Research Article

MEMORY ENHANCING EFFECTS OF *FICUS CARICA* LEAVES IN HEXANE EXTRACT ON INTEROCEPTIVE BEHAVIORAL MODELS.

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ABSTRACT

Objective: *Ficus carica* Linn. (Moraceae) commonly known as the fig plant, a native of Carica in Asia is grown in nearly all tropical and sub-tropical countries. Phytochemical screening of the hexane extract confirmed the presence of flavonoids (quercetin) which have been associated with C.N.S. effects and also claimed to be the responsible C.N.S. active moiety, in recent studies. Hence it was thought worthy to investigate the cognitive effects of hexane extract of *Ficus carica* leaves, in normal and memory deficit mice using Y-maze and rectangular maze models (interoceptive behavioral models).

Method: Hexane extracts of leaves of Ficus carica (dosed at 100 and 200mg/kg each) were administered to adult Swiss albino Wistar mice and the acquisition, retention and retrieval of spatial recognition memory was determined, by using Y-maze and rectangular maze models (interoceptive behavioral models). *Bacopa monniera* extract was used as the standard drug while scopolamine hydrobromide as the amnestic agent.

Results: The higher doses of the plant extract, exhibited a more promising nootropic potential. Maximum response was observed in the 200mg/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 where comparable to that achieved with the standard drug.

Conclusion: The hexane extract of Ficus carica afforded mild memory enhancing effects, the higher dose evoking pronounced alteration of behavior and better learning assessments.

Keywords: *Ficus carica*, Anjeer, cognition, H.P.T.L.C., Rectangular maze

INTRODUCTION

Nootropic agents such as piracetam [1] pramiracetam, aniracetam [2] and choline esterase inhibitors like donepezil are being primarily used to improve memory. However, the resulting adverse effects associated with these agents have limited their use [3] and it is worthwhile to explore the utility of traditional medicines in the treatment of various cognitive disorders. *Ficus carica* Linn. (Moraceae) commonly known as the fig plant, a native of Carica in Asia is grown in nearly all tropical and sub-tropical countries. It is now cultivated chiefly in the Mediterranean region, from Turkey in the east to Spain and Portugal in the west; it is also grown commercially in parts of U.S.A. and Chile and, to a small extent, in India, Arabia china and Japan.[4] .The plant has several folkloric uses in many traditional system of medicines like mild laxative, expectorant, diuretic, good nutritional support for diabetics, aphrodisiac tonic and used in checking haemorrhage.[5,6,7] In search for new medicinals, beneficial in improving medical conditions related to dementia and other C.N.S. disorders, herbals provide a safe option hence it was thought worthy to investigate the cognitive effects of hexane extract of *Ficus carica* leaves, in normal and memory deficit mice using Y-maze and rectangular maze models. Phytochemical screening of the extract confirmed the presence of flavonoids (quercetin) which have been associated with C.N.S. effects and also claimed to be the responsible C.N.S. active moiety, in recent studies.[8,9,10,11,12]

MATERIALS AND METHODS

Experimental Animals: Adult Swiss albino Wistar mice of either sex weighing between 25-30 g procured from our animal house 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. 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. (BBDGEI/IAEC/04/2011)

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 dehydrate (SD Fine Chemicals, Mumbai, India), Normal saline (used as vehicle for Scopolamine HBr)

Plant Material & Preparation of Extracts: The plant material was collected from local areas of Lucknow, Uttar Pradesh and was authenticated from National Botanical Research Institute, Lucknow by depositing a herbarium (Voucher specimen Ref No. NBRI/CIF/178/2010) and was identified as *Ficus carica* L. 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 yields was 15.28%.

Standardization 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. Chromatography of flavonoids was carried out using Chloroform 100% as mobile phase and 8 bands were observed in UV (365nm) wavelength. And also compared with standard quercetin and was matched with the test drug. 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 250.95μg/ml.

Experimental Protocol: 50 mice of either sex were trained on

rectangular maze for assessment of learning and memory (Day1-Day5). Those with lower scores were selected and randomly grouped. Five groups of six mice each were used to evaluate their responses on rectangular maze.

Group-1: Positive control; Vehicle; equivolume p.o.

Group-2: Negative control; Scopolamine (amnestic agent); 0.4 mg/kg i.p, [13] dissolved in normal saline.

Group-3: Standard; *Bacopa monniera* extract (Himalaya Herbals); 40 mg/kg p.o., [14] dissolved in double distilled water.

Group-4: Hexane extracts of *Ficus carica*; 100 mg/kg p.o., dissolved in Tween 80

Group-5: Hexane extracts of *Ficus carica*; 200 mg/kg p.o., dissolved in Tween 80

Group-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.

Group-7: Hexane extracts of *Ficus carica*; 100 mg/kg p.o., dissolved in Tween 80 followed by Scopolamine 0.4 mg/kg i.p, dissolved in normal saline.

Group-8: Hexane extracts of *Ficus carica*; 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 itself. 45 mins 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 rehabilitation 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). [15] No death was observed till the end of the study. The test samples were found safe upto the dose of 2000mg/kg and from the results 500 mg/kg was chosen as the maximum dose for further experimentation. [15]

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). [16]

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.

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 x 30 cm x 15 cm (width x length x 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). [17]

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 8 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). [18]

Statistical Analysis

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

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.	83.125± 0.035	78.125± 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 *Ficus carica* [a]	100 mg/kg p.o.	62.5±0.282	59.6±0.412
5	Hexane extracts of *Ficus carica* [a]	200 mg/kg p.o.	51.25±0.257	49.0±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 *Ficus carica* + Scopolamine [b]	100 mg/kg p.o, 0.4 mg/kg i.p.	91.20±0.415	127.25±0.368
8	Hexane extracts of *Ficus carica* + Scopolamine [b]	200 mg/kg p.o, 0.4 mg/kg i.p.	89.4±0.287	76.66±0.219

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: 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 *Ficus carica* [a]	100 mg/kg p.o.	63.42±0.277	69.14±0.260
5	Hexane extracts of *Ficus carica* [a]	200 mg/kg p.o.	62.56±0.371	65.43±0.313
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 *Ficus carica* + Scopolamine [b]	100 mg/kg p.o, 0.4 mg/kg i.p.	57.33±0.346	59.63±0.247
8	Hexane extracts of *Ficus carica* + Scopolamine [b]	200 mg/kg p.o, 0.4 mg/kg i.p.	60.50±0.227	66.66±0.343

Values represent mean ± SEM; n= 6; p < 0.05a (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 *Ficus carica* [a]	100 mg/kg p.o.	24.50±0.757	18.0±0.719
5	Hexane extracts of *Ficus carica* [a]	200 mg/kg p.o.	17.50±0.266	13.50±0.271
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 *Ficus carica* + Scopolamine [b]	100 mg/kg p.o, 0.4 mg/kg i.p.	25.5±0.457	19.5±0.485
8	Hexane extracts of *Ficus carica* + Scopolamine [b]	200 mg/kg p.o, 0.4 mg/kg i.p.	21.5±0.365	15.0±0.439

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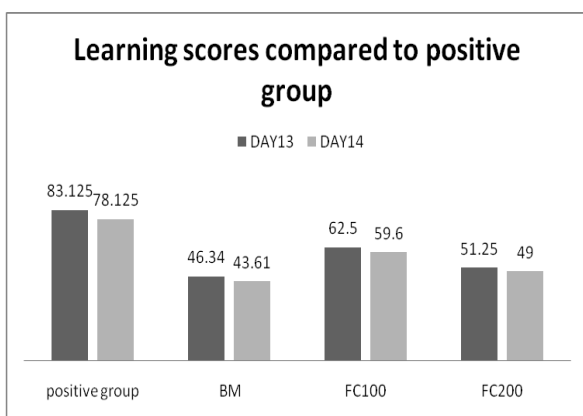

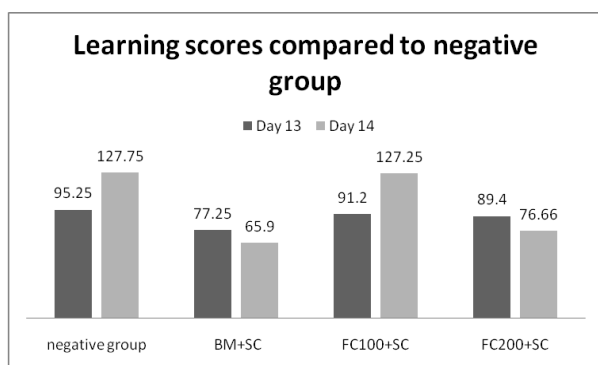**Fig. 1: Learning scores compared to positive group**

Fig. 3: Effect on % Alteration Response

Fig. 2: Learning scores compared to negative group.
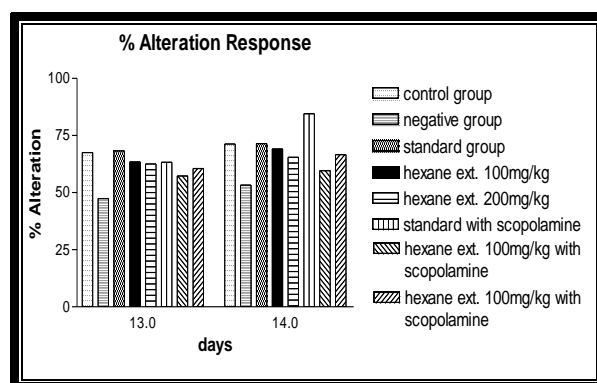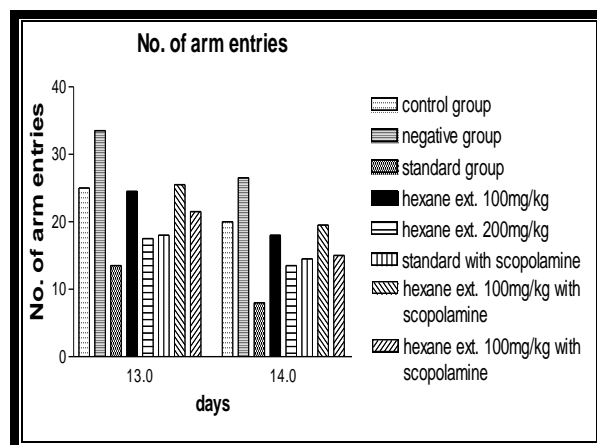

Fig. 4: Effect on No. of arm entries

RESULTS

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 1, 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 them, as compared to the positive control (fig 1). The learning scores observed for the standard Bacopa monniera (Group3) are indicative of a greater nootropic potential than the test drug, however Ficus carica 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, however 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(fig. 2). The higher dose of Ficus carica 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).

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 2 a) and No. of arm entries (Table 2 b).

Effect on % alteration: Normally mice exhibit an alteration of around 60-70% 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 elaborating 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 Ficus carica 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 (fig.3).

Effect on No. 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 (Group 1). Treatment groups 3-5 afforded an increased no. of arm entries; as 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 Ficus carica hexane extracts. There is a significant increase in the no. of arm entries in presence of amnesia (characteristic of amnestic agents). This behavior was well evident in Group 2 because of the amnesic effects of scopolamine, resulting in transient memory impairment. However 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 no. 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). (fig.4). Assuming generalization the no.

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.

DISCUSSION

The finding from the present study are suggestive of the fact that results obtained for the memory enhancing effects of Ficus carica 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 percentage alteration and arm entries criterion in the Y-maze models presents similar results.

Bacopa monniera the standard nootropic gave the most prominent results in both the models, followed by Ficus carica hexane extract dosed at 200mg/kg. The study reveals a dose dependent 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 response 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 (40mg/kg)> Ficus carica hexane extract (200mg/kg)> Ficus carica hexane extract (100mg/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 as stated in some of the recent studies.

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