JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

EVALUATION OF NOOTROPIC ACTIVITY OF POLYHERBAL FORMULATION BY USING OBJECT RECOGNITION TEST AND REVERSAL OF DIAZEPAM INDUCED AMNESIA BY USING Y-MAZE

[1]Mrs. Swati Kolhe, [2]Ms. Aanam Bagwan, [3]Mrs. Nishigandha Yadav, [4]Mr. Aditya Lokhande,[5]Mr. Atharva Suryawanshi, [6]Ms. Ayushi Shah,[7]Ms. Sejal Takawale

[1]Head Of Department, [2]Post Graduate Student, [3]Assistant Professor

[1]Pharmacology Department,

[1]AISSMS College Of Pharmacy, Pune, India

Abstract : Polyherbal formulation consists of plant ingredients of Brahmi (Bacopa monniera), Shatavari (Asparagus racemosus), and Ashwagandha (Withania somnifera).The present study was undertaken to investigate the effects of Polyherbal formulation (PHF) on learning and memory in experimental animals. Object Recognition Test and Y- maze were employed to test learning and memory. Diazepam (1mg/kg p,o) was used as interoceptive (stimulus inside the body) behaviour model. Three doses (100,300 and 600 mg/kg p.o.) of PHF were selected depending upon the acute oral toxicity study observations and were administered in separate groups of animals.Object recognition test results proved the efficacy of the polyherbal formulation as a nootropic by significantly increasing the discrimination index at dose of 300 mg/kg.The Y-maze model results also showed that dose of 300mg/kg of PHF significantly improved learning and memory in rats. Furthermore, this dose significantly reversed the amnesia induced by diazepam (1mg/kg p,o.). Diazepam which is a GABA mimetic agent induces memory impairment and the subsequent inhibition of diazepam induced amnesia by the PHF may be due to inhibition of GABA-B receptors which in turn facilitate learning and memory.

IndexTerms **- Polyherbal Formulation (PHF),Amnesia,Learning,Memory,Nootropic.**

INTRODUCTION

The ability of the brain to encode, store, and recall information is known as memory. The initial perception and registration of information is referred to as encoding. The retention of encoded information over time is referred to as storage. The steps involved in using stored information are referred to as retrieval. When humans recall a previous event successfully, they must have encoded, stored, and retrieved information about the experience (Fotuhi M.,2004); on the other hand, forgetting a crucial fact implies a failure in one of these phases of memory. Memory and learning are intimately linked, and the two phrases generally refer to the same thing. Learning is frequently used to refer to the processes involved in the initial acquisition or encoding of knowledge, whereas memory is more frequently used to refer to the subsequent storage and retrieval of information (Baddeley AD., 1997). In a broad sense, cognition refers to the act of digesting information. It refers to a high level of processing of specialised information, such as thinking, memory, perception, motivation, skilled motions, and language. The hippocampus houses the neuronal circuitry required for cognitive activities such as learning and remembering. Mental functioning encompasses both perceptual and cognitive elements (Trivedi JK.,2006). One of the most functionally damaging aspects of cognitive impairment, a major health problem in the twenty-first century. Memory loss, amnesia, anxiety, high blood pressure, dementia, and more serious neurodegenerative disorders like schizophrenia, depression, Alzheimer's disease, dementia, cerebrovascular impairment, head injury, parkinsonism, Huntington's disease, Down's syndrome, Pick's disease, trauma, chronic insomnia, and attention deficit disorders can all be caused by age, stress, and emotion

(Ingole SR et al , 2008). Memory complaints and disorders have recently become more common as a result of a variety of factors, including natural (ageing, physical, and mental stress), environmental (excess levels of carbon monoxide, carbon dioxide, methyl mercury in the atmosphere, and aluminium in foods), and iatrogenic (electroconvulsive shock therapy, and use of certain central nervous system depressants) (Annapurna A et al, 2004). In both humans and animals, the cholinergic neuronal system plays a crucial role in learning and memory. The enzyme acetylcholinesterase (AChE) converts acetylcholine (Ach) to choline. Brahmi (Bacopa monniera) is utilised as a nerve tonic, antiepilepitic (Kokate CK et al,2001, Russo A et al, 2005), diuretic (Nadakarni KM., 1954), to relieve stress-induced anxiety, nootropic (Achliya G et al,2004, Singh HK and Dhawan BN,1996), sedative, anti-inflammatory (Nadakarni KM.,1954, Channa S et al,2006), antidepressant, and for adaptogenic actions in the Indian ayurvedic school of medicine (Ashram K et al, 2002, Rai D et al,2003). Antioxidant, anti-inflammatory, anti-tussive, anti-diarrhoeal, diuretic, anti-ulcer, anti-aging, anti-depressant, and immunological agent Shatavari (Asparagus racemosus) (Goyal RK, 2003). Ashwagandha (Withania somnifera) is a stress reliever, sedative, hypnotic, anthelmintic, diuretic, and immunomodulator (Dhingra D et al,2003).In the present study, we have focused upon exploring the potential of an Indian ayurvedic poly-herbal formulation, for its efficacy as nootropic by using the object recognition test and in reversing the memory deficits in diazepam induced amesia using Y-maze.

MATERIAL AND METHODS

Drugs and chemicals

Polyherbal Syrup (Shamantak Enterprises, Herbal extract plant supplier, Pune), Piracetam (Nootropil®, Dr.Reddy's Labs), Diazepam ('Calmpose' Ranbaxy, India) Acetylthiocholine iodide (Loba Chemie Pvt.Ltd), Disodium hydrogen phosphate, Sodium dihydrogen phosphate and DTNB (Analab Fine Chemicals) were used in the present study.

Experimental animals

The study employed male Wistar rats measuring 150-300 grammes. The animals were kept in regular conditions. All of the studies took place during the daylight hours (09:00–17:00 h). For the two models, different rat groups were used. The rats were separated into experimental and control groups and kept in sanitised polypropylene cages with sterile paddy husk as bedding. They had unlimited access to standard pellets as a base diet and ad libitum water. Animals were acclimated to laboratory conditions for 48 hours before to the experiment to reduce any non-specific stress. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under ministry of Animal Welfare Division, government of India, New Delhi IAEC. (CPCSEA/IAEC/PT-01/02-2K21)

Acute toxicity study

The Acute Toxicity of PHF was performed as per OECD guideline 425 using Male Wistar rats maintained under standard dietary conditions. The animals were fasted for 3hr before experiment. Animals were administered with single dose of PHF. Maximum dose of PHF administered was 3000 mg/kg.

Animals treated with Polyherbal formulation showed no signs of any toxicity or mortality at the dose of 3000 mg/kg.

EVALUATION OF NOOTROPIC ACTIVITY

Experimental protocol

Male Wistar rats weighing 150-300g were randomly divided into groups (n = 6) and treated as follows ;

Groups Substance administered

Group I: **Control** Water (*p.o.)*
Group II: **Standard** Piracetam (200 mg/kg, *p.o*.)
Group III: **Test group I** *Polyherbal formulation* (100 mg/kg, *p.o*.)
Group IV : **Test group II** *Polyherbal formulation* (300 mg/kg, *p.o*.)
Group V: **Test group III** *Polyherbal formulation* (600 mg/kg, *p.o*.)

Object Recognition Test

During the light phase of the light/dark cycle, a plastic container (35cm35cm35 cm) was used in low light conditions. A habituation phase, an acquisition phase, and a retention phase were the three steps of the technique in general. Rats were individually subjected to a single familiarisation session of 10 minutes on the first day (habituation phase), during which they were presented in the empty arena to become familiar with the equipment. The animals were treated to a single 10-minute session on the second day (acquisition phase), during which floor-fixed two objects (A and B) were placed in a symmetric position in the centre line of the arena, 10 cm from each other and 8 cm from the nearest wall. The two things, which were made of the same material and had a similar colour and smell, had different shapes but were the same size. Rats were free to investigate the things in the open field. To show rat's investigating behaviour, the exploration time on each object was displayed (in seconds). Rats were allowed to roam the wide field on the third day (retention phase) in the presence of two objects: the familiar object A and a novel object C of different forms but comparable colour and size to A. For each rat, the discrimination index (for retention session) was determined as the ratio (TC100)/(TA + TC), where TA and TC represent the time spent during the retention phase on object A and object C, respectively. The amount of time spent studying any object (nose pointing toward object at a distance of less than 1 cm, but not mounting on or playing with the object) was tracked (Tursun Alkam et al,2011, Takeshi Murai et al,2007).

Diazepam induced amnesia

Experimental protocol

Male Wistar rats (150-300 g) were randomly divided into groups (n = 6) and treated as follows for 14 days;

Groups Substance administered

Group I: **Control** Water (*p.o.)*

Group II: **Negative control** Diazepam (*1 mg/kg, p.o*)

Group III: **Standard** Piracetam (200 mg/kg, *p.o*.) + Diazepam (1mg/kg, p.o)

Group IV: **Test group I** *Polyherbal formulation* (100 mg/kg, *p.o*.) + Diazepam (1mg/kg)

Group V: **Test group II** *Polyherbal formulation* (300 mg/kg, *p.o*.) Diazepam (1mg/kg)

Group VI: **Test group III** *Polyherbal formulation* (600 mg/kg, *p.o*.) Diazepam (1mg/kg)

Y-maze (spontaneous alternation behavior)

The Y-maze task is used to assess spatial working memory in rats by observing spontaneous changes in behaviour. Animals are either needed to execute a specified search sequence or minimise errors in the quest for food when food is used as an incentive. As a result, the important characteristics recorded for the evaluation of medication effects delivered after training are temporal measurement and error scoring (Vasudevan M and Milind P,2009). The Y-maze is a three-arm maze with a 120° angle between each of the two arms made of black painted wood. Each arm measures 40 cm in length, 3 cm in width, and 13 cm in height. The three similar arms are designed at random: the start arm, in which the animal begins to explore (A); the reward arm, which contains food stimuli (B); and the other arm, which contains no food stimuli (C). Initially, each rat was put at the end of arm A, free to walk around, and the sequence and number of arm entries were manually recorded throughout an 8-minute period. Rats tend to go through the maze in a systematic manner, entering each arm one at a time. To be able to alternate, the rats needed to know which arm they had previously visited. To evaluate short-term memory, the percentage of triads in which all three arms were represented, i.e., ABC, CAB, or BCA but not BAB, was recorded as a 'alternation.' Between tests, the arms were sprayed with water to eliminate odours and residues. The percent alternation score for each animal was calculated by multiplying the actual number of alternations by the possible number (specified as the total number of arm entries minus two) and multiplying by 100.

% alternation = [(number of alternations) / (total arm entries - 2)] x 100

The number of arm entries was used as an indicator of locomotor activity (Se JP et al,2010).

Histopathological evaluation

The brains of the diazepam induced amnesia animals were excised and fixed in 10% formalin. The brains were then given for histopathological assessment.

Statistical Analysis

All the results were expressed as mean± standard error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett's t-test. *P*-values <0.05 were considered as statistically significant.

RESULTS

Effect on Discrimination index using Object Recognition Test

PHF 1 in a dose of 100mg/kg did not produce any significant change in discrimination index.PHF 2 (300 mg/kg) and PHF 3 (600mg/kg) treated mice showed significant increase in discrimination index ($p<0.0001$) when compared against vehicle treated mice.Piracetam (200mg/kg) was also significant ($p<0.0001$) in both the tests.

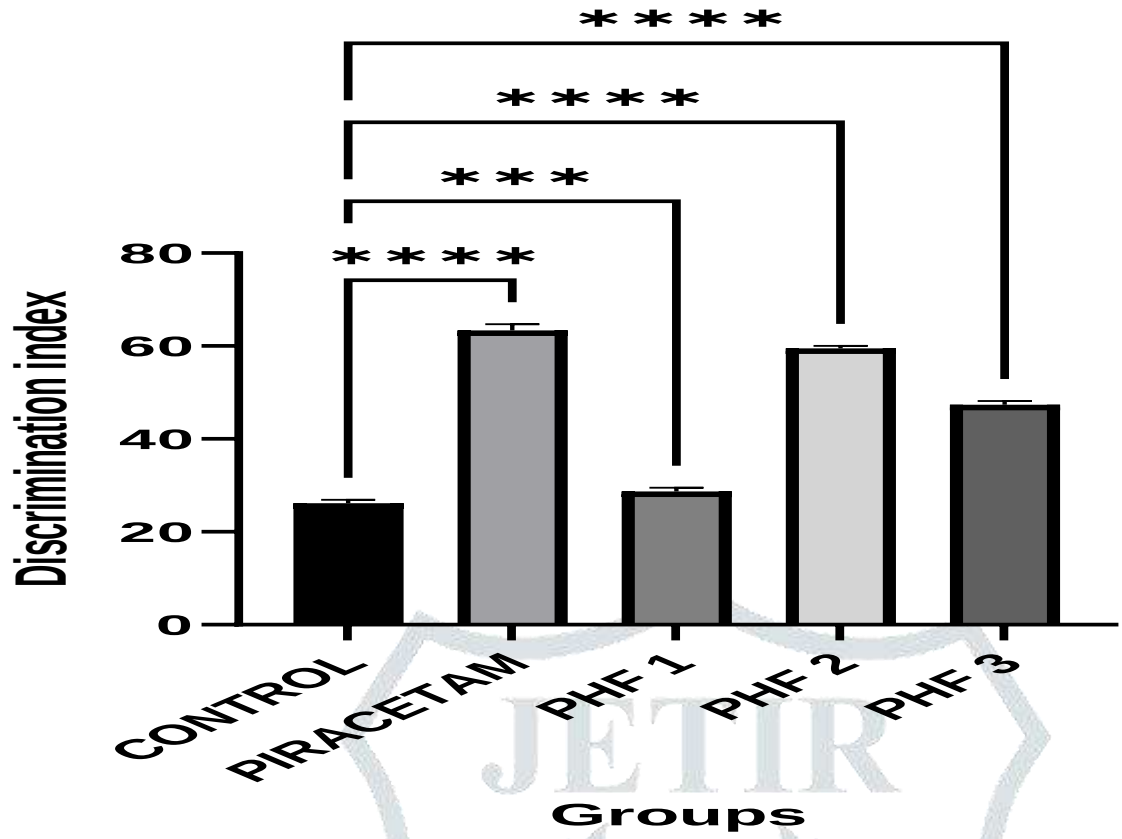

figure 1. effect of phf on object recognition test. values are expressed as mean ± standard error of mean. data were analyzed by one way analysis of variance (anova) followed by dunnett test. p values: *** < 0.001; **** < 0.0001

Effect on percent alteration using Y-maze

To evaluate the effect of PHF administration on reversal of Diazepam induced deficit and other groups, increase in percent alteration was calculated and plotted. Administration of Diazepam (1mg/kg, p.o) induced significant deficit in alteration behavior when compared with vehicle – treated control. However, pre-treatment with PHF protected the rats from spatial memory deficit caused by Diazepam and showed a significant increase in percent alteration when compared with vehicle treated group. Moderate dose of PHF 2 (300mg/kg) produced similar effects and closely approximated to standard Piracetam group. Where it afforded significant (****<0.0001) alteration response compared to lower dose of PHF 1 (100mg/kg). Higher dose of PHF 3 (600mg/kg) also produced significant increase in percent alteration (****<0.0001) but did not closely approximate with the standard drug Piracetam.

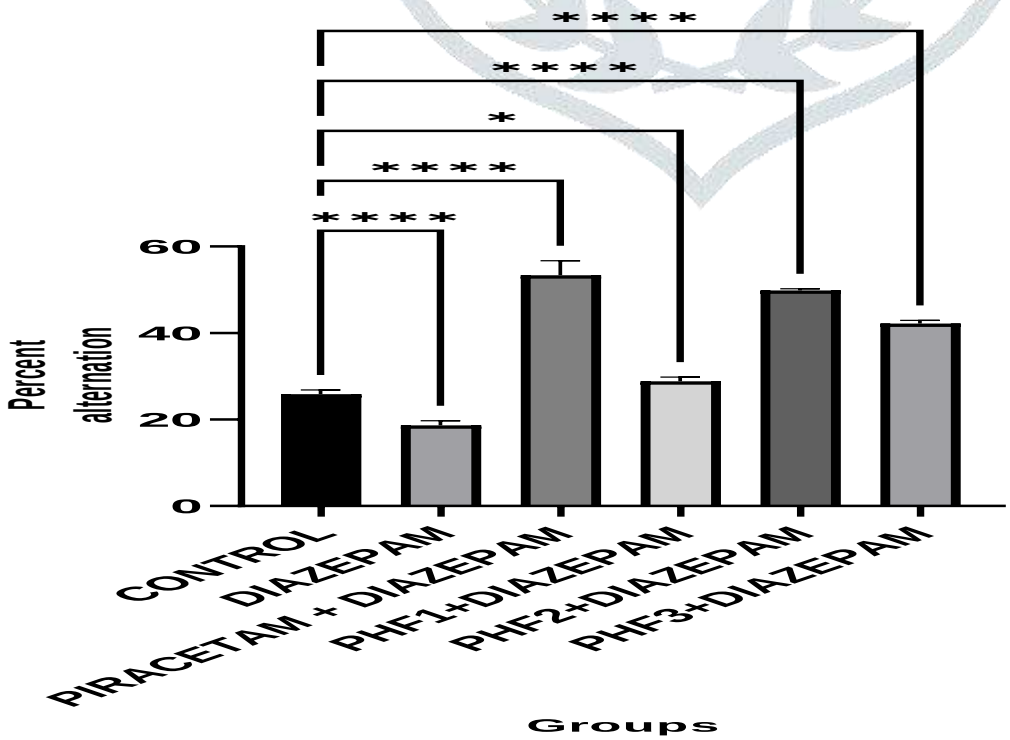

figure 2. effect of phf on object recognition test. values are expressed as mean ± standard error of mean. data were analyzed by one way analysis of variance (anova) followed by dunnett test. p values: *<0.05 ; ****<0.0001 when compared to control.

Histopathological assessment of brain sample

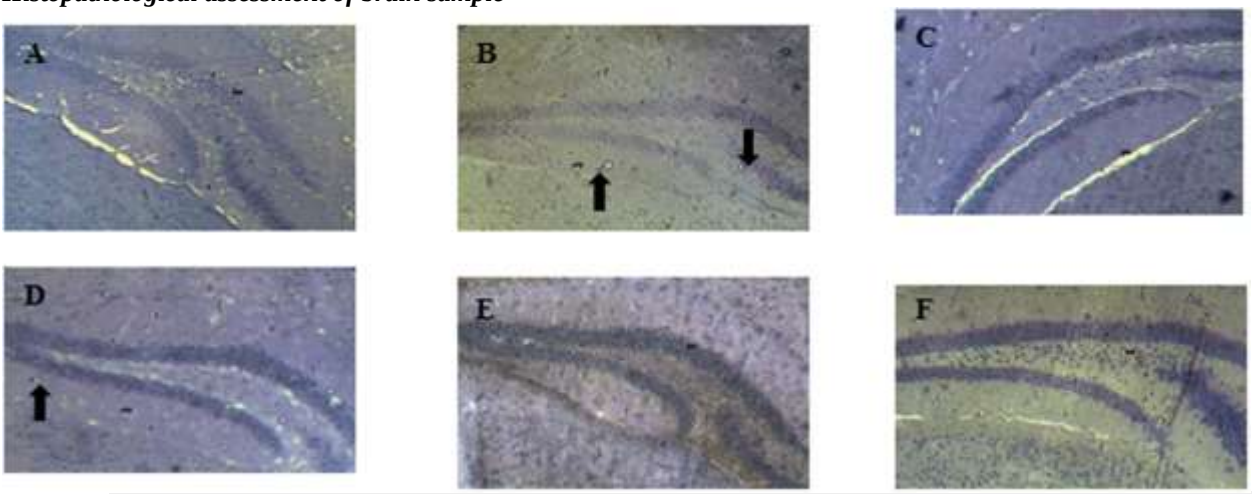

figure 3. effects of phf on neuronal loss in the hippocampus dentate gyrus induced by diazepam. distilled water group **(a)**; diazepam alone treated group **(b)**; *phf 1 (100* mg/kg) **(c)**; *phf 2(300* mg/kg) **(d)**; *phf 3(600* mg/kg) **(e)**; piracetam **(f)**. nissl stain magnification (40).

The histopathological analysis show that the dentate gyrus of distilled water group of rats is normal without any sign of neurodegeneration or necrosis (Figure 3A). The hippocampal sections of diazepam-treated group show a significant reduction in the density of cells of all the layers of the dentate gyrus associated with the presence of apoptotic cells (Figure 3B). The Ethanolic extract of Polyherbal Formulation at the doses of 100, 300, and 600 mg/kg show a normal architecture of the cells layer of the dentate gyrus (Figures 3C – E, respectively). Piracetam group shows a dentate gyrus without any sign of necrotic or apoptotic cells (Figure 3F).

Discussion

Impairment of memory may be defined as an organic brain disorder involving "loss of intellectual ability of sufficient severity to interfere either with occupational functioning, usual social activities or relationship of a person in the absence of gross clouding of consciousness or motor involvement (Singh N et al, 1997).Decreased cholinergic firing in the brain, rise in oxidative stress, hypercholesterolemia, neuro-inflammatory reactions have been demonstrated to play an etiological role in memory decline (Parle M and Bansal N, 2010). The aim of the present study was to investigate the Synergistic effect of polyherbal formulation containing Brahmi, Ashwagandha and shatavari on memory impairment and learning dysfunction in various models.There are many plants with proven nootropic activity used in Ayurveda, siddha and unani system of medicines, Hence the purpose of study was to combine Ethanolic extract of the three plants (Brahmi-whole plant, Ashwagandha-roots, Shatavari-roots) and prove its synergistic effect in enhancing the short term and long term memory. Brahmi contains Bacoside A and Bacoside B in form of saponin alkaloids (Rai D,2003, Goyal RK, 2003),Ashwagandha contains Withanolide as the phytoconstituent (Dhingra D et al, 2003)and shatavarin in shatavari roots contributes to the nootropic activity (Goyal RK, 2003).According to the acute toxicity study conducted, the highest dose of 3000 mg/kg did not cause mortality in animals, Hence the doses selected for evaluation of nootropic activity were 100 mg/kg, 300 mg/kg and 600 mg/kg.

In object recognition test, PHF significantly ($p<0.0001$) caused improvement in discrimination index by 300mg/kg, thereby proving that Polyherbal formulation met the major criteria for nootropic activity, improvement in memory in absence of cognitive deficit (Poschel BPH.1988)

This observation has been strengthened by the finding that PHF has increased the percent alteration behavior in Y-maze model indicating improvement in memory. Y-maze spontaneous alternation is a behavioral test for measuring the willingness of rodents to explore new environments. Rodents typically prefer to investigate a new arm of the maze rather than returning to one that was previously visited. Many parts of the brain, including the hippocampus, septum, basal forebrain, and prefrontal cortex, are involved in this task (Cognato G.P.et al,2012). In the Y-maze, spontaneous alterations indicate working memory. In the present study, treatment with ethanolic extract of polyherbal formulation resulted in significant differences in spontaneous alteration behavior, compared to diazepam treated group. Our data suggest that the extract had protective effect against diazepam-induced memory loss, which was due to its inhibition of GABA-B receptor activity.

In agreement with previous studies, diazepam administration (1 mg/kg, *p.o*) impaired spatial working memory evidenced in the significant reduction in percent alternation behaviour.However, the pre-treatment of rats with PHF (100, 300 and 600 mg/kg, *p.o*) improved spatial working memory evidenced in the significant ($P<0.0001$) increase in relative proportion of spontaneous alternation percentage as compared with diazepam treated rats. These results suggested that PHF could enhance short term or working memory.

The results of histopathological studies demonstrated that administration of diazepam resulted in neurodegenerative processes in the dentate gyrus when compared to naïve rats.

This cell death in the hippocampus dentate gyrus was significantly prevented by a pretreatment with *PHF*. The dentate gyrus is the part of the brain where adult neurogenesis takes place and it is also implicated in hippocampal neurogenesis and plasticity (Kempermann G et al.,2015). Piracetam against Diazepam induced amnesic model may be due to indirect release of acetycholine in the brain, it can be also assumed that Diazepam impairs neurogenesis in the brain which in turn leads to cognitive deficits as in AD (Demars M et al.,2010, Lazarov O. and Marr R, 2013). By antagonizing the cell death in the dentate gyrus induced by diazepam, *Polyherbal formulation* can be a good treatment for cognitive deficits and AD. Diazepam is a GABA mimetic agent that decreases the percent alteration behavior in rats. By counteracting the

effect of diazepam, PHF can have the same mechanism of action, as Piracetam wish is a cholinergic enhancer widely used in the treatment of AD. This property of the herbal plants may also be strongly involved in its neuroprotective effects observed in our study. This study shows that *PHF* has an ability to improve learning of information, ameliorates spatial short-term and long-term memory and recognition memory. The mechanism by which *Polyherbal Formulation* exerts its effects may be related to the indirect release of acetycholine in brain associated with improvement of adult neurogenesis.

Acknowledgements

Authors are thankful to the principal and management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

References

[1] Fotuhi M. The Memory Cure: how to Protect your Brain against Memory Loss and Alzheimer's Disease. New York: McGraw-Hill Professional; 2004

[2] Baddeley AD. Human Memory: theory and Practice. Hove, UK: Psychology Press; 1997.

[3] Trivedi JK. Cognitive deficits in psychiatric disorders: current status. Indian J Psychiatry. 2006;48:10-20.

[4] Ingole SR, Satynaran K, Rajput Sharma SS. Cognition enhancer: current strategies and future perspectives. CRIPS. 2008;9:42-8.

[5] Annapurna A, Ramakrishna V, Murali KK, Krishnakumar V, Prasad KB. Studies on nootropic activity of ramipril and losartan on scopolamine-induced amnesia in rats. Indian J Pharm Sci. 2004;66(1):31-5.

[6] Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy. 17th ed. Nirali Prakashan Pune.2001; 218.

[7] Russo A, Borreli F, Bacopa monniera, a reputed nootropic plant: an overview, Phytomed 2005; 12:305-317.

[8] Nadakarni KM. Indian materia medica, Popular Prakashan Vol. 1. 3rd ed, 1954. Bombay. 582, 1260, 1261, 1292.

[9] Achliya G, Barabde U, Wadodkar S, Dorle A. Studies on the effects of Brahmi ghirta, an polyherbal formulation on learning and memory paradigms in experimental animals. Ind J Pharmacol 2004; 36:159- 162.

[10] Singh HK and Dhawan BN. Pharmacological Studies on Bacopa monniera, an ayurvedic nootropic agent. Eur Neuropsychopharmacol 1996; 6(3):144.

[11]Channa S, Dar A, Anjumb S, Yaquis M. Atta-Ur- Harman. Anti- inflammatory activity of Bacopa monniera in rodents. J Ethnopharmacol 2006; 104:286–89.

[12] Ashram K, Dorababu M, Goel RK and Bhattacharya SK. Antidepressant activity of standardized extract of Bacopa monniera in experimental models of depression in rats. Phytomedicine 2002; 9:207–11.

[13] Rai D, Bhatia G, Palit G, Pal R, Singh S, Hemant K Singh. Adaptogenic effect of Bacopa monniera. Pharmacol Biochem Behav 2003; 75(4):823-30.

[14] Goyal RK, et al. (2003). Asparagus racemosus - an update. bioline.org.br/request?ms03025

[15] Dhingra D, Parle M, Kulkarni SK. "Medicinal plants and memory." Indian drugs 2003, 40(6):313-19.

[16] Tursun Alkam, Masayuki hiramatsu, Takayoshi Mamiya, Yuki Aoyama Atsumi Nitta. Evaluation of object based attention in mice. Behavioral Brain Research 220, 2011, 185-193.

[17] Takeshi Murai, Shoki okuda, Takeshi Tanaka, Hisashi Ohta. Charecteristics of objective location memory in mice: Behavioral and pharmacological studies.Physiology and Behavior, 2007, 90, 116-124

[18] Vasudevan M, Milind P. Memory-enhancing activity of Coriandrum sativum in rats. *Pharmacologyonline.* 2009;2:827–839.

[19] Se JP, Dog HK, Il KL, Won YJ, Dong HP, Jong MK, et al. The ameliorationg effect of the extract of the flower of Prunell vulgaris var. lilacina on drug-induced memory impairment in mice. *Food Chem Toxicol.* 2010;48:1671–6.

[20] Singh N, Sharma A, et al. Effects of BN-50730 and physostigmine (AChE inhibitor) on learning and memory in mice, Methods and Findings in Experimental and Clinical Pharmacology, 1997,19, Pages 585-588.

[21] Parle M and Bansal N, Antiamnesic activity of an Ayurvedic Formulation Chyawanprash in mice,Evidence-Based Complemetary and Alternative Medicine, 2010, Volume 2011, Pages 1-9

[22] Poschel BPH. In: Iversen LL, Iversen SD, Snyder SH, editors. Handbook of psychopharmacology, New York: Plenum; 1988. 437– 45.

[23] Cognato G.P., Bortolotto J.W., Blazina A.R., Christoff R.R., Lara D.R., Vianna M.R., Bonan C.D. 2012. Y-Maze memory task in zebrafish (Danio rerio): the role of glutamatergic and cholinergic systems on the acquisition and consolidation periods. Neurobiol. Learn. Mem. 98: 321–328.

[24] Kempermann G., Song H., Gage F. H. (2015). Neurogenesis in the Adult Hippocampus. *Cold Spring Harb. Perspect. Med.* 5:a018812. 10.1101/cshperspect.a018812

[25] Demars M., Hu Y. S., Gadadhar A., Lazarov O. (2010). Impaired neurogenesis is an early event in the etiology of familial Alzheimer's disease in transgenic mice. *J. Neurosci. Res.* 88 2103–2117. 10.1002/jnr.22387

[26] Lazarov O., Marr R. (2013). Of mice and men: neurogenesis, cognition and Alzheimer's disease. *Front. Aging Neurosci.* 5:43 10.338