Bilosomes as a Potential Carrier to Enhance Cognitive Effects of *Bacopa monnieri* Extract on Oral Administration

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Abstract

The Indian system of medicine, Ayurveda employs *Bacopa monnieri* extract (BME) for memory enhancement. This study attempts to prepare and test a more potent formulation by incorporating BME in nanovesicles. BME-loaded liposomes and bilosomes (bile salt-stabilized liposomes) were formulated using soy phosphatidylcholine. Liposomes and bilosomes had homogeneous size distribution and an average size of 285.7 nm and 84 nm, respectively, with satisfactory zeta potential. Spherical multilamellar bilosomes and unilamellar liposomes were observed under transmission electron microscope (TEM), with BME entrapment efficiency of 85% and 45%, respectively. During a 72 h interval, bilosomes and liposomes released 78% and 65% of the loaded BME, exhibiting a biphasic release, following the Higuchi model diffusion. Both liposomes and bilosomes were stable in simulated gastric and intestinal fluids. When tested on dementia-induced Swiss albino mouse models using the Y-maze apparatus, the bilosome-treated group showed significant cognition enhancement activity than those treated with liposomal vesicles. The better pharmacological effect shown by bilosomes may be attributed to better bioavailability, possibly augmented by higher entrapment efficiency, and improved vesicle integrity afforded by bile salts. Likewise, bilosomes were more stable than liposomes in simulated gastric and intestinal fluids.

Keywords

- brain delivery
- *Bacopa monnieri*
- liposome
- bilosome
- nootropic
- cognitive enhancement

Introduction

A cognitive decline challenges the aging brain. In the context of a steadily growing geriatric population, it is essential to address this emerging public health problem with indigenous solutions.¹ It has been reported that 50 to 60% of those above 65 years of age suffer from neurodegenerative disorders that signal the primary signs of Alzheimer’s disease (AD), namely, a decline of cognitive function or dementia.² AD development is associated with extracellular amyloid (A) plaques and intracellular neurofibrillary tangles, composed of a hyperphosphorylated protein in the cortical and limbic regions of the brain.³ As per...
reports, 55.2 million people are affected by dementia globally, and 60% are in low- and middle-income countries. Dementia cases may rise to 78 million by 2030 and 139 million by the end of 2050. Dementia associated with various neurological conditions remains the seventh leading cause of death worldwide. The World Health Organization (WHO) has called for urgent action in dementia management and risk reduction.4

Nootropic formulations are expected to address the above concerns. Nootropics are cognition enhancers, but a cognition enhancer is not necessarily a nootropic.5 A cognition enhancer improves memory and attention, whereas nootropics enhance mental abilities. Both learning and memory are variations in synaptic neural connectivity and psychological processes. In recent times, there has been a tremendous demand for herbal products to treat chronic ailments in developing countries and the West.6

Bacopa monnieri (BM), family Scrophulariaceae, is known as Brahmi in India and has been used since 500AD. In Ayurveda BM is known as “Medha Rasayana” to treat anxiety, impaired memory, epilepsy, and cognitive processes such as understanding, memory, and recall.7 Bacopa monnieri extract (BME) scavenges free radicals and protects against deoxyribonucleic acid (DNA) damage in a dose-dependent manner. BME is an antioxidant (half as potent as vitamin E) and exhibits anti-stress, immunomodulatory, anti-aging, and anti-inflammatory effects. The memory-enhancing property of BM is credited to its antioxidant property and has been prescribed for long-term therapy at low doses.8 The pharmacological activities of BM have been extensively studied and have been linked to the saponin constituents known as “bacosides.” Bacosides are a complex mixture of structurally related compounds such as jujubogenin or pseudojujubogenin glycosides.7 Alkaloids brahmine and herpestine have been isolated from Brahmi. Tetra cyclic triterpenoid saponin, Bacoside A, and B (crystalline mixture of several saponins) are the main bioactive constituents of the plant. Bacoside A is the most abundant of these. Other saponins include bacoside A1, A3, bacoside saponins A, B, C, D, E, and F. The plant constituents also include d-mannitol, hersaponin, betulic acid, and flavonoids.9

The phytoconstituents often fail to offer therapeutic benefits owing to their poor bioavailability caused by hepatic first-pass metabolism or incomplete absorption. The technological advancements in drug delivery can overcome these challenges enabling the phytonutrients to achieve therapeutic concentrations at the site of action effortlessly. A novel vesicular drug delivery system aims to deliver the active ingredient to the site of action at a rate determined by the body’s need during treatment.10 Nanomedicine is likely to offer better therapeutic success than conventional drug delivery approaches.11 Several studies on tumors and central nervous system (CNS) disorders such as Alzheimer’s disease (AD) use nano-vesicular formulations.12 The therapeutic index, solubility, stability, and rapid degradation of drug molecules have been improved using vesicular drug delivery systems.13

Liposomes entrap the compounds, avoid fast removal or degradation from the systemic circulation, and facilitate their penetration through the blood–brain barrier (BBB) and distribution in the brain tissue, making liposomes an efficient drug delivery system for the brain.12,14 Liposomal delivery generally requires lower doses because of improved bioavailability and tissue distribution. Liposomes contain multiple components, the most important being phospholipids and cholesterol. Soy phosphatidylcholine (SPC) is the most widely used phospholipid in liposomal preparations.10

Liposomes mimic cell membranes by exhibiting excellent entrapment ability, safety, and biocompatibility. Although parenteral delivery of liposomes has been successful, oral delivery is thwarted by various factors such as gastrointestinal (GI) instability. Liposome stability and permeability can be significantly improved for oral administration by modifying the composition of the lipid bilayers or by adding polymers or ligands for targeting.15 Liposomes administered orally would be destroyed partially by gastric acid, leading to the release of the drug payload. Surviving liposomes would subsequently move from the stomach into the small intestine, where intestinal enzymes break down liposomes. Liposomes that survive this stage penetrate the mucus layers and contact intestinal epithelia. Even at this point, liposomes can still be destroyed. Only liposomes that survive the digestive process would be absorbed by M cells as integral vesicles to the lymphatic system.16

A variety of methods are employed to improve liposomal stability. Adding bile salts enhances lipid biomembranes’ flexibility, allowing them to survive the adverse effects of bile acids in the GI tract. Imaging studies have shown that bilosomes that survive the GI environment can be absorbed as entire vesicles.17 Bilosomes are vesicular carriers used to protect proteins and peptides from degradation in the gastrointestinal tract (GIT) to a certain extent. Vesicles are developed by incorporating bile salts into the lipid bilayers, making them resistant to the disruption in GIT. As a result, the vesicle protects the entrapped agents from the harsh GI environment.18 In this novel drug delivery system, carriers such as deoxycholic acid are introduced into the membrane of liposomes.19 Bile salts improve the penetration of lipophilic drugs across biological membranes, thereby enhancing oral bioavailability.20 Here we attempt to enhance the nootropic activity of BM through bilosomal delivery, facilitating value addition to the traditional knowledge system in Ayurveda.

Materials and Methods

Materials

The alcoholic extract of BME was a gift sample from Elixir Extracts Pvt. Ltd., Kochi, Kerala. Soya phosphatidylcholine (SPC or soy lecithin) was procured from Hi-Media Laboratories, Mumbai. Cholesterol was purchased from Merck, Mumbai. Sodium deoxycholate, pepsin (from porcine gastric mucosa), pancreatin (from the porcine pancreas) was purchased from Sigma–Aldrich. Chloroform was purchased from

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Loba Chemie, Mumbai. All chemicals used were of analytical or pharmaceutical grade.

**Froth Test for Saponins in the Bacopa monnieri Extract**

BME (50 mg) was mixed with 5 mL of distilled water in a stoppered test tube, shaken well for ~5 minutes, and allowed to stand for 30 minutes. The honeycomb-like froth formation indicated the presence of saponins.²¹

**Fourier Transform Infrared Spectroscopy Studies**

The Fourier transform infrared spectroscopy (FT-IR) spectra of BME and extract-loaded nanoformulations were recorded in a wavelength range of 4000 to 500 cm⁻¹ on a M-15, Bruker Alpha Fourier transforms infrared spectrophotometer, USA, employed with an ATR crystal. Transmittance peaks were identified and compared with the literature reports.

**Formulation of Liposomes**

**Method 1: Solvent Evaporation Technique**

To prepare liposomes (LS11, LS12, and LS13), equal volumes of BME, SPC, and various amounts of cholesterol were dissolved in 10 mL of ethanol in a beaker. → Table 1 shows the ratio of materials used in the formulation. The mixture was added to 80 mL of distilled water under stirring at 15000 rpm on a magnetic stirrer (on a magnetic stirrer) maintained at 80°C. Stirring was continued for 15 minutes, maintaining the temperature to evaporate the ethanol. The mixture was allowed to cool and was sonicated for 10 minutes (50% amplitude) using a probe sonicator (Sonic, USA).²²

**Method 2: Thin-film Hydration Method (LS2)**

BME-loaded liposomes (LS21, LS22, and LS23) were prepared following a thin-film hydration process in a rotary flash evaporator. BME, SPC, and cholesterol were dissolved in 10 mL of chloroform in a round bottom flask; the quantities are represented in → Table 1. A rotary evaporator was used to evaporate the organic solvent, maintained at 65°C under reduced pressure for 30 minutes. The resulting thin, dry film was left overnight for complete evaporation of organic solvents and then hydrated using a phosphate buffer (pH 7.4) to obtain a crude dispersion of BME-loaded liposomes. Then crude dispersion was further sonicated for 10 minutes (50% amplitude) using a probe sonicator.²³

**Formulation of Bilosomes by Thin-film Hydration Method**

BME-loaded bilosomes (BS1, BS2, and BS3) were prepared using a rotary flash evaporator by a thin-film hydration process, slightly modifying the previous method. As given in → Table 1, BME and SPC were dissolved in 10 mL of chloroform in a round bottom flask. The organic solution was evaporated at 65°C under reduced pressure for 30 minutes until a thin, dry film was formed in a rotary flash evaporator. It was left overnight to achieve complete evaporation of the organic solvent. Crude dispersion of BME-loaded bilosome was achieved by hydrating the thin, dry film with 10 mL of distilled water containing bile salt (sodium deoxycholate). The dispersion was then sonicated for 10 minutes using a probe sonicator.²⁴

**Characterization of Liposomes and Bilosomes**

**Vesicle Size and Zeta Potential Analysis**

The average vesicle size and size distribution (polydispersity index, PDI) of formulated liposomes and bilosomes were analyzed by dynamic light scattering (DLS) using Nano ZS, Malvern Instruments, UK. The surface charge of vesicles was measured in terms of zeta potential (Nano ZS, Malvern Instruments, UK). Only those formulations with desirable size distribution were considered for further characterization.

**High-Resolution Optical Microscopy**

On account of the acceptable size range, formulations LS23 and BS3 were taken for further studies. The vesicles formed were examined for their morphology under a high-power optical microscope (BIOVIS particle analyzer, India). A drop of the liposome/bilosome sample was sufficiently diluted

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**Table 1** Formulation chart of liposome and bilosome formulations showing the method of preparation and their composition

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Formulation type</th>
<th>Method used</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>BME (mg)</td>
</tr>
<tr>
<td>LS11</td>
<td>Liposomes</td>
<td>Solvent evaporation</td>
<td>100</td>
</tr>
<tr>
<td>LS12</td>
<td></td>
<td></td>
<td>100</td>
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<tr>
<td>LS13</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>LS21</td>
<td></td>
<td>Thin-film hydration</td>
<td>100</td>
</tr>
<tr>
<td>LS22</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>LS23</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>BS1</td>
<td>Bilosomes</td>
<td>Thin-film hydration</td>
<td>100</td>
</tr>
<tr>
<td>BS2</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>BS3</td>
<td></td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>
with distilled water, smeared on a glass slide, protected with a coverslip, and inspected under microscopic magnification (100 x objective), and the images were recorded.

**Transmission Electron Microscopy of Formulations**
The morphology and vesicular size of BME-loaded liposomes LS23 and bilosomes BS3 were further analyzed by high-resolution transmission electron microscopy (HR TEM) using JEM-2100 Electron Microscope, Jeol, Japan. After suitable dilution, a drop of the formulation was adsorbed on a copper grid, negatively stained with 1% phosphotungstic acid, and air-dried at room temperature for 10 minutes. The morphological appearance of the selected vesicles was recorded by TEM.23

**Extract Entrapment Efficiency of Vesicles**
Flocculation was induced for LS23 and BS3 by adding potassium chloride solution (1 mL of 3M KCl) before centrifugation. The unentrapped BME in the formulations was removed by ultracentrifugation (Beckman, USA) at 40,000 rpm for 1 hour at 4°C. The concentration of unentrapped extract in the supernatant was analyzed by UV-Vis spectrophotometry at 321 nm (Spectroquant Prove 600, Merk, Germany).25,26

The percentage entrapment efficiency was determined using the following equation:

\[
\% \text{ Extract entrapment efficiency} = \frac{\text{Amount of extract added} - \text{amount of free extract}}{\text{Amount of extract added}} \times 100
\]

**In Vitro Drug Release Study**
Drug release from nanovesicles was studied using the dialysis method. Dialysis kits (Pur–A-Lyzer Maxi Dialysis Kit, Sigma-Aldrich, India) of 12 to 14 kDa molecular weight cut-off were used for this method. Dialysis kits were pre-soaked in distilled water for 10 minutes and later loaded with 3 mL of the formulations. Loaded dialysis kits were fixed on the floating rack and placed in a beaker containing 200 mL of phosphate-buffered saline (PBS; pH 7.4) as a release medium, maintained at 37 ± 0.5°C with continuous magnetic stirring at 50 rpm. Samples of 5 mL were withdrawn at different intervals of 0.5, 1, 2, 3, 4, 6, 7, 9, 24, 48, 69, 72 h, and replaced with the medium. The concentration was determined by UV-Vis spectrophotometry by measuring the absorbance after suitable dilution.27

**In Vitro Gastrointestinal Stability of Nanovesicles**
Simulated gastric fluid (SGF) was prepared by dissolving 2 g of NaCl and 7 mL of concentrated HCl in 1 L of deionized water, then adjusting the pH to 1.2. To mimic gastric digestion, pepsin (0.032 mg/mL) was added to the above solution. Simulated intestinal fluid (SIF) was prepared by dissolving 6.8 g of dipotassium phosphate, 190 mL of 0.1 M sodium hydroxide, 3.32 g of sodium chloride, 8.76 g of calcium chloride, and 0.1 g of sodium deoxycholate (bile salt); in water. The solution was diluted to 1 L, and the pH was adjusted to 7.4. Pancreatin was added to the above solution at a 0.032 mg/mL concentration for intestinal digestion. The liposomes and bilosomes were added to SGF and SIF in separate standard flasks, mixed well, and incubated in the water bath for 30 minutes at 37 ± 0.5°C. Samples were withdrawn after 1 minute and 30 minutes. Vesicle size, PDI, and zeta-potential of liposomes and bilosomes were determined during GI digestion.28

**In Vivo Cognitive Enhancement Activity Studies of Nanovesicles**

**Selection of Dose**
To assess the nootropic activity of BME, the dose was fixed at 200 mg/kg, (1/10th of the previously reported maximum dose in acute toxicity studies, i.e., 2000 mg/kg). Formulations equivalent to the above amount of drug were used for evaluation.29

**Treatment Schedule and Evaluation of Nootropic Activity**
Nootropic activity was evaluated with the help of a Y maze spontaneous alternation test using Male Swiss albino mice. The animals were housed and maintained in large, spacious polycrystalline cages at ambient room temperature with a 12-hour light/12-hour dark cycle. The animals were fed with water and a standard rat pellet diet *ad libitum*. The study was approved by the Institutional Animal Ethics Committee (IAEC) of NGSM Institute of Pharmaceutical Sciences with approval number NGSMIPS/IAEC/JUNE-2020/191.

Male Swiss albino mice (22 to 30 g) were randomly divided into five groups of five animals each.

- **Group I:** Scopolamine (0.5 mg/kg)
- **Group II:** Normal control (saline)
- **Group III:** Scopolamine + BME (200 mg/Kg)
- **Group IV:** Scopolamine + Liposome (LS23)
- **Group V:** Scopolamine + Bilosome (BS3)

The animals in Group I and II were administered orally once daily with vehicle (0.6% w/v sodium carboxymethyl cellulose [Na CMC], 10 mL/kg) for 7 days. Group III was administered with BME (200 mg/kg) orally once daily for 7 days. For seven days, groups IV and V were administered with the formulation LS23 and BS3 containing BME equivalent to 200 mg/kg. On the seventh day, after half an hour of BME administration, each group except Group II received scopolamine (0.5 mg/kg) intraperitoneally.30 After 45 minutes of scopolamine administration on day 7, the nootropic activity was evaluated using the Y maze. To evaluate short-term memory, the percentage of trials in which the mice entered all three arms on consecutive choices alternatively (without returning to the previous arm) was recorded as an alternation.31

**Statistical Analysis**
The data for nootropic effect of BME, LS23 and BS3 formulations are expressed as mean ± standard error of the mean (SEM). The data obtained were subjected to a one-way analysis of variance (ANOVA) followed by Dunnett’s test using GraphPad Prism software. *p*-Values less than 0.05 are considered statistically significant (indicated by ‘*‘).
Results and Discussion

Identification of Bacopa monnieri Extract
The procured BME was brownish-green with a characteristic odor and powdery appearance. In the froth test, a characteristic honey-comb-like froth was formed, indicating the presence of saponin glycosides. Steroid saponin glycoside, such as bacoside A₃ in the extract, helps with memory enhancement.

Formulation of Liposomes and Bilosomes
BME-loaded liposomes and bilosomes were prepared as described in the methodology section. The BME was dissolved in ethanol to solubilize lipophilic active constituents, improving the entrapment. Table 1 depicts the formulation chart of liposomes and bilosomes, showing the preparation method and composition. Solvent evaporation yielded larger vesicles; therefore, the thin-film hydration method prepared the final formulations of liposomes and bilosomes. The difference between the formulation of liposomes and bilosomes was the incorporation of bile salt in the latter during their hydration process. Adding cholesterol modulates membrane permeability and elasticity, reducing vesicle size.

Characterization of Liposomes and Bilosomes
Vesicle Size, Zeta-potential, Morphology
We used Malvern Zetasizer, which employs the dynamic light scattering technique, to determine the vesicle size of the formulations. Results are shown in Table 2. Although liposomes prepared by solvent evaporation method are known for their high entrapment efficiency and ease of preparation, they yielded large vesicles even after sonication. Vesicle size and entrapment efficiency are directly related because increasing the vesicle size increases the volume of extract encapsulated. Additionally, increasing the amount of soy lecithin enhances the medium’s lipophilicity, increasing the entrapment efficiency and vesicle size. Due to the large vesicle size, liposomes prepared by solvent evaporation were excluded from advanced characterization.

The amount of cholesterol affects the vesicle size and drug release from liposomes. Cholesterol increases membrane hydrophobicity, favoring the inclusion of hydrophobic molecules. Furthermore, the increased vesicle size for SPC liposomes could be attributed to the packing parameter and phase transition temperature. Saturated phospholipids with shorter carbohydrate chains have lower packing parameters, favoring smaller vesicles’ formation. On the contrary, phospholipids with monounsaturated carbohydrate chains have the highest packing parameter and lower phase transition temperature, helping the formation of larger liposomes.

Sodium deoxycholate or bile salts impart a negative charge to the vesicles, increasing the zeta potential. Smaller vesicles are observed in bilosomes with better zeta potential, possibly due to bile salt’s presence. Also, the vesicle size is known to increase due to the repulsion between the bilayers in multilamellar bilosomes, which was not observed here.

A detailed comparison of vesicle size is given in Table 2. The polydispersity index (PDI) is an essential measure of dispersion characteristics related to size uniformity. PDI values of more than 0.4 imply high heterogeneity, and those 0.2 or less indicate homogeneity. PDI of LS23 was found to be 0.5 ± 0.15, and BS3 was found to be 0.386 ± 0.01, showing moderate homogeneity in size distribution.

The zeta potential values for BME-loaded liposomes LS23 and bilosomes BS3 were satisfactory, with values of −16.8 and −15.1 mV, respectively, implying moderate stability of the formulations due to electrostatic repulsion. The incorporation of bile salts and cholesterol showed increased zeta potential. Formulations with lower zeta potential and high polydispersity were excluded from further evaluation.

FT-IR Analysis
Infrared spectroscopy helps determine or predict any physicochemical interactions between different components in a

Table 2 Data for estimation of vesicle size, polydispersity index (PDI), zeta potential and extract loading of vesicles

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Vesicle size (nm)</th>
<th>Polydispersity index (PDI)</th>
<th>Zeta potential (mV)</th>
<th>Extract loading efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS11</td>
<td>1585.5 ± 2.0</td>
<td>0.608 ± 0.12</td>
<td>−8.45</td>
<td>–</td>
</tr>
<tr>
<td>LS12</td>
<td>1011 ± 0.9</td>
<td>0.51 ± 0.08</td>
<td>−11.4</td>
<td>–</td>
</tr>
<tr>
<td>LS13</td>
<td>899 ± 3.1</td>
<td>0.661 ± 0.11</td>
<td>−14.5</td>
<td>–</td>
</tr>
<tr>
<td>LS21</td>
<td>652.4 ± 2.2</td>
<td>0.630 ± 0.07</td>
<td>−7.8</td>
<td>–</td>
</tr>
<tr>
<td>LS22</td>
<td>533.2 ± 1.8</td>
<td>0.506 ± 0.01</td>
<td>−12.5</td>
<td>–</td>
</tr>
<tr>
<td>LS23</td>
<td>285.7 ± 3.0</td>
<td>0.5 ± 0.15</td>
<td>−16.8</td>
<td>45%</td>
</tr>
<tr>
<td>BS1</td>
<td>132 ± 2.2</td>
<td>0.722 ± 0.2</td>
<td>−9.2</td>
<td>–</td>
</tr>
<tr>
<td>BS2</td>
<td>149.6 ± 1.8</td>
<td>0.66 ± 0.08</td>
<td>−10.4</td>
<td>–</td>
</tr>
<tr>
<td>BS3</td>
<td>84.0 ± 0.7</td>
<td>0.386 ± 0.01</td>
<td>−15.1</td>
<td>85%</td>
</tr>
</tbody>
</table>

Table 1 Data for estimation of extract load and characterization

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Extract loading (mg/mL)</th>
<th>Lipid load (mg/mL)</th>
<th>Lipid to extract ratio</th>
<th>Zeta-potential (mV)</th>
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<tbody>
<tr>
<td>LS23</td>
<td>4.5</td>
<td>2.5</td>
<td>1.8</td>
<td>−16.8</td>
</tr>
<tr>
<td>BS3</td>
<td>5.2</td>
<td>3.5</td>
<td>1.5</td>
<td>−15.1</td>
</tr>
</tbody>
</table>

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formulation. Therefore, infrared spectroscopy can be a tool to select chemically compatible excipients. The compatibility between soy lecithin, cholesterol, sodium deoxycholate, and BME was established using an FTIR spectrophotometer. Absorption peaks are shown in Fig. 3A. FTIR spectra obtained with the alcoholic BME were concordant with the literature available. The prominent peaks associated with BME, namely O-H bonded (3650–3600), C-H stretch (3000–2850), C=O (Ketone) (1725–1705), C=C (Alkene) (3319–3383), CH3-R(Alkyl) (2944–2882) and C-O bond (furanosyl, pyranosyl) (1075–1018) were observed. The constituent peaks were present in both formulations (Fig. 3B and 3C), thereby ruling out the possibility of any chemical incompatibility between the extract and the lipids used.

Encapsulation Efficiency
Evaluating extract loading efficiency helps estimate the amount of BME entrapped in the vesicles. The encapsulation efficiency was determined to be 45% for LS23, and 85% for BS3. The phospholipid composition of the liposomal membrane influences the drug loading efficiency and drug release kinetics. The membrane’s hydrophobicity, thickness, and compactness determine the drug loading efficiency and leakage of molecules entrapped in liposomes. As far as BS3 is concerned, the encapsulated fraction of extract increased proportionately with an increase in SPC concentration. It may be concluded that SPC concentration has a two-way effect on entrapment efficiency.

Encapsulation of extract was lower in LS23 without bile salt in the bilayers. Nevertheless, the entrapment efficiency of BS3 increased with increased SPC in the presence of bile salt. Reports state that the surface-active properties enable sodium deoxycholate to perturb the acyl chains of the lipid matrix and incorporate them into the surface of the membrane bilayer perpendicularly. Thus, sodium deoxycholate increases membrane flexibility, thereby improving the solubility of a highly lipophilic drug in the membrane. Thus, incorporating bile slats might facilitate BME penetration into the lipid bilayer.

Entrapment efficiency increased significantly with bile salts and high lipid content, implying that lipid and bile salts are the main factors influencing entrapment. The change in the vesicle size also affects the entrapment efficiency as a function of the sodium deoxycholate-to-PC molar ratio. Sodium deoxycholate in the exterior aqueous medium can decrease the surface tension of the vesicles, possibly resulting in a reduction in vesicle size. Increased bile salt concentrations would result in the development of micelles in the dispersion medium, improving drug solubility in the medium and lowering percentage entrapment. Furthermore, high concentrations of bile salts have a fluidizing effect on lipid bilayers, resulting in the loss of the encapsulated medication. Raising the molar...
ratio of SPC would increase the lipophilicity of the systems, increasing the amount of entrapped drugs.\(^{24}\)

**In Vitro Drug Release Studies**

Fig. 4 shows the in vitro drug release pattern of BME-loaded liposomes LS23 and BME loaded bilosomes BS3. A biphasic release profile was observed in nanovesicular formulations, i.e., a rapid initial burst release followed by sustained release. The burst release of BME might be due to the detachment of surface-loaded extract and unentrapped one. In contrast, the sustained release period could be due to BME release from the vesicular bilayer into the release medium over time.\(^{40}\)

**In Vitro Release Kinetics**

The release rates are shown in Table 3. The optimized liposomal and bilosomal suspensions (LS23 and BS3) followed first-order release kinetics with a significant regression coefficient. The drug release process was analyzed using the exponential model Korsmeyer–Peppas and Higuchi. The mechanism of drug release followed the Higuchi model with a higher regression coefficient \(R^2\) than the Korsmeyer–Peppas model. The Higuchi model showed drug release by diffusion through the bilayers.\(^{41}\)

**In Vitro GI Stability of Liposomes and Bilosomes**

**Change in Vesicle Size and PDI of Nanovesicles during Gastric Digestion**

Fig. 5 A shows the stability of BME-loaded liposomes LS23 and bilosomes BS3 in the gastric fluid. In the absence of pepsin, the vesicle size of BME-loaded liposomes was 84 nm with a PDI of 0.5. After 1 minute digestion with SGF, vesicle size increased to 1421 nm, which dropped to 210 nm after 30 min. PDI was 0.23 after 1 minute of digestion and did not decrease much after 30 minute. According to the findings, liposome’s increased size during digestion suggests vesicle aggregation or fusion. The significant drop in pH during the gastric phase, from neutral (pH 7.4 liposomal formulation) to severely acidic (pH 1.5); changes in pH alter the strength and extent of colloidal interaction between particles, permitting liposome coalescence.

Additionally, an osmotic effect due to the pH gradient across the phospholipid membrane (inside–outside the vesicles) could promote destabilization and fusion.\(^{28}\) Studies to stabilize liposomes with bile salts and other surfactants reported an initial increase in the size followed by a decrease in the present study.\(^{42}\) The reduction in the vesicle size after 30 minutes of digestion may correspond to the detachment of

**Table 3** In vitro drug release kinetics of BME-loaded liposome and bilosome formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero-order</th>
<th>First-order</th>
<th>Higuchi</th>
<th>Korsmeyer–Peppas</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(R^2)</td>
<td>(K)</td>
<td>(R^2)</td>
<td>(K)</td>
</tr>
<tr>
<td>LS23</td>
<td>0.929</td>
<td>0.759</td>
<td>0.966</td>
<td>-0.005</td>
</tr>
<tr>
<td></td>
<td>0.978</td>
<td>7.085</td>
<td>0.976</td>
<td>0.759</td>
</tr>
<tr>
<td>BS3</td>
<td>0.952</td>
<td>0.951</td>
<td>0.980</td>
<td>-0.008</td>
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Fig. 5 (A) Stability of BME loaded liposomes LS23 and bilosomes BS3 in gastric fluid, (B) stability of BME-loaded liposomes and BME-loaded bilosomes in intestinal fluid.
loose aggregates formed initially, and this phenomenon rules out the possibility of a fusion of vesicles.

**Change in Vesicle Size and PDI of Nanovesicles during Intestinal Digestion**

Fig. 5 illustrates the stability of BME-loaded liposomes LS23 and bilosomes BS3 in simulated intestinal fluid. In the absence of pancreatin, the vesicle size of BME-loaded liposomes was 285 nm with a PDI of 0.5. After 1 minute of digestion with SIF, the vesicular size decreased to 282 nm and 276 nm after 30 min. PDI decreased to 0.37 after 1 minute and further reduced to 0.33 after 30 minute. In the absence of pancreatin, the vesicle size of BME-loaded bilosomes was 84 nm with a PDI of 0.38. After 1 minute of digestion with SIF, the vesicular size increased to 814.7 nm, then decreased to 651 nm after 30 minute. PDI was 0.43 after 1 minute of digestion and 0.74 after 30 minutes. Previous studies showed that the pancreatic enzyme in SIF could modify the organized structure of assembled lipids or damage the liposomal membrane severely, indicating that the liposomes exhibited lower stability in SIF than in simulated gastric fluid. The kinetic stability of the liposomal bilayer can be influenced by its composition and surface properties. Cholesterol, a principal constituent in many biological membranes, can increase bilayer thickness and membrane ordering and even reduce membrane permeability. Incorporating cholesterol has been assumed to provide physicochemical stabilization to liposomes.

**In Vivo Cognitive Enhancement Activity Studies of Nanovesicles**

BMs memory-enhancing and cognitive effects are believed to be mediated by bacoside A (bacoside A3, bacopacide II, bacopasaponin C, and bacopaside X). A recent report shows that BME inhibits selected human cytochrome P450 (CYP) drug metabolizing enzymes, in addition to altering the expression of rat hepatic and intestinal CYP drug metabolizing enzymes and intestinal P-glycoprotein. Thus, it is conceivable that the bacoside constituents in BME may be metabolized in vivo to active forms before exerting their pharmacological effects. Fig. 6A and 6B depicts the nootropic effect of BME-loaded liposomes and bilosomes on Swiss albino mice, compared with a formulation. Bilosomal delivery of BME significantly improved behavioral characteristics in dementia-induced mice than the extract, possibly because of the better absorption from GIT and better permeability via the BBB.

Table 4 provides the number of Y maze alternations reflecting the nootropic effect of BME and nanovesicles (LS23 and BS3). The bilosomes treated group showed more alternations in the Y-maze apparatus than the BME and liposomal vesicles. Better nootropic performance might be attributed to higher entrapment efficiency and, smaller bilosomes enhanced GIT penetration.

The protective effects of sodium deoxycholate on vesicle integrity might have improved BME’s oral absorption and bioavailability. Including negatively charged lipids changes the vesicle surface charge, affecting biodistribution, and clearance.

Scopolamine, a muscarinic antagonist, phosphorylates tau proteins leading to the formation of β-amyloid in the brain, resulting in memory impairment. Studies have shown that ethnollic extract of BM could prevent β-amyloid-induced toxicity in cultured neurons by inhibiting acetylcholinesterase enzyme and lipid peroxidation. In transgenic mice bearing human PS-1 and AβPP mutations, long-term treatment of BME reduces β-amyloid in different brain regions, possibly the reason behind cognitive enhancement. In the animal model of dementia, Brahma Ghrita (an ayurvedic preparation containing BM in ghee base) has shown a positive effect, possibly by reversing the depletion of acetylcholine, decreasing the choline acetyltransferase activity, and reducing muscarinic, cholinergic receptor binding in the frontal cortex and hippocampus, but also by alleviating cholinergic degeneration, lowering norepinephrine, and increasing 5-hydroxytryptamine levels in the hippocampus, hypothalamus, and cerebral cortex.
Conclusions

Due to suboptimal bioavailability, many phytoconstituents fail to validate ethnopharmacological claims in full measure. One cannot employ higher doses to overcome obstacles such as incomplete absorption, first-pass effect, etc. In this context, the present study has demonstrated the possibility of enhancing the pharmacological effects of BME by loading them in bilosomes. Various phytoconstituents with promising therapeutic or nutritional benefits can be formulated as vesicles. High-quality bioavailability evidence would entail using more advanced and direct techniques such as radio labeling.

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Conflict of Interest

None declared.

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Bacopa monnieri Extract-Loaded Bilosomes for Cognitive Enhancement

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