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Research Paper

Nootropic activity of lipid-based extract of *Bacopa monniera* Linn. compared with traditional preparation and extracts

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Abstract

Objectives The aim was to design an alternative solvent-free extraction method using the hydrophilic lipid Gelucire (polyethylene glycol glycerides) for herbal extraction and to confirm the efficacy of extraction using biological screening.

Methods *Bacopa monniera* Linn. (BM) was selected for the study. Conventional methanolic extract (MEBM), Ayurvedic ghrita (AGBM) and lipid extracts (LEBM) were prepared and standardised by high-performance thin-layer chromatography (HPTLC). Nootropic activity in rats was evaluated using the two-trial Y-maze test and the anterograde amnesia induced by scopolamine (1 mg/kg i.p.) determined by the conditioned avoidance response. The extracts were administered daily at doses of 100, 200 and 400 mg/kg orally. At the end of the conditioned avoidance response test, brain monoamine levels were estimated by HPLC.

Key findings The LEBM, MEBM and AGBM contained 3.56%, 4.10% and 0.005% bacoside A, respectively. Significantly greater spatial recognition was observed with LEBM ($P < 0.001$ at 400 and 200 mg/kg) and MEBM ($P < 0.001$ at 400 mg/kg, $P < 0.01$ at 200 mg/kg) than AGBM. The conditioned avoidance response was significantly higher in the groups treated with high doses of LEBM and MEBM than AGBM. There were significant decreases in brain noradrenaline ($P < 0.001$) and 5-hydroxytryptamine ($P < 0.01$) levels and an increase in dopamine levels ($P < 0.05$) in the LEBM-treated groups compared with the stress control group.

Conclusions The proposed LEBM is solvent free, does not have the shortcomings associated with conventional extraction, and had comparable nootropic activity to the MEBM.

Keywords *Bacopa monniera* Linn.; Gelucire; memory; ghrita; nootropic activity

Introduction

Plant use for medicinal purposes is probably as old as the history of mankind. Extraction and characterisation of several active phytoconstituents from these green factories has given birth to some high activity profile drugs. Extraction forms the first basic step in medicinal plant research. Different extraction methods such as percolation, infusion, maceration and successive solvent extraction are in practice but the utility of these conventional methods is limited by their shortcomings. In conventional extraction, the repeated solvent extraction leads to crude complex products that have to be purified by multiple-step techniques such as chromatography and crystallisation to enrich the active constituents. Also, traces of residual solvents, sticky extracts, difficulties in handling the large volumes of inflammable organic solvents, time-consuming processes and high cost of the solvents limit the use of conventional extracts. There is therefore a need for alternative methods of extraction that overcome these shortcomings.

Dementia is a mental disorder characterised by loss of intellectual ability sufficiently severe to interfere with occupational and social activities. Since the allopathic system of medicine has yet to provide a radical cure, there is a continuous search for herbal remedies. Herbs such as *Ginkgo biloba*, *Glycyrrhiza glabra* and *Bacopa monniera* Linn. (BM) have been used for memory-enhancement in Indian traditional medicine for centuries.[1] Brahmi or BM has been extensively reported in the Ayurveda-Charaka Samhita (compilation of

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Charaka around 6th century AD) as a medicinal plant that rejuvenates intellect and memory.[2] BM (family: Schopulariaceae) has been reported to have pharmacological effects *in vivo* such as sedative,[3] tranquilising,[4] anti-inflammatory[5] and memory-enhancing[6] activities and has been used as a nerve tonic.[7] *In vitro* it has antioxidant activity[8] and activity against *Helicobacter pylori*.[9] The alcoholic extract of BM has been extensively investigated for anti-amnesic activities.[10,11] It improves the performance of rats in various learning situations[2,12] and reverses the anterograde amnesia induced by scopolamine and sodium nitrite in mice.[13] The memory-enhancing effects have been attributed to the active constituent saponins, bacosides A and B.[14–17]

From the available literature it is evident that most studies have used either methanol or ethanol as solvents for extraction of water-insoluble bacosides. In Ayurvedic practice, typical traditional procedures are used for the preparation of herbal formulations. Among the Ayurvedic formulations, ghrita is a preparation in which ghee is boiled with the prescribed kashaya (decoction) or svarasa (fresh juice) and kalka (paste of crude plant powder in water) of a drug according to the Ayurvedic formula.[18] This process ensures absorption of the therapeutic principles from the plant materials. BM is available in the form of Brahmi ghrita, which is commercially used as memory enhancer. Ghee, which is purely lipophilic in nature, is used as the main extracting medium in ghrita. However, ghee is 100% fat and may increase the prevalence of coronary artery disease.[19–21] Although the ghrita claims various medicinal uses and is free from solvents, there is no reason to believe this potential benefit outweighs the increased risk of cardiovascular disease. So it would be beneficial if BM could be extracted using an alternative method that is free of the shortcomings of the conventional extracts and ayurvedic ghrita.

Various techniques to improve the solubility of poorly water-soluble drugs are used, such as micronisation, formation of amorphous drugs and use of lipid-based formulations.[22] Among these new types of excipients is Gelucire: polyethylene glycol glycerides composed of mono-, di- and triglycerides and mono- and diesters of polyethylene glycol.[23] These glycerides are available with a range of properties depending on their hydrophilic–lipophilic balance over the range 1–18 and melting points between 33 and 77ºC.[24,25] Gelucire is used as a solubility enhancer,[26] and the hydrophilic properties of Gelucire are useful in controlled-release formulations.[27] Recent work in our laboratory has shown that Gelucire solid dispersions enhance the dissolution rate of poorly water-soluble synthetic drugs.[22]

To the best of our knowledge, the use of Gelucire in herbal extraction has not yet been established. This inspired us to achieve an effective method of extraction for BM using Gelucire. We have confirmed the efficacy of extraction by biological screening for nootropic activity. The proposed lipid-based extract of BM (LEBM) was compared with conventional extracts and Ayurvedic ghrita of BM (AGBM) for bacoside A content and nootropic activity.

Materials and Methods

Plant material

Plant materials of BM were collected from Pune, India in September to November. The samples were authenticated by Dr P.G. Diwakar, Joint Director, Botanical Survey of India (BSI), Pune, India and a voucher specimen (voucher no. SLBM1) kept in the departmental herbarium of BSI.

Standard bacoside A was a generous gift from Laila Impex R&D Centre, a division of Neutraceuticals, Vijyawada, Andhrapradesh, India. All other chemicals used were of analytical grade. Different grades of Gelucire (39/10, 44/14, 50/13 and 43/01) were generous gifts from Gattefosse (Saint-Priest, France) and were supplied by Colorcon Asia Pvt Ltd, Mumbai, India. Ayurvedic brahmi ghrita was a kind gift from the Department of Rasasala, Ayurvedic College, Bharati Vidyapeeth University (BVU), Pune.

Animals

Male Wistar rats (150–200 g) were procured from Yash Farm (Pune, India). Animals were housed six per cage, with access to water and food *ad libitum*, and were maintained under a constant temperature (23 ± 1ºC) and humidity (60 ± 10%) and a 12 h light–dark cycle (lights on 07:30–19:30 h). All the behavioural tests were performed between 9:00 and 17:00 h in a semi-soundproof laboratory.

All procedures were reviewed and approved by the Institutional Animal Ethical Committee. Animal treatment and maintenance were carried out in accordance with the principle of CPSCEA guidelines.

Preparation of extracts

Methanolic extract

The whole plant was washed thoroughly, shade dried for 15 days and pulverised into coarse powder; 500 g of this powder was subjected to cold maceration with methanol for 10 days. The methanolic extract (MEBM) was then obtained after filtration and concentration under vacuum in a rotary evaporator and refrigerated until further use.

Lipid extract

The fine-powdered plant material (1 g) was extracted with different grades of Gelucire (39/10, 44/14, 50/13 and 43/01) with drug and lipid in the ratios of 1 : 0.25, 1 : 0.5, 1 : 0.75, 1 : 1, 1 : 1.25, 1 : 1.5, and 1 : 2. The lipids were melted at their respective melting temperature. To this the drug powder was added, mixed then cooled. Water (10 ml) was added to disperse the lipid and filtered through filter paper. The filtrate was used for the study. Selection and optimisation of the grade of lipids and the proportion of drug and lipid was done using high-performance thin-layer chromatography (HPTLC) analysis of the extracts on the basis of bacoside A content.

Ayurvedic ghrita extract

The formulation was prepared by an expert Ayurvedic practitioner of Ayurvedic College, BVU, Pune. The fresh whole-plant material was washed and squeezed in a muslin cloth to get the juice called svarasa. This svarasa and kalka (paste of the crude plant in water) were boiled with cow ghee

(ghee : svarasa : kalka 4 : 16 : 1) as per the Ayurvedic formula until the water was completely evaporated. The ghee was then stripped off and used.

Standardisation of extracts

The MEBM, LEBM and AGBM were standardised for content of the active constituent bacoside A using HPTLC.[28] In addition to this, an aqueous extract (AEBM) and ghee extract (GEBM) of BM were also prepared and standardised. A Camag HPTLC system (Camag 100 μl sample syringe; Hamilton, Bonaduz, Switzerland) and Camag Linomat IV sample applicator were used. The mobile phase was ethyl acetate : methanol : water (60 : 14 : 10 v/v/v). Densitometric scanning was performed after derivatisation with a mixture of vannilin (1 g), sulfuric acid (5 ml) and ethanol (95 ml), using a Camag TLC scanner III in the reflectance-absorbance mode at 615 nm and operated by CATS software (V 3.15, Camag).

On the basis of results, the lipid extract was optimised and the LEBM selected as the lipid extract for use in further pharmacological studies.

Experimental design

The animals were divided into 12 groups of six animals. Groups I–III were given MEBM suspended in 1% gum acacia in water at doses of 100, 200 and 400 mg/kg p.o. Groups IV–VI were given LEBM at doses of 100, 200 and 400 mg/kg p.o. Groups VII–IX were given AGBM suspended in 1% gum acacia in water at doses of 100, 200 and 400 mg/kg p.o. Group X was the vehicle control group and was given vehicle only. Groups XI and XII served as the stress-control (subjected to conditioned avoidance response (CAR)) and non-stress control (not subjected to CAR) respectively, for estimation of brain monoamines. The day after achieving 95–100% CAR, groups I–X were given scopolamine butyl bromide (1 mg/kg i.p.) before continuing with daily dosing of the BM extracts.

Acute toxicity test

Acute toxicity studies were carried out for MEBM, LEBM and AGBM following OECD guidelines.[29] Overnight fasted healthy mice (n = 3) were given the extracts orally at doses up to 2000 mg/kg and observed continuously for 4 h and 24 h for mortality.

Two-trial Y-maze test

A two-trial Y-maze test was used to study recognition processes in rats.[30] The automatic Y maze (Techno, Lucknow, India) was used for the study. This was a horizontal maze 40 cm long and 10 cm wide, with walls 12 cm high with three arms. The floor and walls of the maze were constructed from dark opaque polyvinyl plastic. Visual cues were located outside the maze. The floor of the maze was covered with soiled animal bedding that was mixed between trials to reduce the utility of odour as a cue. The three arms of the maze were designated as the start arm, the other arm and the novel arm.

The Y-maze test consists of two trials separated by an inter-trial interval to assess spatial memory. Training was conducted before administration of the test samples. For training, rats were placed inside the start arm and the novel arm was blocked with a block. Rats were allowed to explore the maze for 15 min. At the end of 15 min the animals were given the relevant treatment. The test trials were conducted 2 and 4 h after the training. For these, the block was removed and rats were placed in the start arm and were allowed free access to all three arms for 5 min. The number (index of locomotor activity) and duration (index of spatial recognition) of visits in each arm were recorded. The percentage time spent and number of entries in each arm were analysed to measure spatial recognition.[31] All readings were taken from the automatic digital display of the maze by an observer blinded to the treatment. The experimenter was never present near the maze during the experiment.

Conditioned avoidance response

The avoidance response was evaluated using the conditioned avoidance response (CAR) described by Cook and Weidley.[32] Rats were divided into 12 groups of six animals, which were treated as per the protocol outlined above. A pole-climbing apparatus was used (Techno), which consisted of a chamber with a grid floor through which electric shocks can be given. The rats have to jump on to the wooden pole placed in the middle of the chamber to avoid the foot shock. A 50 Hz buzzer was given as the conditioning stimulus for 15 s, followed by a shock of 1 mA for 15 s as the unconditioned stimulus. Responses were classified as 'escape' when rats climbed the pole after the shock, 'avoidance' when they did so after the buzzer but before the shock, and 'secondary' if they climbed the pole before the buzzer. One hour after the drug treatment, the animals were placed individually in the chamber and allowed to explore the chamber for 1 min during the training schedule. Then the buzzer and foot shock were given and the behaviour was noted. The session ended after the animals responded by climbing or after the 15 s shock, whichever occurred first. The training schedule consisted of ten trials at intervals of 20–30 s on the first day. A single trial was then repeated every 24 h until the 95–100% avoidance response was achieved by all animals in the same treatment group. The group was then given scopolamine butyl bromide (1 mg/kg i.p.) 30 min before the next day's dose of extract. The dosing and screening were continued until the animals returned to 95–100% CAR.

Estimation of brain monoamines

Brain monoamine levels were estimated using HPLC as described by Lakshmana and Raju.[33] Once the animals showed recovery from scopolamine-induced anterograde amnesia and reached 95–100% CAR they were decapitated under anaesthesia and the heads dropped into ice-cold 0.1 mol/l perchloric acid (PCA). The brains were removed on an ice-chilled Petri dish. The brain tissues were weighed, homogenised in 2 ml 30 ng/ml isoprenaline in 0.1 mol/l PCA and centrifuged at 12 000 rpm for 15 min at 4ºC. The supernatant was filtered through a 0.45 μm membrane (Sartorius, Göttingen, Germany) and 20 μl of the filtrate injected onto the HPLC column. A Jasco (Tokyo, Japan) HPLC system was configured for dynamic mixing with a two-pump system. The mobile phase was delivered at 0.9 ml/min. An AS-1555 autosampler (Jasco) was used to introduce

the sample to the column. Detection was with a fluorescent detector (Jasco). The column was an RP-18 250 mm (5 μm) Vydac (Grace, IL, USA) protected by an RP-18 33 mm Kromasil guard column, at ambient temperature. Borwin chromatographic software (Jasco International, Tokyo, Japan) was used to record peaks and for data integration. After separation, noradrenaline (NA), dopamine (DA), isoprenaline and 5-hydroxytryptamine (5-HT) were detected at the excitation wavelength of 280 nm and an emission wavelength of 315 nm. The slit width was kept at 10/10 for excitation/emission respectively. The slit width is expressed as length and width of the sample-plane zone being quantified.

Statistical analysis

All data are expressed as means ± SEM. Two-way ANOVA followed by Bonferroni post-hoc tests was performed for the Y-maze task and CAR. The Student–Newman–Keuls test was used for estimation of monoamines. $P < 0.05$ was considered significant. GraphPad Instat and Prism 5 Demo software (GraphPad Software Inc., La Jolla, CA, USA) were used for the statistical analysis.

Results

Standardisation

The HPTLC results of the various grades of Gelucire showed that the 50/13, 44/14 and 39/10 extracts contained more bacoside A than 43/01 Gelucire. The 50/13 grade Gelucire at the proportion of 1 : 1 crude drug and gelucire 50/13 showed the highest percentage of bacoside A (3.56%) overall and was therefore selected for further studies.

MEBP and AGBM contained 4.1% and 0.005% bacoside A, respectively. GEBM contained 0.5% bacoside A; the aqueous extract did not show the presence of bacoside A. The chromatogram and the overlay spectra of the extracts and standard bacoside A are given in Figures 1 and 2.

Acute toxicity test

The MEBM, LEBM and AGBM were safe up to 2000 mg/kg and no abnormal changes or mortality occurred during the 24-h post-dose period.

Y-maze test

The results of the Y-maze test after 2 h are shown in Tables 1 and 2. A significant increase in the number of entries in the novel arm was observed with MEBM and LEBM at a dose of 400 mg/kg compared with control ($P < 0.01$). The percentage of total duration spent in the novel arm was significantly increased with LEBM at both 400 and 200 mg/kg ($P < 0.001$). Significant increases were observed with

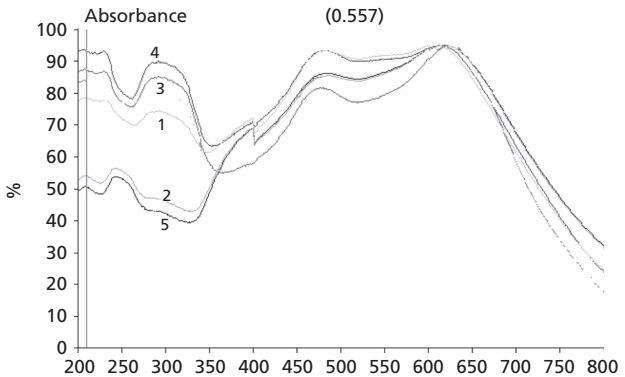

Figure 2 Spectral overlay of different samples of *Bacopa monniera* Linn. 1 = standard bacoside A; 2 = MEBM (methanolic extract); 3 = AGBM (Ayurvedic ghrita); 4 = GEBM (Ghee extract); 5 = LEBM, lipid extract.

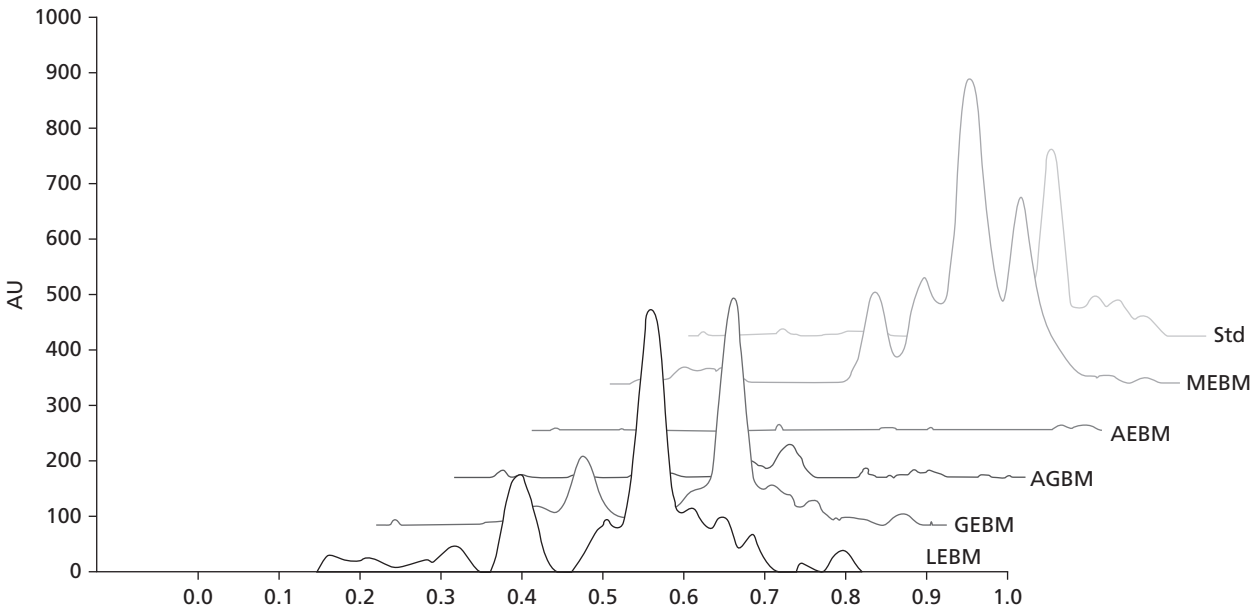

Figure 1 HPTLC Chromatogram overlay of different samples of *Bacopa monniera* Linn. Std, standard bacoside A, MEBM, methanolic extract; AEBM, aqueous extract; AGBM, Ayurvedic ghrita; GEBM, ghee extract; LEBM, lipid extract.

Table 1 Effect of the methanolic, lipidic and Ayurvedic ghrita extracts of *Bacopa monniera* Linn. on number of entries in Y-maze task after 2 h

Treatment	Vehicle	MEBM (mg/kg)			LEBM (mg/kg)			AGBM (mg/kg)		
		100	200	400	100	200	400	100	200	400
Novel arm	6.50 ± 0.42	6.83 ± 0.16	7.50 ± 0.22	8.16 ± 0.30**	7.00 ± 0.25	7.66 ± 0.33	8.33 ± 0.33**	6.66 ± 0.21	6.33 ± 0.33	6.66 ± 0.21
Other arm	3.66 ± 0.33	3.83 ± 0.3	2.83 ± 0.3	2.83 ± 0.4	3.33 ± 0.42	2.83 ± 0.3	2.66 ± 0.21	3.83 ± 0.47	3.66 ± 0.49	4.16 ± 0.3
Start arm	3.50 ± 0.22	2.50 ± 0.22	3.16 ± 0.47	2.83 ± 0.6	3.16 ± 0.3	3.00 ± 0	2.66 ± 0.33	3.33 ± 0.49	3.33 ± 0.21	2.83 ± 0.3
Total arm	13.66 ± 0.61	13.16 ± 0.4	13.50 ± 0.42	13.83 ± 0.3	13.50 ± 0.5	13.50 ± 0.5	13.66 ± 0.42	13.83 ± 0.6	13.33 ± 0.55	13.66 ± 0.42

AGBM, Ayurvedic ghrita; LEBM, lipid extract; MEBM, methanolic extract. Values are number of entries in each arm, mean ± SEM (n = 6). **$P < 0.01$ vs vehicle control group (two-way analysis of variance followed by Bonferroni's post-hoc test).

Table 2 Effect of the methanolic, lipidic and Ayurvedic ghrita extracts of *Bacopa monniera* Linn. on percentage time spent in Y-maze task after 2 h

Treatment	Vehicle	MEBM (mg/kg)			LEBM (mg/kg)			AGBM (mg/kg)		
		100	200	400	100	200	400	100	200	400
Novel arm	39.00 ± 4.74	41.77 ± 5.38	53.55 ± 3.70*	58.50 ± 2.48***	43.72 ± 2.14	57.83 ± 1.93***	59.11 ± 2.83***	42.94 ± 1.75	45.66 ± 3.85	47.44 ± 3.85
Other arm	30.11 ± 1.84	31.77 ± 3.01	27.83 ± 2.8	24.33 ± 3.73	29.22 ± 0.36	24.33 ± 2.95	24.27 ± 1.94	21.22 ± 2.72	29.22 ± 0.36	29.22 ± 0.36
Start arm	30.88 ± 6.14	26.44 ± 6.74	18.61 ± 4.85*	22.61 ± 2.48	27.05 ± 2.33	17.83 ± 4.47*	16.61 ± 3.49*	35.83 ± 4.06	25.11 ± 3.79	23.33 ± 3.72

AGBM, Ayurvedic ghrita; LEBM, lipid extract; MEBM, methanolic extract. Values are % time spent (in s), mean ± SEM (n = 6). *$P < 0.05$, ***$P < 0.001$ vs vehicle control group (two-way analysis of variance followed by Bonferroni's post-hoc test).

MEBM at 400 and 200 mg/kg ($P < 0.001$ and $P < 0.01$ vs control). The 100 mg/kg dose of LEBM and MEBM did not have significant effects. None of the AEBM groups showed significant increases in the number of entries or total duration. Although there was an increase in the percentage time spent in the novel arm with all other groups, this was not statistically significant. In the trial after 4 h, none of the groups showed a significant increase in the percentage time spent in the novel arm and there were no significant differences in the numbers of entries in the novel arm (data not shown).

Conditioned avoidance response

The results for the CAR using the pole-climbing apparatus are shown in Figure 3. The avoidance response of the animals

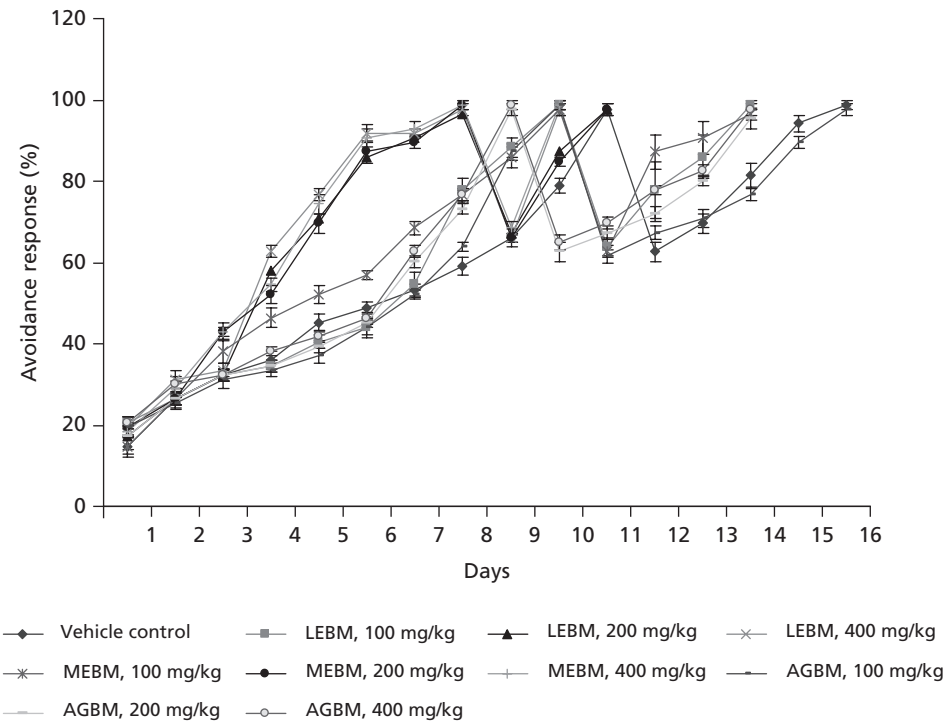

Figure 3 Effect of the methanolic, lipidic and Ayurvedic ghrita extracts of *Bacopa monniera* Linn. on the conditioned avoidance response using pole climbing apparatus. AGBM, Ayurvedic ghrita; LEBM, lipid extract; MEBM, methanolic extract. Values are doses in mg/kg.

increased gradually to 97–98% on day 8 with the higher (400 mg/kg) and medium (200 mg/kg) doses of LEBM or MEBM and on day 9 for the groups treated with the higher and medium doses of AGBM. The % CAR was significant ($P < 0.001$) for the LEBM and MEBM at higher and medium doses from days 5–8 compared with the control and AEBM. Animals given the lowest dose showed maximum percentage activity on day 10; the control groups showed avoidance response only on day 11. Acquisition was quicker for the LEBM- and MEBM-treated groups than the AGBM-treated group, and was dose dependent. Administration of scopolamine butyl bromide produced marked reduction of avoidance response in all groups and was reversed by continued administration of the test extracts, in a dose-dependent manner. The groups that received the highest doses of LEBM and MEBM dose showed recovery on day 10; those receiving the medium dose showed recovery on day 11. Rats given the higher and medium doses of AGBM showed recovery on day 14. The lowest dose of LEBM and MEBM produced recovery on day 14; the control and AGBM groups showed response only on day 16.

Brain monoamines

Levels of monoamines in the brains of rats treated with 400 mg/kg LEBM and MEBM after the CAR test are shown in Figure 4. The stress-control animals subjected to the CAR procedure showed a significant increase in NA compared with non-stress control rats ($P < 0.001$). There was a significant decrease in the levels of NA with LEBM compared with the stress control ($P < 0.001$). There were significant decreases in 5-HT with both LEBM and MEBM ($P < 0.01$ and $P < 0.05$). Although the level of DA was higher than the control in all the groups, the increase was not significant. The LEBM-treated group showed a significant increase in the DA level compared with the stress-control group ($P < 0.05$).

Discussion

Bacoside A, the major active constituent of BM, is insoluble in water and is usually extracted using either ethanol or methanol by various conventional methods of extraction. The present study demonstrated a solvent-free method for the extraction of bacoside A. In the Ayurvedic preparation of ghrita, the process of svarasa (juice) and kalka (paste) may lead to the extraction of water-insoluble components to a lesser extent. This is clearly evident from the HPTLC results, which show lower concentrations of bacoside A in AGBM (0.00549%) than the MEBM (4.1%) and LEBM (3.56%). The lower concentration of bacoside A in GEBM (0.5%) and its absence from AEBM further support the above concept. In the present study, extraction of bacoside A was better with the LEBM than the AGBM and GEBM.

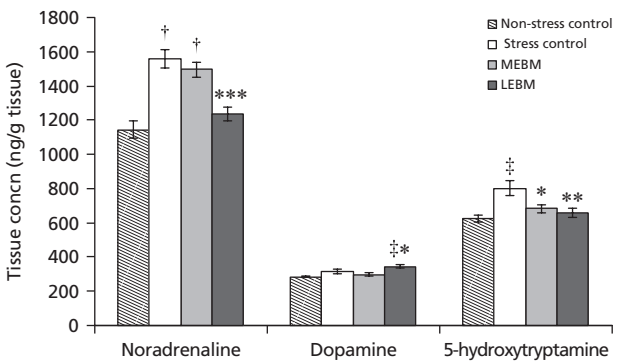

Figure 4 Effects of the lipidic and methanolic extracts of *Bacopa monniera* Linn. (400 mg/kg) on brain monoamine levels after the conditioned avoidance response study. LEBM, lipid extract; MEBM, methanolic extract. $^{\dagger}P < 0.001$; $^{\ddagger}P < 0.01$ vs non-stress control; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs stress control group (one-way analysis of variance followed by the Student–Newman–Keuls test).

In the spatial memory recognition test using the two-trial Y-maze study, the rats treated with LEBM showed a significant dose-dependent increase in time spent and number of entries in the novel arm compared with the MEBM- and AGBM-treated groups after the 2 h interval trial. This indicates the high potential of LEBM. The number of total arm entries did not show any significant changes in the 2 h and 4 h interval trials, which indicates that the activity is not due to a locomotor effect. Memory consolidation in the Y-maze tests is reported generally to remain for only a short period (much less than 4 h).[34] Nevertheless, some of the treated groups showed increases in time spent in the novel arm after 4 h but the changes were not significant. These results are consistent with previous studies.[35] Although the administered LEBM contains a lower concentration of bacoside A (3.56% w/w) than MEBM (4.10% w/w), memory recognition was higher with LEBM. This was further confirmed by the activity observed from the CAR. The groups that received the medium and high doses of LEBM and MEBM showed quicker acquisition (97–98%) and recovery from scopolamine-induced amnesia than AGBM-treated rats. Thus, the LEBM- and MEBM-treated rats showed similar types of activity and avoidance was always higher than in the AGBM and control groups.

Studies have reported that gelucire 50/13 is capable of forming a sub-micron emulsion when it comes into contact with the physiological fluids in the small intestine.[36] It is also reported that lipid excipients solubilise hydrophobic drugs within the dosage form matrix and improve drug absorption in gastrointestinal fluids.[37–39] In the present study, the enhanced activity provided by the LEBM may be due to the above properties of Gelucire, although there is no direct evidence. Moreover, since Gelucire can extract both lipophilic and hydrophilic components, there may be a synergistic activity of (an)other component(s) extracted along with bacoside A that contributes to the enhanced activity. As Gelucire is lipidic in nature, it is possible that penetration of bacosides across the blood–brain barrier (BBB) is higher with LEBM than MEBM.

P-glycoprotein is a key element of the BBB that can actively transport a huge variety of lipophilic drugs out of the brain capillary endothelial cells that form the BBB. Co-administration of drugs with compounds that inhibit P-gp-mediated efflux or the incorporation into specific lipid excipients alters the pharmacokinetics of the administered compound.[40,41] Kristina *et al.* reported the P-gp inhibiting property of Gelucire 44/14.[25] Although there are no reports of the P-gp inhibitory activity of Gelucire 50/13, this lipid

excipient may improve the kinetics of the active constituents of BM, which requires further study.

Brain monoamine levels were measured on the day when the drug-treated groups showed maximum activity in the CAR. BM was shown to normalise the stress-induced elevation of brain monoamine levels in rats.[42] According to Essman, the use of aversive stimuli such as pain to motivate behaviour has stressor components.[43] In the present study, the electric shock involved in the CAR test acts as the stressor. The LEBM- and MEBM-treated groups (400 mg/kg) showed maximum avoidance on day 10. They were compared with an exclusive stress control and non-stress control groups involved in the study until day 10. The increased levels of NA and 5-HT in the stress-control group compared with the non-stress control may be due to the shock acting as a stressor. The groups treated with LEBM and MEBM showed significant decreases in the levels of NA and 5-HT; significance was higher with LEBM. The results are consistent with previous reports.[42,43] The decrease in 5-HT levels coincides with the concept of earlier studies in which the enhanced learning ability was observed with decreased brain 5-HT level.[44] However, there was no significant change in the levels of DA, except for the LEBM-treated group which showed an increase in DA. It is difficult to conclude the mechanism for these changes in the monoamine levels from the present data.

The observations described above establish that even though the concentration of bacoside A is lower in LEBM than MEBM, it showed better therapeutic activity in the Y-maze exploration test than MEBM and AGBM, and activity comparable to MEBM in CAR and monoamine levels.

Conclusions

The proposed LEBM is free from solvents and shortcomings of conventional extracts and ghrita. It was shown to be comparable to MEBM in nootropic activity, as evaluated by the Y-maze test and CAR and reversed scopolamine-induced memory impairment. It reduced the stress-induced elevation of NA and 5HT. Further studies to elucidate the exact mechanism of extractability of Gelucire and the enhanced pharmacological actions are underway.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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