ISSN 0975-6299 Vol.1/Issue-3/Jul-Sep.2010

International Journal of Pharma and Bio Sciences

# HEMIDESMUS INDICUS: EVALUATION OF ITS NOOTROPIC EFFECT IN MICE

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## ABSTRACT

Dementia is one of the age-related mental problems, and a characteristic symptom of Alzheimer's disease. Nootropic agents are clinically used in situations where there is organic disorder in learning abilities and for improving memory, mood and behavior, but the resulting side-effects associated with these agents have made their utility limited. Ayurveda emphasizes use of herbs, nutraceuticals or life-style changes for controlling age related neurodegenerative disorders. The present study was undertaken to assess the potential of an ayurvedic *rasayana* (rejuvenator) drug *Hemidesmus indicus* roots as a memory enhancer. Elevated plus maze and passive avoidance paradigm were employed to evaluate learning and memory parameters. The chloroform and *n-butanol* fractions of ethanolic extract *H. indicus root* (3, 10 and 30 mg/kg, p.o.) were screened for claimed potential in mice. The *n-butanol* fraction of *H. indicus* extract significantly improved learning and memory at all doses mice. Hence, *H. indicus* might prove to be a useful memory restorative agent in the treatment of dementia seen in the Alzheimer's disease.

## KEYWORDS

Alzheimer, *Hemidesmus indicus,* Nootropic, Mice

## INTRODUCTION

Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities; patients also frequently have non-cognitive symptoms, such as depression, apathy and psychosis that impair daily living[1]. It is the most common form of onset of adult dementia and attention deficit disorders[2]**.** Centrally acting anti-muscarinic drugs (like scopolamine) impaired learning and memory of rats[3] and human beings[4]**.** Benzodiazepine receptor agonists such as diazepam and alprazolam have been shown to produce anterograde amnesia in rodents[5] and human beings[6]**.** Nootropic agents such as Piracetam[7], Donepezil® are used primarily for improving memory, mood and behavior. However, the resulting side-effects associated with these agents have limited their use[8]**.**

Ayurveda, the Indian system of medicine, is gaining greater attention and popularity in many parts of the world. The disease preventive and health promotive approach of ayurveda, which takes into consideration the whole body, mind and spirit while dealing with the maintenance of health promotions, now enjoys increasing acceptability. The ancient ayurvedic physicians understood the delicate cellular mechanisms of the body and the deterioration of the functional efficiency of the

body tissues. These ayurvedic scholars had thus developed certain dietary and therapeutic measures to delay ageing and rejuvenating whole functional dynamics of the body organs. This revitalization and rejuvenation is known as the 'Rasyana chikitsa' (rejuvenation therapy)[9]**.** *Rasayana* drugs act inside the human body by modulating the nuero-endocrino-immune systems and have been found to be a rich source of antioxidants[10]**.** According to ayurveda, Alzheimer's disease is an imbalance of *vata, pitta* and *kapha.* Medhya (intellectual promoting) herbs such as, *Convolvulus microphyllus (C. pluricaulis), Centella asiatica, Bacopa monnieri, Acorus calamus* and *Celastrus paniculatus* are beneficial in cognitive disorders[11, 12, 13]

*Hemidesmus indicus* (HI) R. Br. (Family: Asclepiadaceae), commonly known as Indian sarsaparilla or Anantmool is a slender, laticiferous and twining shrub, occurs over the greater part of India and some costal districts of Orissa.[14] It is widely recognized in folk medicine and as ingredient in Ayurvedic and Unani preparations against disease of biliousness, blood diseases, diarrhea, skin diseases, respiratory diseases, fever, bronchitis, eye diseases, burning sensation, rheumatism and gastric disorders.[15] The root is said to be tonic, diuretic, and alterative. Root decoction helps in skin diseases, syphilis, elephantiasis, loss of appetite, blood purification and for kidney and urinary disorders .[16] Several biological activities like hepatoprotective, antioxidant, antithrombotic, anti-ulcerogenic, anti-inflammatory, immunomodulatory, antidiabetic etc. have been reported from various root extracts.[17-22] Inspite of vast reports in scientific texts on the applications of HI very meager work has been carried out on the aspects of central nervous system. In view of this the n-butanol and chloroform fraction from ethanolic extract of HI was investigated for nootropic potential in mice using various standard paradigms.

## MATERIAL AND METHODS

***Preparation of fractions of H. indicus (HI)***: The plant was collected from near by region of Pune and was authenticated at Botanical Survey of India, Pune with voucher specimen no. 4. TKDE. The roots were dried under shade, powdered and passed through 40 mesh sieve. The powdered material was successively extracted with petroleum ether and ethanol using soxhlet apparatus. The ethanolic extract (EHI) obtained was dried in rotary vacuum evaporator at $40^0$C, yielding a reddish brown colored viscous mass (8.40 % w/w).

The ten gm of dry powder of ethanolic extract of *HI* was dissolved in 100 ml of distilled water and transferred to a separating funnel. To this 50 ml of chloroform was added slowly. This mixture was then shaken for 01 hour. The pressure was released intermittently. After 01 hour the mixture was allowed to settle for minimum half an hour. The chloroform layer was carefully decanted, collected in a china dish and evaporated to dryness to obtain chloroform fraction of *H. indicus* (HICF).

The 50 ml of *n-butanol* was added to the remaining dry powder of ethanolic extract of *HI* and the mixture was then shaken for 01 hour. The pressure was released intermittently. After 01 hour the mixture was allowed to settle for half an hour. The n-butanol layer was carefully decanted and collected in a china dish and evaporated to dryness to obtain n-butanol fraction of *H. indicus* (HIBF). Accurately weighed quantity of dry powder of respective fractions was suspended in 1% w/v acacia mucilage individually to prepare the appropriate stock solution. The piracetam suspension was diluted with the distilled water. The doses were administered by selecting the appropriate concentration of the stock solution.

***Preliminary phytochemical and high performance thin layer chromatography (HPTLC) analysis of fraction:*** The following procedures were adopted for testing the presence of various chemical constituents in the extracts.[23] HPTLC system of CAMAG, Muttenz, Switzerland, Anchrome Enterprises (I) Pvt. Ltd, Mumbai, consisting of sample applicator (Linomat IV), Twin trough chambers with lid, UV viewing cabinet with dual wavelength (254/366 nm), HPTLC plates scanner III controlled by CATS software (version 4.06). The system of Anchrome Enterprises, Mumbai was used for HPTLC video

documentation (CAMAG, Switzerland). Sample was applied by the 100 micro-liter syringe (Hamilton) with help of sample applicator (Linomat IV) in the form of bands. Specifications were as follows (unless specified): Application position, 8 mm above the lower edge; start position, 10 mm; doses speed, 150nl/sec; band length, 8 mm; space between bands, 4 mm; quantity applied, 10 µl. The composition of mobile phase was acetonitrile: water (1:3) for HI. Chromatograms developed from each band (the track) were observed in daylight and then in UV viewing cabinet with dual wavelength (254/366 nm). Plates were scanned in HPTLC plate scanner III controlled by software (Version 4.06).[24]

***Animals:*** Swiss mice of either sex weighing around 18 - 25 g were used in the present study. Mice were divided into eight groups; each consisting of six mice for each experiment and received the drug treatment as shown in Table 1 for HI fractions. They were acclimatized to the laboratory conditions for 5 days before behavioral studies. The animals had free access to food and water and maintained under 12:12 h light and dark cycles. All experiments were carried out during day time from 09:00 to 14:00 h. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Dept. of Animal Welfare, Govt. of India.

***Acute toxicity study:*** Healthy adult male albino mice (18-22 g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the Organization for Economic Co-Operation and Development (OECD-2001). The mice were administered with the different doses of the chloroform or n-butanol fractions of HI. The dose progression or reduction was carried out as suggested by the AOT-425 guidelines. The mice were observed continuously for 2 hrs for behavioral and autonomic profiles and for any sign of toxicity or mortality up to a period of 7 days.

***Nootropic activity object recognition test*:** The apparatus, fabricated locally, consisted of white colored plywood (70 × 60 ×30 cm) with a grid floor. It was illuminated by a 40 W lamp suspended 50 cm above the apparatus. The object to be discriminated was also made of plywood in two different shapes of 10 cm height and coloured black. One day before the test, mice were allowed to explore the box without any object for 02 min.

On the day of test, the first trial ($T_1$) was conducted 60 min after respective treatment. Two identical objects were presented in opposite corners of the box and the time taken by each mouse to complete 20 seconds of object exploration was recorded (Exploration was considered as directing the nose at a distance less than 2 cm to the object and/or touching with nose). Second trial ($T_2$) was performed 90 min after first trial ($T_1$) and a new object replaced one of the objects presented in $T_1$ and mice were left in the box for next 05 min.

The time spent for exploring the familiar (F) and the new object (N) was recorded separately and discrimination index (D) was calculated as (N-F)/ (N+ F). The object was changed randomly and apparatus was cleaned with hydrogen peroxide or damp cloth after each trial to avoid place preference and the influence of olfactory stimuli, respectively[25,26]

***Step down type of passive avoidance response (SDPAR)***

***Preselection test:*** In the pre selection trial, each mouse was gently placed on the platform, when the mouse stepped down from the platform and placed all its paws on the grid floor. An electric shock (5mA, 20 V, AC) was delivered maximum for 15 seconds and step down latency (SDL) was noted down. Mice showing step down latency (SDL) between 02- 25 seconds were selected for the study.

***Step down type of passive avoidance response:*** The above selected mice were allowed to habituate in a shock chamber 10 minutes daily for next 03 days without the application of shock. From the 4th day, mice from different groups were treated with the respective drugs for a period of 07 days. The step down type of passive avoidance response was studied on 7th day. On the 7th day, 60 minutes after the last dose, training session consisting of two trials with 60 seconds inter-trial

interval was conducted. Acquisition test was conducted 60 minutes later with the upper cut off time of 60 seconds. The step down latency was noted when the mouse stepped down before 60 seconds and electric shock was delivered for maximum 15 seconds and retention test was carried out after 24 hours in a similar manner with upper cut off of 300 seconds without electric shock. Evaluation was carried out by comparing step down latency to that of vehicle treated control group.[27,28]

***Radial arm maze task performance:*** Locally fabricated wooden radial arm maze elevated 50cm above the floor consisted of an octagonal central hub 36cm in diameter with eight radial arms. Each arm was 43 cm long, 15 cm wide with 12 cm sides, and had small black plastic cups mounted at 30cm from the central hub. Each mouse, maintained at 85% of its total diet, was exposed to the maze daily with the food pellet in a fixed arm followed by respective drug treatment for the period of 07 days. The apparatus was cleaned with damp cloth after each trial to avoid place preference and the influence of olfactory stimuli.

The evaluation was carried out on $7^{th}$ day, 60 minutes after the respective drug treatment wherein a food pellet was placed in a variable arm for evaluation of working memory. Each mouse placed on the central hub was allowed to choose any of the arms freely to get the food. Latency to find food was recorded as a measure of working memory evaluation.[29] The comparison was made against the vehicle treated control group and the data was expressed as mean ± SEM.

The data was analysed by one way ANOVA followed by post hoc Dunnett's test using INSTAT software. The level of significance was $P<0.05$ (GRAPH PAD INSTAT, 2000).

## RESULTS

***Preliminary phytochemical and HPTLC screening of fractions of EHI:*** The preliminary phytochemical evaluation and HPTLC screening of HIBF confirms presence of alkaloids, saponin, triterpenoids and carbohydrates.

***Acute toxicity study:*** All animals treated with n-butanol and chloroform fraction of *H. indicus* (HIBF/HICF) were free of any toxicity as per acceptable range given by the OECD guidelines and no mortality was found up to 2000 mg/kg. Hence three different doses 03, 10 and 30 mg/kg were selected for further study.

***Nootropic activity by object recognition test***: Increase in the discrimination index indicates nootropic activity. The mean discrimination index of vehicle treated control group was 0.17±0.02 and 0.27±0.02 on $2^{nd}$ and $7^{th}$ day of the treatment respectively. On $7^{th}$ day of the treatment with HIBF (10 and 30 mg/kg) was significantly ($p<0.01$) increased the discrimination index, whereas on $2^{nd}$ day of the treatment 30 mg/kg was significant ($p<0.05$) in this regard. The effect of HICF at all doses tested on both the experimental days was insignificant in this regard. Piracetam 150 mg/kg was significantly ($p<0.01$) increased the discrimination index on both the experimental days. The effect of HIBF 10 and 30 mg/kg was statistically equipotent to that of piracetam 150 mg/kg on $7^{th}$ day of the treatment. (Figure: 1)

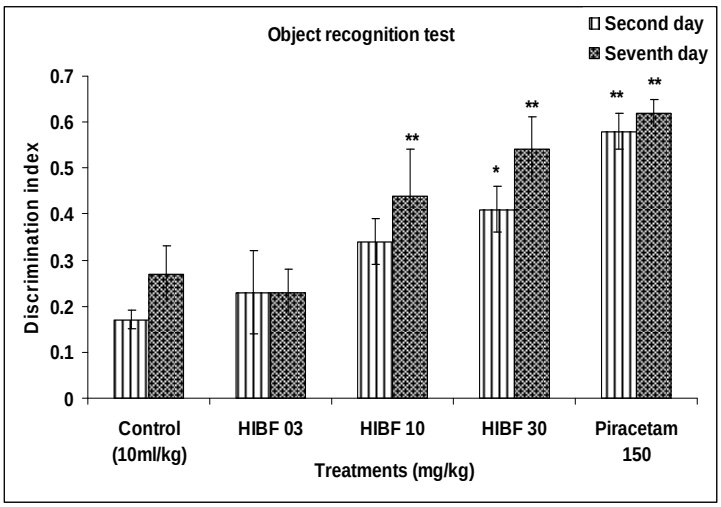


**Figure 1**
***Effect of HIBF on discrimination index in object recognition test***

Data expressed as mean ± SEM (n = 5). *P<0.05 & **P<0.01 as compared to vehicle treated control group.

***Step down type of passive avoidance response (SDPAR)*:** The mean step down latency of vehicle treated control group was 38.54±3.65 seconds and 123.88±4.98 seconds during acquisition and retention test respectively. Piracetam 150 mg/kg showed significant (p<0.01) increase in the step down latency during the acquisition and retention test. Pretreatment with HIBF significantly (p<0.01) increased the step down latency during the acquisition and retention test at 10 and 30 mg/kg.

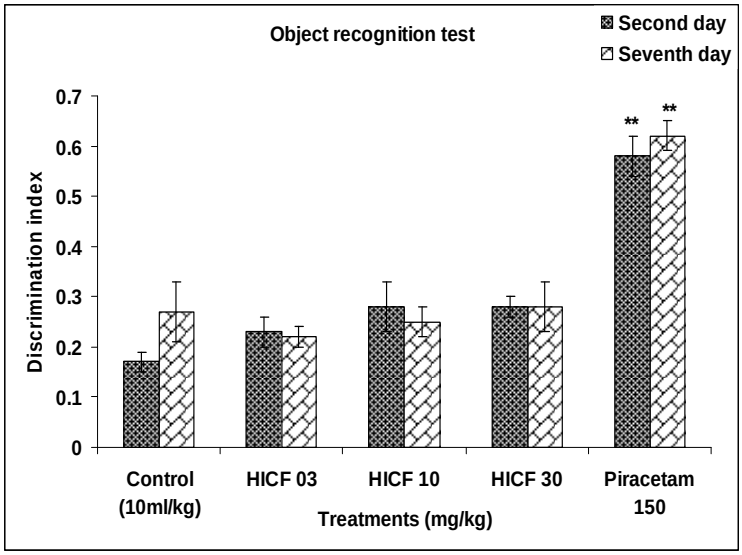


**Figure 2**
***Effect of HICF on discrimination index in object recognition test***
Data expressed as mean ± SEM (n = 5). *P<0.05 & **P<0.01 as compared to vehicle treated control group.

The effect of HICF at all doses tested on both the experimental days was insignificant in this regard. The effect of HIBF 10 and 30 mg/kg was statistically equipotent to that of piracetam 150 mg/kg during the acquisition and retention test. (Table 1)

Hence HIBF 10 and 30 mg/kg was found to be effective in the aversively motivated learning and memory processes.

***Radial arm maze task performance*:** The mean latency to find food in the vehicle treated control group was 55.90± 1.90 seconds. Piracetam 150 mg/kg was significantly ($p<0.01$) decreased the latency to find food. The treatment with HIBF was significantly decreased the latency to find food at 10 and 30 mg/kg. The effect of HIBF 30 mg/kg was more significant ($p<0.01$) than HIBF 10 mg/kg. The effect of HIBF 30 mg/kg was statistically equipotent to that of piracetam 150 mg/kg. The effect of HICF at all doses tested was insignificant in this regard. (Table 2) Hence HIBF 10 and 30 mg/kg was found to be effective towards the appetitively motivated task which evaluates the spatial learning and memory.

**Table 1.**
**Effect of *HIBF* & *HICF* on step down latency for SDPAR**

| Treatments (mg/kg) | Step down latency (Seconds) | |
|---|---|---|
| | Acquisition | Retention test |
| Control (10ml/kg) | 38.54±3.65 | 123.88±4.98 |
| HIBF 03 | 46.39±2.30 | 146.62±4.58 |
| HIBF 10 | 55.10±2.60** | 179.74±5.29** |
| HIBF 30 | 55.71±4.07** | 189.78±4.29** |
| HICF 03 | 38.94±3.71 | 124.14±4.45 |
| HICF 10 | 40.79±4.93 | 141.01±5.49 |
| HICF 30 | 40.34± 4.23 | 142.91±3.77 |
| Piracetam 150 | 58.01±7.64** | 201.72±3.97** |

Data expressed as mean ± SEM (n = 5). *P<0.05 & **P<0.01 as compared to vehicle treated control group.

## DISCUSSION

Memory is the ability of an individual to record sensory stimuli, events, information, etc., retain them over short or long periods of time and recall the same at a later date when needed. Poor memory, lower retention and slow recall are common problems in today's stressful and competitive world. Age, stress, emotions are conditions that may lead to memory loss, amnesia, anxiety, high blood pres-sure, dementia, or to more ominous threats like schizophrenia and Alzheimer's diseases (AD). AD is a neurodegenerative disorder characterized by a progressive loss of memory and cognition. Reducing oxidative stress by anti-oxidants, protecting brain inflammatory lesions using anti-inflammatory drugs and facilitation of brain cholinergic neurotransmission with anti-cholinesterases are some positive approaches to management of AD. The nature provides a new opportunity to regain one's full mental capacity.

**Table 2.**
**Effect of *HIBF* & *HICF* on latency to find food in radial arm maze task performance test**

| Treatments mg/kg | Latency to find food (Seconds) |
|---|---|
| Control (10ml/kg) | 55.90± 1.90 |
| HIBF 03 | 47.70± 1.99 |
| HIBF 10 | 41.37± 2.02* |
| HIBF 30 | 36.66± 1.80** |
| HICF 03 | 58.80± 2.04 |
| HICF 10 | 61.42± 1.12 |
| HICF 30 | 49.88± 2.17 |
| Piracetam 150 | 33.05±2.09** |

Data expressed as mean ± SEM (n = 5). *P<0.05 & **P<0.01 as compared to vehicle treated control group.

AD is a progressive and fatal neurodegenerative disorder manifested by cognitive and memory deterioration, progressive impairment of routine activities of living, and a variety of neuropsychiatric symptoms and behavioral disturbances. The clinical features of AD are an amnesic type of memory impairment, deterioration of language and visuospatial deficits. Motor and sensory abnormalities, gait disturbance and seizures are uncommon until the late phases of the disease. Despite the severity and high prevalence of this disease, Allopathic system of medicine is yet to provide a satisfactory antidote.

Learning and memory involve mechanisms like acquisition, storage, consolidation and recall. Active avoidance learning is reasonably good tests for cognitive function.[30] The ability of the animal to identify the conditioning stimuli (buzzer) as precursor of the unconditioned stimulus (shock) involves recall of task and may implicate long term memory.

The *n-butanol* fraction from ethanolic extract of HI (HIBF) was found to be significant in conditioned avoidance response (CAR) as well as radial arm maze (RAM) task performance. These models are predictive of aversion induced motivation[31,32] and appetite induced motivation[27]. The agent effective in CAR suggest possible use in improvement in more complex task of CNS usually seen in severe neurological complication[32] whereas agent effective in RAM indicate improvement in spatial learning and memory processes.[33,34]

Immunohistochemical studies suggested the existence of chronic inflammation in certain regions of the brain in Alzheimer's disease patients. Since inflammation can be damaging to host tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and the progression of Alzheimer's disease. This hypothesis is supported by the observation that indomethacin (NSAID) halted the progressive memory loss seen in Alzheimer's disease patients. Epidemiological studies have almost confirmed that non-steroidal anti-inflammatory drugs reduce the incidence of AD. Moreover, it has also been observed that elderly patients suffering from Alzheimer's disease showed reduction in symptoms of Alzheimer's disease upon chronic use of anti-inflammatory drugs. Indomethacin, a non-steroidal anti-inflammatory drug, exhibited a memory protective effect against electro-convulsive shock induced retrograde amnesia and also against amyloid deposits in the brain. Anti-inflammatory action of *HI* might have offered protection from the development of inflammatory lesions in brain and contributed to the observed memory-enhancing activity.

Oxygen free radicals, the harmful by-products of oxidative metabolism are known to

cause organic damage to the living system, which may be responsible for the development of Alzheimer's disease in the elderly. *HI* is reported to possess antioxidant activity[15] which might protect the susceptible brain cells from oxidative stress resulting in reduced brain damage and improved neuronal function. The preliminary phytochemical evaluation and HPTLC analysis showed prominent presence of saponins and triterpenoids in HIBF indicating role of these phytochemicals in the observed effect. [38-42] Thus, a combination of anti-inflammatory and antioxidant of *HI* could all be leading to the net memory enhancing effect of HI. Hence HI may be useful as a nootropic agent in the treatment of various cognitive disorders.

## REFERENCES


1. Jewart, RD, Green J, Lu CJ, Cellar J. and Tune LE, Cognitive, behavioral, and physiological changes in Alzheimer disease patients as a function of incontinence medications. Am J Geriatr Psychiatry, 13: 324-328(2005).
2. Robert K and Claudia K, Risk factors for Alzheimer's disease. Neuro Science News 1(4): 27-44, (1998).
3. Higashida A and Ogawa N, Differences in the acquisition process and the effect of scopolamine on radial maze performance in the strains of rats. Pharmacol Biochem Behav, 27: 483-489, (1987).
4. Sitaram N, Weingartner H and Gillin JC, Human serial learning. Enhancement with arecholine and choline and impairment with scopolamine. Science, 201: 247-276(1978).
5. Singh N, Sharma A and Singh M, Possible mechanism of alprazolam induced amnesia in mice. Phrmacology, 56: 46-50, (1998).
6. Lister RG, The amnesic action of benzodiazepines in man. Neurosci. Biobehav Rev, 9: 87- 94(1985).
7. Schever K, Rostock A, Bartsch P and Muller WK. Piracetam improved cognitive performance by restoring neurochemical deficits of the aged rat brain. Pharmaco psychiatry 32: 10-16, (1999).
8. Rogers SH, Farlow, M. R., Doody, R. S., Mohs, R. and Friedhoff LI, Donepezil study group. A 24 week, double blind, Placebo-controlled trial of donepezil in patients with Alzheimer's disease. Neurology 50: 136-145, (1998).
9. Govindarajan R, Vijayakumar M, Pushpangadan P. Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. J Ethnopharmacol 99: 165-178, (2005).
10. Brahma SK and Debnath PK Therapeutic importance of Rasayana drugs with special reference to their multi-dimensional actions. Aryavaidyan 16: 160-163, (2003).
11. Sharma PV, Dravyaguna Vijnan, Medhya Varga, Chaukambha Bharati Academy, Varanasi. Hindi, (1987).
12. Govindadasa, Bhaisajyaratnavali, Varanasi, Chaukambha Sanskrita Academy, (1984).
13. Lolamba, Raj Vaidya Jivanam, Chaukambha Sanskrita Academy, Varanasi, Sanskrit, (1947).
14. Anonymous. The Wealth of India. Raw materials, Vol. Ill, V and X, CSIR: New Delhi, India, (1997).
15. Austin A. A review on Indian sarsaparilla, hemidesmus indicus (L.) R. Br. J Bio Sci; 8 (1): 1-12, (2008).
16. Anonymous. Indian Pharmacopoeia. Indian Pharmacopoeia Committee, Ministry of Health and Family Welfare, Government of India: New Delhi; (1996).
17. Baheti JR, Goyal RK, Shah GB. Hepatoprotective activity of *Hemidesmus indicus* R. br. in rats. Indian J Exp Biol,44(5):399-402, (2006)
18. Verma PR, Joharapurkar AA, Chatpalliwar VA and Asnani AJ. Antinoceceptive activity of alcoholic extract of *Hemidesmus indicus* R. br. in mice. Fitoterapia, 71(1), 55-59 (2000).
19. Mary NK, Achuthan CR, Babu BH and Padikkala J. In vitro antioxidant and antithrombotic activity of *Hemidesmus*

*indicus* (L) R.Br. J Ethanopharmacol, 87 (2-3), 187-191 (2003)
20. Gupta M, Shaw BP, and Mukerjee A. Studies on antipyretic-analgesic and ulcerogenic activity of polyherbal preparation in rats and mice. Int. J. Pharmacol. 4 (2), 1811-7775(2008).
21. Laxman K, Shivaprasad HN, Jaiprakash B and Mohan S. Antiinflammatory and antipyretic activities of *Hemidesmus indicus root extract.* African J Trad. Comp. and Alt Med. 3, (1) (2006).
22. Wadkar KA, Magdum CS, Patil SS and Naikwade NS. Antidiabetic potential and Indian medicinal plants J Herbal Med and Tox. 2 (1) 45-50 (2008).
23. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. 10th edition, Pune: Nirali Prakashan:, 149-156 (2006).
24. Sethi PD, Charegaonkar D. Identification of drugs in pharmaceutical formulations by thin layer chromatography, 2nd edition. CBS Publishers and distridutors: New Delhi2–52, 148-189 (1999).
25. Jain NN, Ohal CC, Shroff SK, Bhutada RH, Somani RS, Kasture VS, Kasture SB. *Clitoria ternatea* and CNS. Pharmacol Biochem Behavior. 75: 529-536(2003).
26. Nikolaos P, Rigamonti A, Cella S, Muller E. The non-NMDA receptor antagonist NBQX does not affect rats performance in the object recognition task. Pharmacological Research; 45 (1): 42-46 (2002).
27. Reddy DS. Assessment of nootropic and amnestic activity of centrally acting agents. Ind J pharmacol. (29): 208-221(1997).
28. Bhattachrya SK and Satyan KS. Experimental methods for evaluation of psychotropic agents in rodents: I – Antianxiety agents. Ind J Expt Biol. 35:565-575(1997).
29. Vyawahare NS, Bodhankar S L, Nikam A P, Sharma R G, Deshpande M M, Tarnalli A D, Effect of Clitoria ternatea extract on radial arm maze task performance and central cholinergic activity in rats J Cell Tissue Res; 7(1): 949-952 (2007).
30. Goswami M, Mund S, Ray A. Effects of some psychotropic agents on cognitive functions in rats. Ind J Pharmacol; 40(1): 75-78(1996).
31. Meneses A, Hong E. Effect of serotonernic compounds on associative learning. Proceeding of western pacific regions of pharmacological society; 34: 461-66 (1991).
32. Meneses A, Hong E. Role of 5-HT1B, 5-HT2A and 5HT2C receptors in learning. Behav Brain Res; 87: 105-110 (1997).
33. Olton DS, Becker JT, Handalmann GE. Hippocampus, space and memory. Behav Brain Sci; 2: 313-66 (1979).
34. Olton DS, Walker JA, Wolf WA. A disconnection analysis of Hippocampal function. Brain Res; 233: 241-53 (1982).
35. Achliya G, Barabde U, Wadodkar S, Dorle A. Effect of brahmi Ghrita, an polyherbal formulation on learning and memory paradigms in experimental animals. Ind J. Pharmacol; 36(3): 159-162 (2004).
36. Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th Edition. New York: McGraw-Hill. (1996).
37. Cha HY, Seo JJ, Park JH, Choi KJ, Hong JT, Oh JK. Anxiolytic Effects of total saponin fraction from Ginseng Radix Rubra on the elevated plus-maze model in mice. Ginseng Res; 28:132-135 (2004).
38. Jin SH, Jin KP, Nam KY, Park SN, Jung NP. Korean red ginseng saponins with low ratios of protopanaxadiol and protopanaxatriol saponins improve scopolamine induced learning disability and spatial working memory in mice. J Ethanopharmacol; 66:123-29 (1999).
39. Nalini K, Aroor A, Karanth K, Rao A. Effect of *Centella asiatica* fresh leaf aqueous extract on learning and memory and biogenic amine turnover in albino rats. Fitoterapia.; 63: 232-237 (1992).
40. Yamaguchi Y, Higashi M, Kobayashi H. Effect of ginsenosides on impaired performance caused by scopolamine in rats Er J Pharmacol; 312(2): 149-151(1996).

41. Beaubrun G, Gray G. A review of herbal medicines for psychiatric disorders. Psychiatr Serv.; 51: 1130-1134 (2000).
42. Mantle D, Pickerins AT, Perry EK. Medicinal plant extract for the treatment of dementia: a review of their pharmacology, efficacy and tolerability. CNS Drugs; 13: 201-13(2000).