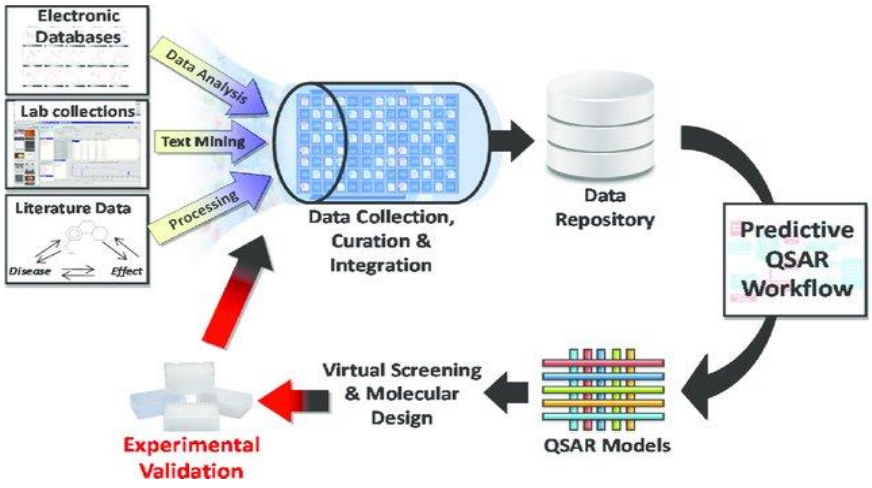

Fig 9 :- Workflow of QSAR

3.1 Types of CADD

The following are the two basic methodologies for drug design with CADD:

1. Direct or structure-based medication design

2. Ligand hased drug design / indirect approach

1)Structure Based Drug Design:

The structure of the target protein is known in structure based drug design (SBDD).These methods are very efficient and alternative approach to the discovery and development of drug design course .The (3D)structure of proteins are provided in SBDD. The availability of 3D structures of therapeutically important proteins favours identification of binding cavities and has laid the foundation for structure-based drug design (SBDD). This is becoming a fundamental part of industrial drug discovery projects and of academic researches.

SBDD is a more specific, efficient, and rapid process for lead discovery and optimization because it deals with the 3D structure of a target protein and knowledge about the disease at the molecular level . Among the relevant computational techniques, (SBVS), molecular docking, and molecular dynamics (MD) simulations are the most common methods used in SBDD. These methods have numerous applications in the analysis of binding energetics, ligand–protein interactions, and evaluation of the conformational changes occurring during the docking process.(Fig.10)

- Important tools include:

. Molecular Docking: Predicts how a small molecule will fit and bind with the target.

. Molecular Dynamics: Studies how stable the drug-target complex remains over time.

. De Novo Design: Builds new molecules directly inside the active site.

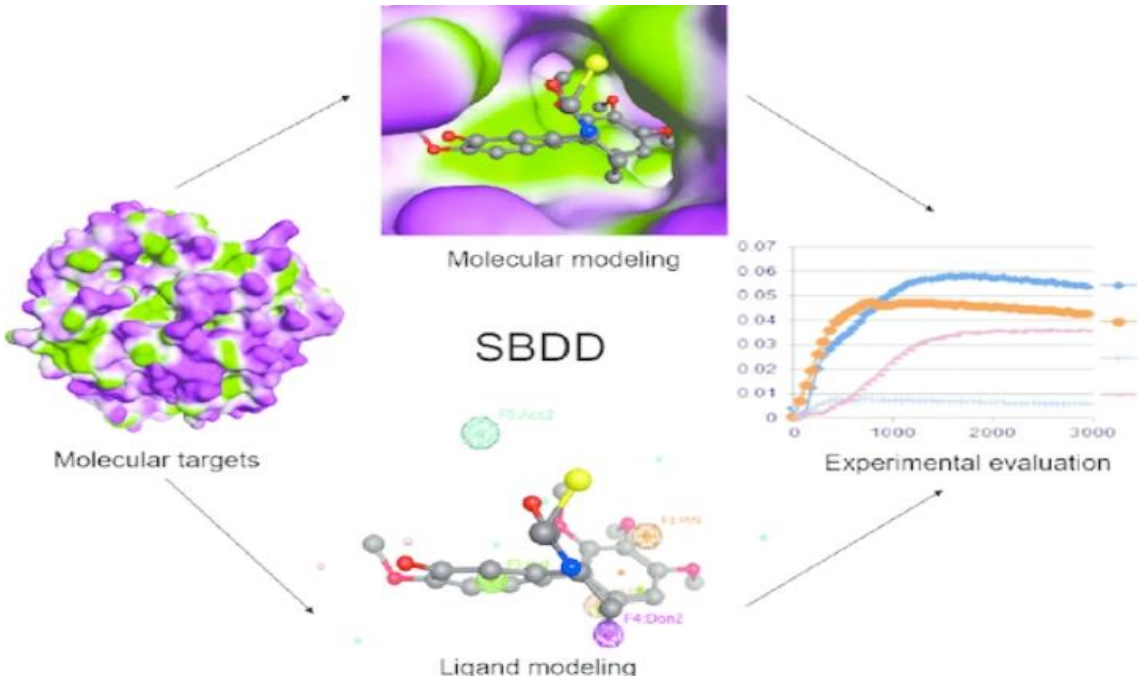

Fig 10:- Structure-Based Drug Design from Target Selection to Drug Candidate

2)Ligand Based Drug Design:

Ligand based drug design is an indirect approach to facilitate the development of pharmacologically active compounds by studying molecules that interact with the biological target of interest. Ligand based drug design methods are useful in absence of an experimental 3D structure. Due to the lack of an experimental structure, the known ligand molecules that bind to the target are studied to understand the structural and physicochemical properties of the ligands that correlate with the desired pharmacological activity of those ligands.

Ligand based method may include natural products or substrate analogues that interact with the target molecule yielding the desired pharmacological effect. In some cases, usually in which data pertaining to the 3-D structure of a target protein are not available, drug design can instead be based on processes using the known ligands of a target protein as the starting point. This approach is known as "ligand-based drug design".(Fig.11)

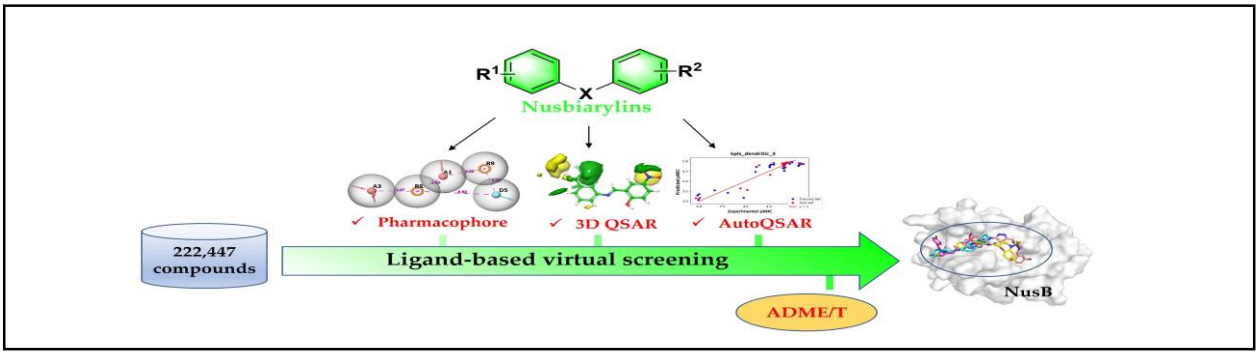

Fig 11 :- Ligand Based Drug Desig

Conclusions

Thymoquinone (TQ), the main compound found in Nigella sativa seeds (black cumin), has shown great potential in protecting the brain from Alzheimer's disease. Its natural antioxidant and anti-inflammatory properties help fight two of the biggest problems in Alzheimer's oxidative stress and brain inflammation. By removing harmful free radicals and reducing the release of inflammatory chemicals, TQ helps protect nerve cells from damage and supports healthy brain function.Studies suggest that TQ can also stop the buildup of toxic amyloid-beta proteins, which are linked to memory loss and brain cell death in Alzheimer's. It supports the brain's energy centers (mitochondria), improves memory, and helps maintain normal communication between nerve cells. Because of these multiple protective actions, TQ is considered a promising natural compound that could help slow down or even prevent Alzheimer's disease. It also offers hope for developing new, safer drugs based on natural products. However, more research and clinical testing are needed to fully understand how TQ works in humans and to confirm its long-term saety and effectiveness.

Till present, there is no confirmatory pharmacotherapeutic approach for AD treatment. Currently available conventional therapeutics revolve around symptomatic relief steps to ameliorate both cognitive and behavioral symptoms. Studies report those treatments that are capable of halting or at least efficiently altering the course of AD as ‘disease-modi fying’ drugs and they are still under extensive research. Ideally, in order to intervene the progression of the disease, drugs need to alter the pathogenic steps involved for clinical symptoms, including the deposi tion of extracellular Aβ, intracellular neurofibrillary tangle formation, oxidative damage, mediation of inflammatory mediators, disruption of calcium homeostasis, loss of ACh stores. These aberrant signaling path ways can be exploited as a promising therapeutic approach for the management of AD both prophylactically and from the curative point of view. Collective evidence-based studies demonstrated that the neuro protective effects of TQ may be associated with the modulatory effects on inflammation, apoptosis and oxidative stress. Considering lesser side effects, good tolerability, ready availability and minimal expenditure, the spice component, TQ from NS exert its inhibitory effects across various mechanistic pathways specifically targeting the pathogenic process of AD development. [47,48]

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