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PHARMACOGNOSTICAL EVALUATION AND BIOACTIVITIES OF ADHATODA VASICA (LEAVES) AND VIGNA MUNGO (SEEDS)

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Abstract:

Background: Cognitive dysfunction, a major health problem in 21st century is one of the most functionally demoralizing characteristic of many neuropsychiatric and neurodegenerative disorders which can be overcome by use of Rasayana plants which are believed to have Neuroprotective property. In the present study, *Adhatoda vasica* (Leaves) and *Vigna Mungo (Seeds)* were selected to assess its memory enhancing potential.

Material and methods: The plant parts were collected; authenticated; shed dried and successively extracted using different solvent according to polarity. The extracts were subjected to various chemical analyses like Estimation of phytoconstituents, Inhibition of lipid peroxidation, antioxidant activity against free radicals and inhibition of Acetylcholine esterase enzymes. The acute and chronic toxicity studies were performed within prescribed guideline set by OECD. The Evaluation of Memory Enhancing Activity was carried out by Morris Water test and Elevated plus Maze test. The Biochemical Estimation was carried out by Acetyl cholinesterase inhibition activity.
The extracts and their fractions were subjected to characterization by different spectroscopic and chromatographic techniques. Finally isolated fractions subjected to pharmacological studies.

Results and Discussion: The results obtained revealed the presence of various phyto-constituents responsible for memory enhancing activity. The antioxidant activities of different extracts were performed by lipid peroxidation, hydroxyl radical scavenging activity and DPPH methods. In all antioxidant assays, the chloroform extract showed better activity as compared to standard. The equivalent results were also obtained in lipid peroxidation assays for both plant extracts. The acute oral toxicity for both plants revealed that, there were no sign and symptoms of toxicity even at highest dose of 2000mg/kg.

Conclusion: Based on the *In-vitro* and *In-vivo* memory enhancing studies, finally we concluded that, the plant parts of *Adhatoda vasica (Leaves) and Vigna Mungo (Seeds)* have manifest nootropic action in experimental animals. The outcomes also proposed that action could be because of cholinesterase inhibitory and antioxidant activity of particular extracts. Moreover, identified plumbagin, ά-amyrin and presence of alkaloids are accountable for accrediting the nootropic action. Additional studies such as dosage form design and clinical trials can be done to well being of humans. A detailed study is also required on structure determination of the compounds from bioactive extracts in arrange to find the structure activity relationship.

Keywords: ***Adhatoda vasica (Leaves) and Vigna Mungo (Seeds)*** Plumbagin, ά-amyrin, Nootropic.

1.0 INTRODUCTION

One of the most hazardous health issues arising in 21st century are related to neuropsychiatry disorders which can be considered most functionally frightening to human well being includes dementia, Alzheimer's Disease (AD), Schizophrenia, cerebrovascular impairment, parkinsonism and head grievance. (Ingole *et al*., 2008).

The most common disorder that solemnly affects personality to do routine behavior is dementia and most common form in elder ones is AD, which includes the area of intellect which is controlling the inspirations, observations, recall and verbal communication which further leads to brutal brain denting. (Joshi *et al*., 2007).

Dementia can be said as severe pathological condition of brain which escort to commotion of manifold of superior cortical role counting memory, logic, orientation, thoughtfulness, learning aptitude and poignant reliability. It is a chief wellbeing catastrophe in ordinary living along with in many pathological environments such as AD, Pick's disease, PD , alcoholism & many more. A clinical condition which overwhelms memory and cognition in elderly is senile dementia. Cognitive disorders like AD and many more are still immobile in the meadow of medication (Ashwalayan & Singh, 2011).

The mean age at origination of AD occurs around the age of 60+ years with overall occurrence as regards of 1% in most urbanized nations. If AD pervasiveness is splited into divergent age crew then we see a speedy supplement from 1% in the 60-64 year age grouping to superior than 25% in entity over 85 years of era (Sutherland & Kril, 2012). The NIH forecast, if progress inclination continues, there is possibility of about 8.5 million AD patients by the year 2013 in U.S.A alone and so on and if this condition persists then at the end of 2050, more than 13,150,000 people will suffer from same. (Joshi & Parle, 2006)

Types of memory

As all knows, some reminiscences remain for few seconds, whereas some lasts for hours to lifetime. So as a principle of discussion, memory is divided into-

Short term memory: It comprises memories which are for seconds or most minutes until and unless transformed into longer phrases.

Midway extended memories: It is for few days to weeks then got lost.

Extended term memory: It is when stored in mind can be recalled anytime

Working memory: It includes short term memory and used during the period of cerebral reasoning but it come to end once trouble get solved (Guyton and Hall, 2006).

Memories are generally differentiated on the basis of kind of information stored. One of the categorization which divides memory into declarative and non declarative is-

Declarative memory:

It comprises memory for truths and proceedings and it relay on veracity of hippocampus & narrated arrays.

Non Declarative memory:

It refers to a mixed compilation of memory abilities where piece alterations but restricted of affording access to the skill that caused the amendments (Zola & Squire, 1990).

Dementia:

Dementia defined as the defeat in logical mind (In scientific technology known as cognitive dysfunction) of adequate aptitude with hindrance of social or occupational functioning (Shivakumar *et al*., 2011). It can result from an assortment of illness which may lead to brain cells damage. There are many poles as part types of dementia, each with its own cause and symptoms. Increasing substantiation from lengthy term epidemiological surveillance and autopsy learning's suggested that dementia is related to peoples brain related problems which can be considered & may show association with one or more types. (Alzheimer's & Dementia, 2013).

Categories of Dementia

Alzheimer's disease:

It is a frequent kind accounting in predictably majority of cases. Involvedness in recalling events & activities is primary quantifiable indication besides lethargy & depression. Later symptoms embrace impaired belief, confusion, behavior transformations, complexity in conversation, ingestion and walking.

Vascular dementia:

It which was previously recognized as multi-infarct or post-stroke dementia is a less widespread as lonely source of dementia than AD. Difficulty in judgments' or to create plans is an initial symptom of AD which progresses in later stages of life. Vascular dementia is a major cause which results due to injury to brain like micro-vascular bleeding & impediment of blood vessels. Spots of brain injury conclude patient's thoughts ability & bodily implementations.

Dementia with Lewy bodies (DLB):

The persons who are suffering from DLB have many functions same as AD such as sleep deprivation, visual hallucinations, muscular rigidity and sometimes tremors also.

LB's are atypical aggregations of protein ά-synuclein & their deposition in cerebral cortex rising dementia. The ά-amyloid also gets deposited in brains of individuals suffering from PD, but cumulative may look in different blueprints.

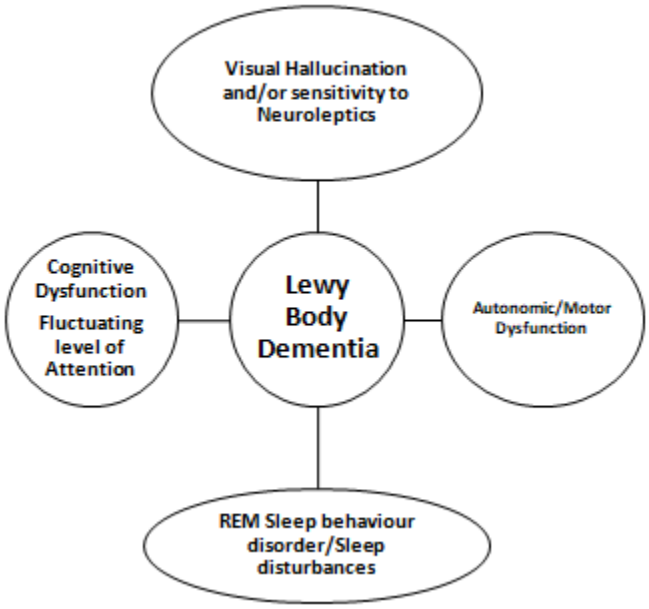

Figure:1 Dementia

Anatomy and physiology associated with dementia:

The dementia can be divided particularly based on twin prototype of brain worsening and these are sub-cortical and cortical regions. A cortical kind of dementia is characterized by marked reminiscence conflict. Dementia with cortical type affects all lobes partially or completely which results in AD. MRI scan gave results regarding prevalence of AD in various areas and its progresses (Scahill *et al*., 2002). Sub-cortical disarray is frequently linked with motor disability.

In conducting all routines by involvement of memory, one has to use whole brain i.e. each & every portion of brain & its parts. It is supposed that, hippocampus & amygdale both are engrossed in configuration, storage & repossession of memory related activities.

Pathophysiology of Alzheimer Disease (AD):

The lesions of AD discovered in cortical area as well as temporal lobe of brain. The lesions may be due to plaques & neurofibrillary tangles leading to development of AD. As it progresses, progressive loss of neurons, synapses observed leading to neuronal-dystrophy. The

plaque formation over neurons is the leading cause of AD but its role is unclear. There are numerous mechanisms which propose to elucidate transformations in brain, which comprises βAP aggregation & deposition which causes plaque deposition, hyperphosphorylation of tau protein, oxidative stress and mitochondrial dysfunction.

Inflammatory Mediators:

Inflammatory pattern always treated as amyloid cascade, positively amyloid saturation acquaintances through local inflammatory and immunologic alterations.

According to hypothesis, β-AP plays straight role in toxicity of neurons which might be an indirect conclusion of β-AP protofibril-induced microglia instigation along with astrocyte staffing. These inflammatory reaction symbolize confront to comprehensible amyloid statement. However, this is connected through discharge of cytokines, NO & supplementary radical varieties & harmonized aspects which can harm neurons & encourage continuing irritation. Certainly, in patients of AD, there is existence of diversity of chemokines & cytokines in brain through incidence of some variety of pro-inflammatory genes polymorphism (Dipiro *et al*., 2008).

An amorphous deposit of β-amyloid peptides on neurons is the most evidenced pathological change responsible for induction of inflammatory reactions. This results in acute phase responses which indulges IL-1 & 6 in microglial cells of brain parenchyma & TGF- β1 in peri-vascular astrocytes. The various secretary products from adjacent astrocytes get discharged due to influencing cytokines which catalyzes the transformations in diffused β-amyloid into fully grown amyloid filaments. The excessive manufacture of β-amyloid & pathological moieties like ACT & Apo-E speed up polymerization of β-amyloid peptides into filaments. Amyloid configuration makes extracellular protein sturdy, which finally leads to cell death (Nilson *etal*.1998).

Genetics of AD:

Mainly 3 genes playing a imperative task in etiology of genetically induced AD and they are: the APP on chromosome 21, the presenilin-1 (PS1) gene on chromosome 14, & presenilin-2 (PS2) gene on chromosome 1. Apo-lipoprotein E on chromosome 19 have significant jeopardy trait for intermittent AD.

A. APP mutations

Extracellular plaques have major protein recognized as β-amyloid & which is a usual element of brain produced by breakdown of bigger APP by two enzymes known to be β & γ-secretase. A substituted proteolysis trail engrosses α-secretase preventing Aβ-generation. The β-Amyloid having so much succession of peptides comprises of 39 to 43 amino acids among which Aβ40 is in profused form. In this regards, Aβ42 and Aβ43 have 42 & 43 amino acids augment formation of amyloid plaques responding to development of AD. A mutation in APP & production of Aβ42 & Aβ43 leads to AD. The APP abnormality also observed with Down syndrome, in which additional copy of chromosome produces defects.

Patients of Down syndrome have low intellectual property with developmental abnormalities in early stage of life. As patient age increases, deposition of β-amyloid plaques also get boosted which are also observed in AD patients (Encyclopedia of Life Science, 2001).

B. Presenilin mutations

The chief cause of genetic AD is – presenilin (PS-1) genes which is located on chromosome-14. It is a trans-membrane protein found in ER & Golgi bodies (Encyclopedia of Life Science, 2001). A mutation of chromosome 14 give rise to presenilin 1. In this context, a structurally alike protein called as presenilin 2, is also generated by a gene on chromosome 1. These membrane proteins -presenilin 1 & 2 are involved in dispensation of APP. It is observed that, approx 160 mutations in have been recognized playing noteworthy task in action of γ-secretase. It is a vital enzyme in the configuration of βAP. (Dipiro *et al*., 2008).

C. Apolipoprotein E-4 allele polymorphism

In cholesterol metabolism, Apo-E is chief serum lipoprotein. There are three unsurprisingly occurring alleles of the Apo-E gene, e2, e3 & e4, conflicting from one another by solitary codons (Encyclopedia of Life Science, 2001).

Genetic vulnerability to intermittent, late -onset-AD is considered as principally linked to Apo E genotype. A gene accountable for creation of APO- E is putted on chromosome-19. The scheme by which Apo-E4 bestow an amplifying risk is unidentified, although Apo E4 is correlated to further aspects contributing to AD pathology, like idiosyncrasy of mitochondria, cytoskeleton dysfunction & diminished glucose uptake. The extent of jeopardy relays on factors like digits of Apo-E4, age, background & sexual sorting. Approximately 40% of patients amid late-onset AD encompasses at least solitary replica of Apo E4.

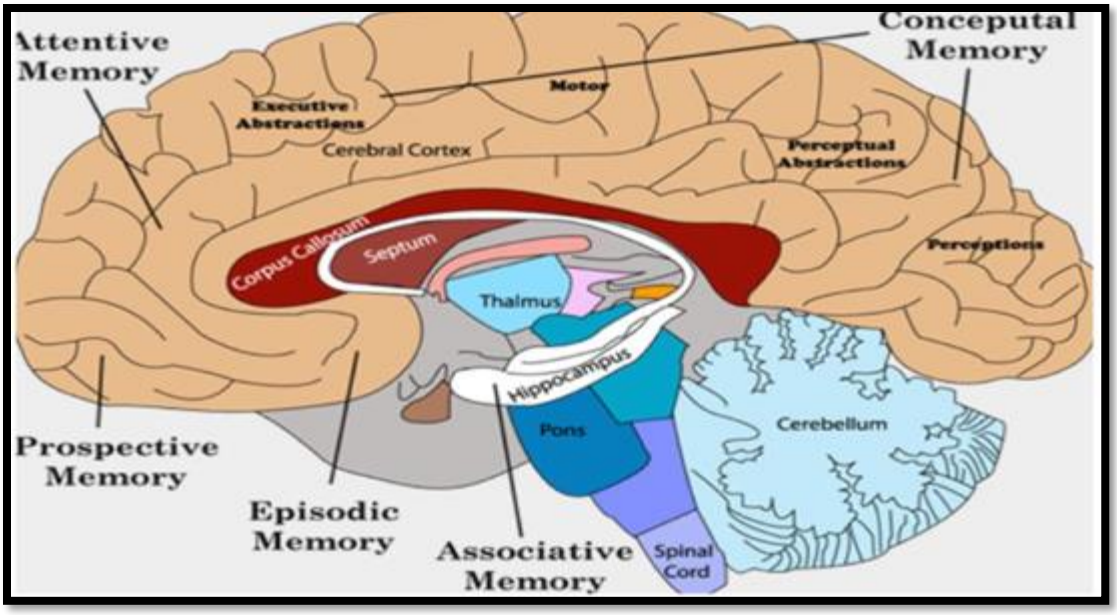

Figure No. 02: Part of brain involved in different types of memory

The symptoms of AD like wandering, urinary incontinence sleep disturbances, agitation, and hostility can be managed at primary level by regular communication with patients to avoid the stress on patient as well as society.

As all knows, personal uneasiness triggers behavior and so it is most important thing for supervision of pain, hunger, constipation, thirst, fatigue, bladder fullness, fears, frustrations etc.

2. MATERIAL & METHODS

Collection of Plant Material & Authentification

The plant parts were purchased from local market of Bundelkhand region. They were taxonomically identified by Head of the Department of Botany, Apex University, Jaipur and voucher specimens were deposited in laboratory.

Preparation of Extract by Successive Solvent Method

All the plant materials were dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of *Adhatoda vasica* (Leaves) and *Vigna Mungo (Seeds)* were extracted using petroleum ether, chloroform, methanol, and butanol and finally by water using Soxhlet's device. The whole extracts were completely dried by applying pressure, the respective extracts were subjected to weighing and % yield was determined (Mukherjee, 2002).

Figure No.03: Soxhlet's apparatus for extraction

Preliminary Phytochemical Tests

Qualitative chemical tests of chloroform, methanol, butanol and water extracts were taken for various physic-chemical tests to distinguish a range of phyto-constituents present in them. (Kokate, 2003; Khandelwal 2006).

Test for Alkaloids:-

- ✓ **Dragendorff's test: -** Take extract in test tube and add Dragendorff's reagent presence of reddish brown precipitate indicating alkaloids.
- ✓ **Mayer's test: -** Take extract and add Mayer's reagent, presence of cream colored precipitate indicates presence of alkaloids.

Test for Amino acids:-

- ✓ **Millon's test: -** In extract add about 2ml of Million's reagent presence of white precipitate indicating occurrence of amino acids.
- ✓ **Ninhydrine test: -** In extract add Ninhydrine solution, boil and observance of violet color indicate presence of amino acid.

Test for Flavonoids: -

- ✓ **Zinc hydrochloride test: -** In extract, insert a blend of zinc dust & conc. HCl. Presence of red color after a minute indicate flavonoid presence.

Test for Phenolics compounds (Tannins):-

- ✓ **Ferric chloride test: -** Treat the extract with $FeCl_3$ solution, blue color emerge out if hydrolysable tannins are present & green color appears if condensed tannins are there.

Test for Proteins:-

- ✓ **Biuret test: -** Take extracts about 2ml & put in Biuret reagent about 2ml, indication of violet color confirms presence of proteins.

Test for Steroids and Triterpenoids:-

- ✓ **Libermann-Burchard test: -** Treat the extract with few drops of $C_4H_6O_3$, then boil & cool. Afterwards, add H_2SO_4 from side of tube, formation of brown ring at junction of both layers, of which upper layer turns green indicating steroids & lower layer having red color shows occurrence of triterpenoids.

Test for Anthraquinone glycosides: -

- ✓ **Borntrager's test: -** Take the test sample, add 1 ml of H2SO4, filter while hot then cool it & shake with equal volume of CHCl3. Split the lower layer of CHCl3 & shake with half of its volume of dilute ammonia. Presence of Anthraquinone glycosides confirmed by presence of pink or red color.

Test for Coumarin glycosides: -

- ✓ Get small amount of extract in test tube & wrap with moist filter paper which was damped in dilute NaOH solution. Keep test tube on water bath for few minutes and expose this paper to UV light, observance of green fluorescence indicates existence of Coumarin glycosides.

Test for Saponin glycosides: -

- ✓ **Froth formation test:** -Add 2 ml of extract in water; shake well, if steady foam is formed indicating presence of saponins.

Estimation of different constituents

- **Determination of total Phenolic Content (TPC):**

The TPC of test extracts of plant parts measured using Folin-Ciocalteu reagent. The extracts of both plants were solubilized in distilled water. After that 100 µl of sample was mixed with Folin-Ciocalteu reagent (500 µl) with sodium carbonates (400 µl) and distilled water (5 ml). This solution was set aside at room temperature for 30 min, and the absorbance of solution was measured at 760 nm. Total phenolic content was completed using gallic acid as standard (Soni *et al*., 2018; Madaan *et al*., 2011).

- **Determination of total flavonoid content:**

The extracts of both plant parts in a quantity of 500 µl were mixed with ethanol (1.5 ml), aluminum nitrate (100 ml, 10 %), potassium acetate (100 ml, 1 M) and water (2.8 ml). The solution was reserved at ambient temperature for 40 min, and calculated the absorbance of solution using spectrophotometer. Total flavonoid content was recorded according to a standard established curve with Quercetin (Soni *et al*., 2018; Mohsen & Ammar 2008).

- **Determination of crude saponin content:**

In flask, about 20 gm of powder extract taken along with 10 ml of 20 % aqueous ethanol. This solution was frenzied for 4 hour with continuous stirring at 55^0 C. Then it was filtered and marc was extracted with 200ml of 20% ethanol. Afterwards, both extracts were mixed and let the solvent to evaporate till 40 ml remains. The remained concentrate was extracted with 20 ml of diethyl ether using separating funnel. Then aqueous layer was recovered while the ether layer was superfluous. Finally aqueous extract was purified by adding 60 ml n-butanol. Then it was washed twice with 10 ml of 5 % aq. NaCl. It was dried and percentage of saponin content was noted (Soni *et al*., 2018; Eleazu *et al*., 2012).

- **Determination of tannin content:**

Tannic acid stock solution (1mg/ml) was prepared by dissolving 100 mg of precisely weighed tannic acid in water. In this, 1-10ml of aliquots were prepared in which 0.5 ml of Folin-Denis reagent, 1 ml of Na2CO3 solution were added to each tube. All tubes adjusted to 10 ml with distilled water and mixed well then kept for 30 min. The absorbance was made at 760 nm against blank reagent (Soni *et al*., 2018; Polshettiwar *et al*., 2007).

- **Determination of alkaloid:**

Plant extract approx 1mg was liquefied in DMSO, in which 1 ml of 2N HCl was added & then filtered. Solution was taken to separating funnel and 5 ml of bromocresol green solution and 5 ml of phosphate buffer were mixed in it. This total content was shaken by adding 1, 2, 3 and 4 ml

chloroform with continuous trembling with chloroform. Reference standard solution of atropine was prepared in same manner. The absorbance's for test & standard solutions were resolute beside the reagent blank at 470 nm with an UV- spectrophotometer (Soni *et al*., 2018; Tambe & Bhambar 2014)

3.5 Assessment for antioxidant activity

- **Lipid Peroxidation Inhibitory Test**

➢ **β-carotene bleaching inhibition assay**

It is calculated as capability of β-carotene oxidative bleaching in carotene/linoleic acid combination with or without adjoining of a variety of extractives of both plants. In this, 6.0 mg ß-carotene was liquefied in 10 ml of $ChCl_3$ then 1ml was pipetted in glass filled of 20 mg of linoleic acid. From this, approx. 5 ml pipetted to reaction tube filled of extract in a diversity of concentration then blended homogenously. Sample absorptions were carried prior to and following incubation at 50^oC for 30, 60 & 120 minutes (Murdifin *et al*., 2017).

The β-carotene bleaching inhibition is estimated as-

% Inhibition = [1- (AA(120) - AC(120)) / (AC(0) - AC(120))] X 100

AA(120): sample absorbance at t = 30, 60 or 120 minute

AC(120): control absorbance at t = 30, 60 or 120 minute

AC(0): control absorbance at t = 0 min

➢ **Hydroxyl-radical ($OH^{\cdot}$) scavenging method**

The 1 ml of the reaction blend contained 100 µL of 2.8 mM 2-deoxyribose dissolved in phosphate buffer (10 mM) having pH 7.4, 500 µL solution of various concentrations of the extract (500n1000 µg/mL), 200 µL of 200 µM $FeCl_3$ and 1.04 µM EDTA (1:1 v/v), 100 µL of H_2O_2 (1.0 mM) and 100 µL of ascorbic acid (1.0 mM). After incubation time of one hour at 37^OC, the amount of deoxyribose deprivation was measured by TBA reaction (Badmus *et al*., 2011; Halliwell *et al*., 1987).

The % inhibition was checked by using formula given below-

$$\text{Percentage Inhibition} = \frac{(100 - A_{Sample}) \times 100}{A_{Control}}$$

➢ **DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical scavenging activity**

In 2001, Mensor and collogues explained the free radical quenching potential of drugs by use of 1, 1-Diphenyl-2-picrylhydrazyl i.e. DPPH. Here, DPPH get adhered to antioxidant

molecules and get reduced. The Color changes from deep violet to golden/light yellow which can be easily measured at 518 nm.

Here, 1 mL of 0.3 mM of DPPH solution was incorporated to 1mL of each and every test solutions and then incubated for 30 min at room temperature and absorbance was taken at 518 nm. (Badmus *et al*., 2011). The % anti-oxidant potential was computed using formula-

$$\text{DPPH scavenging effect (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Preliminary *In-Vivo* Evaluation of different extracts

Animal Selection

The male/female wistar mice of amid 1 to 2 months of age weighing between 25-35 g were used procured from the approved animal house The rodents let liberated and fed with pallet diet (Lipton India Ltd, Mumbai, Ind.) with water *ad libitum.* All the laboratory conditions and animals were maintained as per CPCSEA guidelines throughout the experiments. The study design was as per CPCSEA guidelines.

Drug Safety Profile

The short and long term toxic effects of both drugs and their extracts were performed within prescribed guideline set by OECD guideline no. 423.

The albino rats of 150-200 Gms were employed for study and kept in 12hour day-night cycle with *ad libitum.* The principle was based on use of minimal animals. The extracts were suspended in 1% Tween 80, prepared in distilled water. Animals were fasted for 12 hours with *ad libitum* and extracts were admistered orally with increasing doses to 2000 mg/kg considering as maximum weight. The crucial observation made in first 4 hours to check for any bizarre behaviors like changes in skin and fur, eye, hyperactivity, grooming, convulsions, sedation, hypothermia, salivation, tremor, coma, lethargy, body weight, and mortality. From the observations and study, $1/10^{th}$ and $1/5^{th}$ of the LD used as therapeutic dose & cut-off values were chosen as 200 & 400 mg/kg to evaluate dose dependent action for the assessment of nootropic activity (OECD guidelines, 2001).

Evaluation of Memory Enhancing Activity by Different Models Morris

Water test

The various parameters and procedure for assessment of nootropic potential of mice were followed as reported by Domange *et al*., 2013; Morris, 1984; Parle & Singh, 2007.

The animals were divided in 10 groups with 6 in every cluster. The group 1 treated as control and group 2 as standard drug receiving physostigmine, 0.1 mg/kg *i.p.* The next groups 3 to 10 were treated with different extracts (chloroform, methanol, butanol and water) with a dose of 200 and 400 mg/kg, respectively for 15 successive days. On 11^{th} to 14^{th} days, EL was documented subsequent to 120 min of moieties/extracts administration. On day 15^{th}, after 120 min of drug administration, TSTQ was observed and calculated. Animals administered with physostigmine, EL and TSTQ were noted after 30 min of drug administration.

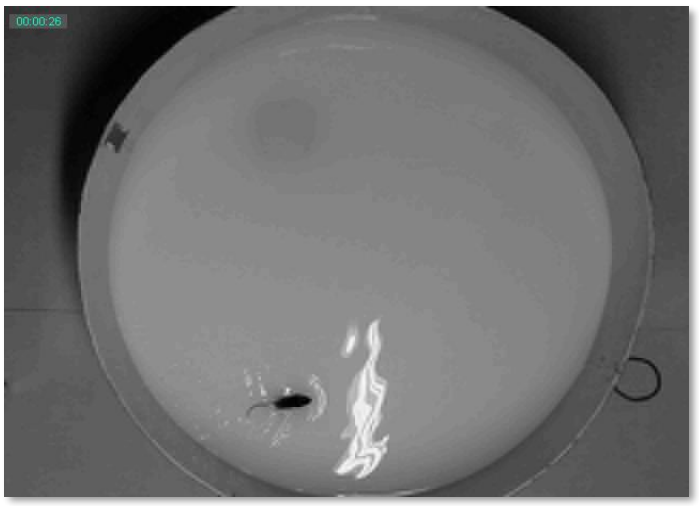

Fig. No.4: Morris water maze

The treatment schedule was as follows-

Group-1 control vehicle

Group-2 Standard drug (physostigmine, 0.1 mg/kg *i.p.*)

Group-3 chloroform extract of *Vigna Mungo :*200 mg/kg

Group-4 chloroform extract of *Vigna Mungo* : 400 mg/kg

Group-5 methanolic extract of *Vigna Mungo* : 200 mg/kg

Group-6 methanolic extract of *Vigna Mungo* : 400 mg/kg

Group-7 butanolic extract of *Vigna Mungo* : 200 mg/kg

Group-8 butanolic extract of *Vigna Mungo* : 400 mg/kg

Group-9 water extract of *Vigna Mungo* : 200 mg/kg

Group-10 Mice were treated by water extract of *Vigna Mungo* : 400 mg/kg

In case of *Acorus calamus*, the procedure was same as followed above.

The various parameters and procedure for assessment of nootropic potential of mice were followed as reported previously (Domange *et al*., 2013; Morris, 1984; Parle & Singh, 2007).

The treatment schedule was as follows-

Group-1 control vehicle

Group-2 Standard drug (physostigmine, 0.1 mg/kg *i.p.*)

Group-3 Chloroform extract of *Adhatoda vasica* : 200 mg/kg

Group-4 Chloroform extract of *Adhatoda vasica* : 400 mg/kg

Group-5 Methanolic extract of *Adhatoda vasica* : 200 mg/kg

Group-6 Methanolic extract of *Adhatoda vasica* : 400 mg/kg

Group-7 Butanolic extract of *Adhatoda vasica* : 200 mg/kg

Group-8 Butanolic extract of *Adhatoda vasica* :400 mg/kg

Group-9 Water extract of *Adhatoda vasica* : 200 mg/kg

Group-10 Water extract of *Adhatoda vasica* : 400 mg/kg

Elevated-Plus-Maze (EPM)

In this model, the equipment have 2 open and 2 close arm of equal dimensions (20cm X 7cm) with close arm have 30 cm elevations. The all arms are joined centrally in a square. All the walls and base are colored with black paint and placed in sound proof room. During assessment, rodents were placed with their head facing open arm. During whole experimentation, trouble from external stimuli are avoided which may cause anxiety.

The calculations were made for percentage of time given in the open arms and number of open arm entries using formula-[100 × open/ (open + enclosed)] and (100 × open / total entries), respectively (Nishino *etal*., .2008).

One hour after administration of vehicle, diazepam & different extracts of *Adhatoda vasica (Leaves) and Vigna Mungo (Seeds)* (chloroform, methanol, butanol and water) were assessed for memory enhances or behavior studies using elevated plus-maze test. Test extracts of both plants were used in a dose of 200 & 400 mg/kg.

In this study, vehicle and diazepam treated mice groups were same in case of *Adhatoda vasica* (Leaves) and *Vigna Mungo (Seeds)*. No separate mice or groups were used in case of both plants.

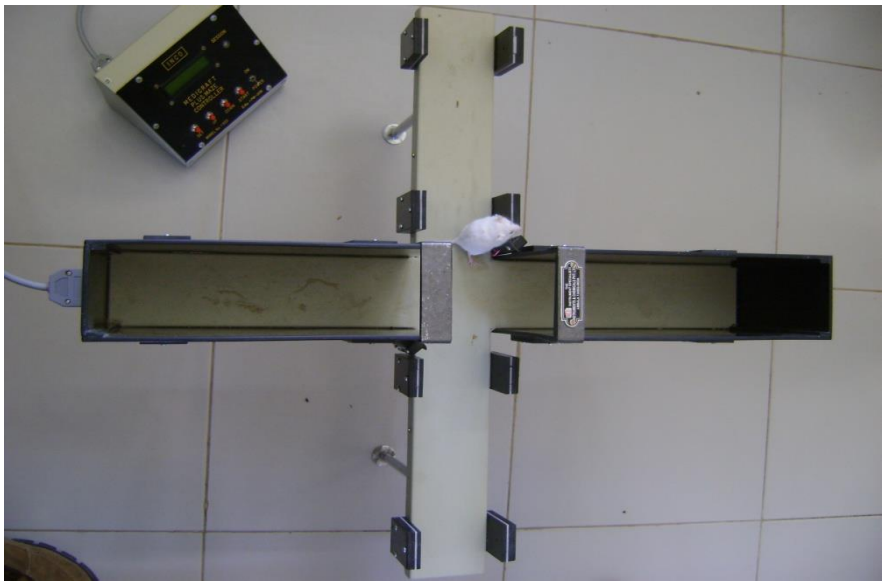

Fig. No. 5: Elevated Plus Maze

Biochemical Estimation

Collection of Brain Sample

The animals were sacrificed after 15th day of Morris water maze by cervical dislocation method. The brains were carefully removed and were weighed immediately. The brain was homogenized at 3000 rpm for about 10 min using temperature 4^0 C by addition of 10 vol. of 0.1M phosphate buffer (pH-8) using refrigerated centrifuge (Remi, Mumbai). The obtained unclear liquid was used in the estimation of brain acetyl cholinesterase activity (Ellman. *etal*., 1961.).

Assessment of Acetyl cholinesterase effect

In glass test tube, having 2.6ml of phosphate-buffer and 5,5-dithiobis-2-nitrobenzoic acid reagent (0.1 ml), 0.4 ml of brain homogenate was added and the absorbance was noted at 412 nm. To this, 0.02 ml of Ach-iodide solution added and one more absorbance was taken 15 min afterwards. The alterations in absorbance/ minute were computed. (Ellman *et al*., 1961).

Chromatographic study

In chromatographic techniques the components of an extracts are scattered between two phases, i.e. stationary and mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be crowded in a column, spread as a layer, or dispersed as a film, etc. The mobile phase may be gaseous, liquid or supercritical fluid. The separation method based on adsorption or may be based on dissimilarity in the physico-chemical properties of the molecules such as size, mass, volume etc (Mukherjee, 2002).

Pharmacological Evaluation of isolated α-Amyrin

Effect of Plumbagin on EL & TSTQ

EL and TSTQ is associated with learning and memory and decrease of EL and increase of TSTQ by mice in MWM indicates development of learning and memory. In our study, α-Amyrin (10 & 20 mg/kg) and Physostigmine (0.1mg/ kg, *i.p.*) administered for 15 succeeding days. After administration, they significantly decreased EL of mice from 11^{th} to 14^{th} day and augmented TSTQ by mice on 15^{th} day as compared to the control, thus illustrated learning and memory enrichment result. Rat's treated by α-Amyrin in a dose of 20 mg/kg notably upturned scopolamine-and diazepam-induced amnesia as compared to scopolamine and diazepam treated groups

Table No. 1: Effect of α-Amyrin on EL of mice using Morris water Maze

Treatment Schedule	EL (Sec) Day 11	EL (Sec) Day 12	EL (Sec) Day 13	EL (Sec) Day 14
Normal Control	92.24 ± 1.29	94.44 ± 1.23	94.31 ± 1.36	93.34 ± 2.19
Physostigmine, 0.1 mg	93.22 ± 1.31	88.14 ± 1.26*	84.33 ± 1.53**	77.42 ± 2.29 ***
α-Amyrin (10 mg/kg)	91.21± 1.33	86.31 ± 1.25 *	81.53± 1.44 **	76.31 ± 1.64 ***
α-Amyrin (20 mg/kg)	92.33 ± 1.64	83.41 ± 1.34*	79.36 ± 1.45**	73.52 ± 1.66 ***

Values are expressed as mean± SEM, n=6 in each group; * p <0.05, compared to control ** p<0.01, compared to control. *** p <0.001, compared to control

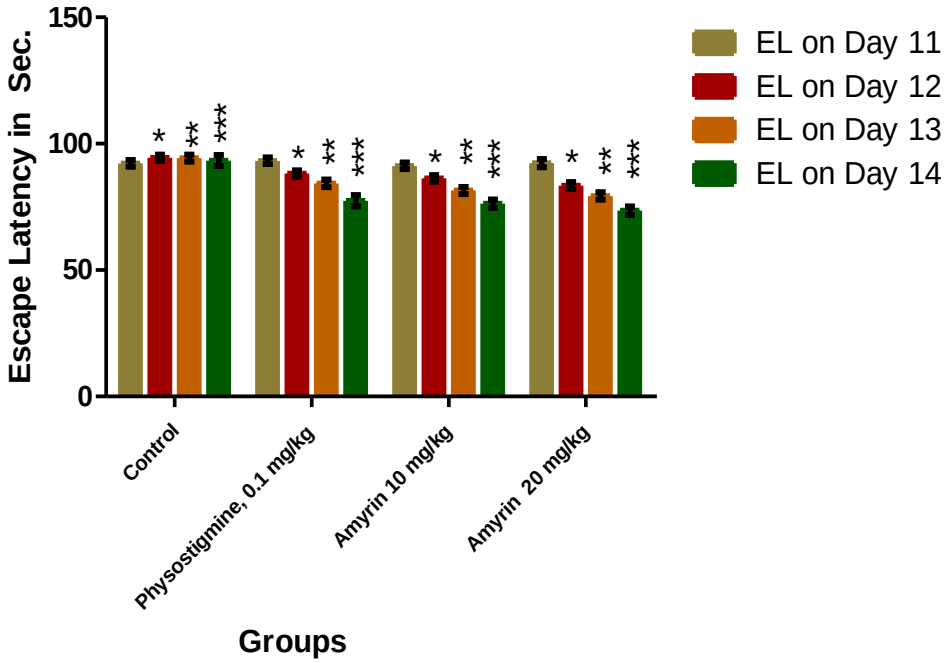

Figure No. 7: Effect of α-Amyrin on Escape Latency (sec) on different days.

Table No. 2: Effect of α-Amyrin on TSTQ of Morris Water Maze

Treatment Schedule	TSTQ (Sec) (15th day)
Normal Control	46.72 ± 2.51
Physostigmine, 0.1 mg	102.21±2.55***
α-Amyrin (10 mg/kg)	89.12±3.41***
α-Amyrin (20 mg/kg)	107.43±3.66***
Scopolamine (0.4 mg/kg)	55.21±1.56*
Diazepam (1 mg/kg)	58.21±1.37*
α-Amyrin + scopolamine (20+0.4 mg/kg)	80.87±3.88**
α-Amyrin + diazepam (20+1 mg/kg)	86.76±3.91**

Values are expressed as mean± SEM, *n*=6 in each group; * *p* <0.05, compared to control ** *p*<0.01, compared to control. *** *p* <0.001, compared to control

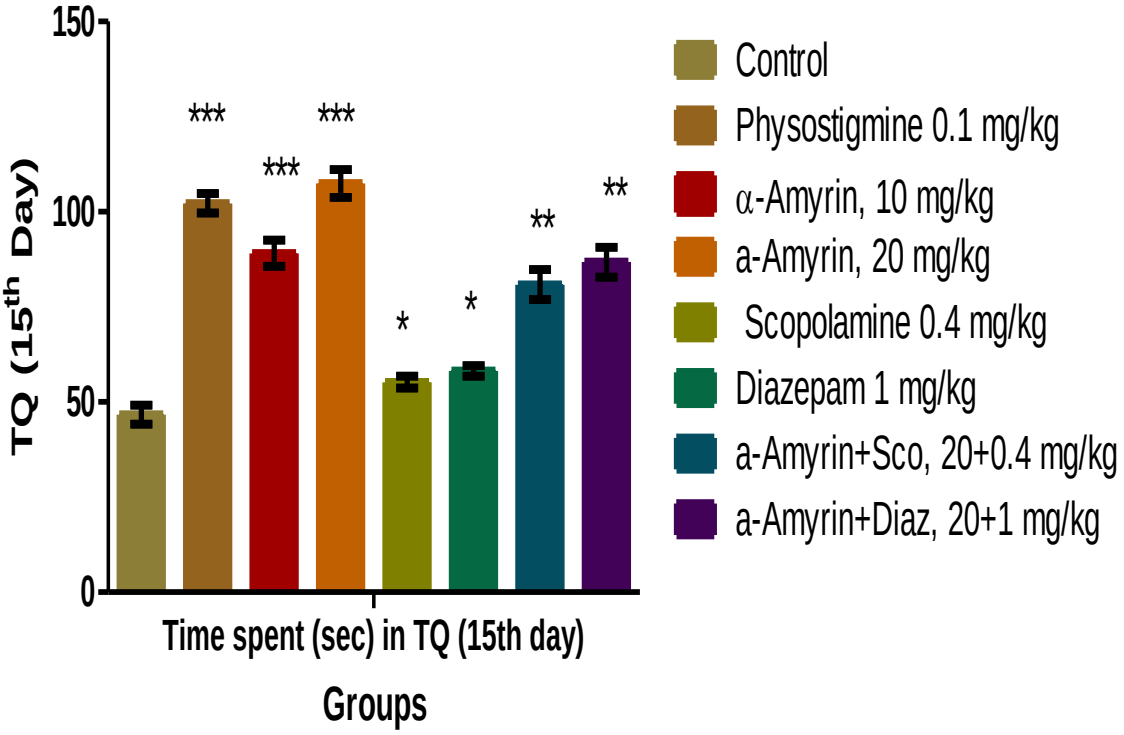

Figure No. 8: Effect of α-Amyrin on TQ on 15th Day

Effect of α-Amyrin on Locomotor activity of mice

There was a noteworthy transformation in Locomotor activity in mice treated by α-Amyrin (10 & 20 mg/kg) and physostigmine as compared to vehicle treated control

Table No. 3: Effect of α-Amyrin on Locomotor activity of mice

Treatment Schedule	Locomotor activity counts / 5 min
Normal Control	210.40 ± 2.14
Physostigmine, 0.1 mg	410.15±2.31***
α-Amyrin, 10 mg/kg	288.36±3.63**
α-Amyrin, 20 mg/kg	332.64±3.66***

Values are expressed as mean± SEM, *n*=6 in each group; * p <0.05, compared to control ** p<0.01, compared to control. *** p <0.001, compared to control

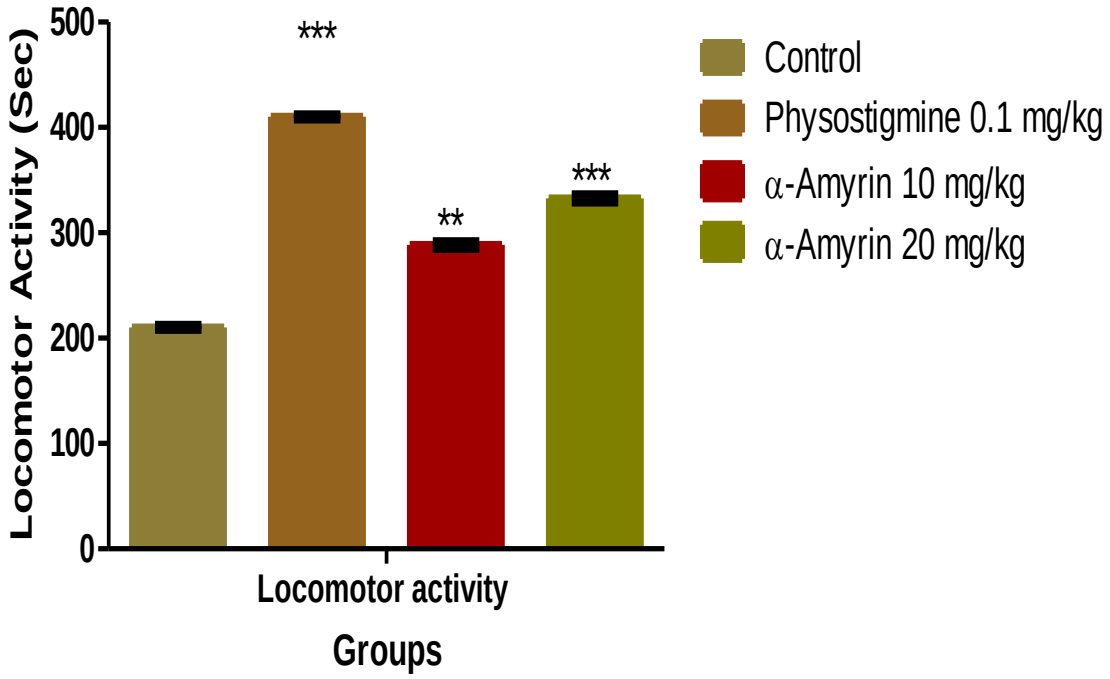

Figure No.9: Effect of α-Amyrin on Locomotor activity in mice

Effect of α-Amyrin on brain Acetylcholine esterase activity of mice

Animals treated by α-Amyrin and physostigmine for 15 successive days created a noteworthy decrease in brain Acetyl cholinesterase activity as compared to control group. Mice fed with α-Amyrin in a dose of 20 mg/kg showed a exceedingly noteworthy decreasing effect on brain Acetyl cholinesterase activity compared to rats of control group.

Table No.4: Effect of α-Amyrin on brain Acetyl cholinesterase activity of mice

Treatment Schedule	**Acetyl cholinesterase activity (mol/l per min × 10^{-6}/g of tissue)**
Normal Control	0.068 ± 0.010
Physostigmine, 0.1 mg	0.021±0.007***
α-Amyrin, 10 mg/kg	0.032±0.014***
α-Amyrin, 20 mg/kg	0.025±0.012***

Values are expressed as mean± SEM, *n*=6 in each group; * *p* <0.05, compared to control ** *p*<0.01, compared to control. *** *p* <0.001, compared to control

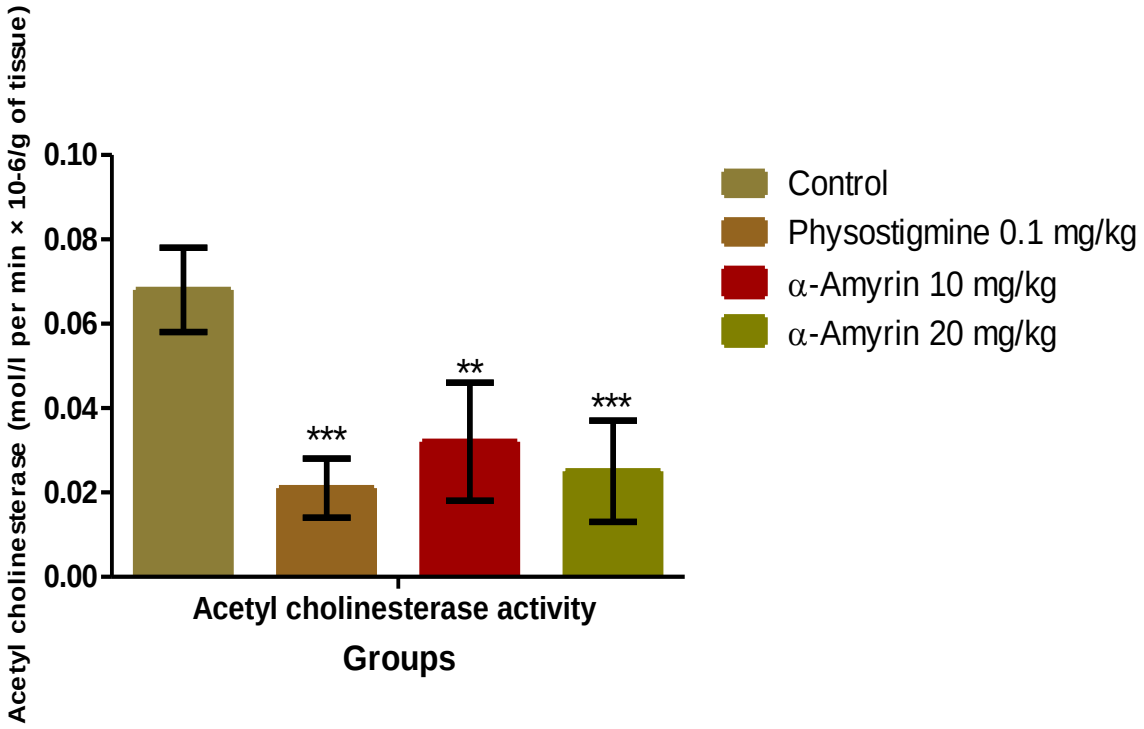

Figure No. 10: Effect of α-Amyrin on Acetyl **cholinesterase level in brain**

3.0 CONCLUSION

The demand for herbal medication isolated from plant parts are being increasing to treat ample diversity of diseases, although relatively modest associate about their mode of action is existing. The Ayurveda had come in light and developed interest to researchers for phyto-pharmacological evaluation.

However there are numerous others herbal drugs claimed to possessed beneficial activity in treating neurodegenerative disorders but they need to separate and characterize the bioactive molecule responsible for therapeutic efficacy to potentiate their effect, diminish the side effect and explore it in modern era.

Hence, by keeping in view, the two plants i.e. *Adhatoda vasica (Leaves)* and *Vigna Mungo (Seeds)* were selected for evaluation of memory enhancing activity (Nootropics).

In our beginning study, the shed dried powder of both plants were extracted sequentially using petroleum ether, chloroform, methanol, butanol and water. After drying, it was evaluated for presence of various constituents. From study, we found the presence of various essential phytoconstituents which may responsible for memory enhancing such as terpenoids, alkaloids glycosides, phenolic compounds, flavonoids, tannins fatty acids and steroids. After that, antioxidant assays were carried out for different extracts by hydroxyl radical scavenging activity, lipid peroxidation and by DPPH methods

β-carotene bleaching inhibition method

In case of *Vigna Mungo* , we found that, among all extracts, chloroform extract showed best inhibitory activity. Then methanolic extract showed higher activity as compared to butanolic and water extracts. Vitamin E as standard was used in this assay and 84% inhibition was found to be at 30 minutes. Chloroform extract also showed 70% inhibition at 30 minutes which was reduced to 45% at the time of 120 minutes.

In case of *Adhatoda vasica,* the same i.e. chloroform extract showed best inhibitory activity. Then methanolic extract showed higher activity as compared to butanolic and water extracts. Vitamin E as standard was used in this assay and 84% inhibition was found to be at 30 minutes. Chloroform extract also showed 71% inhibition at 30 minutes which was reduced to 46% at the time of 120 minutes.

Hydroxyl Scavenging Activity

In case of *Vigna Mungo*, Hydroxyl radical scavenging capability calculated as that chloroform, methanol, butanol and water extracts having IC_{50} values of 47 µg/mL, 100µg/mL, 200 µg/mL and 150µg/mL, respectively. From the observations, it was concluded that chloroform and methanol extracts possesses highest OH° radical scavenging activity when compared with water and butanolic extracts.

In case of *Adhatoda vasica*, IC_{50} reveals that chloroform; methanol, butanol and water extracts have IC_{50} values of 50 µg/mL, 100µg/mL, 200 µg/mL and 150µg/mL, respectively. These observations implied that chloroform and methanol extracts have highest OH° radical scavenging capability as compared to butanolic and water extracts.

DPPH Free Radical Activity

In case of *Vigna Mungo*, DPPH scavenging ability calculated as IC_{50} shows that chloroform extract has IC_{50} of 36 µg/mL trail by ascorbic acid (3.2 µg/mL), gallic acid (3.5µg/mL), methanol (40µg/mL), butanolic (47µg/mL) and water (45µg/mL) extracts. The result revealed that chloroform extract had the highest DPPH scavenging ability.

In case of *Adhatoda vasica* , DPPH scavenging ability calculated as $IC_{50,}$ shows that chloroform extract has IC_{50} of 36 µg/mL followed by ascorbic acid (3.2 µg/mL), gallic acid (32µg/mL), methanol (40µg/mL), butanolic (42µg/mL) and water (44µg/mL) extracts. The result revealed that chloroform extract had the highest DPPH scavenging ability among all extracts.

The acute oral toxicity for both plant extracts were studied and observed for any sign and symptoms of toxicity like hypersensitivity reactions, diarrhea, itching, behavioral changes and mortality at the utmost fatal dose of 2000 mg/kg body weight. Therefore, $1/10^{th}$ (200 mg/kg) and $1/5^{th}$ (400 mg/kg) of the lethal dose were picked up as effectual measure for evaluation of memory enhancing activity.

Memory may be looked upon as skill to maintain an intelligence precedent event. It is a multifaceted progress involving diverse parts of the brain, numerous neurotransmitters and sensory organs. Here, the MWM was used for spatial learning and memory in laboratory rodents.

Learning and memory are associated with escape latency (EL) and time spent in target quadrant (TSTQ). The *Vigna Mungo* produced decline of EL and augment of TSTQ by rodents in MWM indicated upgrading of learning and memory and vice-versa. Various extracts and Physostigmine (0.1mg/ kg, *i.p.*) given for 15 consecutive days appreciably decreased EL of mice from 11^{th} to 14^{th} day and augment TSTQ by mice on 15^{th} day as compared to the control, thus showed significant enhancement of learning and memory. Among all the extracts, chloroform extract showed an exceedingly noteworthy outcome on EL and TSTQ. Chloroform extract significantly declined ($P<0.001$) EL and significantly improved TSTQ as match up to vehicle treated control.

In our study, all the extracts in both doses were administered for 15 consecutive days significantly pick up learning and memory of rodents. Memory improving effects of extracts were comparable to physostigmine. MWM was used as a behavioral model for assessment of learning and memory. Chloroform extract of both plants significantly decreased EL during training and it significantly increased TSTQ during recovery, indicating enhancement of learning and memory.

The introductory phytochemical study showed the burly proof of alkaloids and triterpenes in chloroform extract. So, the presence of Plumbagin and α-amyrin were employed to detect the probable mechanism of memory enhancing.

Acetylcholine is measured as the very imperative neurotransmitter engaged in the functioning of cognition. In previous discussion regarding plumbagin, which is the main active constituent of chloroform extract may be responsible for enhancement of memory. This is due to its inhibition of acetyl cholinesterase which is accountable for augment in echelon of brain acetylcholine.

In case of *Adhatoda vasica*, chloroform extract also significantly reduced the cholinesterase enzymes in brain. From the phytochemical screening, it was cleared that chloroform extract shows the presence of Triterpenoids, and the α-amyrin is the main triterpenoids present in chloroform extract. So from study, it may be concluded that memory enhancing effect of chloroform extract may be due to availability of these triterpenoids.

Anxiety, akin to all sentiment, has cognitive, neurobiological and behavioral means. It is an unconstructive emotion that happens in respond to perceived risk that can come from internal or external sources and can be genuine or probable.

In our study of *Vigna Mungo*, the vehicle-treated mice (10 ml/kg, p. o. normal saline) gave more time in closed arm and illustrated fewer entries in open arm as compared to closed arm of the maze in period of 5 min. Rodents treated with diazepam (1 mg/kg, p. o.) showed noteworthy ($P < 0.001$) augment in the proportion of open arms entries as well as time spent in open arm whereas, in closed arm number of entries and time spent were significantly ($P < 0.001$) decreased. The chloroform extract of *Vigna Mungo* in 200 and 400 mg/kg, p.o. respectively exhibited noteworthy ($P < 0.01$) boost in the percentage of number of open arm entries and time spent in open arm while, in the closed arm number of entries and time spent was significantly ($P < 0.01$) reduced as compared to vehicle-treated group.

Bioactivity guided isolation was also performed for *Adhatoda vasica* and fraction 104-123, eluted with Petroleum ether: Dichloromethane (9:1) were mixed together to give an active compound, which was crystallized out as compound on addition of a few drop of cold methanol.

In our investigations, mice treated by Plumbagin (1.5 & 3 mg/kg, *p.o.*) administered for 15 consecutive days appreciably upturned amnesia induced by scopolamine and diazepam in mice. The plumbagin (1.5 & 3 mg/kg) drastically reduced acetyl cholinesterase level in brain of mice as compared to the control. In whole investigation, the behavioral model employed was Morris

water maze for assessing memory and learning. The α-amyrin considerably picks up learning and memory of experimental animals in pertinent test along with decreased the altitude of acetyl cholinesterase enzyme. The results and observations from this study have exposed that extracts and phytoconstituents exhibited significant memory enhancing activity.

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