Elucidation of role of 3-O-Acetyl-11-Keto-β-Boswellic Acid (AKBA) on Peroxisome proliferator-activated receptor-γ in scopolamine induced cognitive impairment model in rats

A Dissertation Submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI - 600032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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CERTIFICATE

This is to certify that the dissertation work entitled **" Elucidation of role of 3-O-Acetyl-11-keto-β-Boswellic Acid (AKBA) on Peroxisome proliferator activated receptor-γ in scopolamine induced cognitive impairment model in rats "** is a bonafide research work carried out by the candidate **JINU AVARACHAN (Reg.No:261825804)** and submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in partial fulfilment for the Degree of **Master of Pharmacy** in **Pharmacology** , KMCH College of Pharmacy, Coimbatore, Tamil Nadu during the academic year 2019- 2020.

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DECLARATION

I hereby declare that the dissertation work entitled **"Elucidation of role of 3-O-acetyl–11-keto-β-boswellic acid (AKBA) on Peroxisome proliferator-activated receptor-γ in scopolamine induced cognitive impairment model in rats "** submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfilment for the Degree of **Master of Pharmacy** in **Pharmacology** was carried out under the guidance of **DR.G.VENKATESH M.Pharm.,Ph.D.**, at, KMCH College of Pharmacy, Coimbatore, Tamil Nadu during the academic year 2019-2020.

This research work either in part or full does not constitute any of other thesis / dissertation.

Date:

Place: Coimbatore

JINU AVARACHAN

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EVALUATION CERTIFICATE

This is to certify that the research work entitled **"Elucidation of role of 3-O-Acetyl-11-keto-β-boswellic acid (AKBA) on Peroxisome proliferator-activated receptor-γ in scopolamine induced cognitive impairment model in rats"** submitted by **Ms. JINU AVARACHAN (Reg.No:261825804)** to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, in the partial fulfilment for the Degree of **Master of Pharmacy** in the Department of **Pharmacology**, is a bonafide work carried out by the candidate at KMCH College of Pharmacy, Coimbatore, Tamil Nadu during the academic year 2019-2020 and the same was evaluated.

Examination Centre: KMCH College of Pharmacy, Coimbatore

Date:

Internal Examiner **External Examiner**

Convener of Examination

Dedicated to Almighty,

My Beloved Parents

Teachers ,Brothers

& Sisters

ACKNOWLEDGEMENT

Life is beautiful when one's dreams paves the way for the destiny; as my alma mater caters the inner development and exponential growth in my potentials to tread confidently through the path of knowledge. My heart remains with boundless gratitude to my professors, mentors and friends whose encouragement, support, guidance and blessings helped me to overcome all challenges and thereby leading to the successful completion of this project.

At the outset I would like to express my sincere gratitude and my obligations to my esteemed research guide **DR. G. Venkatesh M.Pharm., Ph.D., Associate Professor** , Department of Pharmacology, who by his constant evaluation assured that I stayed focused on my work progressively, his relentless encouragement and sharing of insightful suggestions motivated and helped me to learn more in my studies.

It is a great privilege for me to thank **DR. A. Rajasekaran M.Pharm.,Ph.D., Principal, KMCH College of Pharmacy**, Coimbatore, who has provided excellent facilities to do research in this institution.

I proffer my sincere thanks and remain beholden to our most beloved Managing Trustee, Hon'ble **Dr. Nalla G. Palaniswami** and respected Trustee Madam **Dr. Thavamani D. Palaniswami,** Kovai Medical Center Research and Educational Trust, Coimbatore for all the facilities provided to me at the institution.

I owe unlimited gratitude to our esteemed Head of the department ,**DR. G. Ariharasivakumar M.Pharm.,Ph.D.,** and our teaching staff **DR.R Arivukarasu M.Pharm., PhD., DR. Sengottvel M.Pharm.,Ph.D., DR. Dithu Thekkara M.Pharm.,Ph.D., Mr. G. Sivakumar M.Pharm** and **Mrs. K. Sanju M. Pharm.,** for their immense support, timely help and valuable suggestions.

I express my sincere thanks to **DR.K.S.G. Arulkumaran, M. Pharm., Ph.D.,** for the helps offered during my work.

I am indebted to my friends, **Anitta Augustine**, **Haritha P.H, Anu Anna Abraham, Sumitha M. Das, Dharshan Unni, P. Maharaja, V. Pavithra, S. Anjaladevi, B. Rajesh, K. Balamurugan, Sundar Rajan, K. NIthish, M. Sreedevi, B. Bhaskar, Palanichami and Nithyakala** for the dedication, love and support they gave me during the hard times. Their selfless support and motivation throughout the research is beyond word

It was indeed awesome to share Master studies with wonderful people. I wish to thank my loving friends **Kanupriya.J, Treesa Pearl, Cuckoo, Reshma, Keerthana, Athira, Nayanthara, Solly and Henna.**

I take this opportunity to extend my indebtedness, gratitude to all my juniors for their memorable company and involvement – especially to **Vishali, Kavitha, Pavithra, Jayanthi, Saranya, Nadhiya, Nivetha, Nishanth, Nijanthan, Rajesh, Raj** and soon…

I express my special thanks to **Mrs. Muneeshwari (Lab technician, Dept. of Pharmacology)**, **Mr. Tamilarasan (Lab technician, Dept. of Pharmacology), Mr. Saravanan (Lab technician, Dept. of Pharmacology), Ms. Jeeva (Dept. of Pharmaceutics)**, Librarian and other Lab technicians of KMCH College of Pharmacy for their valuable support and timely help during the course of entire work.

I have great gratitude to **Mrs. Dhanalakshmi** for helping in animal maintenance during the study.

I would like to express my whole hearted gratitude to my Papa **Mr. P. V. Avarachan**, my Mummy **Sani Avarachan**, my sisters **Anu & Binu and my brother-in-law Ebin Johny,** who remembered me in their prayers for the ultimate success. They gave me incessant moral support, encouragement and motivation in my academical pursuits to realize my dreams. My parents – my two life lines supported me financially and thus enabled me to concentrate fully on my studies without any worries.

Last but not least, I would like to thank each and everyone who was important to the successful realisation of this thesis.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORM
AKBA	3-O- Acetyl -11-Keto β Boswellic Acid
PPAR-γ	Peroxisome proliferator activated receptor-gamma
HPMC	Hydroxypropyl Methyl Cellulose
i.p	Intra peritoneal
p.o	Per oral
EDTA	Ethylene Diamine Tetraacetic acid
DMSO	Dimethyl sulfoxide
AchE	Acetylcholinesterase
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
ATCI	Acetylthiocholine
rpm	Rotations per minute
GABA	Gama Amino Butyric Acid
HPTLC	High Performance Thin Layer Chromatography
w/v	Weight/volume
Mg/kg	Milligram/kilogram
ELT	Escape latency time
MMP	Matrix metalloproteinase
SD	Standard deviation
MWM	Morris water maze
OFT	Open field test
NOR	Novel object recognition

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INTRODUCTION

Cognitive impairment is a troubling comorbid consequence of many neurodegenerative diseases that often negatively impacts day-to-day activities, results in unemployment and leads to reductions in quality of life *(Sandry J et al.,2015).*Cognitive decline is found to be a common feature of various neurological disorders such as Alzheimer's disease (AD), schizophrenia, epilepsy and depression*(Puri et al.,2014).*These cognitive impairments may be caused by changes in morphology such as structural changes in neurons, synapses and nerve fibers; changes in neurotransmitter levels; or oxidative brain damage*(Haideret al.,2016).*Various neurodegenerative disorders are accompanied by memory-related neuronal degeneration in the brain and the progression of memory deficits*(Lee B et al .,2015).*It occurs due to various neurochemical and morphological alterations in the brain bringing about severe cognitive and behavioral deficits *(Stasiak et al .,2014).*

Memory involves the retention of information in the brain over a period of time. It is extremely complex in terms of the kind of information that is represented in the brain, the processes associated with it, and its distribution across a variety of neural systems. Memory includes storing, retaining, and later recalling information. Memory consolidation and recall are dependent upon complex mnemonic mechanisms, which vary based on the type of memory representation. The mnemonic processes underlying memory are multifaceted in that information is distributed across a variety of neural systems. For example amnesia results in impairment of such memory processes as pattern separation and pattern association for place, time, affect, response, sensory, perceptual, and language information*(Kesner et al ., 2010).*

There are separate long- and short-term memory (LTM and STM) systems subsequently became a core assumption of modern cognitive psychology *(Norris, 2017).* STM is an essential component of cognition and is defined as the maintenance of information over a short period of time (seconds). STM can remain unimpaired in amnesic patients who show distinct LTM impairments. STM (primary memory) involves a conscious maintenance of sensory stimuli over a short period of time after which they are not present anymore. LTM refers to the mechanism by which acquired memories gain stability or are strengthened over time, and become resistant to

interference. LTM (secondary memory) involves the reactivation of past experiences that were not consciously available between the time of encoding and retrieval *(Anna et al., 2001).*

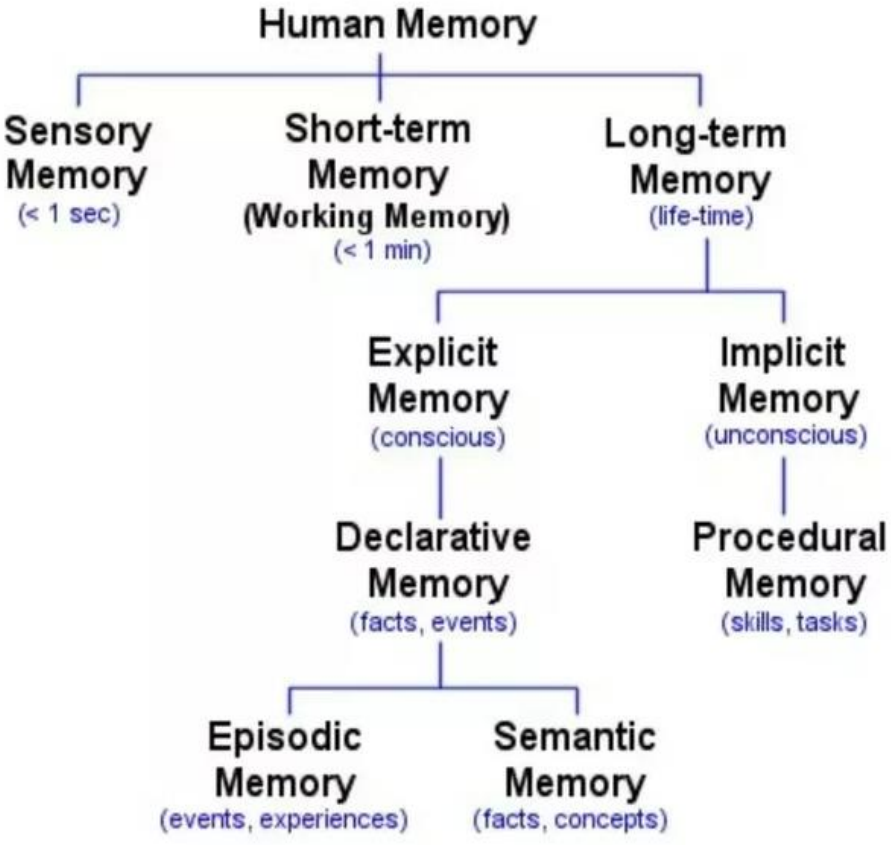


Figure 1: Types of human memory

AMNESIA

Amnesia refers to a deficit of memory due to a specific cause. It is a disorder that arises as a consequence of more than 15 different types of diseases and injuries that affect the brain, such as neurodegenerative and neurological diseases, vascular disorders and traumatic lesions. It is often the earliest and most persistent symptom of dementia. Amnesia has a huge clinical significance its effects on the daily life of patients who suffer from it can be enormous. As a result, there are many efforts towards developing successful treatments. Currently, therapeutic interventions are limited by the lack of understanding of how memory functions in both health and disease *(Ortega et al., 2018).*

There are mainly two types of amnesia

1. Anterograde amnesia [AA]
2. Retrograde amnesia [RA]

Anterograde amnesia refers to the inability to acquire and retain new information. Retrograde amnesia affects the recall of past or recently learned memories*(Markowitschet al., 2016)*.It occur after direct brain damage, but which occurs more frequently as a result of a psychiatric illness*(Smith et al ., 2013)*.RA can sometimes appear disproportionately severe in comparison to AA. Moreover, AA can sometimes occur in the absence of RA. Patients with damage to both the hippocampus and para hippocampalgyrus exhibited the most severe AA and the most severe RA. Patients with damage limited largely to the hippocampus exhibited less severe AA and less severe RA *(Ritchieetal ., 2015).*

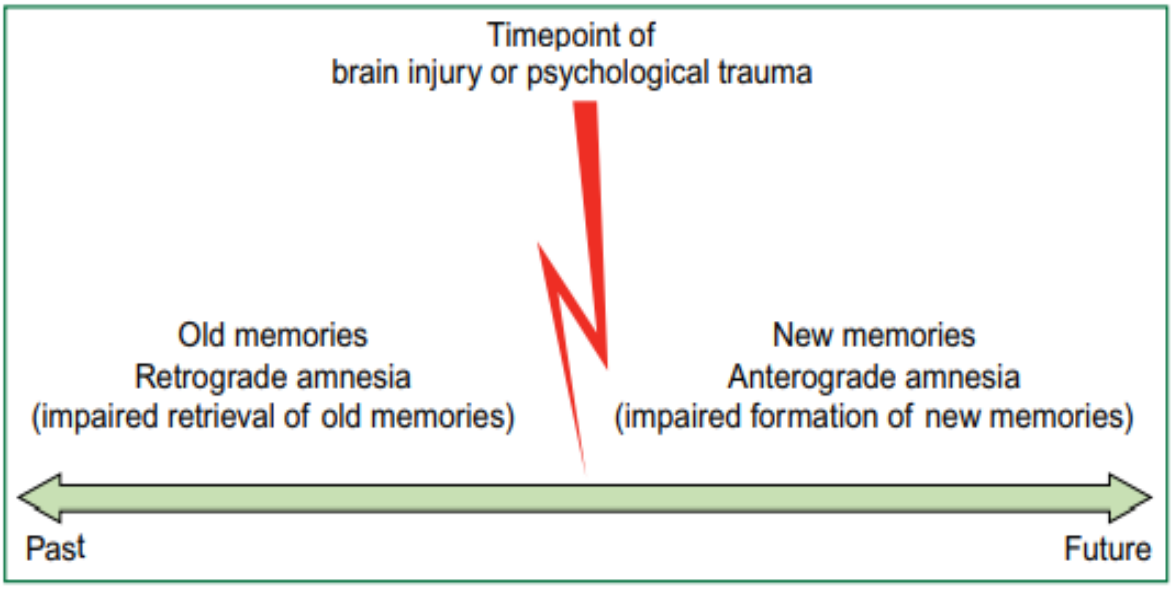


Figure 2: Types of amnesia

DEMENTIA

Dementia, a state of cognitive decline, has recently been described as a neurological disorder itself by American Psychiatric Association Diagnostic and Statistical Manual of mental

disorders (DSM-V) *(Van De et al .,2015)*and considered as a one of the major cause of morbidity and mortality among older people.

The ICD-10 Classification of Mental and Behavioral Disorders (World Health Organization (WHO) 1992) describes dementia as a syndrome occurring as a result of disease of the brain, which is usually chronic or progressive in nature. It consists of impairment of several higher cortical functions, which include memory, thinking, comprehension, calculation, learning, language and judgment. These impairments often occur alongside changes in emotional control, social behavior or motivation. Alzheimer's disease and cerebrovascular disease are among the causes of dementia (WHO 1992). People who develop dementia before the age of 65 years are said to have early-onset (or working age) dementia and those affected after that age to have late-onset dementia. The causes of dementia are not fully understood, but the result is always structural and chemical changes in the brain, leading to neuronal loss and shrinkage of brain volume.

Main causes of dementia

Dementia is not itself a single disease but rather, a clinical syndrome – that is, a collection of symptoms and other features that exist together and form a recognized pattern. The syndrome of dementia has several causes, although some are more common than others.

The main causes include

1. Alzheimer's disease
2. Vascular dementia
3. Dementia with Lowy bodies
4. Front temporal dementia
5. Mixed dementia

Less common causes of dementia

1. Huntington's disease
2. Corticobasal degeneration
3. Creutzfeldt-Jacob disease

4. Multiple sclerosis
5. Normal pressure hydrocephalus
6. Human immunodeficiency virus-related dementia

1. Alzheimer's disease

Alzheimer's disease is the most common form of dementia and is responsible for up to 75% of cases. There is abnormal deposition of insoluble 'plaques' of a fibrous protein called amyloid and twisted fibres called 'neurofibrillary tangles' in the brain. These abnormal plaques and tangles interfere with normal functioning of brain cells. There is also deficiency of the neurotransmitter acetylcholine, which is important for learning and memory.

2. Vascular dementia

Vascular dementia is the second most common type of dementia after Alzheimer's disease. It occurs when blood supply to the brain is compromised by arterial disease. Vascular dementia may develop following a stroke, although progression is more often gradual than step-wise. It may have many manifestations depending on the nature and location of the pathology. In addition to memory and language difficulties, as in Alzheimer's disease, slowing of cognitive processes, depression, anxiety and apathy are common.

3. Dementia with Lewy bodies

Dementia with Lewy bodies is the third most common type of dementia, accounting for around 10% of cases. It is closely associated with Alzheimer's and Parkinson's diseases because it shares several characteristics with these conditions. Characteristic features of dementia with Lewy bodies are visual hallucinations, recurrent falls, and marked fluctuations in levels of conscious awareness and disturbed sleep and/or nightmares.

4. Fronto temporal dementia

Front temporal dementia is a relatively uncommon type of dementia, and the term covers a range of conditions that affect regions in the front of the brain responsible for planning, emotion,

motivation and language. There are several types of frontotemporal dementia, depending on which part of the frontal or temporal lobe is most affected.

5.Mixed dementia

This refers to a condition where more than one type of dementia exists. The most common type is mixed Alzheimer's and vascular dementias, where there are clinical characteristics and brain changes common to both conditions. Mixed dementia is often characterized by a gradual decline in abilities, as in Alzheimer's disease, but with additional mini-strokes or strokes contributing to the overall picture. Alternatively, the person has a history of vascular disease or vascular risk factors, for example ischemic heart disease, hypertension, diabetes, raised lipid levels or smoking.

Epidemiology and risk factors

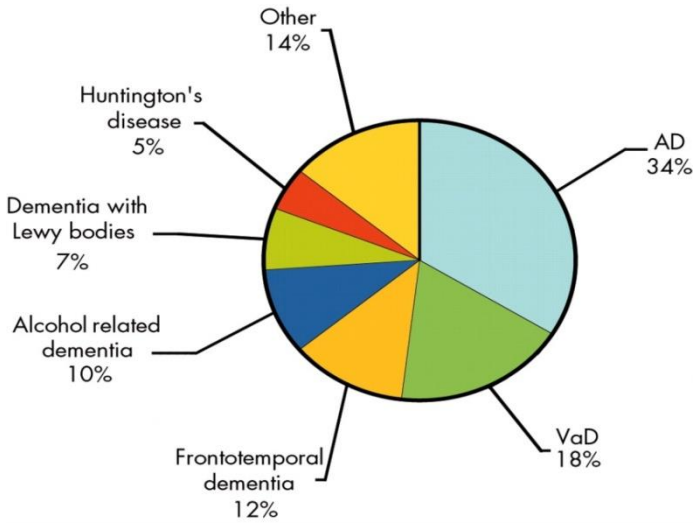


Figure3: Epidemiology of dementia

Risk factors are those features that either increase or decrease the chances of someone developing the condition. They do not mean the person will definitely develop the condition.

1. Age
2. Gender
3. Genes
4. Depression

5. Down's syndrome
6. Vascular risk factors
   a) Blood pressure
   b). Diabetes mellitus
   c). Stroke
   d). Heart disease
7. Lifestyle factors
   a).smoking
   b).Alcohol
   c). Exercise and mental and social stimulation *(Dening et al .,2015)*

VARIOUS MODELS

Various animal models of dementia and cognitive decline have been developed and these may be broadly classified based on diverse pathophysiological basis.

Table 1: Various models of cognitive impairment

Models	Use
Spontaneous models • Aging induced dementia • Senescence accelerated mouse (SAM) models of dementia	Alzheimer's disease Familial Alzheimer's disease
Chemically induced memory deficits • Scopolamine induced memory deficits • IntracerebroventricularStreptozotocin induced dementia • Alcohol induced memory deficits • Aβ infusion induced memory deficits	Alzheimer's disease Sporadic Alzheimer's disease Retrograde amnesia Alzheimer's disease

• Methionine induced dementia • Colchicine induced dementia • Okadaic acid induced memory • Excitotoxins, neurotoxins and choline toxins induced memory deficits • Benzodiazepines induced dementia • Heavy metals induced and • Sodium azide induced dementia	Vascular dementia Sporadic Alzheimer's disease Alzheimer's disease Alzheimer's disease Retrograde or anterograde amnesia Alzheimer's disease
Miscellaneous chemically induced models • Lysophosphatidic acid induced memory deficits • PAF antagonists induced memory deficit • NG-nitro-L arginine methyl ester (L-NAME) and N-omega-nitro-L-arginine induced memory deficits • Clonidine-induced memory deficits • Clozapine-induced memory deficits • Lignocaine-induced memory deficits • Cycloheximide-induced memory deficits • Phenytoin induced memory deficit	Alzheimer's disease Amnesia Alzheimer's disease Alzheimer's disease Alzheimer's disease Alzheimer's disease Alzheimer's disease Alzheimer's disease Alzheimer's disease
Transgenic animal models of dementia • Amyloid β-peptide related animal models • Neurofibrillary tangles related animal	Alzheimer's disease Alzheimer's disease

models • Apo-E mouse models • Secretase transgenic mouse models • Presenilin related animal models • Axonal transport models • Knock out animal models	 Alzheimer's disease Alzheimer's disease Familial Alzheimer's disease Alzheimer's disease Alzheimer's disease
Miscellaneous transgenic animal models • Mutated human α-synuclein mouse model • Mouse models overexpressing human cyclooxygenase-2 (COX-2) • Anti-nerve growth factor (ngf) mice	Alzheimer's disease Alzheimer's disease Alzheimer's disease
High fat diet induced dementia	Alzheimer's disease
Hypoxia induced memory deficits • Hypoxia induced by chemicals • Hypoxia induced by surgery	Vascular dementia, amnesia Alzheimer's disease
Glutamatergic denervation	Alzheimer's disease
Brain injury induced animal model • Concussion like brain injury induced	

animal model • Electrolytic lesion induced memory deficits	Alzheimer's disease Alzheimer's disease
Thiamine deficiency induced animal models • Thiamine deficiency induced memory deficits • Pyrithiamine induced thiamine deficiency dementia.	Alzheimer's disease Wernicke–Korsakoff syndrome *(Sodhi et al .,2014)*

Several studies suggested that among all dementia-inducing scopolamine-induced amnesic condition has more pronounced significant deficits in performance on behavioral tasks of cognitive functioning along with corresponding signs of neurodegeneration in rats which are the characteristic of dementia state. Scopolamine-induced dementia is a widely used animal model for investigating cognitive enhancing drugs *(Haider et al., 2016)*. Scopolamine is basically a muscarinic cholinergic receptor antagonist that causes disturbances in cholinergic system as well as in associated neurochemical cascades resulting in cognitive decline. It is capable of producing memory deficits in the processes of learning acquisition and consolidation. (*Du CN et al .,2015).*Along with neurochemical alterations in CNS scopolamine-induced cognitive decline may also be associated with changes in oxidative status of brain. Elevated oxidative stress triggers a vicious cycle of synaptic dysfunction, memory deficit, neuroinflammation and apoptosis *(Ohno et al .,2007).*

Scopolamine-induced dementia resulted in cholinergic system dysfunction as evidenced by elevation in AChE activity, an important enzyme which hydrolyses ACh, an essential neurotransmitter involved in learning and memory*(Yang et al .,2015 and Qu Z, et al ., 2017).*It also increasescortical and hippocampal MDA content, (The final product of lipid peroxidation and subsequent reduction of the endogenous antioxidant namely GSH, due to elevated Reactive Oxygen Species (ROS) and implies that scopolamine associated oxidative stress accounts for

memory impairment)*(Asgharzade et al .,2015and Rabiei et al .,2015)*, increases GFAP protein expression (implies that scopolamine unregulated inflammatory cascade via astrocytic activation) *(Xu T et al.,2016)*andNF-κB protein expression (indicating that elevated inflammatory response resulting from oxidative stress might account for cognitive deficit in amnestic rats)*(El-Marasy SA et a .,2018).*

Scopolamine treatment resulted in hippocampal MMP-2 and MMP-9 decline (MMP-2 and MMP-9 plays role in modulation of hippocampal synaptic physiology and plasticity.) and GABA and dopamine contents*(Moosavi et al.,2018).*(GABA and dopamine have a greater impact on memory retrieval and consolidation than 5-HT and NE.GABAergic and DAergic deficits contribute to memory impairment*(Gueli MC et al 2013).*

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARS)

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors, belonging to the nuclear receptor family and they are responsible for regulating gene expression. They initially found in the Xenopus frog which induces proliferation of peroxisomes in the cells *(Swati et al., 2016).*

There are three PPAR isotypes which include PPARα, PPARβ /δ, and PPARγ and are structurally homologous. Three isotypes display different physiological functions depending upon their target genes and their tissue distribution *(Anna et al., 2016).* PPARs have been studied as targets for treating multiple diseases *(Ruscicaet al., 2017).*PPARs are similar to steroid or thyroid hormone receptor and they get stimulated by small lipophilic ligands. In case of rodents chemicals including herbicides, industrial solvents, and hypolipidemic drugs lead to increase in the number and size of peroxisomes in the liver and may cause liver hypertrophy, liver hyperplasia, hepatocarcinogenesis, and transcription of genes encoding proximal enzymes. Activated PPARs are also capable of transcriptional repression through DNA-independent protein-protein interactions with other transcription factors such as NFκB signal activators and transducers of transcription STAT-1 and AP-1 signaling *(Oliveira et al., 2007).*

PPAR-α activation reduces triglyceride le PPARs are present mainly in the kidney, liver, small intestine, heart, brain etc. and are responsible for the metabolism of fat and

carbohydrate homeostasis, cell proliferation and differentiation, inflammation, vascular biology, and cancer. PPAR-α are expressed mainly in the liver, kidney, heart etc. *(Marion et al .,2016)*. PPAR-δ/β are expressed majorly in the brain, adipose tissue, and skin, and PPAR-γ is expressed in different tissues.*(Chigurupati et al .,2015)*.In numerous chronic diseases such as, diabetes, cancer, inflammation and atherosclerosis the role of peroxisome proliferator-activated receptors (PPARs) is well established .PPARs are also expressed in the cardiovascular (endothelial cells, vascular smooth muscle cells, and monocytes/macrophages) system and its ligands have shown a role in different cardiovascular risk factor*(Agha et al .,2019).*

Activation of PPAR-γ leads to insulin sensitization and improves glucose metabolism, whereas activation of PPAR-β/δ mainly increases fatty acids metabolism.*(Tyagi et al ., 2011)*.PPARs can be activated by wide range of ligand such as products derived from diet or from intracellular signaling pathway.*(Krey et al.,199)* Several anti-inflammatory drugs like indomethacin, fenoprofen, ibuprofen can also activate PPAR-α and PPAR-γ . Therefore, we can conclude that anti-inflammatory action of these drugs may be due to their ability to bind PPAR*(Jiang et al.,2008)*.Expression of PPARS in the brain cells like neurons and glia, suggested its role in in neuroprotection neurodegenerative disorders *(Escribano et al .,2009).*

Structure of PPAR

Like other receptors PPARs possess four domains. They have a The N-terminal A/B domain contains a weak ligand-independent transactivation function called AF-1. The DNA binding domain (DBD) contains two zinc-fingers that is the particularity of the nuclear receptor (NR) family. Through hinge region the DBD is connected to the Ligand binding domain (LBD). Ligand binding domain (LBD) possesses a ligand dependent transactivation function AF-2. The LBD comprises a fold made by 12α helices and 4β sheets which forms a large hydrophobic pocket for legends to bind.LBD serves as site for dimerization and cofactor interaction *(Nolte et al., 1998)*.Binding of a ligand to the receptor changes the dynamic behavior of PPAR helix12, suggesting that helix 12 plays an important role in PPAR activity (*Kallenberger et al., 2003).*

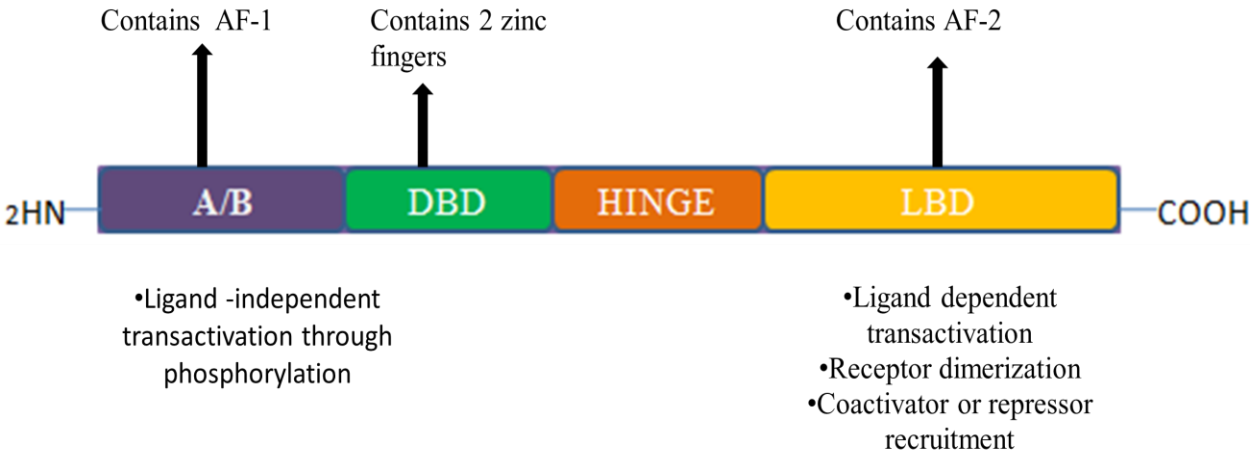


Figure 4: Structure of PPAR

PPAR signaling

Expression of different genes through PPARs is regulated by complex mechanisms. With an another class of nuclear receptor called retinoid X receptor (RXR),the homo PPAR make a heterodimer*(Desvergne et al .,1999)*.Retinoid X receptor (RXR) also exist in three isoforms (α, β, and γ,) and these isoforms are activated by 9-cis retinoic acid.RXR-α shows wide distribution pattern when compared with RXR β, and γ*(Mangelsdorf et al .,1992)* and also RXR-α found to be more promiscuous as a heterodimeric partner for numerous nuclear receptors.*(Chandra et al.,2008)*

The binding of ligands to the PPARs make changes in the expression level PPAR target genes encoded mRNAs and is called as 'transactivation'.PPAR/RXR complex acts as the functional transcription factor*(Kliewer et al., 1992)*.If there is no stimuli gene transcription is suppressed by the association of PPARs-RXR complex with a corepressors such as NCoR and SMRT*(Desvergne et al ., 1999)*.Binding of a ligand to the LBD of the PPAR receptor changes the conformation of structure and this binding cause the release of co-repressors leads to activation of the receptor. Ligand binding domain get stabilized by releasing of the corepressor which result in the binding of co-activators such as CBP/P300, p160/SRC-1, PGC-1*α* etc. Cognition is mainly mediated by the coactivatorPGC-1*(Mattson et al., 2012)*.Co-repressors repress Target gene transcription by the formation of multiprotein complex containing histone deacetylase (HDAC) activity*(Guan et al., 2005)*.Coactivators also forms multiprotein complex

which possess histone acetyltransferase (HAT) activity or methyltransferase activities.*(Yu Set al .,2007)*

Some proliferator response elements (PPRE) are present in the promoter region of target genes and are activated by the PPAR/RXR complex. This brings RNA polymerase complex and allows gene transcription *(Lin J et al.,2005).*

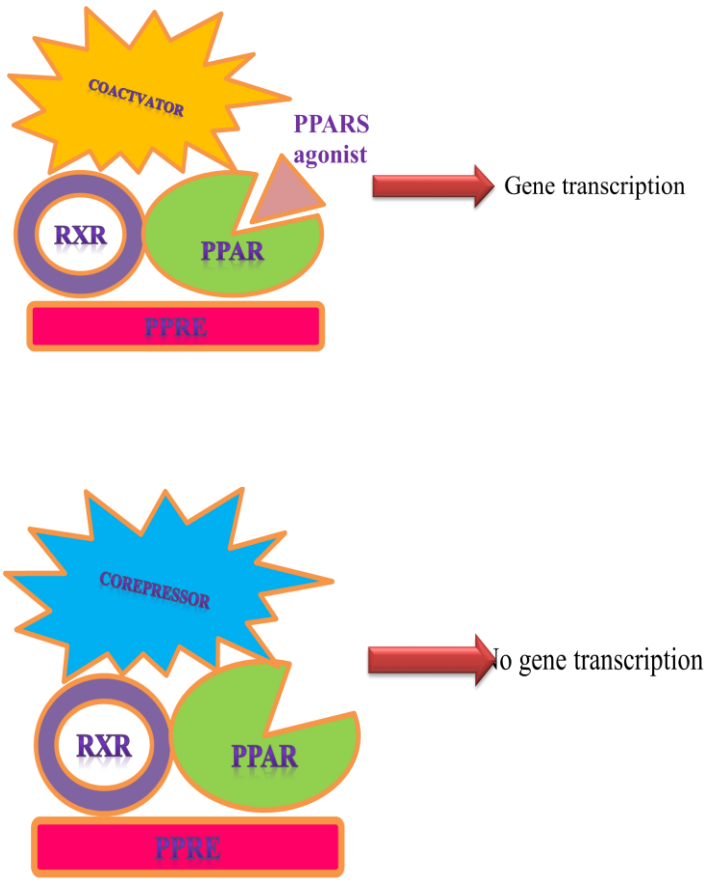


Figure 5 : PPAR signaling mechanism

Synthetic agonists of RXR are able to activate the complex PPAR:RXR and thereby produce antidiabetic actions similar to those actions seen with PPAR agonists in mouse models of type 2 diabetes.*(Mukherjee et al.,1997)* Transcriptional control of PPRE containing target gene for a particular cell type may be exerted according to the level of PPAR and RXR isofroms ,the lipid and retinoid ligands and competition for a PPRE binding site among different PPAR ligand:RXR ligand heterodimer combinations.

Three isoforms of PPAR-γ have been detected in human PPARγ-1, PPARγ-2 and PPARγ-3*(Zhu Y, et al.,1995)*Among the three isotypes PPARγ-1 and 3 code for the same protein while PPARγ-2 code for a different protein. PPARγ-1 has broad tissue distribution (heart, large and small intestines, colon, kidney, pancreas, spleen, and skeletal muscle, brain etc).PPARSγ-2 present in adipose tissue only. PPARγ-3 found only in adipose tissue, macrophages, and colon epithelium.*(Fajas et al.,1998)*

The essential fatty acids like linoleic acid, linolenic acid, arachidonic acid, and eicosapentaenoic acid (EPA) have been shown to bind PPARγ*(Xu HE et al.,1999)*.The first synthetic ligand compounds reported with high-affinity for PPARγ agonists known as thiazolidinediones and are used to treat type 2 diabetes*(Lehmann et al.,1995)*.PPAR-γ agonist overcomes insulin resistance by opposing the effect of TNF-α in adipocytes*(Kubota et al., 2006)*.PPAR-γ also increases the expression of a number of genes code for proteins involved in glucose and lipid metabolism*(Balakumar et al.,2007)*.PPAR- γ has the highest expression in CNS (neurons, astrocytes, and glial cells) than other two types*(Moreno et al., 2004)*.

PPAR-γ is a master regulator of cerebral physiology and potential therapeutic target for the treatment of several pathological conditions associated with neuro inflammation within CNS. Inflammation within the CNS contributes to many acute and chronic degenerative disorders such as PD and AD*(Gonzalez- et al.,1999)*.Inflammation is also under study for a role in the onset of some psychiatric diseases (i.e., depression, post-traumatic stress disorder [PTSD], schizophrenia) *(Dantzer et al., 2008)*.The anti-inflammatory function of PPAR-γ has attracted many attentions since its agonists exert a broad spectrum of protective effects in several animal models of neurological diseases (AD, multiple sclerosis) *(Feinstein et al., ,2003)*.Similar effects have been also described in animal models of psychiatric diseases: studies have shown that stress enhances the production of 15d-PGJ2(natural ligand for PPAR-γ) and increases the expression of PPAR-γ in cerebral cortex as a counterbalancing anti-inflammatory/antioxidant mechanism*(García-Bueno et al.,2008)*.

PPAR-γ and its ligands is master regulators of cerebral physiology so that pathological conditions associated with neuro inflammation can be potentially treated targeting this receptor with inCNS *(Bright et al., 2008)*.

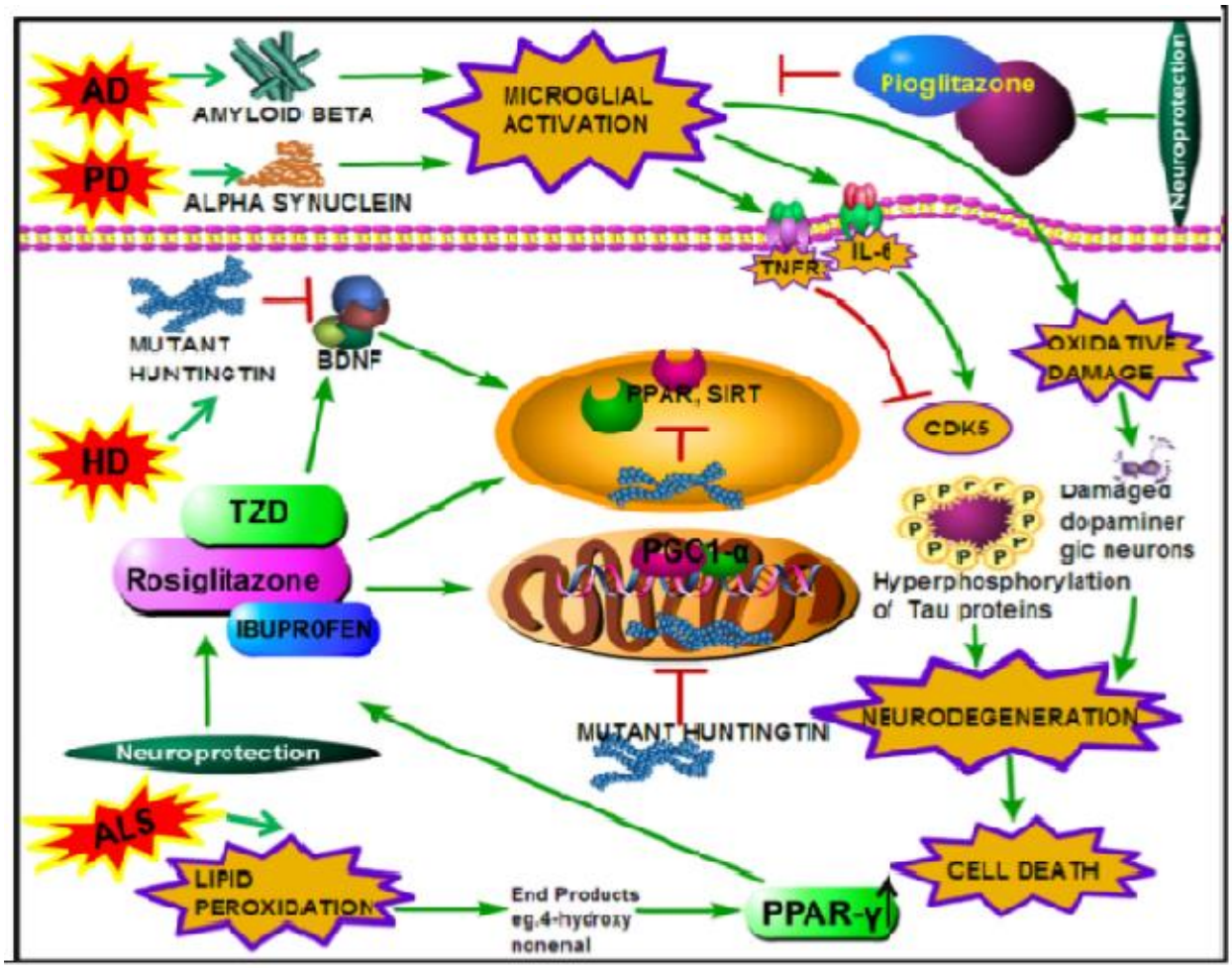


Figure 6: Mechanism of PPAR-γ in neuronal diseases

The resinous part of *Boswellia serrata* possesses monoterpenes, diterpenes, triterpenes, tetra cyclic triterpenic acids and four major pentacyclictriterpenic acids (i.e. B-boswellic acid, acetyl-B-boswellic acid, 11-keto-B-boswellic acid and acetyl-11-keto-B-boswellic acid) (*Siddiqui MZ et al.,2011*). In this line, Boswellic acids have been thoroughly investigated for their possible role in neuroprotection in in-vivo model (*Hamidpour R et al.,2016*). Hence it is essential to develop a molecule that can ameliorates the cognitive deficit is noteworthy.

The Combination of *Boswelliaserrata* (BS) with Melisa officinalis extract showed auditory, visual and working memory improvement in rats(*Iram F et al.,2017*).Studies also stating that BS treated aged rat had greater dentate gyrus ,increased dentate granule cells with more hippocampal density and dendritic spines than untreated rats. which are considered to be a unique region for memory processing(*Taghizadeh M et al.,2018*).Recent studies demonstrated

that Peroxisome proliferator- (PPAR-γ) activated receptor has been identified as a novel target for dementia and Alzheimer's(*Ebrahimpour S et al.,2017, Govindarajulu M et al.,2018*).

Interestingly Boswellic acid had promising interaction with Peroxisome proliferator-activated receptor (PPAR- γ), evident from 3D-pharmacophore study (Swiss Target Prediction tool).Though Boswellic acid have possible physiological, anatomical and docking evidence with PPAR- γ relation with cognitive functionality, no study have substantiated yet. Hence we hypothesis that, treatment with Boswellic acid can be a new zone of approach for treating dementia by activating PPAR- γ receptor.

REVIEW OF LITERATURE

Chao Wei et al., (2020) stated that AKBA (Acetyl-11-keto-β-boswellic acid) a novel candidate, could protect against cognitive and neuro pathological impairments in AD. They found that AKBA treatment resulted in a significant improvement of learning and memory deficits, a dramatic decrease in cerebral amyloid-β (Aβ) levels and plaque burden, a profound alleviation in oxidative stress and inflammation, and a marked reduction in activated glial cells and synaptic defects in the APPswe/PS1dE9 mice. Furthermore, amyloid precursor protein (APP) processing was remarkably suppressed with AKBA treatment by inhibiting beta-site APP cleaving enzyme 1 (BACE1) protein expression to produce Aβ in the APPswe/PS1dE9 mice brains. Mechanistically, AKBA modulated antioxidant and anti-inflammatory pathways via increasing nuclear erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) expression, and via declining phosphorylation of inhibitor of nuclear factor-kappa B alpha (IκBα) and p65.

Aghaei et a.,l **(2019)** evaluated Peroxisome proliferator-activated receptor-γ activation attenuates harmaline-induced cognitive impairments in rats. Male Wistar rats were divided into vehicle (normal saline), PIO (20 mg/kg i.p.), harmaline (10 mg/kg, i.p.) and PIO + harmaline (PIO injected 2 h before harmaline) groups. Open field, rotarod, wire grip, foot print and Morris water maze tests were used to evaluate the motor and cognitive performance. The results indicated that administration of PIO attenuated harmaline-induced locomotor, anxiety-like behaviors, and spatial learning and memory impairments, but it partially decreased the tremor score. The neuroprotective and anxiolytic effects of PIO can offer the PPAR-γ receptor agonism as a potential therapeutic agent in the treatment of patients with tremor that manifest mental dysfunction.

Govindarajulu M et al.,(2018) stated thatPeroxisome proliferator-activated receptor gamma (PPARγ) agonists such as rosiglitazone and pioglitazone reduce amyloid and tau pathologies, inhibit neuroinflammation, and improve memory impairments in several rodent models and in humans with mild-to-moderate AD. However, these agonists display poor blood brain barrier permeability resulting in inadequate bioavailability in the brain and thus requiring high dosing with chronic time frames. Furthermore, these dosing levels are associated with several adverse effects including increased incidence of weight gain, liver abnormalities, and heart failure. Therefore, there is a need for identifying novel compounds which target PPAR-

γ more selectively in the brain and could provide therapeutic benefits without a high incidence of adverse effects.

Salma A et al., (2018) evaluated the Ameliorative Effect of Silymarin on Scopolamine-induced Dementia in Rats and stated that Scopolamine-induced dementia is a widely used animal model for investigating cognitive enhancing drugs. Scopolamine, a muscarinic receptor blocker that disrupts cholinergic neurotransmission leading to memory impairment. Scopolamine-induced dementia resulted in cholinergic system dysfunction as evidenced by elevation in AChE activity, elevation of cortical and hippocampal MDA content, increased protein expression of GFAP, and NF-κB in the brain cortex and hippocampus, indicating that elevated inflammatory response resulting from oxidative stress might account for cognitive deficit in amnestic rats.

Ebrahimpour S et al., (2017) stated that *Boswellic acid* has a significant inhibitory effect against AChE and controls degeneration of the pyramidal neurons in the rat hippocampus and the cortical areas (pyriform cortex, entorhinal cortex and subiculum) connected to the hippocampus, as well as neuronal loss in associated areas. Therefore BA may be used as a potential natural resource for mitigating learning and memory dysfunction caused by aging and neurodegenerative diseases.

Min Zhang.et al., (2017)stated that Peroxisome proliferator activated receptor γ (PPAR-γ) is a prototypical ligand activated nuclear receptor that coordinates lipid, glucose and energy metabolism, and modulates the inflammatory response. Activation of PPAR-γ may have an impact on the pathogenesis of AD by reducing Aβ accumulation, inflammatory markers and the phosphorylation of tau. PPAR-γ agonists improve memory and cognition in patients with mild to moderate AD and in animal models of the condition. Activation of PPAR-γ resulting in inhibition of p38MAPK activation,which is a part of a complex intracellular signaling pathway that is mainly involved in inflammatory responses and apoptosis.

Aya Shoukry Sayed et a.,l(2016) stated that oral administration of Boswellia, low polar gum resin extract, significantly improved the learning capabilities in rats performing elevated radial arm maze which may reflect an enhancement of spatial learning and memory retention. AKBA showed protection against neuron ischemic injury in a rat stroke model which was caused by the activation of nuclear factor erythroid-2-related factor 2/heme oxygenase-1 pathway. This

protection was shown against transient oxygen and glucose deprivation . It was found that the systemic administration of the boswellic acids fraction of Boswellia papyrifera enhanced spatial memory retention using Morris water maze.

C. Jalili et al., (2014) stated that Boswellic acid plays a role in the synaptic enhancement in hippocampus. Also, it can be assumed that Boswellia extracts and various kinds of boswellic acids improve spatial memory through affecting the metabolism of arachidonic acid. Different doses of hydroalcoholic extract of *Boswellia papyrifera* enhanced spatial memory, which is in line with the findings of the present study. Hippocampal neurogenesis occurs following Boswellia extract administration. Boswellia extract can exert protective effects on the brain neurons in kindled rats. Thus, it can be assumed that Boswellia is a protective factor against neuronal damages during seizure. It seems that Boswellia decreases expression of Cyclin B1 and Bax protein (involved in cell cycle and apoptosis). Also, potent anti-inflammatory effects are found in Boswellia.

Nemat A.Z. Yassina et al., (2013) evaluated the Effect of Boswellia serrata on Alzheimer's disease induced in rats and stated that Boswellia serrata shows satisfactory antioxidant activity in the cerebrovascular system. B. serrata has protective and therapeutic effects on AD-induced rats. Administration of the extract to AD rats improved the pathogenesis of AD as demonstrated by an improvement in the behavioral stress tests (levels of activity and motor coordination) and cognitive abilities, increased brain Ach levels, and decreased AChE levels in the brains.It could ameliorate the neurodegenerative characteristics of AD. The effects of B. serrata at higher doses are better compared with those at lower doses. These results represented satisfactory therapeutic approaches for inter vention against the progressive neurological damage associated with AD, with special reference to oxidative insults.

Siddiqui et al., (2011) stated that the resin of *Boswellia* species has been used as incense in religious and cultural ceremonies and in medicines since time immemorial. *Boswellia serrata*(*Salai/Salai guggul*), is a moderate to large sized branching tree of family Burseraceae (Genus Boswellia), grows in dry mountainous regions of India, Northern Africa and Middle East. Oleo gum-resin is tapped from the incision made on the trunk of the tree and is then stored in specially made bamboo basket for removal of oil content and getting the resin solidified. In addition to its beneficial use for arthritis, this gummy resin is also mentioned in traditional Ayurvedic and Unani texts as an effective remedy for diarrhoea, dysentery, ringworm, boils,

fevers (antipyretic), skin and blood diseases, cardiovascular diseases, mouth sores, bad throat, bronchitis, asthma, cough, vaginal discharges, hair-loss, jaundice, hemorrhoids, syphilitic diseases, irregular menses and stimulation of liver. It is also diaphoretic, astringent, diuretic and acts both as nternal and external stimulant. Modern medicine and pharmacology strongly point out to its use as an antiarthritic, anti-inflammatory, anti-hyperlipidemic (controls blood lipids), anti-atherosclerotic (anticoronary plaque), analgesic (pain-reliever) and hepatoprotective (protects the liver).

Farah Iram et al., (2007) stated that pentacyclic triterpenoids, are the bioactive phytoconstituents of boswellia of which AKBA has shown promising results in experimental and clinical studies. It is considered as the potential pharmacophoric molecule of natural origin that can play a vital role in drug discovery of anti inflammatory and chemotherapeutic agent .Boswellia species and their active constituents Boswellic acids have been thoroughly investigated for their possible role in neuroprotection owing to their potent anti-inflammatory actions. Frankincense (oilbanum) is reported to protect against the streptozotocin induced AD in a rat model by virtue of their antioxidant, anti-inflammatory and anti acetylcholinesterase activities.

AIM AND OBJECTIVES

Aim

Elucidation of role of 3-O-Acetyl-11-Keto-β Boswellic Acid (AKBA) on PPAR-γ in scopolamine induced cognitive impairment model in rats.

Objectives

- To study the effect of 3-O-Acetyl-11-Keto-β Boswellic acid on behavioural changes in scopolamine induced cognitive impairment in rats.
- To study the effect of 3-O-Acetyl-11-Keto-β Boswellic acid on acetylcholinesterase activity.
- To study the effect of 3-O-Acetyl-11-Keto-β Boswellic acid on glutamate and GABA level.
- To study the effect of 3-O-Acetyl-11-Keto-β Boswellic acid on PPAR-γ MMP2 and MMP9 gene expression.
- To assess the histological role of 3-O-Acetyl-11-Keto-β Boswellic acid on scopolamine induced changes in hippocampus and pre frontal cortex.

DRUG PROFILE

3-O-Acetyl-11-keto-β-boswellic acid (AKBA)

Molecular Formula : $C_{32}H_{48}O_5$

Molecular Weight : 512.7g/mol

Chemical name : 3-acetyloxy-heptan ethyl-14-oxo-tetradcahydropicene- 4 carboxylic acid

Structure:

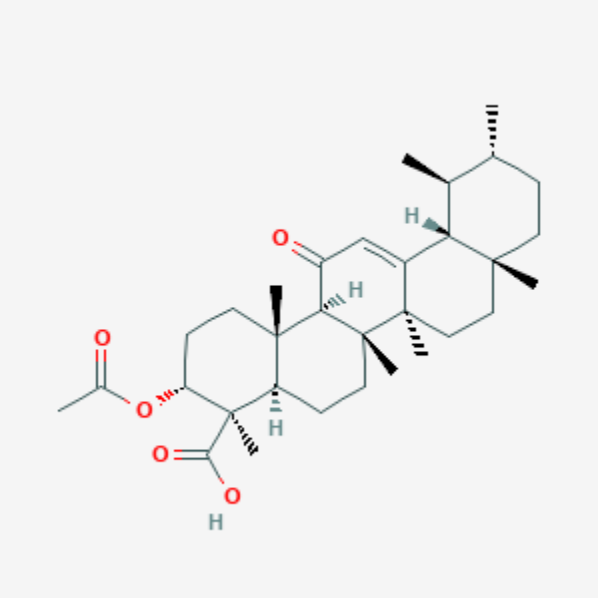


Fig: 7 Structure of AKBA

Physical properties

Physical Description : Solid

Color : White crystalline powder

Solubility : Soluble in methanol & chloroform and insoluble in water

PLAN OF WORK

Phase l

- Literature Review
- Ethical Committee approval (IAEC Reg.No.685/PO/Re/S/2002/CPCSEA. IAEC No.KMCRET/M.Pharm/06/2019-20)

Phase II

Behavioral study

- Open field test
- Y-maze
- Morris water maze test
- Novel object recognition test

Phase III

- Biochemical analysis in rat brain and plasma
- Gene expression study in rat brain
- Histopathological study in rat brain

Phase IV

- Tabulation, compilation of results and statistical analysis of data obtained

STUDY PLAN

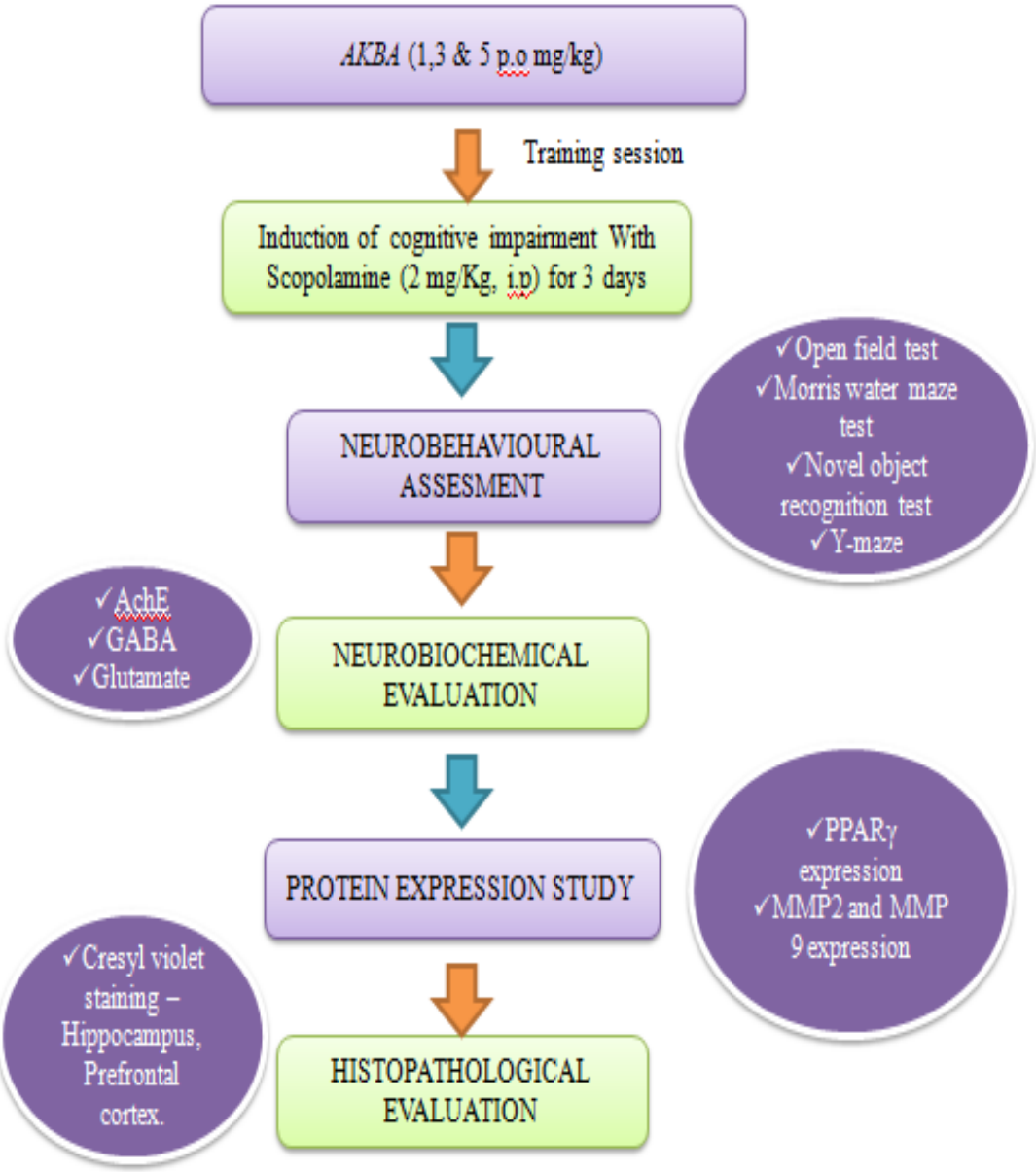


Figure: 8 Study plan

MATERIALS

Table : 2 List of major chemicals

Sl.No	Name of drugs	Manufacturer
1	AKBA(3-O-Acetyl-11-keto-β-boswellic Acid)	Natural remedies
2	Donepezil	Sigma Aldrich
3	Scopolamine	Sigma Aldrich

Table :3 List of instruments

Sl.No	Name of Instruments	Manufacturer
1	Analytical weighing balance	Shimadzu
2	Open field apparatus	Inco manufacturers
3	Morris water maze	Inco Manufactures
4	Y-maze	Inco manufactures
5	Cooling centrifuge	REMI
6	HPTLC	Camag
7	UV-spectrophotometer	PharmaSpec UV-1700, Shimadzu
8	Homogenizer	REMI
9	Magnetic stirrer	REMI
10	pH meter	Eutech

METHODOLOGY

Animals

Female Wister rats (n=36) weighing 150- 200g were obtained from Biogen laboratory animal facility, Bangalore (Biogen Laboratory Animal Facility, Bangalore,Reg. No:971/bc/06-CPCSEA). Rats were kept in separate cages (6 animals per cage) and housed with husk as a bedding material under normal room temperature (25±2º C), 12/12 hr light – dark cycle as well as constant relative humidity (55±5%) throughout the experimental period according to CPCSEA guidelines.

Table :4 Animal Grouping

Groups	**Treatment**	**No. of animals**
1	Normal saline (0.9% w/v,NaCl, p.o)	6
2	Scopolamine (2 mg/kg i.p.)	6
3	Scopolamine+Donepezil (2.5 mg/kg. p.o)	6
4	Scopolamine+AKBA (1mg/kg p.o)	6
5	Scopolamine+ AKBA (3mg/kg p.o)	6
6	Scopolamine+ AKBA (5mg/kg p.o)	6

Preparation of drug solution:-

Scopolamine was dissolved in distilled water for intraperitoneal injection. Donepezil used as a standard dissolved in distilled water and administered orally using a gavage needle at a dose of 2.5 mg/kg.3-O-Acetyl-11-keto-β-boswellic Acid (AKBA) was prepared by suspending in 0.5% HPMC solution and administered orally(*Bairwa K et al.,2015*).

Induction of memory impairment:-

Scopolamine was administered through i.p after one hour of test /standard drug administration to all groups undergoing except the normal control group .

BEHAVIORAL STUDIES

1.Open field test: Exploratory response

The open field test (OFT) is a common measure of exploratory behavior both qualitatively and quantitatively. It is used to assay general locomotor activity levels, anxiety and willingness to explore in animals.

Animals were removed from the home cage and placed directly into one corner of the open field (60cm×60cm). The floor was divided into a grid of 4×4 squares. Movement of the animal in the arena during the 5-minute testing session was recorded. After 5 minutes,the animal was removed and returned to the home cage, and the walls and floor surfaces were thoroughly cleaned with 5% ethanol between the tests to prevent olfactory cues from affecting. The behavior of subsequently tested rats. Parameter like grooming, rearing, ambulation were noted (*Shaahin et al.,2015)*

2.Y maze Test : Simultaneous Discrimination Learning and memory

The 'Y' MAZE is designed for studying Shock Motivated Brightness Discrimination Response, that is, Simultaneous Discrimination Learning in rats. The 'Y' Maze has been designed to make the animal to learn and to discriminate between two 'Arms' -one illuminated, without shock and other non illuminated with shock - and learn to reach the correct 'Arm', the illuminated one.

Three identical, removable sunmicalined chambers arranged in 'Y' shape connected to the central chamber. Each arm has a working dimension of approx. 30 cm x15cm x15 cms., with electrifiable grill, and has chamber light or Cue light with indicator, grill charge indicator, rat presence indicator and hinged top. The Central compartment also contains a wire grill. The Separate Control unit, having a replica of the maze along with long connecting cables.

At the beginning, every rat, in turn, is placed in one of the chambers for a period for 5 minutes, without any stimulus, to allow an accommodation to the situation. The naive animal was allowed to explore the 'Y' Maze apparatus for 5 minutes at the start of their training. The rats were then put into the Starting chamber (as per program switch). After 5 seconds (approx.) shock was applied by switching the unit on or by the rotary program selector till the animal reaches the goal in the illuminated Arm. The animal was then allowed to stay in that Arm for the entire inter- trial period. Then the next program was selected from the Program Selector Switch and the process was repeated. This training continued till the animal attains 9 out of 10 correct choices

(An error is counted when the animal enters the wrong compartment during the entire trial.). After training the animal was placed in any of the arm and applied shock after 5 seconds. The time taken by the animal to reach the illuminated arm was noted (*Ru M et al.,2018*).

3.Novel object recognition test: Recognition memory

The NOR task is very useful to study short-term memory, intermediate-term memory, and long-term memory, through manipulation of the retention interval, i.e., the amount of time animals must retain memory of the sample objects presented during the familiarization phase before to the test phase, when one of the familiar objects is replaced by a novel one

The test consists of three sessions separated by 24 h. In session (habituation); animals were allowed to freely explore the open field for 10 min. In session 2 (familiarization); rats were allowed to interact with two identical objects placed in the centre of the open field during two 5 min periods. During inter period time (1 hour), rats were placed in their home cage. In session 3 (test); after 24 h, rats were presented with 1 familiar object and a novel object that differs in shape, colour, and texture during 10 min session. The initial position of the animal facing the objects unchanged throughout the sessions. Contacts with objects defined as when the animal's nose less than 1 cm from the object. No of contact with objects,time to reach the object and time spent in the vicinity of objects were noted(*Venkatesh G et al.,2019*).

4.Morris water maze Test: spatial memory

Morris water maze (MWM) test used to check spatial learning of rodents which relies on distal cues to navigate from start locations around the perimeter of an circular pool to locate a escape platform. Spatial learning is assessed across repeated trials and reference memory is determined by preference for the target area without platform.

The test was carriedout in a black circular pool (160 cm in diameter and 80 cm in height) filled with water to a depth of 40 cm (21±2 °C). The pool divided into four equal quadrants. A platform (10 cm in diameter) submerged 1.5 cm below the surface of the water in the center of one of the quadrants. The experiment was performed in a dimly lit room with some visual cues around the maze

The animals were received four trials per day. The rats were trained to find the hidden platform. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive training trials on each day. The rat was gently placed in the water between quadrants, facing the wall of pool and allowed 60 sec to locate submerged

platform. Then, it was allowed to stay on the platform for 20 sec. If it failed to find the platform within 60 sec, it was guided gently onto platform and allowed to remain there for 20 sec. Day 6 escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition or learning. Animals were subjected to training trials for six consecutive days. Between one trial and the next, water was stirred to erase olfactory traces of previous swim patterns. Time spent in the target quadrants and the number of crossings while searching the target quadrant was also noted as index of memory(*El-Marasy SA et al.,2018*).

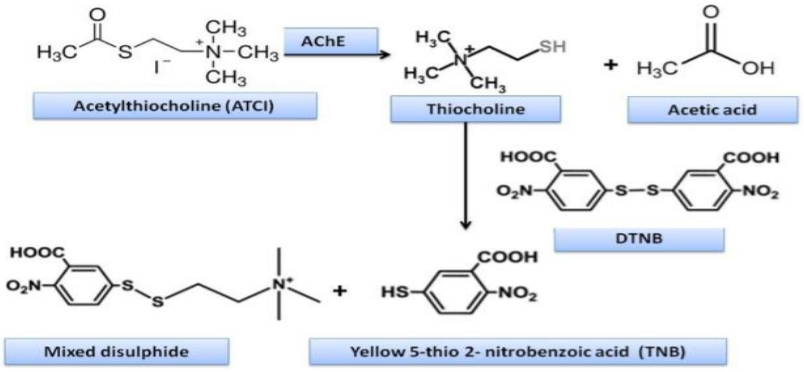
BIOCHEMICAL ESTIMATIONS

Blood and Brain sample Collection

Immediately after the last behavioral test, animals were anaesthetized and blood was collected through retro-orbital route in 0.2 % ETDA containing tubes kept in ice and Centrifuged at 3000 rpm for 15 min. Plasma was separated and aliquots were stored at −80°.Animals were sacrificed by decapitation and collected the brain sample in the containers kept on the ice.

1.In vitro acetylcholinesterase inhibition assay

Ellman's procedure is commonly used for the determination of cholinesterase activity and also for monitoring of the ACh hydrolysis by acetylcholinesterase (AChE) or butyrylcholinesterase. The is substrate acetylthiocholine is hydrolysed by the enzyme resulting in the product thiocholine and it reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2- nitrobenzoate which can be detected at 412 nm.



0.4 mL blood plasma was added to a test tube containing 2.6 ml of 0.1M phosphate buffer, 100 µL of DTNB reagent (10 mg DTNB in 100 mL of phosphate buffer, pH8.0) The substrate acetylthiocholine iodide 20 µL(75mg of acetylcholine iodide per 50 mL of distilled water) was added and change in optical absorbance was measured every 2 min for 10 min at 412 nm to provide a measure of enzyme activity. The AChE activity was determined by following formula:

$R=5.74\times10^{-4}\times\Delta A/C$

Where,

R= Rate of enzymatic activity (in µmoles of acetylthiocholine hydrolysed /min/mg protein)

A=Change in absorbance /min

C = Concentration of tissue homogenate

2. Glutamate and GABA estimation in brain tissue by HPTLC method

Every 10 mg brain tissues were homogenized with 200µl of 0.1 N Hcl in 70% ethanol, then transferred to polypropylene tubes and centrifuged at 4300 rpm for 15 min in a cooling centrifuge. The supernatant are collected into micro centrifuge tubes and used immediately for glutamate and GABA estimation by HPTLC (CAMAG) at 550 nm wavelengths. Standard solutions of glutamate (10–100µmol /spot) and GABA (5-80ng/spot) will be prepared for plotting the calibration curve.

Chromatographic Conditions:

- ✓ Stationary phase : HPTLC Silica gel GF254 plates
- ✓ Mobile phase : n-butanol: glacial acetic acid: water (60:15:25 v/v)
- ✓ Chamber saturation time : 15mts
- ✓ Prewashing : Methanol
- ✓ Instrument : HPTLC (Camag)
- ✓ Applicator : Linomat V
- ✓ Scanner : Camag TLC scanner III
- ✓ Developing chamber : Twin trough glass chamber (20 × 10)
- ✓ Developing mode : Ascending mode (multiple development)
- ✓ Detection reagent : 0.2% Ninhydrin in n- butanol
- ✓ Scanning wavelength : 550 nm
- ✓ Experimental condition : Room temperature

- ✓ Temp/RH-55 : 65%.

Preparation of standard solution:-

0.1N HCl was prepared using 80% ethanol. This was used to dissolve L-glutamic acidand GABA.

Preparation of 0.2%Ninhydrin solution:-

In 100 ml standard flask, 200 mg of ninhydrin was taken and dissolved in 100 ml of n-butanol(*Venkatesh G et al.,2019*).

3.Polymerase Chain Reaction (PCR)- Gene expression study

Preparation of brain samples

1A. Tissue:

Homogenized tissue samples in TRI Reagent (1 ml per 50–100 mg of tissue) in a Polytronor other appropriate homogenizer

1B. Monolayer cells:

Lyses cells directly on the culture dish. Use 1 ml of the TRI Reagent per 10 cm^2 of glass culture plate surface area. After addition of the reagent, the cell lysine was passed several times through a pipette to form a homogenous lysate.

1C. Suspension cells:

Isolated cells by centrifugation and then lyse in TRI Reagent by repeated pipetting. One ml of the reagent was sufficient to lyse $5–10\times10^{-6}$ animal, plant, or yeast cells, or 10^7 bacterial cells.

2. Phase Separation:

To ensure complete dissociation of nucleoprotein complexes, allowed samples to stand for 5 minutes at room temperature. Added 0.1 ml of 1-bromo-3-chloropropane or 0.2 ml of chloroform per ml of TRI Reagent used. Covered the sample tightly, shook vigorously for 15 seconds, and allowed to stand for 2–15 minutes at room temperature. Centrifuged the resulting mixture at 12,000 × g for 15 minutes at 2-8 °C. Centrifugation separated the mixture into 3 phases: a red organic phase (containing protein),an interphase (containing DNA), and a colorless upper aqueous phase (containing RNA).

RNA Isolation

1. Transfered the aqueous phase to a fresh tube and add 0.5 ml of 2-propanol per ml of TRI Reagent used in Sample Preparation, step 1 and mix. Allowed the sample to stand for 5–10

minutes at room temperature. Centrifuged at 12,000 ×g for 10 minutes at 2-8 °C. The RNA precipitated and formed a pellet on the side and bottom of the tube.

2.Removed the supernatant and washed the RNA pellet by adding a minimun of 1 ml of 75% ethanol per 1 ml of TRI Reagent used in Sample Preparation,step 1. Vortex the sample and then centrifuged at 7,500 ´ g for 5 minutes at 2-8 °C.

The RNA was quantified by NanoDrop (Thermo Scientific Wilmington, DE, USA) and whose 260/280 ratio found >1.8, were used for cDNA conversion. The RNA was converted to cDNA by high capacity cDNA conversion kit (Applied Biosystems). Expressions of PPARy MMP2 , MMP9, and were studied. Primers were designed using Primer3 software. PCR reactions were run in qPCR (Applied Biosystems) system. Reactions were initiated with denaturation at 95 °C for 30s, followed by 40 cycles of two-step reaction, denaturation at 95 °C for 5s, and annealing and extension for 30s. Gene expression were normalized by reference gene GAPDH. The experiments were conducted in duplicates.

Table :5 PCR Primer details

GENEs	**PRIMER /SEQUENCE**
MMP-2	Forward- TGGTGTGGCACCACCGAGGA Reverse - CCTTGCCATCGCTTCGGCCA
MMP-9	Forward- AGCCGGGAACGTATCTGGA Reverse - TGGAAACTCACACGCCAGAAG
PPAR-γ	Forward- TAGGTGTGATCTTAACTGTCG Reverse – GCATGGTGTAGATGATCTCA
GAPDH	Forward- CAACTTTGGCATCGTGGAAG Reverse- CTGCTTCACCACCTTCTT

HISTOPATHOLOGICAL EVALUATION

Histopathology is the microscopical study of tissues for pathological alterations. This involves the collection of morbid tissues from biopsy or necropsy, fixation, preparation of sections,staining and microscopical examination.

Collection of materials

Thin pieces of 3 to 5 mm, thickness were collected from tissues showing gross morbid changes along with normal tissue.

Fixation

Kept the tissue in fixative for 24-48 hours at room temperature

The fixation was useful in the following ways:

a) Serves to harden the tissues by coagulating the cell protein,

b) Prevents autolysis,

c) Preserves the structure of the tissue, and

d) Prevents shrinkage

Common Fixatives: 10% Formalin

Haematoxylin and eosin method of staining: Deparaffin the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol. Then cleaned the section in tap water and stained with haematoxylin for 3-4 minutes and again cleaned under tap water. Allow the sections in tap water for few minutes and counter stained with 0.5% eosin until section appears light pink (15 to 30seconds), and then washed in tap water. Blotted and dehydrated in alcohol and cleared with xylol (15 to 30 seconds). Mounted on a Canada balsam or DPX Mounted and kept the slide dry and remove air bubbles*(Nimish L et al.,2011).*

STATISTICAL ANALYSIS

Data were expressed as Mean±SD. Statistical analysis was carried out by one way ANOVA followed by post hoc analysis Tukey's multiple comparison test and in two-way ANOVA followed by Bonferroni post test using prism 5.0.

RESULTS

EFFECT OF AKBA ON BODY WEIGHT

There were gradual increase in the body weight of animals but he scopolamine induced group of animals showed a comparatively slow rate of increase as compared to other groups.

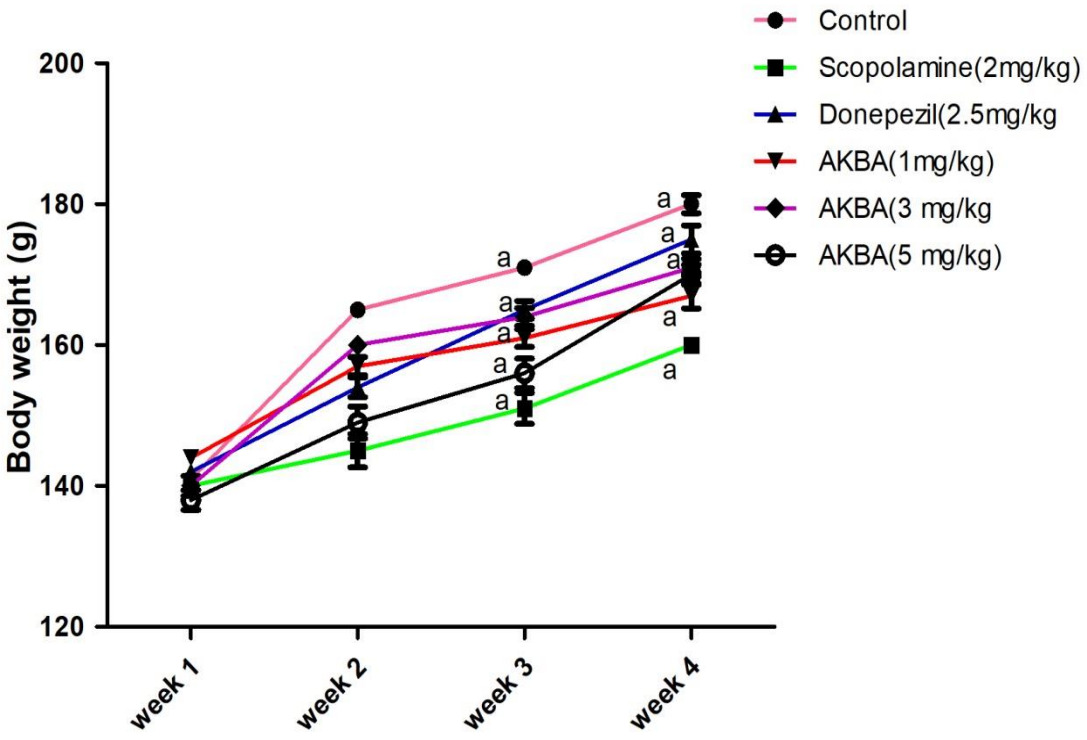


Figure9:Body weight Mean±SD, n = 6. Statistical analysis was carried out in two-way ANOVA followed by Bonferronipost test using prism 5.0. Statistical significance $^{a}P< 0.001$ vs scopolamine treated group

OPEN FIELD TEST

1.Effect of AKBA on rearing activity

As compared to control group scopolamine treated group showed a decrease in rearing activity F (5, 30) =6.29 P <0.01].Treatment with donepezil and AKBA did not show any significant change in rearing activity when compared with scopolamine treated group.

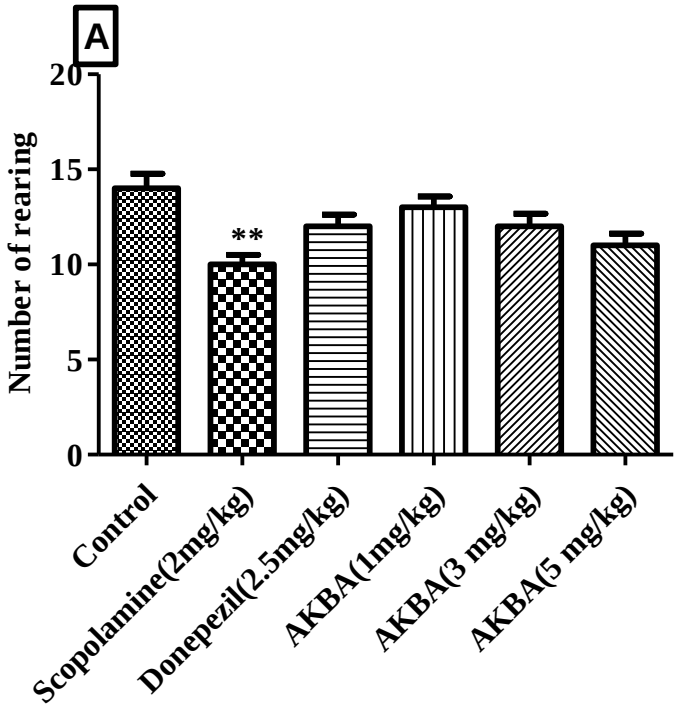


Figure:10Effect of AKBA on open field test Mean±SD, n = 6. Statistical analysis was carried out in one-way ANOVA followed by post hoc analysis Tukey's multiple comparison tests using prism 5.0. Statistical significance $^{**}P < 0.01$, vs control

2. Effect of AKBA on grooming activity

Treatment with scopolamine,donepezil and AKBA (1,3 and 5mg/kg) did not show any significant results on grooming acivity.

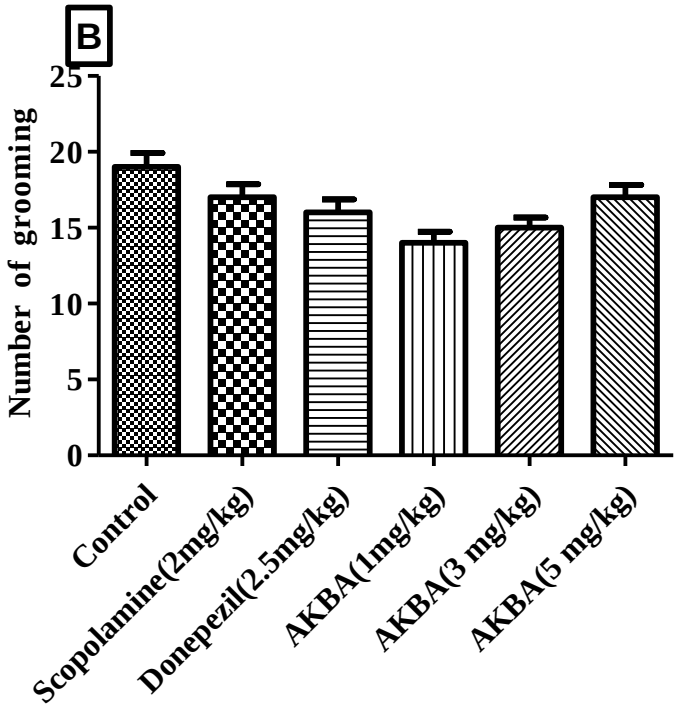


Figure11:Effect of AKBA on open field test Mean±SD, n = 6. Statistical analysis was carried out in one-way ANOVA followed by post hoc analysis Tukey's multiple comparison tests using prism 5.0.

3. Effect of AKBAon ambulation activity

Treatment with scopolamine,donepezil and AKBA (1,3 and 5mg/kg) did not show any significant results on ambulation acivity.

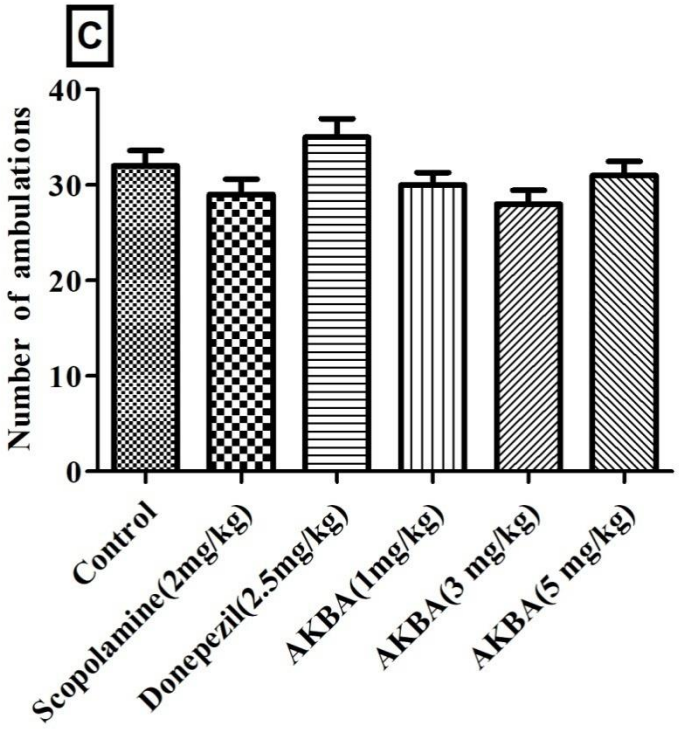


Figure 12:Effect of AKBA on open field test Mean SD, n = 6. Statistical analysis was carried out in one-way ANOVA followed by post hoc analysis Tukey's multiple comparison tests using prism 5.0.

Y-MAZE TEST

Effect of AKBA on escape latency time

Scopolamine treated rats showed [F (5, 30) = 31.80, P <0.001] increased escape latency time when compared to control.Treatment with donepezil [F (5, 30) = 24.61, P < 0.001] and AKBA at 1mg/kg, [F (5, 30) = 12.33, P < 0.001], 3mg/kg [F (5, 30) = 15.92, P < 0.001] and 5mg/kg [F (5, 30) = 19.42, P < 0.001] markedly decreased ELT when compared to scopolamine treated group.

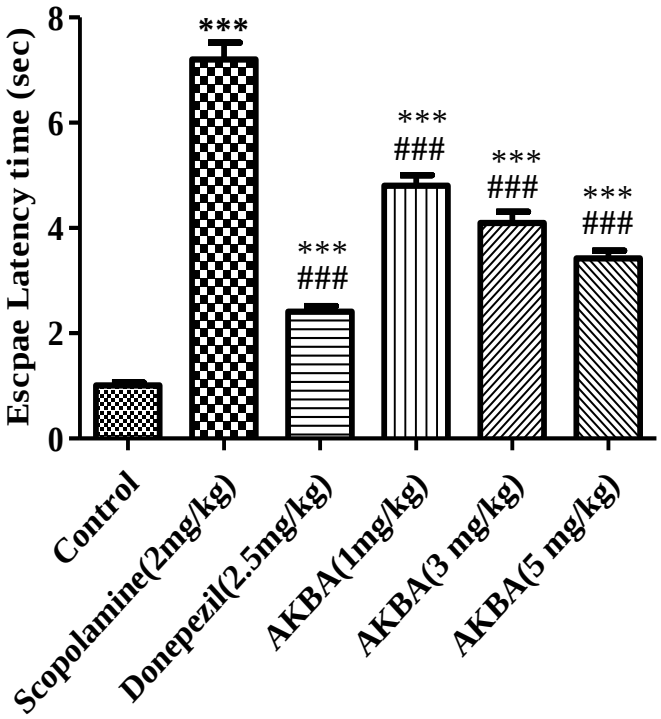


Figure 13:Effect of AKBA on Y maze performance.Mean SD, n = 6. Statistical analysis was carried out in one-way ANOVA followed by post hoc analysis Tukey's multiple comparison tests using prism 5.0. Statistical significance.***P < 0.001, vs control. $^{###}$ P < 0.001 vs scopolamine treated group.

NOVEL OBJECT RECOGNITION TEST

1.Effect of AKBA on number of contacts.

Scopolamine treated rats significantly decreased the number of contacts with novel object [F (5,30) =13.90 , P < 0.001] and increased number of contacts with familiar object [F (5, 30) = 19.25, P < 0.001] when compared to control rats. In comparison with negative control group number of contacts were found to be increased with novel object [F (5, 30) = 10.69, P < 0.01] and decreased with familiar object [F (5, 30) = 11.76, P < 0.001] for donepezil treated group. AKBA (1,3 and 5 mg/kg) significantly increased the number of contacts with novel object [F (5, 30) = 3.208, P < 0.001) ,[F (5, 30) = 6.416,P < 0.001] and [F (5,30) =8.020, P < 0.001], and significant decrease in number of contacts with familiar object [F (5, 30) = 3.743, P < 0.001) ,[F (5, 3;0) = 6.416,P < 0.001]and [F (5,30) = 9.411, P < 0.001]was observed as comparable to scopolamine treated rats.

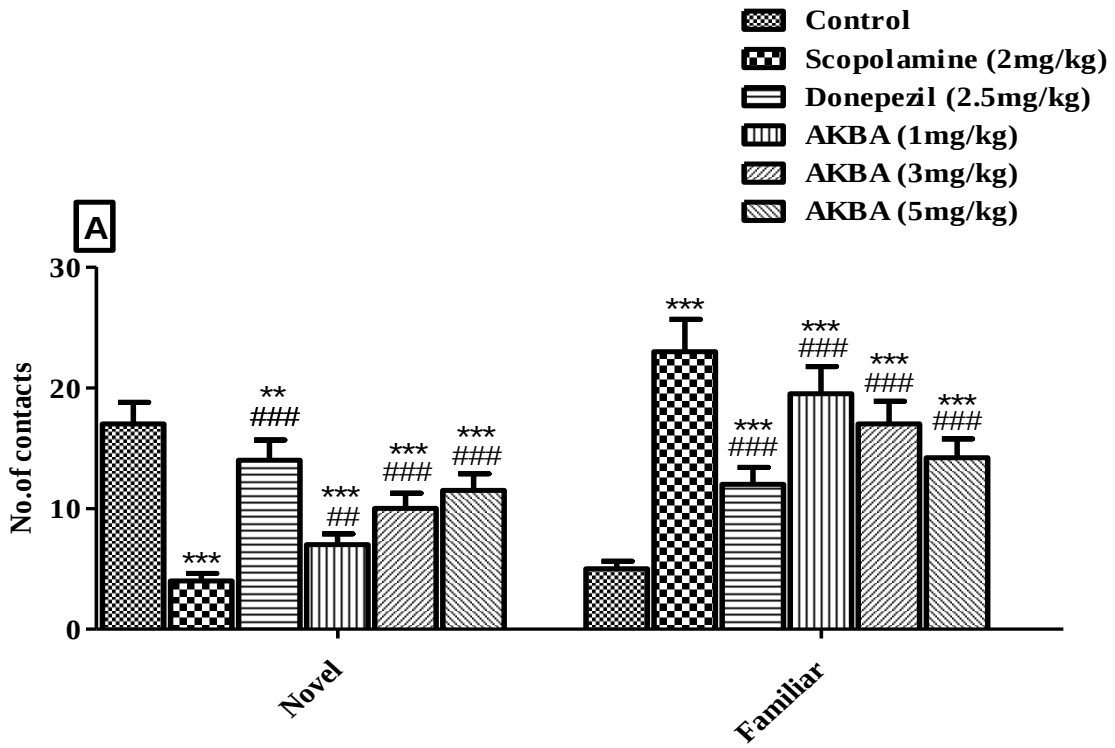


Figure 14: Effect of AKBA on Novel object recognition test. Mean±SD, n = 6. Statistical analysis was carried out in two-way ANOVA followed by Bonferronipost test using prism 5.0. Statistical significance $^{***}P < 0.001$, $^{**}P < 0.01$vs control.$^{###}$ P < 0.001 vs scopolamine treated group.

2.Effect of AKBA on time spent

Scopolamine induction significantly decreased the time spent with the novel object [F (5,30) =16.93 P < 0.001] and increased with familiar object [F (5,30) = 23.94, P < 0.001] as compared to control group. Donepezil treated group increased the time spent with novel object [F (5,30) = 10.88, P < 0.001] and decreased with familiar object [F (5,30) = 21.28, P < 0.05] when compared with scopolamine treated group. AKBA (1,3 and 5 mg/kg) treatment significantly increased the time spent with novel object [F (5, 30) = 4.837, P < 0.001) ,[F (5, 30) = 6.287 ,P < 0.001]and [F (5,30)= 7.738 P < 0.001], and significant decrease in time spent with familiar object [F (5, 30) = 9.915, P < 0.001], [F (5, 30) = 16.44, P < 0.001] and [F (5, 30) =17.41, P < 0.001] was observed as comparable to scopolamine treated rats.

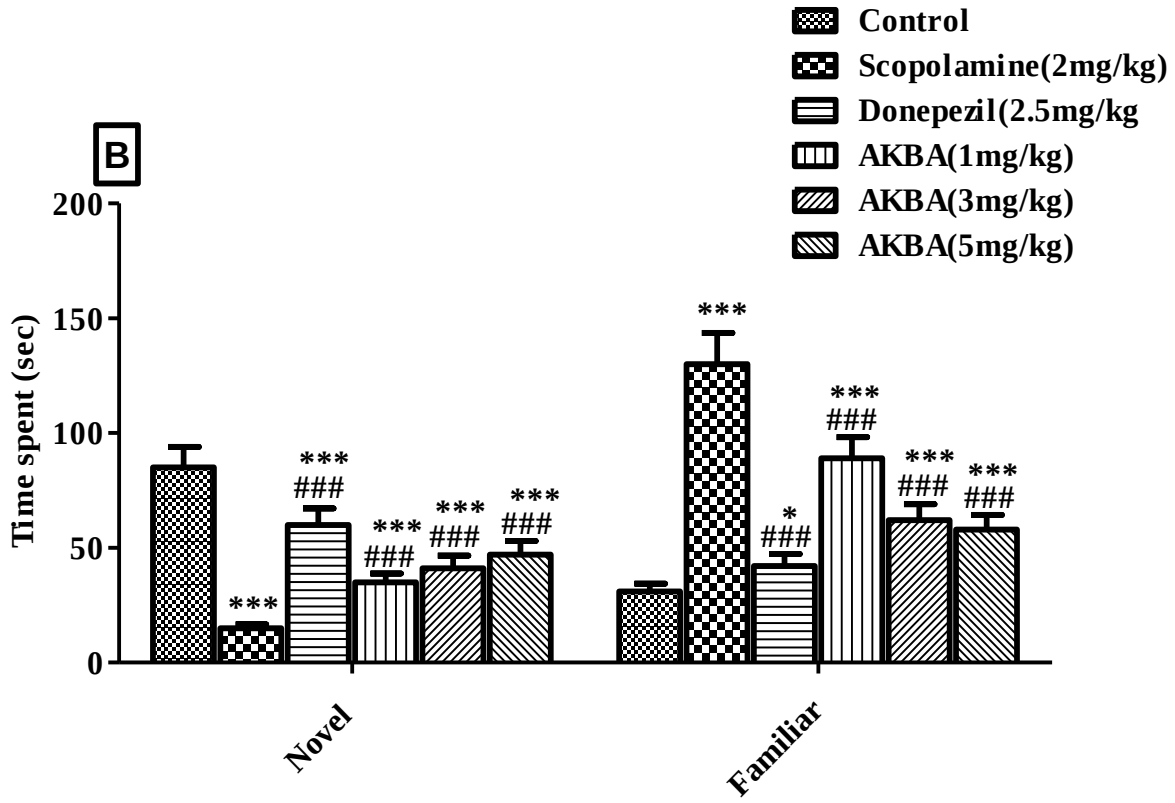


Figure 15 :Effect of AKBA on Novel object recognition test. Mean±SD, n = 6. Statistical analysis was carried out in two-way ANOVA followed by Bonferronipost test using prism 5.0. Statistical significance $^{***}P < 0.001$, $^{*}P < 0.05$ vs control. $^{###}$ P < 0.001,vs scopolamine treated group.

3.Effect of AKBA on time to reach

The time taken by the scopolamine treated group to reach the novel object [F(5,30) = 20.88, P < 0.001] is less than that of familiar object [F(5,30) =8.441 , P < 0.001].For donepezil treated group time to reach novel object [F(5,30) =18.66, P < 0.001] is than that of familiar object as compared to negative control group [F(5,30) =6.664 , P < 0.001]. AKBA (1,3 and 5 mg/kg) treated group takes less time to reach the novel object.[F (5, 30) =11.55 , P < 0.001) ,[F (5, 30) =14.22 ,P < 0.001]and [F (5,30)=16.88 , P < 0.001] than familiar object.[F (5, 30) = 2.221, P < 0.001) ,[F (5, 30) =3.554 ,P < 0.001] and [F (5,30)=4.887, P < 0.001] when compared to scopolamine treated group.

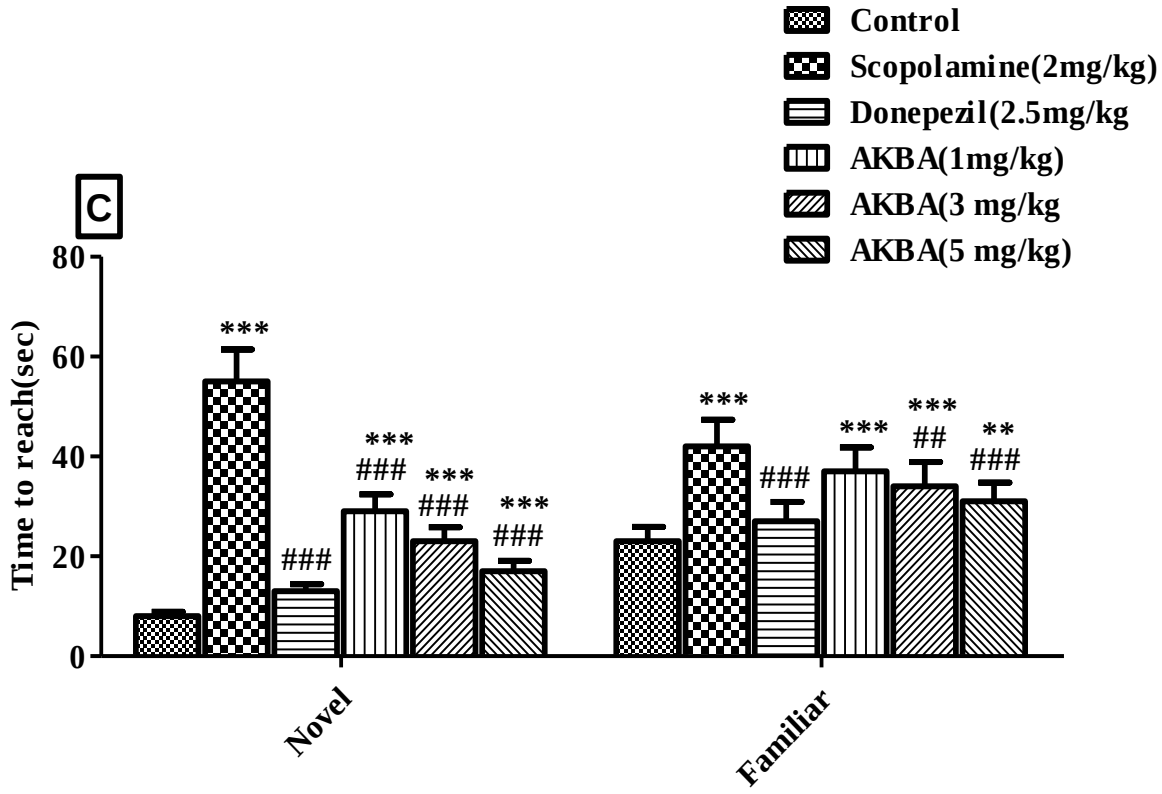


Figure16:Effect of AKBA on Novel object recognition test. Mean±SD, n = 6. Statistical analysis was carried out in two-way ANOVA followed by Bonferronipost test using prism 5.0. Statistical significance $^{***}P < 0.001$, $^{**}P < 0.01$ vs control. $^{###}P < 0.001$, $^{##}P < 0.01$, vs scopolamine treated group.

MORRIS WATER MAZE TEST

1.Effect of AKBA on escape latency time

Effect of AKBA on the scopolamine induced spatial memory was examined by morris water maze.Scopolamine induction markedly increased the escape latency time [F (5,30)=22.3, p < 0.001] when compared with control group.Donepezil at the dose of 2.5 mg/kg significantly [F (5, 30) = 18.73, P < 0.001] reduced ELT in comparison with scopolamine induced group.Treatment with AKBA at 1mg/kg [F (5,30)=11.89,P < 0.001],3mg/kg[F (5,30)=16.35,P < 0.001] and 5 mg/kg [F (5,30)=18.73,P < 0.001] significantly reduced ELT as compared to scopolamine induced group. AKBA at 5mg/kg did not show any significant effects as compared to control and standard group.

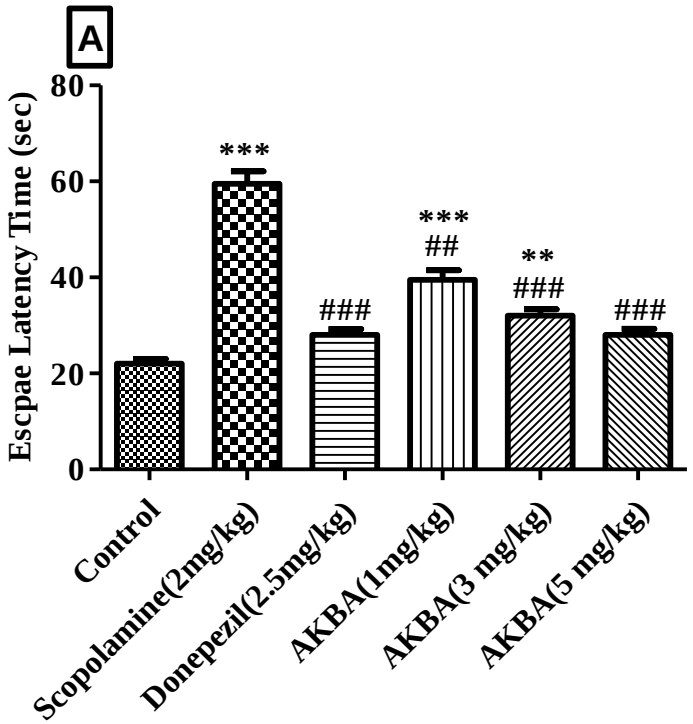


Figure17: Effect of AKBA on Morris water maze: Mean±SD, n = 6. Statistical analysis was carried out in one-way ANOVA followed by post hoc analysis Tukey's multiple comparison tests using prism 5.0. Statistical significance $^{***}P < 0.001$, and $^{**}P < 0.01$ vs control. $^{###}P < 0.001$ and $^{##}P < 0.01$ vs scopolamine treated group.

3.Effect of AKBA on time spent in the target quadrant

Scopolamine induced rats resulted in significant decrease in time spent in the target quadrant in comparison with control rats [F (5, 30) =16.10,P < 0.001].Treatment with donepezil showed significant increase in time spent in the target quadrant when compared to scopolamine treated group [F (5, 30) =13.17,P < 0.001].AKBA treatment at 3mg/kg [F (5, 30) =7.319, P < 0.001] and 5mg/kg [F (5, 30) =10.25, P < 0.001] but not 1mg/kg significantly increased the time spent in target quadrant.

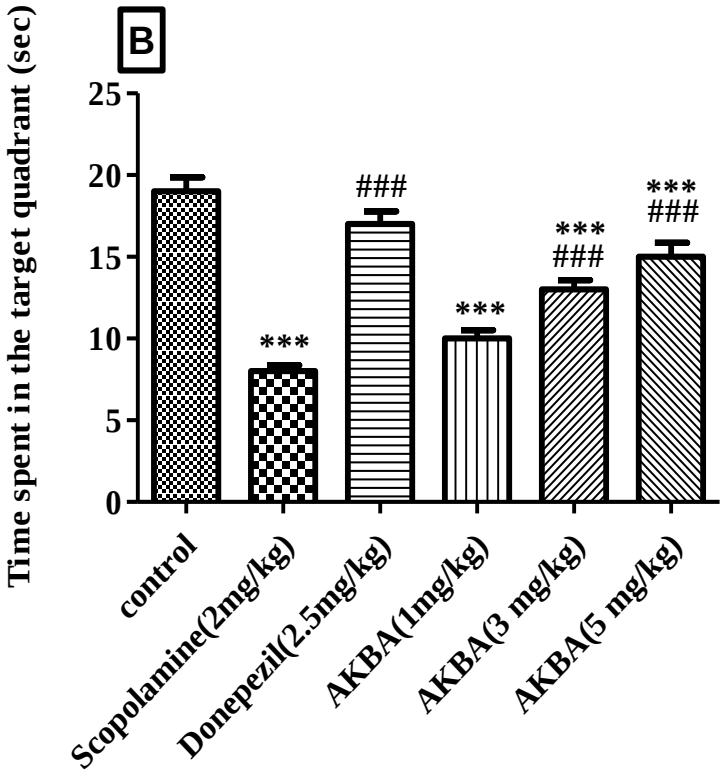


Figure 18:Effect of AKBA on Morris water maze performance.(Mean±SD, n = 6. Statistical analysis was carried out in one-way ANOVA followed by post hoc analysis Tukey's multiple comparison tests using prism 5.0. Statistical significance $^{***}P < 0.001$ vs control.$^{###}$ P < 0.001 vs scopolamine treated group.

3.Effect of AKBA on number of crossings

Number of crossings in the target quadrant decreased for scopolamine treated group when compared to control group [F (5, 30) =16.60,P < 0.001].As compared to scopolamine treated group donepezil significantly increased the number of crossings [F (5, 30) =11.86, P < 0.001].3mg/kg [F (5, 30) =10.60,P < 0.001] and 5 mg/kg [F (5, 30) =10.60,P < 0.001] of AKBA and administered rats exhibited increase in the number of crossings to the target quadrant in comparison with scopolamine group.

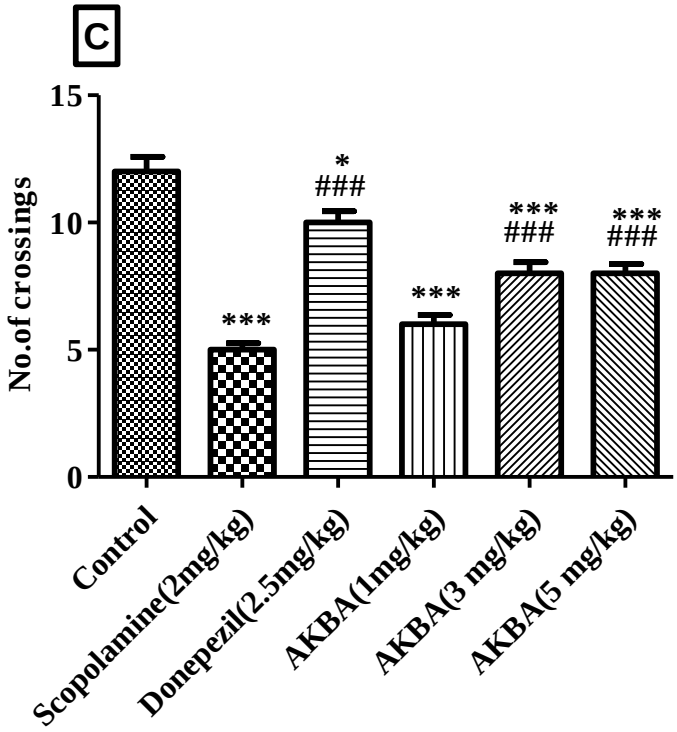


Figure19: Effect of AKBA on Morris water maze performance. Mean±SD, n = 6. Statistical analysis was carried out in one-way ANOVA followed by post hoc analysis Tukey's multiple comparison tests using prism 5.0. Statistical significance $^{***}P < 0.001$, $^{*}P < 0.05$vs control.$^{###}$ P < 0.001vs scopolamine treated group.

EFFECT OF AKBA ON ACETYLCHOLINESTERASE ACTIVITY

When compared with control group scopolamine induced group significantly increased rate of acetylcholinesterase activity [F (5, 30) = 24.19, P < 0.001].Donepezil [F (5, 30) = 17.24, P < 0.001] decreased the rate of enzyme activity in comparison with scopolamine treated group. Administration of AKBA at 1mg/kg [F (5, 30) = 5.88, P < 0.01] 3mg/kg [F (5, 30) = 1298, P < 0.00] and 5mg/kg [F (5, 30) = 16.03, P < 0.001] showed reduction in rate of AchE activity as compared to scopolamine treated group.

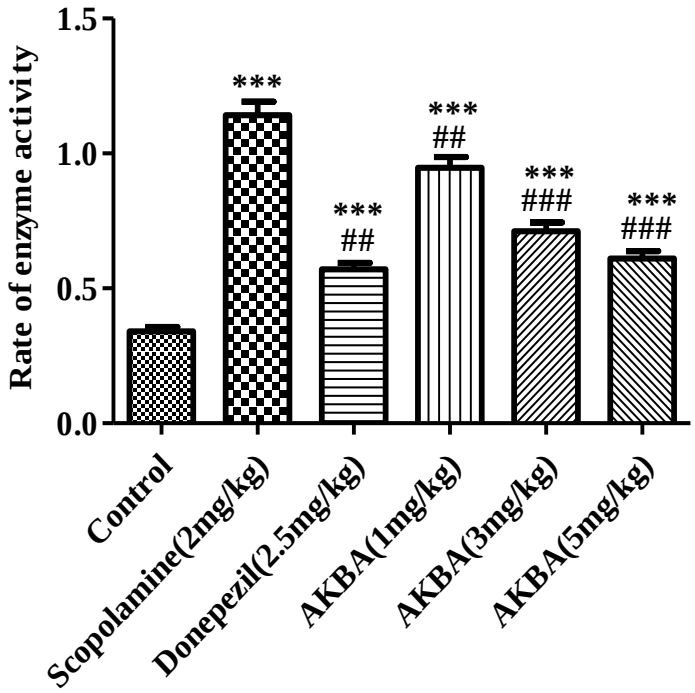


Figure 20: Effect of AKBA onacetylcholinesterase activity in blood plasma.± SD, n = 6. Statistical analysis was carried out in one-way ANOVA followed by post hoc analysis Tukey's multiple comparison tests using prism 5.0. Statistical significance $^{***}P < 0.001$ vs control.$^{##}$ P < 0.01, $^{###}$ P < 0.001 vs scopolamine treated group.

EFFECT OF AKBA ON BRAIN NEUROTRANSMITTER LEVEL

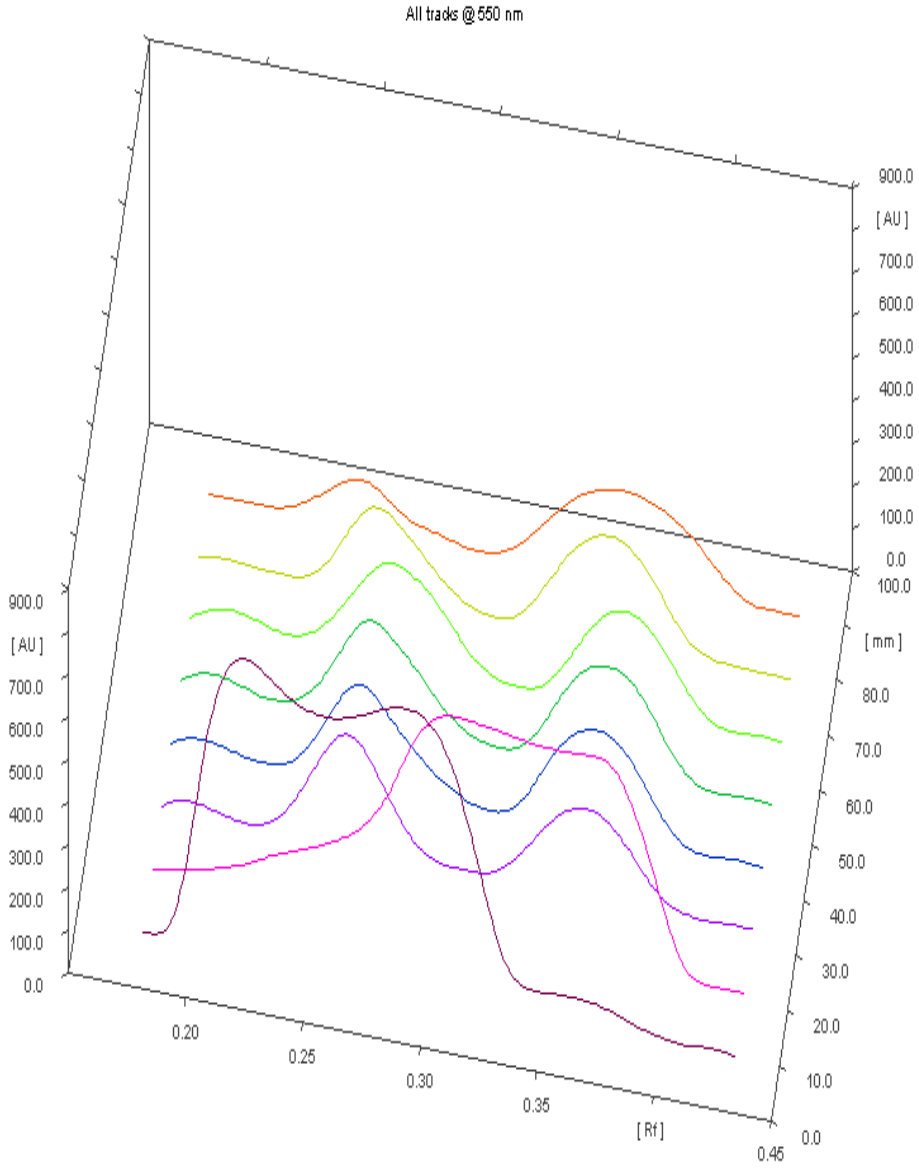


Figure:213D chromatogram view of glutamate and GABA

1.Effect of AKBA on glutamate level

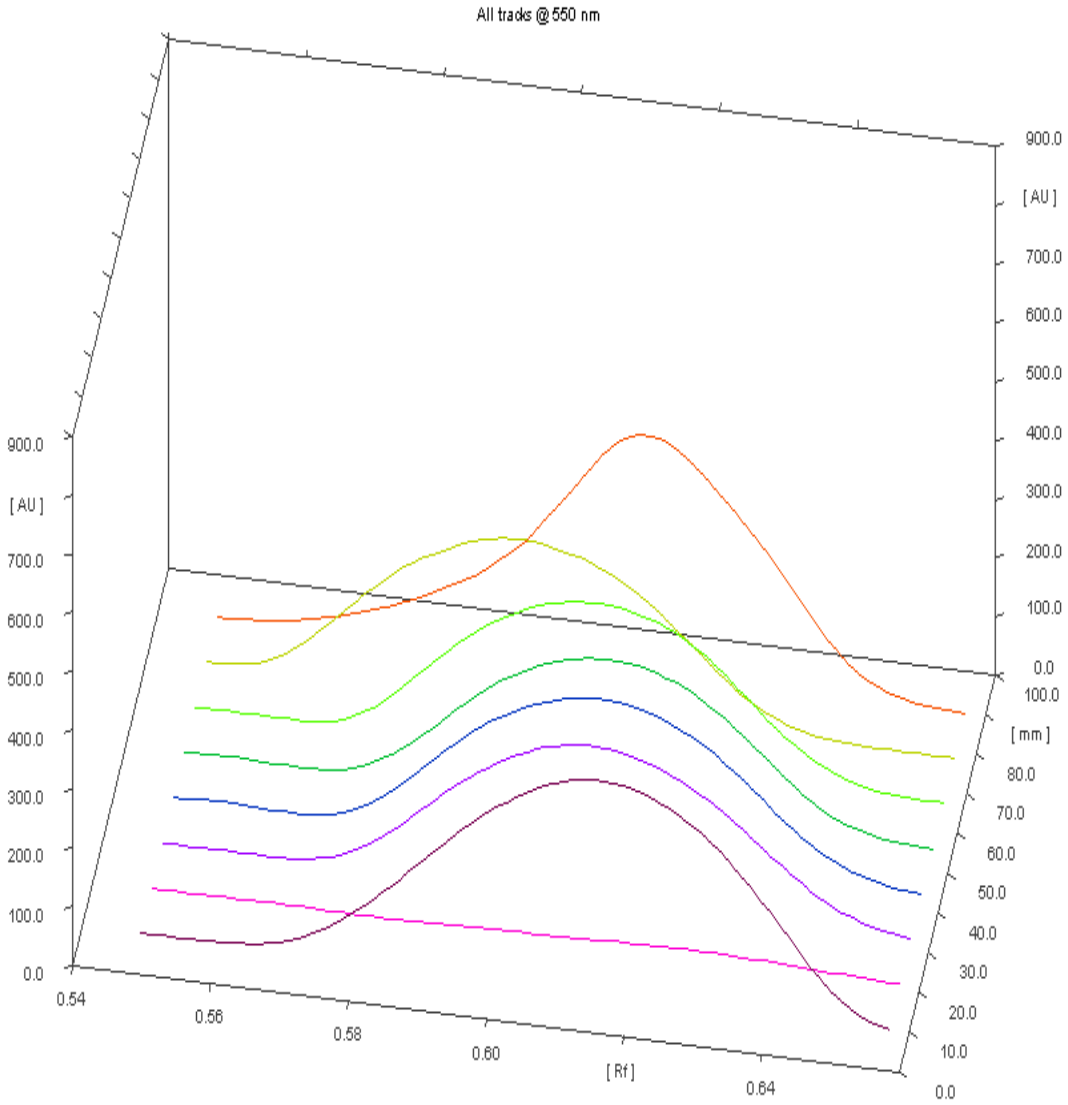


Figure :22Glutamate chromatogram in 3D View

Scopolamine treated group significantly increased the glutamate level when compared to control group [F(5,30) =14.61, P < 0.001].Treatment with donepezil [F(5,30) =10.41, P < 0.001] decreased glutamate level in comparison with scopolamine treated group. AKBA (3 and 5mg/kg)

[F(5,30) =5.163 , P < 0.05] and [F(5,30) =6.650, P < 0.01] treatment reduced the glutamate level as compared to scopolamine treated group.

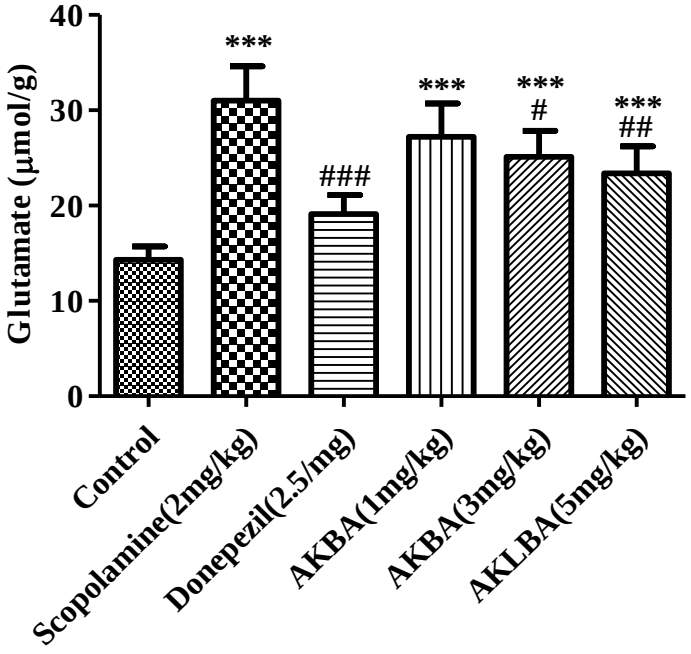


Figure 23:Effect of AKBA on glutamate level in brain homogenate by HPTLC. Mean±SD, n = 6. Statistical analysis was carried out in one-way ANOVA followed by post hoc analysis Tukey's multiple comparison tests using prism 5.0. Statistical significance $^{***}P < 0.001$vs control. $^{###}$ P < 0.001, $^{##}$ P < 0.01 and $^{#}P < 0.05$ vs scopolamine treated group

2. Effect of AKBA on GABA level

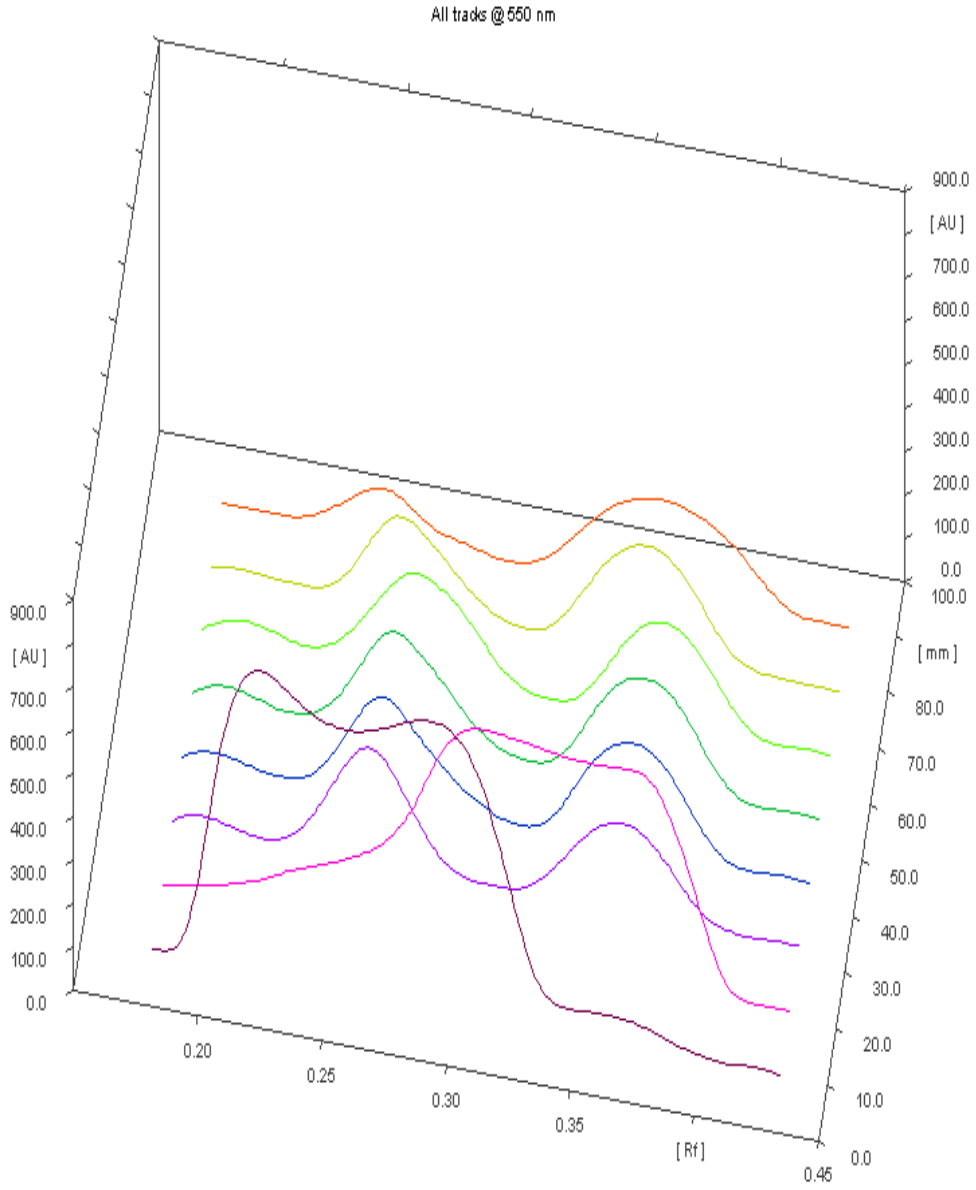


Figure 24:GABA chromatogram in 3D View

Scopolamine treated group significantly decreased the GABA level when compared to control group [F(5,30) =19.53, P < 0.001].Treatment with donepezil [F(5,30) =16.98, P < 0.001]markedly increased GABA level in comparison with scopolamine treated group. AKBA (3

and 5mg/kg) [F(5,30) = 8.491 , P < 0.0] and [F(5,30) = 13.59, P < 0.001] treatment increased the GABA level as compared to scopolamine treated group.

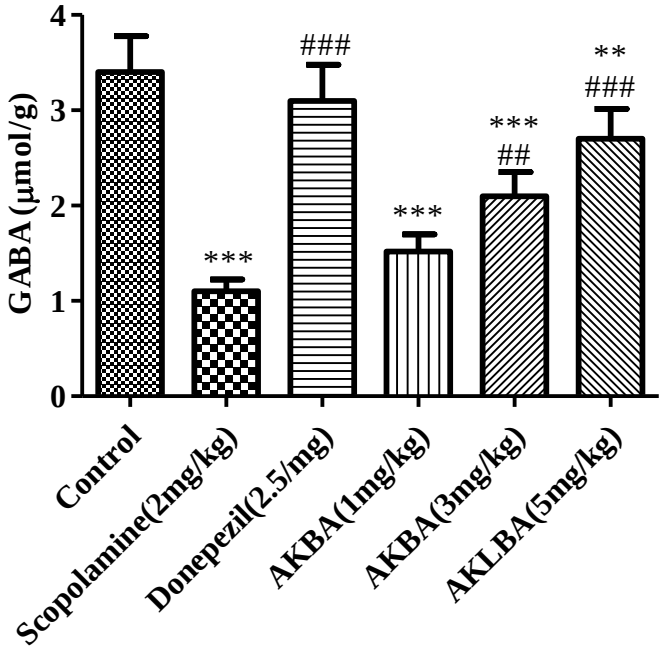
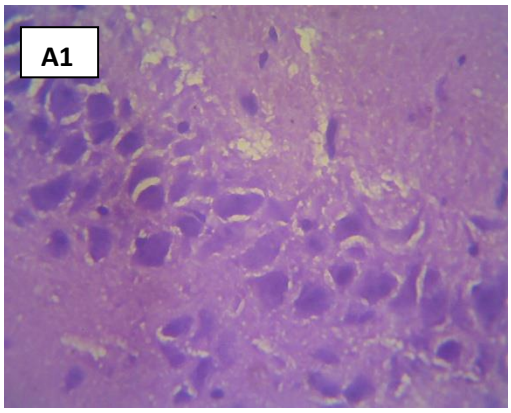
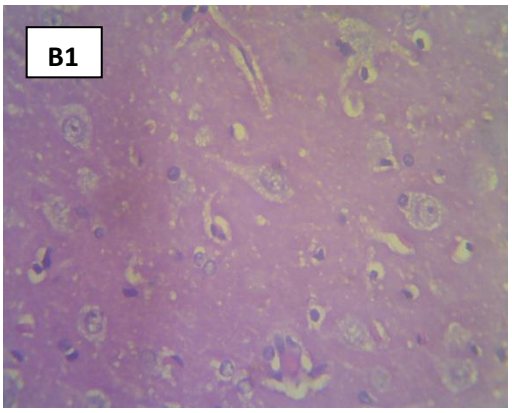
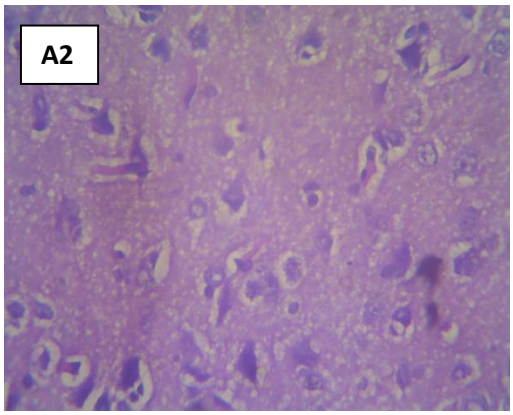


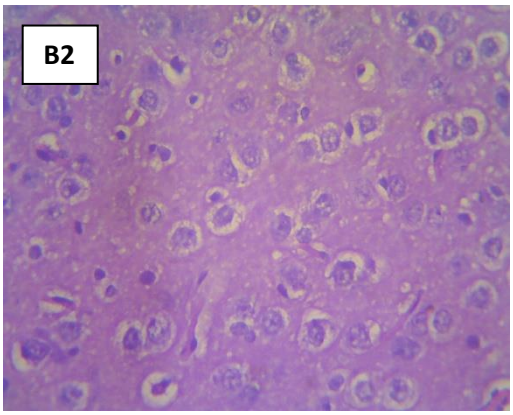
Figure 25:Effect of AKBA on GABA level in brain homogenate by HPTLC. Mean±SD, n = 6. Statistical analysis was carried out one-way ANOVA followed by post hoc analysis Tukey's multiple comparison tests using prism 5.0. Statistical significance $^{***}P < 0.001$, $^{**}P < 0.01$ vs control. $^{###}$ P < 0.001 and $^{##}$ P < 0.001 vs scopolamine treated group.

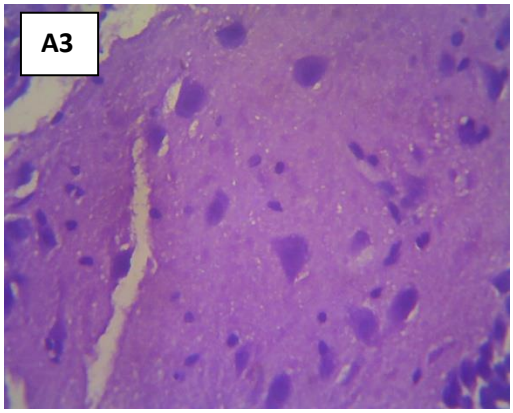
HISTOPATHOLOGY

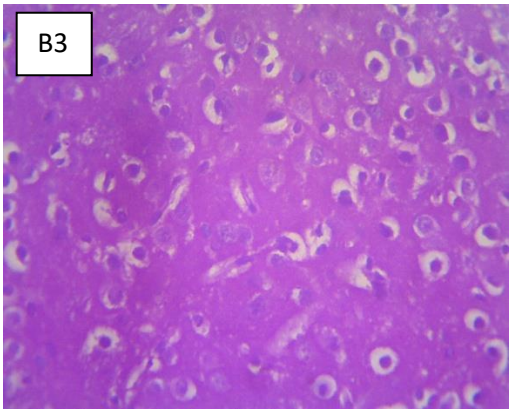












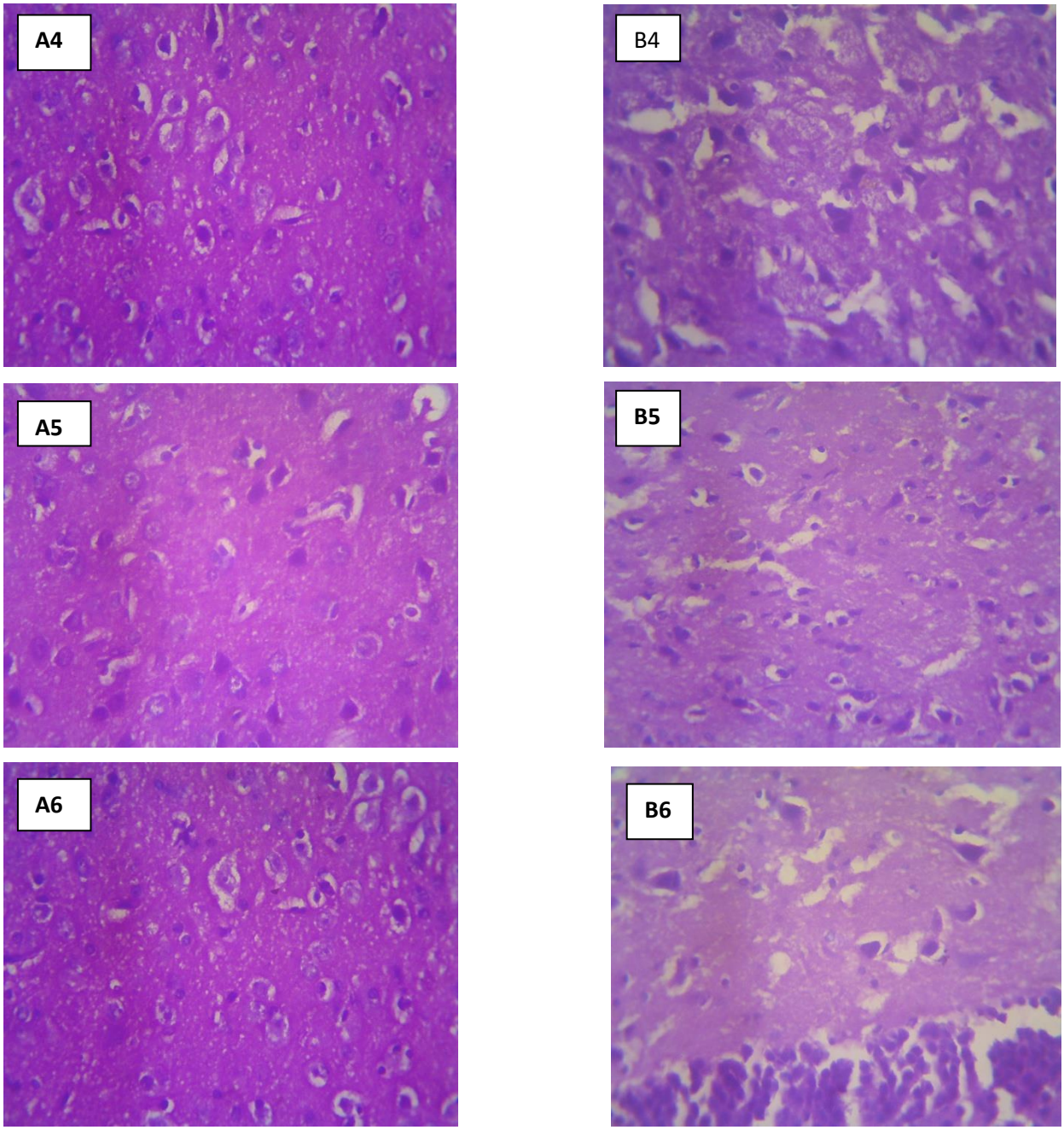


Figure 26: Histopathological analysis of effect of AKBA in scopolamine induced rat brain.

A indicates pre frontal cortex and **B** indicates the hipocampal region of the brain.

A1 – control, A2 – Negative control, A3 – standard, A4 – AKBA 1mg /kg, A5 – AKBA 3 mg/kg, A6- AKBA 5 mg/kg.

B1 – control, B2 – Negative control, B3 – standard, B4 – AKBA 1mg /kg, B5 – AKBA 3 mg/kg, B6- AKBA 5 mg/kg

DISCUSSION

In the present study,we aimed to investigate the effects of AKBA on behavioural (Morris water maze, openfieldtest,Y-maze and novel object recognition test),biochemical (AchE, Glutamate and GABA) parameters and also the effects of AKBA ongene expression in scopolamine induced rats.The results of present investigation indicated that scopolamine induction cause impaired spatial working memory, short-term memory and recognition memory performance. The data from these studies were consistent with previous studies which suggest that induction with scopolamine developes memory impairment by affecting neurotransmitters like cholinergic activity etc.(*El-Marasy et.al.,2018*). The novel object recognition (NOR) task is widely used to study the neurobiology of non-spatial memory in rodent(*Cohen SJ et.al 2013*).The discriminating ability of animals were analysed by novel object recognition test(*Eagle AL et.al., 2013*).Animals treated with scopolamine were not able to remember the similarity between same objects (*Malik J et.al 2013*).Time spent,time to reach the object and no of contacts with novel object were found to be increased than the familiar object for scopolamine treated group. Administration of AKBA to the animals exhibited more interaction with novel object.From this observation we can suggest that AKBA can improve recognition memory.

Y-maze analyses pointssimultaneous discrimination learning in rodents and thus indicates memory acquisition (*McLamb RL et.al.,1988*).The time taken to escape (escape latency time) to the safe zone was recorded. Increased ELT and any escapes to non-safe zones were regarded as error which represents impairment in memory[*Ru M, Liu H.2018*). In present study scopolamine treatment exhibited marked increase in escape latency time.AKBA administration reversed the scopolamine induced memory impairment. MWM test was done to analyze the hippocampus-dependent memory and learning. Reduction in the mean of escape latency time is indicated as improvement in the learning process. While, increased percentage of time spent in target quadrant and higher number of entry to the target quadrant are indicated as improvement in reference memory(*Jafarian et.al 2019*).Administration of AKBA reversed the scopolamine-induced spatial learning and working memory impairment by increasing time spent in the target quadrant and number of crossings and decreasing the escape latency time.

AChE has been strongly related with impairment in memory. AchEdegrades acetylcholine, which is a cholinergic neurotransmitter in the brain (*Hyde C et.al., 2013*).In our

study Scopolamine administration significantly increases the activity of AchE in cortex and hippocampus(*Choi WY et.al.,2018*).AKBA administration showed a significant decrease in the AchElevel and this finding suggest that AKBA has a role in prevention of neuronal loss in cholinergic system. It is reasonable that the observed improvement in learning and memory in the Morris water maze test was associated with the elevation of hippocampal Ach by inhibiting AchE enzyme (*Xiang GQ et.al 2012)*.

Glutamate is the most important excitatory neurotransmitter of the central nervous system (CNS). Glutamate plays a critical role in synaptic maintenance and plasticity. It contributes to learning and memory.Glutamate concentrations are normally maintained at low micromolar levels via the activity of Na+ glutamate transporters expressed by neurons and astrocytes to assure proper synaptic function and to prevent excitotoxic injury of neurons (*Tapiero Het.al., 2002*). Enhancement of the glutamatergic signal may have adverse effects on memory, because high levels of glutamate are neurotoxic.(*Hescham S et.al., 2016*).Scopolamine significantly elevated glutamate levels which suggests that acetylcholine typically regulates glutamatergic transmission.(*Rawls SM et.al., 1998*).This statement coincide with the current study and our test drug significantly reduces the level of glutamate.GABA, a primary inhibitory neurotransmitter of the brain have a greater impact on memory retrieval and consolidation.GABAergicdeficits contribute to memory impairment.The pathological changes due to scopolamine-induction can be coupled with GABA reduction in the cortical and hippocampal GABA *(El-Marasy et.al., 2018*). Treatment with AKBA restored the reduced GABA contents which implies that the test drug preserves neurotransmitters and thereby enhance memory.

Cognitive performance can be improved through PPAR nuclear receptors.PPAR-γ has a prominent role in the regulation of central nervous system (CNS) inflammation and neuroprotection leading to improvement in cognitive performance. Increased expression of PPAR-γ in brain has been proved to improve spatial learning and memory(*Hajjar T et.al., 2012*).Scopolamine mainly affects brain cholinergic system and previous studuies reported that stimulation of PPAR-γ results in the enhancement of the cholinergic nerve and subsequent improvement of learning and memory, implying there might be some connection between PPAR-γ and the cholinergic nerve in the CNS(*Xiang GQ et.al 2012*). In present study we found that scopolamine treatment decreased the PPAR-γ gene expression level and this effect of

scopolamine was reversed by the treatment with AKBA. From this observation we can suggest that AKBA can attenuate memory impairment through the interaction with PPAR-γ.

The histopathological study of rat hippocampus and prefrontal cortex provedthat scopolamine-treated group develops reactive gliosis, extensive areas of necrosis and hemorrhage than the normal rat.The histopathological changes were markedly attenuated in AKBA treated group. From the observations, study concludes that, AKBA can be a potent drug to treat cognitive impairment induced by scopolamine in rats through the regulation of PPAR-γ receptor.

CONCLUSION

In present study the administration of AKBA reversed the changes in behavioral, biochemical parameters and gene expression levels in scopolamine induced animals .The changes observed in the brain treated with AKBA was further confirmed by histopathological analysis. Study concluded that, AKBA can be a potent drug to treat cognitive impairment induced by scopolamine in rats through the regulation of PPAR-γ receptor.

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ABSTRACT

Background

Boswellia serrata have been proved for its role in memory.According to traditional medicine documents, Frankincense has been used for increasing memory. It has been reported to possess cognitive improving properties that may aid in the treatment of common neurodegenerative disorders, such as dementia.

Aim & Objective

To Elucidate the of role of 3-O-Acetyl-11-Keto-β Boswellic Acid (AKBA) on PPAR-γ in scopolamine induced cognitive impairment model in rats.

Methodology

Totally 6 groups of rats were used for the study. control, scopolamine (2mg/kg), donepezil (2.5mg/kg) and 3 test groups (AKBA at 1,3 and 5 mg/kg).Behavioral studies such as, Open field test,Y-maze, Novel object recognition test and Morris water maze and biochemical studies like plasma AchE level, glutamate and GABA level in brain estimation were performed. Moreover, gene expression MMP2, MMP9 and PPAR-γ were studied along with brain histology.

Results

The administration of AKBA (1,3 & 5 mg/kg) reversed the changes in behavioral, biochemical and gene expression levels in scopolamine induced rats. Histopathological analysis were demonstrated the changes in the brain tissue on AKBA administration when compared to control and negative control group.

Conclusion

Study concludes that, AKBA can be a potent drug to treat cognitive impairment induced by scopolamine in rats through the regulation of PPAR-γ receptor.