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An Update Review on Natural Product Inspired Cholinesterase Inhibitor for the Treatment of Alzheimer's Disease

Chavan Kaushal Madhukar[1], Chaudhari Yogeshwar Rajendra[2], Rathod Kiran Bhaidas[3]

[1,2,3]Dr. Uttamrao Mahajan College of B. Pharmacy, Chalisgaon

ABSTRACT:-

Alzheimer's disease (AD) is a progressive, neurodegenerative pathology that primarily affects the senior population, and is estimated to regard for 50- 60 of madness cases in persons over 65 times of age. The main symptoms associated with AD involve cognitive dysfunction, primarily memory loss. Other features associated with the after stages of AD include language poverties, depression, behavioural problems including agitation, mood disturbances and psychosis. One of the most promising approaches for treating this complaint is to enhance the acetylcholine position in the brain using acetylcholinesterase (AChE) impediments.

Introduction :-

Acetylcholine (ACH) **1** is found in vertebrates and arthropods and is one of the major compounds by which electrical impulses carried by nerve cells are transmitted to another nerve cell or to voluntary and involuntary muscles. It was first discovered in 1867 as a synthetic compound and detected in human tissue in 1906 in extracts of the adrenal gland transmitted to another from which the adrenalin had been removed **(1)**. Two major types of receptor sensitive to ACh are recognised, muscarinic and nicotinic.

Muscarinic receptors are chiefly associated with the peripheral nervous system and with smooth and cardiac muscle. The effect of binding with ACh is generally associated with stimulation of the parasympathetic nervous system. Classical symptoms ofparasympathetic stimulation are decreased heart rate and blood.

pressure, constriction of bronchi, increased salivation, promotion of digestion and increase in intestinal peristalsis, release of fluids from the bladder and accommodation of the eyes for near vision, with contraction of the pupils.

The nicotinic receptors are found in the central nervous system (CNS) and in the motor end plates which are the synapses between nerves and skeletal muscle. In the CNS. Ach stimulation of the nicotine receptors appears to be associated with cognitive process and memory, whilst in skeletal muscles it causes contraction.

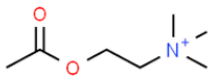


Fig.1 Acetylcholine

ACh is stored in the nerve terminals in structures called vesicles. The contents of those vesicles are released from the nerve endings when the nerve terminal is depolarised, and the Ach thus released enters the synapse and binds to the receptor. The ACh which is released has a very short half-life due to the presence of large amounts of acetylcholinesterase (AChE), an enzyme which hydrolyses the ester bond in the molecule, thus leading to loss of stimulatory activity. Inhibition of AChE will, therefore, as far as nervous transmission is concerned, result in a prolongation of the existence, and therefore the activity, of ACh This concept has been employed in medicine for treating disease states associated with inadequate levels of ACh, and toxicologically to cause illness of death by means of excess cholinergic stimulation **(2)**.

It should be noted that, in recent years, AChE has been found to be involved in a number of other functions besides nerve transmission. These include a role as an adhesion protein, a bone matrix protein, in neurite growth and in the production of amyloid fibrils, which are characteristically found in the brain cells of patients with Alzheimer's disease **(3)**.

Structure and mechanism of acetylcholinesterase (AChE).-

It consists of a complex protein of the α/β hydrolase fold type having an overall ellipsoid shape containing a deep groove, usually called the gorge, which is about 20A deep Hydrolysis of ACh appears to take place at the bottom of the gorge and the mechanism is fairly complex being described in more detail below At present, the structural knowledge concerning AChE is mostly based on work carried out on the enzyme *Tc*AChE.

Although the hydrolysis process takes place in the base of the gorge, initial binding of ACh is thought to occur at its outer rim in a region called the "peripheral site''. At the bottom of the gorge, where the actual hydrolysis occurs, there are four main subsites, these being the "esteratic site'', the ''oxyanion hole'', the ''anionic subsite'' and the "acyl pocket": The substrate enters the enzyme gorge and the product is released in conjunction with defined conformational changes. The esteratic site contains the catalytic machinery of the enzyme, which is dependent on a catalytic triad of Ser200-His440-Glu327**(4)**, Cholinesterases use this Ser-His-Glu catalytic triad to enhance the nucleophilicity of the catalytic serine since the strong hydrogen bond between His and Ser improves the ability of Ser to mount a pictophilic attack on the substrate while Glu stabilizes the histidinium cation of the transition state The "oxyanion hole" (OH) (Fig. 2) consist of Gly118, Gly119 and Alx201. The three peptide residues contain hydrogen bond donors and they stabilize the tetrahedral intermediate of Ach (Fig.3) which is formed during the catalytic process**(5)**.

The ''anionic subsite'' (choline-binding subsite or hydrophobic subsite) is largely comprised of aromatic residues and contains Trp84, Phe330 and Glu199, which are believed to bind to quaternary ammonium ligands by p-cation interactions The positive charge of the quaternary ammonium group of Ach can forms a stable interaction with the electron-rich p systems of aromatic rings **(6)**. Trp84 is an important residue for binding ACh. Substitution of Trp84 by an aliphatic residue (alanine) results in a large decrease in reactivity toward Ache, but no decrease for non-charged ligands 5 while site-directed mutagenesis of Glu199 to Asp199 had a similar effect on the binding of charged ligands**(7)**. The ''acyl pocket'' (Acyl binding pocket or acyl binding pocket) consists of Phe288 and Phe290, which are believed to play a role in limiting the dimension of substrate which are able to enter the active site. AChE is one of the fastest hydrolytic enzymes. It has about 103 fold and 1013 fold greater activity than serine proteases and BChE respectively, in the spontaneous hydrolysis of ACh at the same temperature and pH**(8)**. Due to the high turnover of the enzyme and the strong electrostatic dipole caused by the asymmetric charge distribution, it is unlikely that the choline produced by hydrolysis leaves the active site through the same path by which the substrateentered For this reason the "back door'' mechanism has been proposed in order to account for these properties.

It has been proposed based on molecular dynamics and site-directed mutagenesis, that the process occurs through the opening of the bottom of the AChE gorge to provide a channel through which the products can pass, so enabling a part, fem of substrate through the enzyme active site.

A number of studies have been conducted in order to investigate the interaction between inhibitors and AChE. These include X-ray diffraction of the crystal complex between AChE and inhibitors as well as site-directed mutagenesis**(9)**. Inhibitors can be divided between those that bind to the active site at the bottom of the gorge and those that bind to the PAS As far as the alkaloidal inhibitors are concerned, binding takes place at the active site at the bottom of the gorge and the important features of an inhibitor appear to be a positively- charged nitrogen which binds to the oxyanion hole area, especially the Trp84, and a region, separated by a lipophilic area from the positive charge, which confirm hydrogen bonds with the serine200 residue and others such as His440**(10)**.

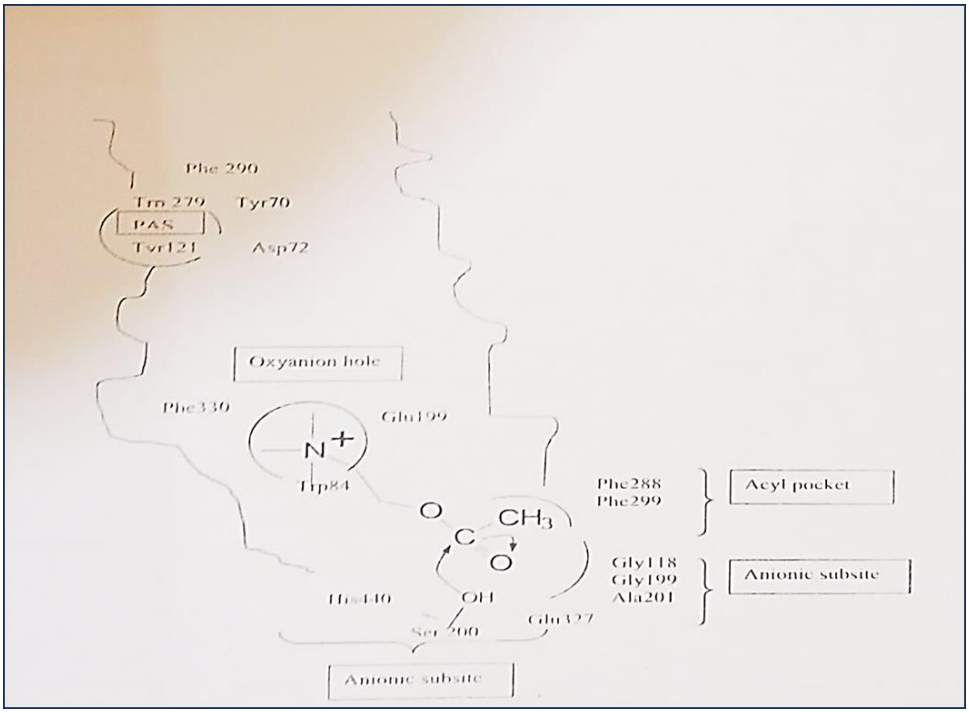


Fig. the binding region of AChE gorge

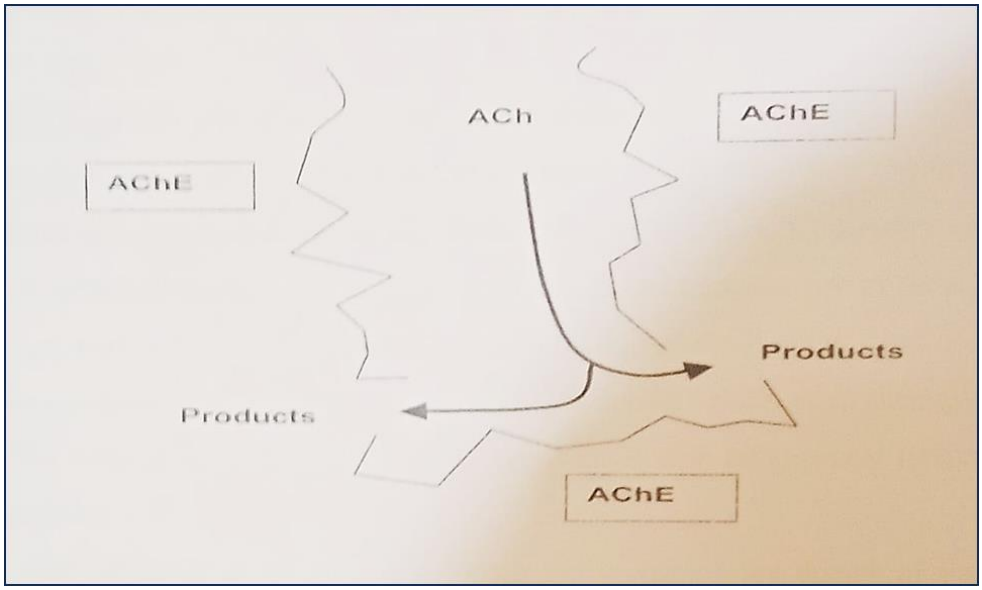
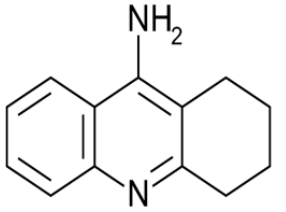
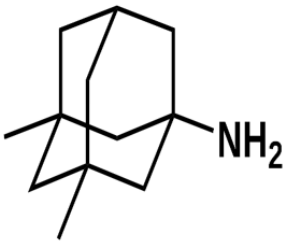


Fig. the release of Ach hydrolysis products from the bottom of the gorge

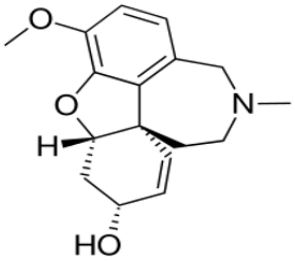
Inhibitors of AChE :-



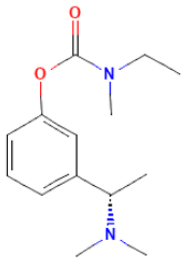
Tacrine



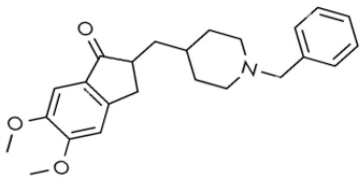
Memantine



Galantamine



Rivastigmine



Doneprezil

There is some indication that some inhibitors bind so both sites Because of the variety of chemical structures involved, few generalisations can be made concerning the detailed interaction between inhibitors and the amino acid residues of gorge and binding sites Consequently, where it known the some of inhibition discussed below for individual compounds.

AChE inhibitors (AChE) are ueed in two major ways, as pharmaceuticals and as pesticides, especially against insects and other arthropod vertebrates. These two applications medicine and pest control, are usually distinct but may be linked in purpose when control of disease vectors a required in *e.g.*malaria control. A more sinister use of these agents is there employment as toxicological agents, either in traditional ritual killings and ordeal trials as practiced in some societies (see notes about physostigmine below) or as chemical warfare agents. The latter group costs of synthetic compounds with no chemical relationship to natural products.

In medicine AChEls are employed mostly for correcting the effects of insufficient levels of ACh. The oldest application in this respect is in ophthalmology for the treatment of glaucoma. Glaucoma is caused by a build-up of fluid in the eye, due to adequate drainage The vessels through which

drainage occurs are dilated by cholinergic stimulation, so allowing faster drainage Physostigmine was formerly used in this way but has now been largely replaced by the use of direct cholinomimetics such as pilocarpine.

The advent of the hypothesis connecting Alzheimer's disease and low levels of Ach in the brain**(11)**. provided a new application for AChEIs, since they could increase the ACh levels. In addition, AChE has been postulated to accelerate the deposition of extracellular plaques of b-amyloid which are characteristic of the abnormal histology of the forebrain of patients with Alzheimer's disease **(12).**Rivastigmine (ExelonR) is used clinically and is an analogue of physostigmine which has been developed to cross the blood-brain barrier and so act mainly in the CNS. The alkaloid galantamine (ReminyIR), was also licensed for use in early stages of Alzheimer's disease in 2001, and is now widely employed in this respect. especially since it appears to have nicotogenergic effects which increase its effectiveness in treating AD**(13).**

Cholinesterase inhibitors have also been used as antidotes to toxins such as the tropane alkaloid atropine which are competitive inhibitors of ACh Intoxication may occur through ingestion of plantmaterial containing these compounds, or from overdosing of medicinal products which contain them as the active ingredients A major use of AChEls is in agriculture where they are used for the control of insects and some other arthropod pests. This use extends into pharmacy since some AChEls are mod as insecticides for treating infections of headlice.

Detection of AChE inhibition:-

ACHE inhibition was initially detected by the use of gutbath pharmacological techniques with preparations such as guinea pig ileum. These methods are costly in several respects, including time, animal tissue and amounts of compound needed so they have been replaced by more sensitive chemical methods.

The method developed by Ellman et al is widely used**(14)**. This is a colorimetric method based on the amount of thiocholine released when acetylthiocholine is hydrolysed by AChE The thiocholine released is quantified colorimetrically by its reaction with 5,5-bisdithionitrobenzoic acid (DTNB), either after or throughout a given time period. The product is an orange-coloured compound with maximum absorbance at 490 nm. The absorbance obtained using a standard volume of a known concentration of the substrate with a fixed dose of AChE is compared with that in the presence of an added compound or extract (all other factors being identical), a significant reduction indicating an inhibitory role for the substance added.

When this technique was first introduced, standard visible spectroscopy procedures requiring cuvettes and therefore several ml of reaction mixture were used and it was sometimes difficult to obtained enough material to show an effect. The development of the method for use on a smaller scale using microlitre wells and a plate reader has been introduced and has enabled determinations to be performed with a much higher throughput the possibility of running several replicates for each determination to improve statistical treatment of results, and the use of smaller amounts of reagents and test substances**(15)**.

The Ellman reaction for detecting AChEl activity has also been adapted for thin layer chromatography plates**(16)**. The plate is developed in the normal way and then sprayed with a mixture of the acetylthiocholine and DTNB, followed by spraying with a solution of the enzyme and incubation. After a suitable incubation time, inhibitory zones are seen as white or pale areas against a yellow orange background. It has been recognized that false positives might arise from inhibition of the reaction between thiocholine and DTNB by naturally- occurring compounds in an extract, rather than inhibition of thiocholine hydrolysis due to the enzyme. To detect such false positives, a test, where the enzyme is emitted from the procedure, must also he carried out in parallel to the test for enzyme inhibitory activity **(17)**. A similar method for TLC detection has been introduced which uses acetylnaphthol as the substrate and measures the amount of naphthol, the reaction product formed, by its chromogenic reaction with Fast Blue B salt(18). An online HPLC detection method has also been developed **(19)**. This uses a reverse-phase column with the column eluate being split into two streams, one with a UV detector and the other connected to a biochemical detection system. This latter system consisted of the eluant being mixed with AChE and DTNB before the on-line introduction of acetylthiocholine. The intensity of the reaction product was measured by at 405 nm by means of a spectrophotometer as an indication of the amount of thiocholine- DTNB product formed.

Natural Inhibitor Obtain From As Follow :-

1) plant

A) Alkaloid as AChE inhibitors

B) Terpenoids as AChE inhibitors

C)Shikimate-derived compounds as AChE inhibitors

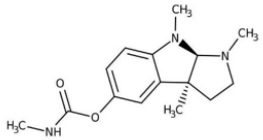
Alkaloids as AChE inhibitors :-

Physostigminic 9 also known as eserine, is the prototype acetylcholinesterase asset. Its isolation in 1864 arose from studies on the Calabar bean. the seeds of *Physostigma venenosum* (Papilionaceae), which was used in southeastern Nigeria as an ordeal poison and for ritual deaths associated with the funeral of a chief**(20)**.

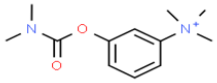
The use as an ordeal poison was carried out using as an extract of the seeds which was given to suspected criminals to assess their guilt. Rapid death was an suggestion that the suspect was shamefaced and innocence was shown by survival. Although this approach might feel to warrant scientific credentials it has been refocused out that differences in immersion might arise due to psychosomatic influences, with nervous belting by shamefaced suspects enabling greater absorption**(21).**

After the isolation of 9, a number of pharmacological studies were carried out but it was not until 1926 that its potentiation of cholinergic effects was shown to be due to its inhibition of cholinesterase The structure of physostigmine 9 was determined in 1935 after numerous times of trouble and it was shown to have an unusual Pyrroloindole skeleton**(22).**

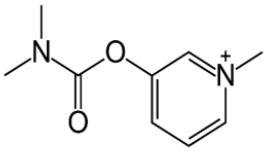
Physostigmine is distributed throughout the body and so gives a general cholinergic effect but it's relatively polar so isn't distributed in large attention in the CNS. It's an effective AChEl with an IC50 of 0.25 IM when tested in bovine erythrocytes**(23).**



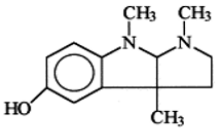
9=PHYSOSTIGMINE



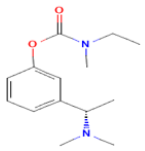
12=NEOSTIGMINE



14=RIVASTIGMINE



11=ESEROLINE



13=PYRIDOSTIMINE

The carbamate portion of the molecule was found to be necessary for cholinesterase inhibitory activity since, when the ester link is hydrolysed the product escroline 11, has no activity. It has been shown that the carbonyl group interacts with the OH of a serine in the AChE to forman ester with the urethane part of the molecule.

This interferes with the AChE *activity* of the enzyme, and the ester is only slowly hydrolysed to regenerate the active parent form. The carbamate moiety has been a key factor in the use of 7 as a lead molecule for derivatisation or de novo synthesis of longer-lasting or more selective drugs, insecticides and other agents. However, the presence of an aromatic ring and a N atom, to facilitate binding and give a good leaving group, also appear to be necessary.

For several decades, physostigmine analogues were developed which were water soluble but had longer half-lives than physostigmine, and were used to treat cholinergic deficiencies in the peripheral nervous system, especially myasthenia gravis, which is characterized by weak muscles. The two major compounds in clinical use are neostigmine 12 and pyridostigmine 13. Both of these compounds contain a quaternary N, as opposed to the tertiary amine form found which makes the compounds very hydrophilic and prevents them fromcrossing the blood-brainbarrier and so exhibiting any CNS effects.

The comparatively recent utilization of AChEIs in treating early symptoms of Alzheimer's disease has stimulated the synthesis of compounds related to physostigmine which are more lipophilic, and so able to cross the blood-brain barrier. Chief amongst these rivastigmine 7 which was licensed for use in Europe in 2000. Another compound eptastigmine 11 was subjected to several clinical trials but these have all now been suspended because of its toxic effects Four novel alkaloids from s. saligna hadmuch less activity with saracocine **(44)** being the most potent with IC50 20 Mm **(48)**. These alkaloids were also shown to inhibit BChE with a dependency on concentration in vitro and were more selective for BChE than AChE(4).

The nitrogen substituents at C-3 and C-20,which are protonated at physiological pH were associated with the inhibitory potency of these alkaloids. The presence of electron withdrawing substituents is reported to be more important for AChE inhibition when positioned at C-3 rather than C-20. This was suggested, since isosarcodine, which has acetamide substitution at C-3 was a more potent inhibitor of AChE (IC50 10.31 IM) than sarcodine 55 (IC50 49.77 IM), which has an acetamide substituent at C-20.60 Molecular docking studies indicated that ring A of the steroidal skeleton (and thus C-3 substituents) of these compounds is situated at the bottom of the aromatic gorge of AChE, while C-20 substituents remain outside the gorge. The hystrophobicity of those alkaloids may also assist with their diffusion made the momatic gorge of Ach.

Two acrdones quinolactacin A1and A2 were isolated from the fungus *Penicillium citrinum* (Trichocomaceae) **(24)**. had the stronger AChEI effect (IC50 19.8IM) compared to which had IC50 280 IM. Another nitrogenous fungal compound vizoltricinie with weak activity (IC30 400 IM against human serum AChE) was isolated from another fungus Fusarium tricinctum **(25)**.

As recounted above, the first AChE inhibitors discovered were alkaloids and, from their structure a hypothesis was developed for the binding site on the enzyme which involved the interaction of the positively charged N with an anionic site in the cleft as an integral and important feature of any inhibitory mechanism It was therefore, somewhat surprising when AChE inhibition by nonalkaloidal compounds began to be reported about twenty years ago and it was these findings which have stimulated the proposals that other binding sites are involved in inhibitory processes which do not necessitate an actual

or potential positively- charged moiety in the inhibitor. The two major types of natural products found in flowering plants which have been found as inhibitors are the terpenoids and a variety of compounds biosynthesised via the shikimic acid pathway terpenoids which have been isolated and which have this effect and the shikimate-derived compounds.

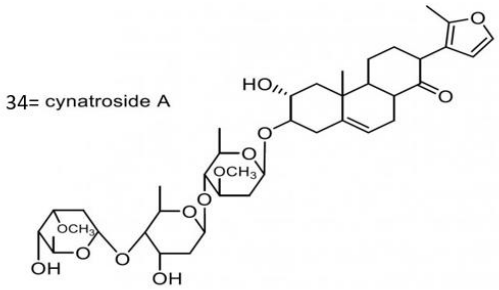
Terpenoids as AChE inhibitors:-

The monoterpenes were the first to be shown to be AChE inhibitors, partly from studies on chemical interactions between plant volatiles and insects and partly from investigations based on English ethnopharmacology that monoterpene containing plants were good for the memory **(26)**.

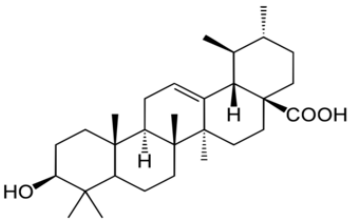
monoterpenes such as camphor and bornyl acetate were present but displayed only very weak activity and it is noteworthy that the activity was 103 weaker than physostigmine More recent studies have shown some synergistic effects between the monoterpenes **(27)**. It is of interest that evidence of improvement of cognitive function after administration of the oil or extracts of S. lavandulaefolia or S. officinalis now exists for in viva animal studies and for clinical studies on healthy humans and those in the early stages of Alzheimer's disease**(32)**. The findings of inhibition of AChE by monoterpenes were similar to those arising from insect plant interaction studies which found that citral was also active**(34)**. Studies on a range of monoterpenes with a p-menthane skeleton also showed that (+)- pulegone was the most potent compound (IC50 890 IM) and that the ketones generally were more active than the corresponding hydrocarbons and alcohols **(36)**. Both (+)- and (-)-carvone have shown some AChE inhibitory effects although in neither case was it Very strong**(38)**.

Several different types of interpenoids and steroids have been shown to inhibit AChE and the activity of the steroidal alkaloids is discussed above. The roots of *withania somnifera* (Solanaceae) are one of the most highly regarded herbs in Ayurvedic medicine where it is known as "ashwagandha'' It has a history of use in treating decline in give function associated with age for almost 4000 years. Pharmacological and clinical studies of extracts of the roots, and of isolated compounds and extracts rich is a type of steroid, known generically as with anolides have produced evidence of enhanced cognitive performance**(43)**. It therefore somewhat surprising that vitro AChEI activity for some withanolides was reported only recently**(45)**.

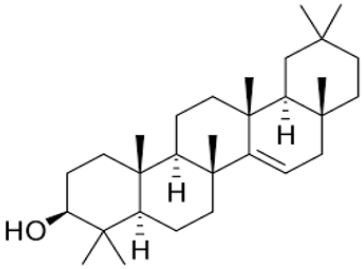
Six compounds were tested two of them being novel of several pregnane glycosides isolated from the roots of Cynanchum atratum (Asclepiadaceae) cyanatroside B 34 was reported to be the most potent AChE inhibitor and its inhibitory action was dose-dependent, reversible and non-competitive in vitro It also ameliorated memory impairment in vivo**(47)**.



Triterpenes appear to be more active than corresponding glycosides Ursolic acid 35, from from *origanum majorana*(lamiceae) was the compound showing the greatest AChE inhibitory activity (IC50 value 75 (IM) when 139 different Indian medicinal plants and spices were screened**(34)**. Taraxerol 36 has a similar structure and was shown to be one of the active compounds, with an IC50 value 79 IM when an exact of the twigs of vaccinium oldhami was investigated**(35).**



35= URSOLIC ACID



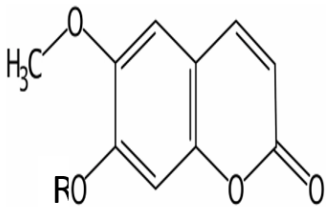
36=TARAXEROL

Shikimate derived compounds as AChE inhibitors :-

The shikimic acid pathway is a major route for the production of a large number of different types of compound in flowering plant Compounds produced me usually phenolic in nature have a skeleton derived from just one phenylpropanoid unit alone, two units combined together or one or more

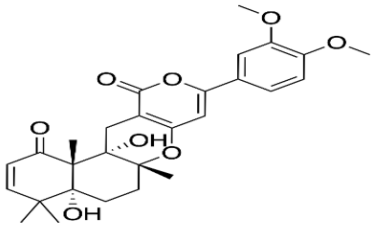
phenylpropanoid units combined with fragments arising from other the metabolic pathways Compounds inhibiting AChE have been reported from all these three types The coumarin scopoletin 37 was isolated as the more active AChEI component (IC50 value 79 IM) of the methanolic extract of vaccinium oldhami (ericaceae) (35). At about the same time, 37, together with scopolin 38 had been discovered to have AChE inhibitory properties using in *silico* approach**(49).**

A model 3D pharmacophore was constructed using a database of known inhibitors and their interaction with AChE from torpedo calefornica. This was Then used to predict compounds from a large database of those with known molecular coordinates, which would be likely to have AChE inhibitory activity. After their activity was predicted 37 and 38 were extracted from Scopolia carniolica (Solanaceae) and shown to be active using the Ellman assay. The activity of 37 was much greater than the corresponding glucoside 38. Interestingly, 37 also showed activity invivo when given to rat.



R=H 37

R=Glucose 38



ARISUGACIN = 39

Compound 39 has been suggested to be biogenetically related to arisugacin meroterpenoids from Penicillium species**(51)**. Arisugacins A and B are selective inhibitors of AChE (and were not selective for BChE) and have been shown to be 200-fold more potent than tactine at inhibiting AChE activity in vitro**(54)**. Several other arisugacins were isolated and tested although not all showed activity(55). Since the antiChE arisugacins lack nitrogen, their mechanism for interaction with AChE is likely to differ fromthat of compounds containing quaternary nitrogen such as ACh. The suggested mechanism for binding to AChE is an electron-donating electron-withdrawing interaction; the donation being from the electron density of the dimethoxy group, coupled with electron-withdrawing of the 2-pyrone ring, a moiety which is considered crucial for antiAChE potency**(56).**

Miscellaneous AChE inhibitors :-

Compounds from **Buddleja crispa** (Buddlejacene) whole plant material have been screened for activity against AChE and BChE. Compounds identified as nonyl benzoate and hexyl phydroxycinnamate weakly inhibited both AChE and BchE**(57).**

Ceramides, tanacetamides A and B, isolated from the whole plants of *Tanacetum artemisioides* (Asteraceae) are reported to inhibit AChE in vitro (IC50 67.1 IM and 74.1 IM, respectively)**(58)**.

Another aromatic alkyl compound with weak activity (IC50 231 IM) which has been isolated recently is 14-(2,3,5-trihydroxyphenyl)tetradecan-2-ol which was obtained from a species of the fungal genus *Chrysosporium***(59)**. Investigations into the constituents of an unknown *Xylaria* species of fungus resulted in the isolation of a series of unique. compounds, the xyloketals, of which xyloketal A showed a strong inhibitory effect against ACHE (IC50 1.5 IM)(1).

Conclusion:-

The usefulness of AChE inhibitors as insecticides and as a treatment for symptoms of the early stages of Alzheimer's disease has stimulated much research into finding natural products with this activity in recent years. This has been facilitated by the introduction of rapid and simple in situ methods for detecting activity. The use of the different enzyme kinetics for an inhibitor employing isoenzymes with known variations and mutations in the amino acid sequence has enabled some attempts at comparison of different areas of binding This has been especially facilitated by the use of molecular modeling programs. In this way. the binding of molecules carrying a positively-charged nitrogen to the "active site'' has been largely clarified and approximately confirms earlier hypotheses about features necessary for a molecule to have inhibitory effects Of more novel interest are the several types of compounds, especially the fungal meroterpenoids, which display very strong inhibitory activity but which do not possess an amine group. Studies into such compounds have revealed the importance of the peripheral binding site in the neck of the gorge.

It is debatable whether new cholinesterases are required as therapeutic agents since cholinesterase inhibition is now being left behind as a therapeutic strategy for Alzheimer's disease. A combination of useful properties, as seen with galantamine, where nicotinergic stimulation occurs as well as AChE inhibition may, however, result in lead molecules for the development of new drugs arising from natural sources. More likely is the fact that discovery of AChE inhibitory compounds in traditional remedies, may explain their use in improving memory and other cognitive functions associated with cholinergic stimulation.

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