Pharmacognosy Communications
Volume 1 | Issue 2 | Oct-Dec 2011
www.phcogcommn.org

Research Article

Procognitive Effects of Hexane Extracts of *Michelia Champaca* Leaves in Normal and Memory Deficit Mice

Hafsa Ahmad, Vasundhara Saxena, Anurag Mishra, Rajiv Gupta*, Shubhini A Saraf

Faculty of Pharmacy, Dept of Pharmacognosy BBDNITM, Lucknow, India

ABSTRACT: **Introduction:** *Michelia champaca* (Magnoliaceae), commonly known as Svarna champa is a glorious ancient Indian medicinal plant, rich in flavonoids and possessing several folkloric uses, traditionally claimed of having CNS effects. This study was undertaken to ascertain the nootropic potential (memory enhancing effects) of the hexane extracts of the leaves of *Michelia champaca* (Magnoliaceae) using rectangular maze and Y maze (interoceptive behavioral models). **Methods:** Hexane extracts of leaves of *Michelia champaca* (dosed at 100 and 200 mg/kg each) were administered to adult Swiss albino Wistar mice and the effect on acquisition, retention and retrieval of spatial recognition memory was determined, by using rectangular maze and Y maze (interoceptive behavioral models). *Bacopa monniera* extract was used as the standard drug while Scopolamine hydrobromide served as the amnestic agent. **Results:** The higher doses of the *Michelia champaca* extract exhibited a more promising nootropic potential. Maximal response was observed in the 200 mg/kg dose of extract, which closely approximated the results for the standard drug Brahmi. The higher dose elicited greater responses in both the models studied and were comparable to that achieved with the standard drug. **Conclusion:** The hexane extract of *Michelia champaca* afforded mild memory enhancing effects, the higher dose evoking pronounced alteration of behavior and better learning assessments.

KEYWORDS: *Michelia champaca*, memory enhancing, cognitive, HPTLC, Quercetin.

INTRODUCTION

According to the world health report (WHO 2001), approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receive even the most basic treatment. This amounts to 12.3% of the global burden of the disease and is expected to rise to 15% by 2020.[1] Drugs acting in the central nervous system were among the first to be discovered by primitive human and are still the most widely used group of pharmacological agents. The CNS altering drugs are invaluable therapeutically, because they can produce specific physiological and psychological effects from the vast array of therapeutic agents mentioned in Materia Medica. From the indigenous system so many plants have been reported to have activity against CNS disorders and thus act as very useful remedies for the alleviation of human suffering.[2] In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research worldwide has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models.[1]

Michelia champaca L. (Magnoliaceae) commonly known as Svarna champa, a tall handsome tree with yellow fragrant blossoms, is commonly used by many traditional healers in many of herbal preparations for kidney diseases and diabetes.[3,4] The plant is also reported to have significant wound healing,[5] antimicrobial,[6] antidiabetic,[7] antitumor,[8] anti-inflammatory,[9] antioxidant,[10] leishmanicidal[11] and anti-infective[12] properties. Traditionally it is used for its astringent, disinfectant, diuretic and cooling properties and in parasitic infections, dysuria and diseases due to vitiated blood.[4] It finds mention as one of the ingredients of the Sarvasugandhi group and is used in psychoneurosis by traditional healers. There is also mention of the different actions it has on CNS, however, these have not been validated scientifically.[4] Therefore, this report aims to validate these claims. After the assessment of the hexane extract of leaves by phytochemical tests, TLC and its standardization by HPTLC, an attempt was made to evaluate its learning and memory

*Correspondence:
E-mail: rajiv961@redillmail.com
DOI: 10.5530/pc.2011.2.6

potential on the interoceptive behavioral models (scopolamine induced amnesia producing transient memory impairment), using rectangular maze and Y maze.

MATERIALS AND METHODS

Experimental Animals

Adult Swiss albino Wistar mice of either sex, weighing between 25-30 g were procured from our animal house, at BBDNITM and were housed under standard environmental conditions (25 ± 1 °C, 55 ± 5% humidity and 12 h/12 h light/dark cycle). The animals were allowed free access to tap water and standard laboratory rat food (obtained from Pranav Agro Foods, New Delhi). The care and handling of mice were in accordance with the internationally accepted standard guidelines for use of animals, and the protocol was approved by our Institutional Animal Ethics Committee under the CPSCEA. (BBDNITM/IAEC/01/2011)

Drugs and Chemicals

Bacopa monniera extract (Brahmi, Himalaya Herbal Healthcare, Bangalore, India), Scopolamine hydrobromide (Sigma Aldrich, USA), Tween 80 (SD Fine Chemicals, Mumbai, India), Quercetin dihydrate (SD Fine Chemicals, Mumbai, India), Normal saline (used as vehicle for Scopolamine HBr)

Plant Material & Preparation of Extracts

The plant material was collected from the local area of Lucknow, Uttar Pradesh and was authenticated from National Botanical Research Institute, Lucknow. A plant specimen was identified as *Michelia champaca* L (by the Taxonomy Division at NBRI) and deposited (Ref. No. NBRI/CIF/176/2010) in the Lucknow herbarium. The leaves were shade dried at room temperature for about two weeks and were powdered finely for extraction. The dried powdered drug (leaves) 200 g was Soxhlet extracted for a 72 hour cycle with n-hexane and the yield was 10.34%.

HPTLC of Plant Extract

The hexane extract was subjected to phytochemical tests, which showed the presence of steroids, terpenoids, fats and flavonoids which were confirmed by TLC and HPTLC. Quantitative estimation of quercetin was attempted with the help of HPTLC system equipped with a sample applicator device Camag Linomat 5. Camag twin trough chamber, Camag TLC scanner and integration software (Wincats). Increasing serial dilutions of quercetin working standards (200-1000 µg mL^{-1}) along with the test extract were scanned at 366 nm (Mobile phase: Toluene: Ethyl acetate-Acetic acid- Methanol 2.5:7:0.25:0.25) to ascertain the amount of quercetin present in the test extract. The estimated value was found to be 682.235 µg mL^{-1} amounting to 68.223 mg/g in the drug sample. The Rf's of the standard and test sample were found to be 0.90, under the chromatographic conditions described above. A Typical HPTLC chromatogram of quercetin (standard) and that obtained for the test sample (leave) is shown in Fig 1.

Experimental Protocol

60 mice of either sex were trained on a rectangular maze (detailed description under the heading **Assessment of learning and memory using Hebb's William Maze (Rectangular Maze)** for assessment of learning and memory (Day1-Day5). Those with lower scores indicating better learning i.e. those which took lesser time to explore the maze were selected and randomly grouped. Eight groups of six mice each (housed according to sex, males together and females together) were used to evaluate their responses in the rectangular maze. The remaining mice were not taken for experimentation.

Groups	Treatments
1	Positive control; Vehicle; equivolume p.o.
2	Negative control; Scopolamine (amnestic agent); 0.4 mg/kg i.p, dissolved in normal saline.
3	Standard; *Bacopa monniera* extract (Himalaya Herbals); 40 mg/kg p.o., dissolved in double distilled water
4	Hexane extracts of *Michelia champaca*; 100 mg/kg p.o., dissolved in Tween 80
5	Hexane extracts of *Michelia champaca*; 200 mg/kg p.o., dissolved in Tween 80
6	*Bacopa monniera* extract (Himalaya Herbals); 40 mg/kg p.o., dissolved in double distilled water, followed by Scopolamine 0.4 mg/kg i.p, dissolved in normal saline.
7	Hexane extracts of *Michelia champaca*; 100 mg/kg p.o., dissolved in Tween 80 followed by Scopolamine 0.4 mg/kg i.p, dissolved in normal saline.
8	Hexane extracts of *Michelia champaca*; 200 mg/kg p.o., dissolved in Tween 80 followed by Scopolamine 0.4 mg/kg i.p, dissolved in normal saline.

The dosing commenced on day 6 for a period of 7 days and on the Day 13, amnesia was induced by administration of scopolamine (0.4 mg/kg i.p.) to Groups 2, 6, 7 and 8, and for Groups 3-5 trials were executed on the rectangular maze. The results for Groups 3-5 were duplicated on Day 14. The negative control group (group 2) received just one dose of scopolamine on Day 13. Forty five minutes after the administration of amnestic agent, trials were taken on rectangular maze and the retention was observed 24 hours after.

The same experimental protocol was followed on the same experimental animals after one month of rehabilization for assessment of learning and memory by Y-maze model.

Acute Toxicity Studies

The acute toxicity studies were performed in accordance with the OECD (Organization for Economic Co-operation and Development) guidelines no. 425 (Up and Down Procedure).[13] No death was observed till the end of the

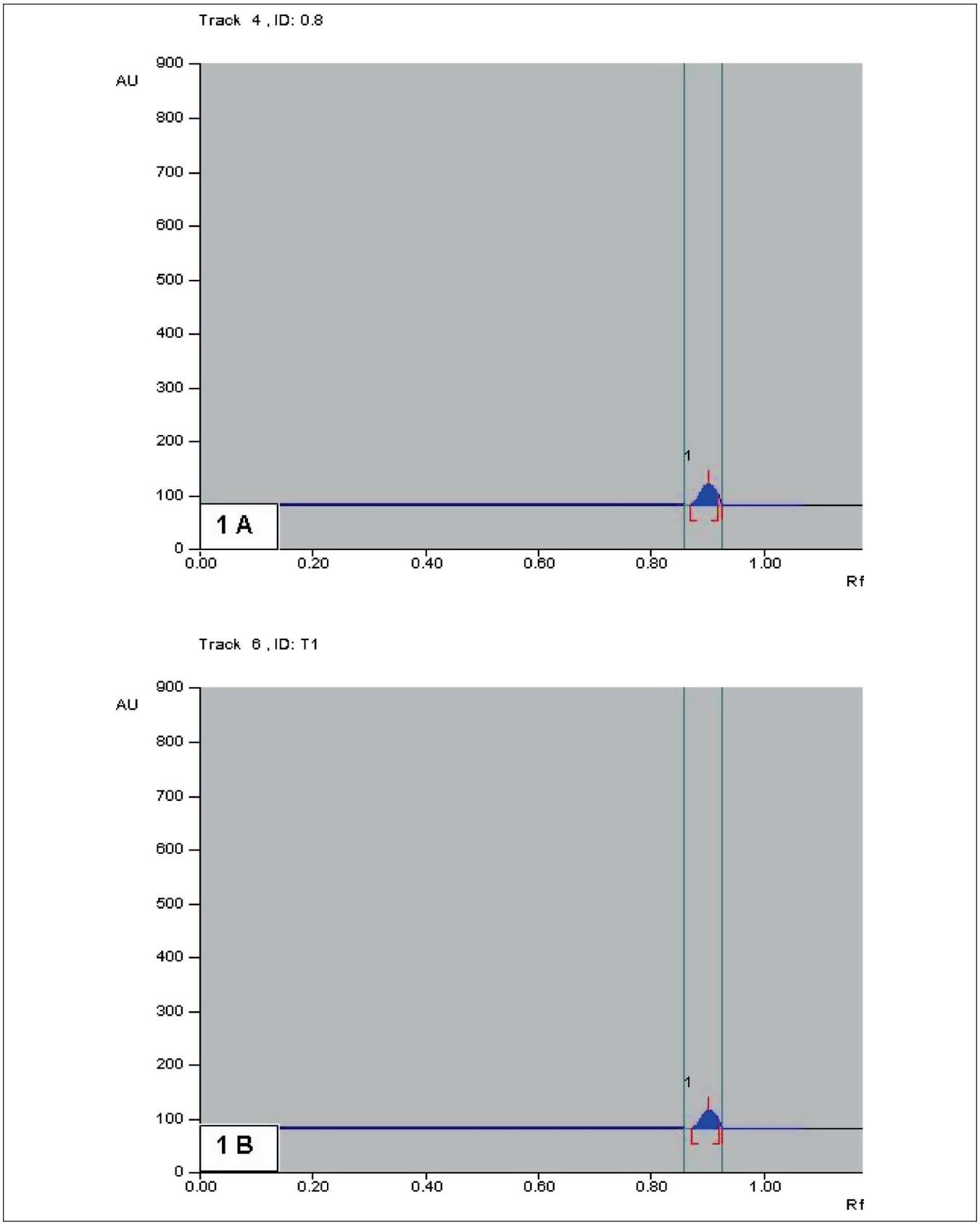

Figure 1: A Typical HPTLC chromatogram of quercetin (A) standard (B) in *Michelia champaca* L. leaves

study. The test samples were found safe up to the dose of 2000 mg/kg. 200 mg/kg was chosen as the maximum dose for further experimentation on mice in the present study.

Assessment of learning and memory using Hebb's William Maze (Rectangular Maze)

The Hebb William maze (Medicraft Rectangular Maze Model No. 511 ER) The maze consists of completely enclosed rectangular box with an entry (A) and reward chamber (B) appended at opposite ends. The box is partitioned with wooden slats into blind passages leaving just twisting corridor (C) leading from the entry (A) to the reward chamber (B).[14] The learning assessment for control and treated mice was conducted at end of treatment. On the first day, all the mice were familiarized with the Hebb William maze for a period of ten minutes. From the 2nd to 5th day the mice received four consecutive trials of training per day in the maze. In each trial the mice were placed in the entry chamber and the timer was activated as soon as the mice leave the chamber. The time taken by the mice to reach the award chamber was taken as the learning score of the trial. The average of four trials was taken as the learning score for the day. Lower scores of assessment indicate efficient learning while higher scores indicate poor learning in animals. During learning assessment the animals were exposed to food and water ad libitum only for 1 hour after the maze exposure for the day was completed to ensure motivation towards reward area (B).

Assessment of learning and memory using Y Maze Apparatus

The Y-maze is a simple two-trial recognition test for measuring spatial recognition memory. It does not require learning of a rule, and thus is useful for studying memory in rodents, and in particular for the study of genetic influences on the response to novelty and recognition processes. The Y-maze, made of wood, consists of three arms with an angle of 120° between each of the two arms. The arm dimensions are 8 cm × 30 cm × 15 cm (width × length × height). The three identical arms were randomly designated: start arm, in which the mice started to explore (A), novel arm (B, with food stimuli), and the other arm (C).[15] Mice tend to explore the maze systematically, entering each arm in turn. The ability to alternate requires that the mice know, which arm they have already visited. On the first day, all the mice were allowed to explore the Y maze apparatus for a period of ten minutes each. From the 2nd to 5th day the mice received four consecutive trials of training per day in the maze of 5 min duration. In each trial, the mice were placed in the entry chamber (A) and the series of arm entries in all the three arms, including possible return into the same arm was recorded visually. Alteration is defined as the number of successive entries into the three arms on overlapping triplet sets. The percentage of alteration is calculated as the total number of arm entries minus two, and multiplied by 100. Pretreatment with amnestic agent 30 min prior to trials induces a marked decrease in spontaneous alteration performance with a concomitant increase in the total number of arm entries. Administration of agents that possesses memory enhancing effects is expected to reverse the changes. During learning assessment the animals were exposed to food and water ad libitum only for 1 hour after the maze exposure for the day was completed to ensure motivation towards reward area (B).[16]

Statistical Analysis

All results were expressed as mean ± standard error of mean (S.E.M.). Data was analyzed using Wilcoxon signed rank test and one-way ANOVA followed by Dunnett's' test. $P < 0.05$ was considered as statistically significant.

RESULTS

The up and down procedure for the acute toxicity studies indicated a reasonably good safety potential for both the parts employed in the study i.e. leaves and stem bark and on this basis 200 mg/kg was chosen as the maximum dose for further experimentation on mice in the present study.

Assessment of learning and memory using Hebb's William Maze (Rectangular Maze)

The learning scores (time in seconds) obtained by each group as presented in Table I, are suggestive of the fact that the mice took lesser time on Day 14 than Day 13. The learning scores obtained by Group 1 (positive control) were higher than those afforded by Groups 3-5, indicating better and efficient learning in these groups compared to the positive control group. The learning scores observed for the standard *Bacopa monniera* (Group3) are indicative of a greater nootropic potential than the test drug. However *Michelia champaca* hexane extract dosed at 200 mg/kg (Group 5) afforded a close comparison with the standard Brahmi. Group 2 (Negative control group) showed an increase in learning score due to the memory deficit induced by scopolamine. In Groups 6-8, there was a significant decrease in learning scores on Day 13 and Day 14, thus elaborating the drugs responses to overcome the learning deficits produced by scopolamine. The higher dose of *Michelia champaca* hexane extract, in presence of amnesia (Group 8) afforded better learning scores as compared to the lower dose (Group 7) and closely approximated the learning scores obtained for the standard drug *Bacopa monniera* (Group 6).

Figure 2 presents a 2D clustered column display of learning scores obtained by Groups 3-5 in comparison to Group 1 and Groups 6-8 in comparison to Group 2.

On the basis of the data obtained the following order of nootropic potential can be traced.

Table 1: Learning Scores of mice on Day 13 (45 min post amnesia) and Day 14 (24 hours after amnesia)

Group	Treatment	Dose	Learning Scores (Time in seconds) Day 13	Learning Scores (Time in seconds) Day 14
1	Positive control; Vehicle	Equivolume p.o.	83.15 ± 0.035	78.12 ± 0.047
2	Negative control; Scopolamine (amnestic agent)	0.4 mg/kg i.p.	95.25 ± 0.471	127.75 ± 0.245
3	Standard; *Bacopa monniera* extract[a]	40 mg/kg p.o.	46.34 ± 0.361	43.61 ± 0.439
4	Hexane extracts of *Michelia champaca*[a]	100 mg/kg p.o.	74.50 ± 0.282	69.60 ± 0.412
5	Hexane extracts of *Michelia champaca*[a]	200 mg/kg p.o.	49.25 ± 0.257	48.0 ± 0.391
6	Standard; *Bacopa monniera* extract + Scopolamine[b]	40 mg/kg p.o., 0.4 mg/kg i.p.	77.25 ± 0.369	65.90 ± 0.513
7	Hexane extracts of *Michelia champaca* + Scopolamine[b]	100 mg/kg p.o, 0.4 mg/kg i.p.	85.67 ± 0.341	79.82 ± 0.418
8	Hexane extracts of *Michelia champaca* + Scopolamine[b]	200 mg/kg p.o, 0.4 mg/kg i.p.	80.40 ± 0.224	70.90 ± 0.345

Values represent mean ± SEM; n = 6; $p < 0.05$
[a]($P < 0.05$ as compared to positive control), [b]($P < 0.05$ as compared to negative control)

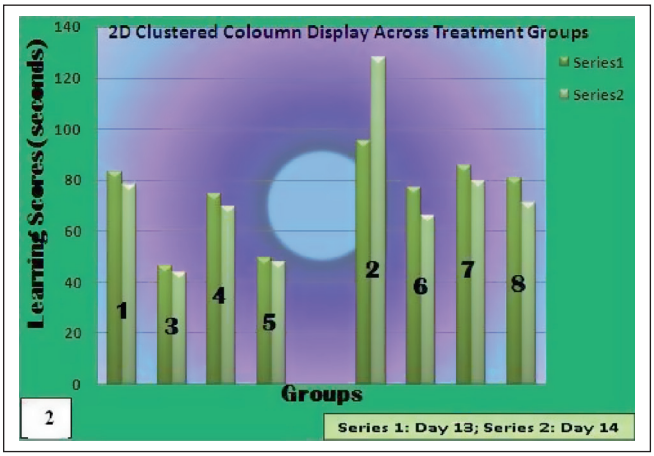

Figure 2: 2D clustered coloumn display of learning scores obtained by Groups 3-5 in comparison to Group 1(Positive control) and Groups 6-8 in comparison to Group 2(Negative control).

In normal mice

Bacopa monniera extract (40 mg/kg) > Hexane extracts of *Michelia champaca* (200 mg/kg) > Hexane extracts of *Michelia champaca* (100 mg/kg) > Vehicle; Positive control (equivolume)

In mice with an induced memory deficit

Bacopa monniera extract (40 mg/kg) + Scopolamine > Hexane extracts of *Michelia champaca* (200 mg/kg) + Scopolamine > Hexane extracts of *Michelia champaca* (100 mg/kg) + Scopolamine > Negative control; Scopolamine (0.4 mg/kg)

Assessment of learning and memory using Y Maze Model

Y maze model used in the present study proved to be a sensitive measure of spatial recognition memory. The effect on alteration behavior was studied on two parameters, % alteration (Table II a) and No. of arm entries (Table II b).

Effect on % alteration

Normally mice exhibit an alteration of around 60-70% (normal rodent behavior) as exhibited by the positive control group (Group 1). Groups 3-5 did not show much difference in alteration response in comparison to the positive control; Group 3 (*Bacopa monniera* extract) achieving a slightly higher alteration than the vehicle group (Group 1; positive control). The alteration showcased by Groups 1, 3-5 is indicative of the natural tendency of mice to exhibit an alteration of around 60-70% in a 5 min session, however the alteration achieved on the second trial (Day 14) was higher exhibiting higher % alteration (ability to alternate) on account of acquisition of memory. Group 2 exhibited a marked decrease in spontaneous alteration (a characteristic feature of amnestic agents), elaborating the amnestic effects of scopolamine. However in Groups 6-8 there was a significant increase in % alteration thus supporting their memory enhancing effects to reverse the effects of scopolamine. The greatest alteration was achieved by the standard *Bacopa monniera* extract; in presence of amnesia (Group 6) followed by the higher dose of *Michelia champaca* hexane extract in presence of amnesia (Group 8). It succeeded in closely approximating the alteration response of the standard *Bacopa monniera*. Assuming generalization % alteration on Day 14 was found to be greater than that observed on Day 13, thus elucidating the retention characteristics of the purported nootropics. A 3D stacked view of % alteration on the two different trials across all treatment groups (against control; and in induced amnesia condition) is demonstrated in Figure 3. The purported order of activity as evident from % alteration in the memory deficit mice is as follows:

Bacopa monniera extract (40 mg/kg) > Hexane extracts of *Michelia champaca* (200 mg/kg) > Hexane extracts of *Michelia champaca* (100 mg/kg) > Negative control; Scopolamine (0.4 mg/kg)

Table 2: Effect on Alteration Behavior in Y Maze in mice (a) % Alteration Response (b) No. of arm entries

Table 2(a)

Group	Treatment	Dose	% Alteration on Day 13	% Alteration on Day 14
1	Positive control; Vehicle	Equivolume p.o.	67.50 ± 0.072	71.25 ± 0.087
2	Negative control; Scopolamine (amnestic agent)	0.4 mg/kg i.p.	47.38 ± 0.594	53.33 ± 0.259
3	Standard; *Bacopa monniera* extract[a]	40 mg/kg p.o.	68.33 ± 0.594	71.49 ± 0.389
4	Hexane extracts of *Michelia champaca*[a]	100 mg/kg p.o.	66.42 ± 0.319	70.34 ± 0.259
5	Hexane extracts of *Michelia champaca*[a]	200 mg/kg p.o.	64.56 ± 0.239	65.23 ± 0.393
6	Standard; *Bacopa monniera* extract + Scopolamine[b]	40 mg/kg p.o., 0.4 mg/kg i.p.	63.33 ± 0.319	84.52 ± 0.296
7	Hexane extracts of *Michelia champaca* + Scopolamine[b]	100 mg/kg p.o, 0.4 mg/kg i.p.	55.33 ± 0.346	59.63 ± 0.247
8	Hexane extracts of *Michelia champaca* + Scopolamine[b]	200 mg/kg p.o, 0.4 mg/kg i.p.	62.50 ± 0.239	66.66 ± 0.393

Values represent mean ± SEM; n = 6; $p < 0.05$
[a]($P < 0.05$ as compared to positive control), [b]($P < 0.05$ as compared to negative control)

Table 2(b)

Group	Treatment	Dose	No. of arm entries on Day 13	No. of arm entries on Day 14
1	Positive control; Vehicle	Equivolume p.o.	25.0 ± 0.035	20.0 ± 0.069
2	Negative control; Scopolamine (amnestic agent)	0.4 mg/kg i.p.	33.50 ± 0.119	26.5 ± 0.257
3	Standard; *Bacopa monniera* extract[a]	40 mg/kg p.o.	13.50 ± 0.218	8.0 ± 0.239
4	Hexane extracts of *Michelia champaca*[a]	100 mg/kg p.o.	22.50 ± 0.716	15.0 ± 0.427
5	Hexane extracts of *Michelia champaca*[a]	200 mg/kg p.o.	16.50 ± 0.254	12.50 ± 0.294
6	Standard; *Bacopa monniera* extract + Scopolamine[b]	40 mg/kg p.o., 0.4 mg/kg i.p.	18.0 ± 0.276	14.50 ± 0.313
7	Hexane extracts of *Michelia champaca* + Scopolamine[b]	100 mg/kg p.o, 0.4 mg/kg i.p.	23.5 ± 0.365	19.0 ± 0.382
8	Hexane extracts of *Michelia champaca* + Scopolamine[b]	200 mg/kg p.o, 0.4 mg/kg i.p.	20.5 ± 0.115	17.0 ± 0.260

Values represent mean ± SEM; n = 6; $p < 0.05$
[a]($P < 0.05$ as compared to positive control), [b]($P < 0.05$ as compared to negative control)

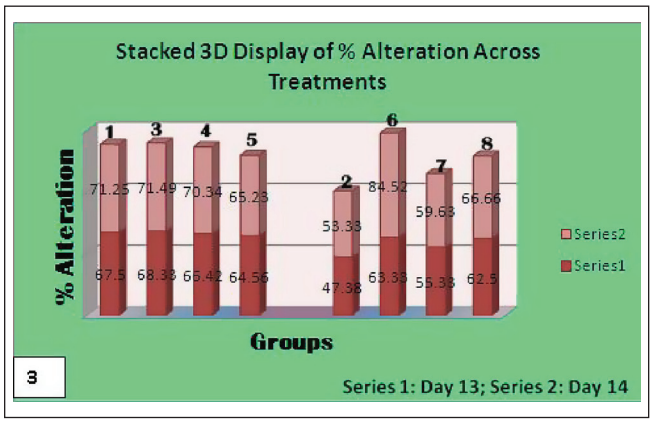

Figure 3: 3D stacked view of % alteration on the two different trials across all treatment groups (against control; and in induced amnesia condition)

Effect on Number of arm entries

Normally mice tend to make 20-25 entries in a five minutes trial on the Y maze, as exhibited by the positive control group (Table IIb Group 1). Treatment groups 3-5 afforded an increased number of arm entries compared to the positive control (Group 1), elucidating their effects on spatial recognition; the highest recognition being achieved by *Bacopa monniera* extract (Group 2) i.e. showcasing the minimum no. of arm entries, followed by the high dose and then the low dose of *Michelia champaca* hexane extracts. There is a significant increase in the number of arm entries in the presence of amnesia (characteristic of amnestic agents). This behavior was evident in Group 2 because of the amnesic effects of scopolamine, resulting in transient memory impairment. However, the presence of memory enhancing effects in a drug tends to reverse the effects brought about by an amnestic agent, as exhibited by Groups 6-8. There was a dramatic decrease in the number of arm entries, the maximum being showcased by *Bacopa monniera* extract in presence of amnesia (Group 6), which is a proven nootropic. The higher dose of the test drug afforded close proximity to the results observed for the standard Brahmi (Group 8). Assuming generalization the number of arm entries on Day 14 was found to be lesser than that recorded on Day 13 in all groups thus serving as an index of their transfer latencies, acquisition and retrieval of spatial recognition memory. A stacked cone view of the number of arm entries made by all treatment groups on two different trial occasions (Day 13 and day 14) are demonstrated in Figure 4.

The purported order of activity as evident from no. of arm entries is as follows:

In normal mice

Bacopa monniera extract (40 mg/kg) > Hexane extracts of *Michelia champaca* (200 mg/kg) > Hexane extracts of *Michelia champaca* (100 mg/kg) > Vehicle; Positive control (equivolume)

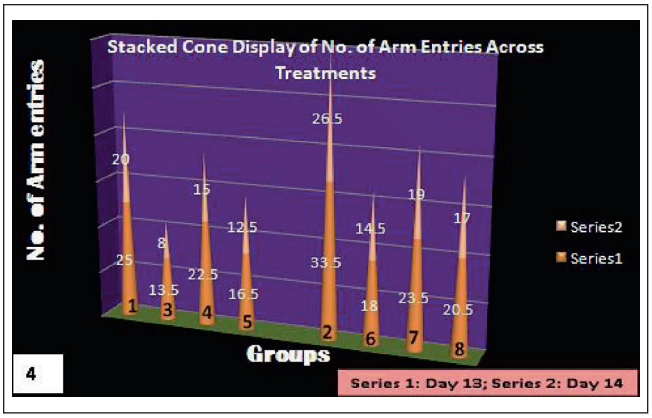

Figure 4: Stacked cone view of the no. of arm entries made by all treatment groups on two different trial occasions (Day13 and Day 14).

In mice with an induced memory deficit

Bacopa monniera extract (40 mg/kg) + Scopolamine > Hexane extracts of *Michelia champaca* (200 mg/kg) + Scopolamine > Hexane extracts of *Michelia champaca* (100 mg/kg) + Scopolamine > Negative control; Scopolamine (0.4 mg/kg)

DISCUSSION

The findings from the present study are suggestive of the fact that results obtained for the memory enhancing effects of *Michelia champaca* hexane extracts and also the standard nootropic agent i.e. *Bacopa monniera* draw similar results through two different interoceptive models having different parameters and methods of evaluation, thus providing enough scientific promise to validate the claims on their nootropic potentials. The learning scores in the rectangular maze and the percent alteration and arm entries criterion in the Y-maze models present similar results. *Bacopa monniera* the standard nootropic gave the most prominent results in both models, followed by *Michelia champaca* hexane extracts dosed at 200 mg/kg. The study reveals a dose dependant effect, the higher dose of the test drug being able to produce better results and even giving a closer comparison with the standard. Treatment groups 6-8 were efficient to overcome the learning deficits created by scopolamine induced amnesia; presenting efficient learning responses than the negative control (Group 2) and treatment groups 3-5 elaborated better responses of learning acquisition, retention and retrieval as compared to the positive control (Group 1) The cumulative order of activity based on both the models is as follows: *Bacopa monniera* extract (40 mg/kg) > Hexane extracts of *Michelia champaca* (200 mg/kg) > Hexane extracts of *Michelia champaca* (100 mg/kg). A literature review as well as the chromatographic studies (HPTLC) on present research work exhibits the presence of quercetin, which may be the bioactive moiety responsible for the C.N.S. effect. The authors also perceive that since plant is exhibiting scientifically justified promising nootropic activity, renewed aggressive stress is to be given on its cultivation as it is one of the exotic reserved species.

ACKNOWLEDGEMENT

The author's are thankful to the All India Council for Technical Education (AICTE) for providing the necessary grants to carry out the present research work. [AICTE-MODROBS Grant (F. No. 8024/RID/BOR/MOD-458/2009-10)]

REFERENCES

1. Ruiz MH, Beltren YG, Mora S, Gabriela D, Glauce SB. Tortoriella J, et al. Antidepressant and anxiolytic effect of hydroalcoholic extract from *Salvia elegans*. J. Ethnopharmacol. 2006; 107(1):53-8.
2. Suba V, Murugesan, Rao RB, Pal M, Mandal SC, Saha B. Neuropharmacological profile
3. of *Barleria lupulina* lindle extract in animal model. J. Ethnopharmacol. 2002; 81:251- 55.
4. Nadkarni K.M., Nadkarni A. K. Indian materia medica. Mumbai, India: Popular Prakashan 1995.
5. Kritikar KR, Basu BD. Indian medicinal plants. Dehradun, India: Oriental enterprises; 2001.
6. Dwajani S, Shanbhag TV. *Michelia champaca*: Wound Healing Activity in Immunosuppressed Rats. The Internet Journal of Alternative Medicine. 2009; 7(2):1540-1545.
7. Khan MR, Kihara M, Omoloso AD. Antimicrobial activity of *Michelia champaca*. Fitoterapia. 2002; 73(7-8):744-48.
8. Jarald EE, Joshi SB, Jain DC. Antidiabetic activity of flower buds of *Michelia champaca* Linn. Indian Journal of Pharmacology 2008; 40(6):256-60.
9. Hoffmann JJ, Torrance SJ, Wiedhopf RM, Cole JR. Cytotoxic Agents from *Michelia champaca* and *Talauma ovata*: Parthenolide and Costunolide. J Pharm Sci. 1977; 66:883-84.
10. Vimala R, Nagarajan S, Alam M, Susan T, Joy S. Antiinflammatory and antipyretic activity of *Michelia champaca* Linn. (White variety), *Ixora brachiata Roxb*. and *Rhynchosia cana* (Wild.) D.C. flower extract. Indian Journal of Experimental Biology. 1997; 35(12):1310-14.
11. Hasan SMR, Hossain MM, Akter R, Jamila M. DPPH free radical scavenging activity of some Bangladeshi medicinal plants. Journal of Medicinal Plants Research. 2009; 3(11):875-79.
12. Takahashi M, Fuchino H, Satake M, Agatsuma Y. *In Vitro* Screening of Leishmanicidal Activity in Myanmar Timber Extracts. Biological and Pharmaceutical Bulletin. 2004; 27(6921):921-25.
13. Oumadevi R, Guy R, Francisco ER, Kiban C. Screening for anti-infective properties of several medicinal plants of the Mauritians flora. J Ethnopharmacol. 2007; 109(2):331-37.
14. W.J. Dixon. "The up and down methods for small animals". J. Amer Statist Assoc. 1965; 60:967-978.
15. Agarwal A, Malini S, Bairy KL, MS. Effect of *Tinospora cordifolia* on learning and
16. memory in normal and memory deficit rats. Indian J. Pharmacol. 2002; 34:339-49.
17. Ma MX, Chen YM, He J, Zeng T, Wang JH. Effects of morphine and its withdrawal on y-maze spatial recognition memory in mice. Neuroscience. 2007; 147:1059-65.
18. Kulkarni SK, Handbook of experimental pharmacology. Delhi, India: Vallabh Prakashan; 2007.