

Integrating Ancient Therapeutics into Nutraceutical Oral Films for Alzheimer's Neuroprotection

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ABSTRACT

Background: There are many popular neuroprotective medicinal plants mentioned in Indian traditional medicine systems which need to be made available to Alzheimer's patients in suitable dosage form. **Objectives:** Herbal nutraceutical in the form of oral fast dissolving film (OFDF) containing bioactive enriched fractions of traditionally well-known Indian nootropic plants will enhance neuroprotective efficacy and quality of life of Alzheimer's patients. **Materials and Methods:** The standardized extracts of Centella and Bacopa are prepared and formulated into oral films which are further evaluated for pharmaceutical parameters, stability, safety and behavioural animal studies. **Results and Conclusion:** The modernised dosage form of traditional medicines in the form of oral films found to reduce dosage frequency due to enhanced bioavailability of bioactive enriched herbal extracts and is easy for administration to Alzheimer's patients. Behavioural animal studies exhibited promising multitargeted neurocognitive efficacy of developed oral films.

Keywords: *Bacopa monnieri*, *Withania somnifera*, *Convolvulus pluricaulis*, Alzheimer's disease, neurocognitive efficacy, Dementia

INTRODUCTION

Alzheimer's disease is a gradually progressing neurodegenerative disorder that affects millions of people worldwide. Popular Indian Traditional nootropic plants like *Bacopa monnieri*, *Convolvulus pluricaulis* and *Withania somnifera* are rich in bioactives like triterpenoidal saponins bacosides, coumarins like scopoletin, and steroidal compounds like withanolides respectively which have proven neuroprotective efficacy¹. Despite of potency, high doses of powders or extracts need to be consumed for long time to get appropriate benefits. Again, geriatric patients especially suffering from Alzheimer's disease are found to be less cooperative to conventional dosage forms like tablets, capsules, syrups or solutions. To address this issue, Oral Fast Dissolving Films (OFDF) containing standardised doses of highly enriched bioactive fractions of Bacopa, Convolvulus and Withania plants developed to target multiple Alzheimer's disease causes; enhance bioavailability, patient compliance, and reduce dose as well as duration.

MATERIALS AND METHODS

Materials

Hydroxy propyl methyl cellulose 15 cps and 50 cps (HPMC) manufactured by CDH, New Delhi, India, Polyethylene glycol-400 (PEG) (CDH, New Delhi, India), Citric Acid, Sucrose, n-butanol, Ethyl acetate, Ethanol, Methanol, disodium hydrogen phosphate, Sodium dihydrogen Phosphate and Sodium hydroxide procured from CDH, New Delhi, India, Scopolamine Hydrobromide Injection manufactured by APP Pharmaceuticals LLC India.

Plant Material

In November 2020, Ayurvedic herbs, namely *Convolvulus pluricaulis* Choisy Convolvulaceae, *Bacopa monnieri* (L.) Wettst., Plantaginaceae and *Withania somnifera* (L) Dunal, Solanaceae procured from the Amravati region of Maharashtra, India. Plant materials authenticated by Department of Pharmacognosy, GCOP, Amravati, Maharashtra. The voucher specimens of selected herbs with accession numbers (GCOPA/CP/19-20/23, GCOPA/BM/19-20/24 and GCOPA/WS/19-20/25) were submitted to the Department of Pharmacognosy at the Government College of Pharmacy, Amravati for future reference.

Preparation and Standardisation of Bioactive Enriched Fractions

In this study, the hydroalcoholic extract (70%) of *Convolvulus pluricaulis* L. (CME) *Bacopa monnieri* L. (BME) and *Withania somnifera* L. (WME) were prepared by dissolving them separately in 100 ml of warm water with continuous stirring. The standardization of the preliminary extracts was designed with reference to the earlier reported Coumarins, triterpenoides and Withanolides separation techniques²⁻⁴. The resulting water-soluble extracts were then subjected to liquid-liquid extractions using 100 ml of n-butanol in each separating funnel for 30 minutes. The n-butanol layers were collected and concentrated using a rotary evaporator at temperatures below 40°C. The concentrated n-butanol fractions of *C. pluricaulis*, *B. monnieri* and *W. somnifera* were further processed with 50 ml of ethyl acetate to eliminate the impurities. Subsequently, the ethyl acetate-treated fractions were dissolved in 95% ethanol and

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filtered, and the filtrates were collected, concentrated, and dried. The resulting bioactive enriched fractions were named CSE (Convolvulus standardized extract), BSE (Bacopa standardized extract) and WSE (Withania standardized extract) and stored in airtight containers away from light for further analysis.

Formulation and optimisation of Oral Fast Dissolving Films

A solvent casting technique was employed with the aid of a film former device to produce herbal oral fast dissolving film dosages (OFDFs)^{5,6}. Placebo film trials were conducted before finalizing the ingredients for the OFDFs (As shown in Table 1). HPMC 15 cps and 50 cps were selected as the film polymers based on the outcomes of the placebo film formulation trials. Polyethylene Glycol-400 (PEG) was used as the film former, while citric acid was used as a salivary agent or disintegrating agent and sucrose was used to mask the bitter taste. The preparation method for the herbal OFDFs involved soaking HPMC 15 cps and HPMC 50 cps in 40 ml of distilled water separately for 24 hours. Standardized extracts of Convolvulus (CSE), Bacopa (BSE), Withania (WSE) and preliminary extracts CME, BME, WME were dissolved in combination (1:1) in an appropriate quantity of a mixture of ethanol and water (70:30) until the extract dissolved completely without forming lumps. The HPMC polymers were homogenized together over a magnetic stirrer at 150 rpm for 30 minutes. Sucrose and citric acid were dissolved in a separate beaker with the help of a small quantity of deionized water. The homogenized polymer was blended with the extract solution under constant stirring. The solution of sucrose and citric acid was added later to the mixture, and PEG-400 was added at the end of the mixture. The entire mixture was stirred using a magnetic stirrer at a speed of 199 rpm for one hour to achieve homogenization. After 1 hour, the mixture was kept still for 4 hours to remove the entrapped air bubbles. The bubble-free oral film formulation mixture was transferred to a dragger, which was previously calibrated with thickness in mm. The film was spread over the film former with the help of a dragger, and the temperature of the film former was optimized during the trial to obtain the best film. After a certain period

and cooling, the film was removed carefully and subjected to further evaluations.

Pharmaceutical Evaluation







Two different fast dissolving film formulations loaded with extracts, CBWM-OFDF (*Convolvulus-Bacopa-Withania* Mother Extract combination film) and CBWS-OFDF (*Convolvulus-Bacopa-Withania* standardized extract combination film), were prepared with a (1:1) ratio of extracts and evaluated for their visual appearance. Measuring the film's thickness was done using a Vernier Calliper, while its surface pH and folding endurance were assessed by repeatedly folding the film until it broke. The film's disintegration time was calculated by subjecting it to the Disintegration test apparatus with a phosphate buffer medium at $37 \pm 3^\circ\text{C}$.

The Dissolution apparatus type II USP paddle type was used to conduct the dissolution test at $37 \pm 3^\circ\text{C}$, with a phosphate buffer medium (pH 7.4) and a constant paddle speed of 50 rpm.⁸ The films were sliced into square shapes ($2 \times 2 \text{ cm}^2$) and put in the basket of the dissolution apparatus containing the buffer medium^{8,9}. Samples were withdrawn at specific intervals (0-10 min). A UV-Visible spectrophotometer was used to measure the drug concentration. The percentage of drug release was calculated using a calibration curve.

The compatibility between the drug and polymer in OFDF was determined using Fourier Transform Infrared UV Spectrophotometer (FTIR) by scanning the wavelength between 500 - 4000 (cm^{-2})⁹. An analysis of the surface and degree of extract blending with the polymer was conducted using SEM. Differential Scanning Calorimeter was used to measure the change in the physical properties of the film with temperature against time.

The developed oral film formulation was evaluated for its stability at an accelerated condition of 45°C temperature and 75% Relative humidity in the Environmental test chamber for three months^{8,9}. The stability of FDOF was determined concerning their weight variation, dissolution time, folding endurance, pH and thickness after storing them for three months.

Table 1. Placebo OFDFs trials and preliminary evaluation by appearance, folding endurance and thickness

Composition	OFDF1	OFDF2	OFDF3	OFDF4	OFDF5	OFDF 6
Polymer (w/w)	HPMC 50 cp - 4 % Sod. Alginate - 1 %	HPMC 50 cp - 1 % Gelatin - 0.5 %	HPMC15 cp - 2.9 % Gelatin - 1 %	Methyl cellulose - 3 %	Sodium alginate - 3 %	HPMC50 cp - 3 % HPMC15 cp - 3 %
Plasticizer (w/v)	Glycerol - 0.4 %	Polyethylene glycol - 400 - 0.4 %	PEG (Polyethylene glycol) - 3 %	Polyethylene glycol - 400 - 0.2 %	Plasticizer - Glycerol - 0.2 %	Polyethylene glycol 400 - 6 %
Salivary Agent (w/w)	Citric Acid - 0.4 %	Citric Acid - 0.3 %	Citric acid - 0.3 %	Citric Acid - 0.4 %	Citric Acid - 0.4 %	Citric Acid - 0.6 %
Taste Masking agent (w/w)	Sucrose - 1 %	Sucrose - 0.5 %	Sucrose - 0.5 %	Sucrose - 0.6 %	Sucrose - 0.6 %	Sucrose - 0.9 %
Solvent (v/v)	Water - 70 ml	Water - 70 ml	Water - 70 ml	Water - 60 ml	Water - 70 ml	Water - 60 ml
Film Appearance	Texture - rough Transparency - Opaque	Texture - Rough Transparency - very opaque, creates bad odor at the time of formulation	Texture - smooth and glossy Transparency - slightly transparent, creates bad odor at the time of formulation	Texture - Rough Transparency - Opaque	Texture - smooth Transparency - slightly transparent, creates bad odor at the time of formulation.	Texture - slightly rough due to bubbles Transparency - slightly transparent
Folding Endurance	76.03 \pm 0.2	57.12 \pm 0.23	84.54 \pm 0.5	85.08 \pm 0.32	72.12 \pm 0.11	92.34 \pm 0.34
Film Thickness (mm)	0.34 \pm 0.5	0.42 \pm 0.23	0.23 \pm 0.5	0.21 \pm 0.22	0.22 \pm 0.4	0.21 \pm 0.5
Observations						

Experiment performed in triplicate; Data expressed in mean \pm SD; data found significant with $p < 0.05$

In Vivo Comparative Neuroprotective Evaluation

Animals

The study was conducted in accordance with appropriate ethical guidelines (IAEC/GCOPA/2021/01, 02). Preclinical evaluation was conducted using Sprague-Dawley rats (male) 10-12 weeks old, weighing 300 - 350 grams. The rats were housed in cages and provided with appropriate diets, while being maintained under specific light and humidity conditions, following established guidelines. The light cycle was set at 12 hours of light and dark, at temperature of $22 \pm 5^\circ\text{C}$ and relative humidity of $65 \pm 5\%$.

Acute Toxicity Studies

Acute toxicity study was conducted as per OECD guidelines for acute toxicity testing¹¹. A total of 10 rats were included in each treatment group, and they were administered with fixed oral film dose of 2000 mg/kg based on their body weight. The films were administered through the use of oral gavages. The rats were then closely monitored for a period of 24 hours and were provided free access to food and water. Any occurrences of mortality were observed and recorded during this period.

Behavioural Studies

The present study investigated the potential neuroprotective effects of orally administered herbal OFDFs using a "Scopolamine-induced Alzheimer's disease-like model" in rats as shown in Figure 1¹². Adult Sprague Dawley rats were grouped (n = 6). A battery of behavioural assessment methods, including the Y-Maze and Morris Water Maze (MWM) (VJ Instruments), was conducted for 14 days. From day 1-14, rats of each group were administered with oral fast dissolving films (dose - 100 mg/kg and 200 mg/kg), extract combinations of preliminary and standardized extracts, standard drug Donepezil hydrochloride (10 and 20 mg/kg b.w) followed by scopolamine hydro bromide injection (10 mg/kg b.w) i.p after 30 minutes. Negative control group was administered daily with 10 mg/kg b.w of scopolamine hydro bromide injection intraperitoneally. From 11th to 13th day animals training for water Maze study was conducted, Actual experiment in Y maze and Morris Water Maze was conducted on 14th day of experiment. Rat activity was monitored using video tracking system (Maze master 5.0.0). The neuroprotective effects on spatial working memory were compared with those of the extracts based on study outcomes.

Statistical Analysis

Values were expressed as mean \pm SD. The experimental data analysis was performed by Single factor ANOVA. Data was considered statistically significant when $p < 0.05$

RESULTS AND DISCUSSION

Preparation and Standardisation of Bioactive Enriched Fractions

The percent yields of standardized extracts of *Convolvulus* (CSE), *Bacopa* (BSE) and *Withania* (WSE) were determined to be 1.9 % w/w, 2.1% w/w and 9.02 % w/w respectively. Upon characterization using HPLC (Figure 2), the percent purity of bacosides in the *Bacopa* standardized extract (BSE) was found to be 13.19 %. The percentage of scopoletin found in the *Convolvulus* standardized extract was 2.10 %, which is significantly higher than the level of bacosides (3.56%), scopoletin (1.05%) in the conventional form of extracts. While in case of *Withania* standardized extract, the level of Withaferin A was estimated by HPLC to avoid any possible cytotoxic effects due to enhanced levels¹². The level of Withaferin A in standardized extract found to be negligible. Therefore, this study confirmed the successful

standardization of *Convolvulus*, *Bacopa* and *Withania* standardized extracts, respectively through HPLC analysis.

Formulation Optimization and Evaluation of Oral Fast Dissolving Films

Choosing the right polymers for the formulation of oral fast-dissolving films (OFDF) is crucial in ensuring even drug release and complete binding of the drug to the polymer¹³. In the present study, various trial batches of placebo OFDFs were prepared and evaluated for their physical characteristics, and OFDF6 composition comprising of HPMC 50 cps and HPMC 15 cps polymers was found to be the most appropriate with a film thickness of 0.21 ± 0.5 mm and 92.34 ± 0.34 times folding endurance. Hence, a mixture of HPMC 50 cps and HPMC 15 cps as a polymer (1:1) ratio, Polyethylene glycol - 400 as a plasticizer, citric acid (salivary agent) and sucrose (film disintegrating as well as sweetening agent) was selected as the final composition for extract loading.

Two different fast-dissolving oral films of combinations of standardized extracts (BCWS-OFDF) (1:1 ratio) and preliminary extracts (BCWM-OFDF) (1:1 ratio) of *Bacopa*-*Convolvulus*-*Withania* herbs were prepared to evaluate the difference in neuroprotective efficacy. In physical characterization, BCWS -OFDF found to be partially opaque and smooth to feel (As shown in Table 2). BCWS - OFDF thickness was found to be within an acceptable range (0.21 ± 0.01 mm). The folding endurance of the film was found to be in the range of 109 -114 times, indicating good flexibility and plasticity of the film. The surface pH of the film is an essential criterion to determine the acidic pH that may irritate the oral mucosa as a side effect. The pH of both films was found near to the neutral pH value ($6.74 - 6.78$)¹¹. The disintegration time was slightly higher than the normal disintegration time of synthetic drug oral fast dissolving films (2.19 ± 0.03 min to 2.29 ± 0.01 min) due to the complex formation between polymers and functional groups of phytochemicals in the film. However, the obtained disintegration time was within an acceptable range as there is no standard disintegration value given for fast-dissolving oral films. In conclusion, the prepared oral fast-dissolving films - BCWS-OFDF and BCWM-OFDF were found to be suitable for further *in vivo* study.

In-vitro Drug Release Studies

The drug release pattern of the developed oral fast-dissolving films *in vitro* was evaluated using a dissolution apparatus in a buffer medium with a pH of 7.2 and a temperature of $37 \pm 3^\circ\text{C}$. The drug release percentage from BCWS-OFDF was found to be 72.72 % after 3 minutes (as shown in the figure 3A), while BCWM-OFDF (as shown in the figure 3B) demonstrated a drug release of 73.60 % after 3 minutes. Both films showed a comparable drug release pattern.

Drug Excipient Interaction Studies

The assessment of drug-excipient interaction was conducted by comparing the FTIR spectra of the placebo film, as well as combination films BCWS-OFDF, BCWM-OFDF, and combinations of preliminary extracts (BCWM) and standardized extracts (BCWS). The IR spectra of the placebo film displayed strong peaks at 1163 cm^{-2} , indicating C-O stretching of the ester group, 1396.52 cm^{-2} , indicating O-H bending of alcohol, 956.73 cm^{-2} , indicating C=C stretching of alkene, 1705.15 cm^{-2} , indicating C=O stretching of carboxylic acid, and 1473.68 cm^{-2} , indicating C-H bending of a methyl group, which are characteristic of the HPMC polymer. When compared to the IR spectra of extracts, the extract spectra lacked the characteristic peak of frequency 956.73 cm^{-2} , but it was present in extract-loaded films, indicating the incorporation of the extract into the polymer matrix. Some variations in the stretching and functional groups of extracts observed in the film suggest that the drug forms a complex and is embedded in the polymer matrix.

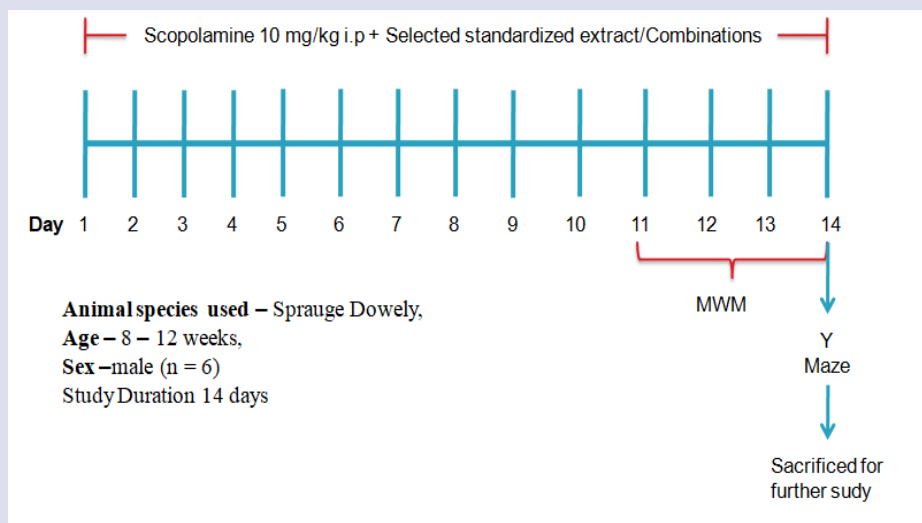


Figure 1. Behavioral Study layout

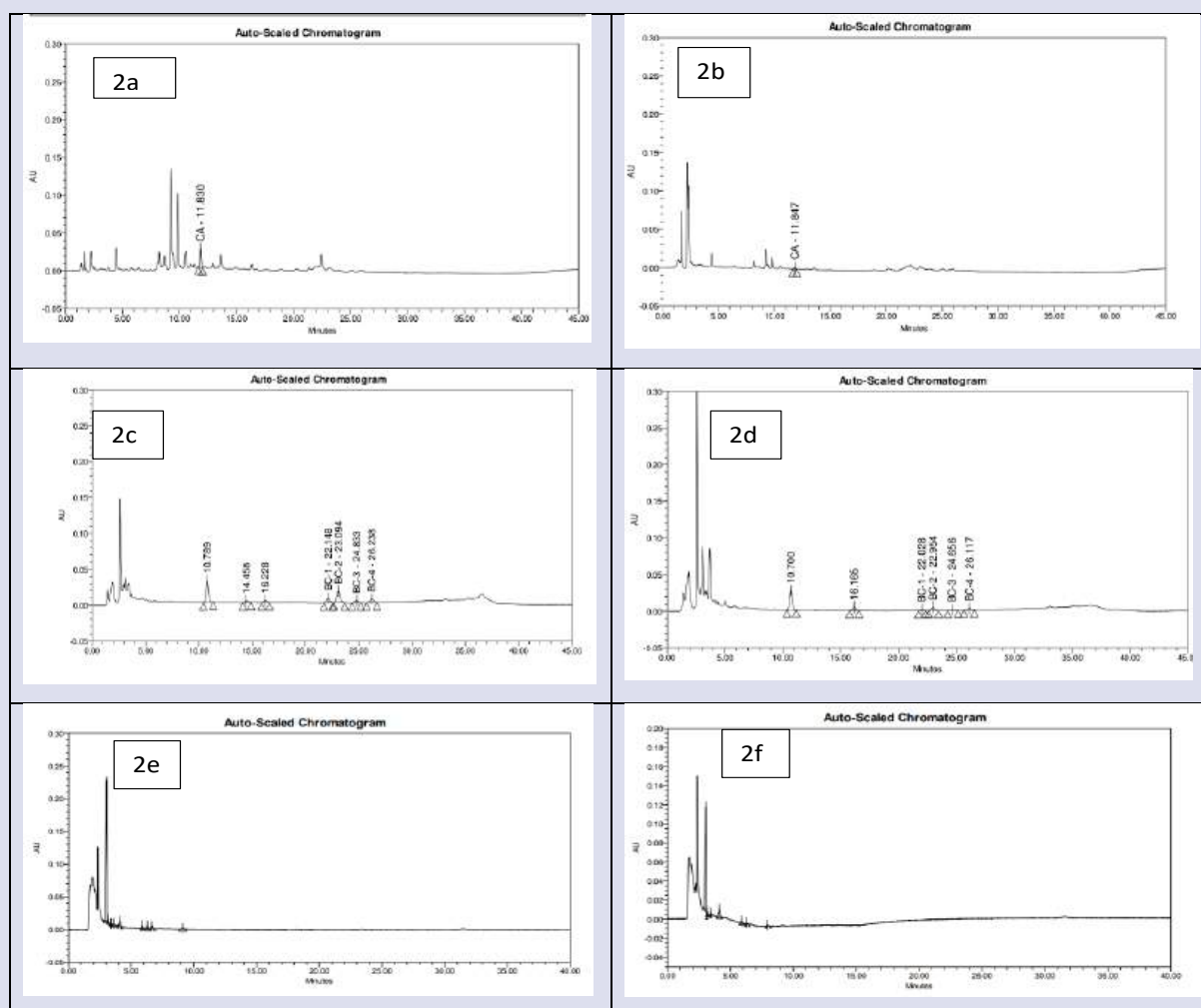


Figure 2. HPLC Chromatograms of bioactive rich fractions where a: Chromatogram of *C. pluricaulis* standardized extract (CSE); RT - 11.3; Peak area – 190185 b: Chromatogram of *C. pluricaulis* Preliminary extract (CME); RT - 11.847; Peak area – 22036 c: Chromatogram of *B. monnieri* standardized extract (BSE); RT - BC1 - 22.14, BC2 - 23.09, BC3 - 24.83, BC4 - 26.23; Peak Area - BC1- 143153, BC2 - 353436, BC3 - 6424, BC4 - 112165 d: Chromatogram of *Bacopa monnieri* Preliminary extract (BME); RT - BC1 - 22.028, BC2 - 22.954, BC3 - 24.656, BC4 - 26.117; Peak Area - BC1- 28646, BC2 - 91990, BC3 - 18649, BC4 - 42653 e: Chromatogram of *W. somnifera* Preliminary extract (WME); RT - 17.083, Peak Area – WA – Negligible f: Chromatogram of *W. somnifera* standardized extract (WSE); RT - 17.083, Peak Area – WA – Negligible

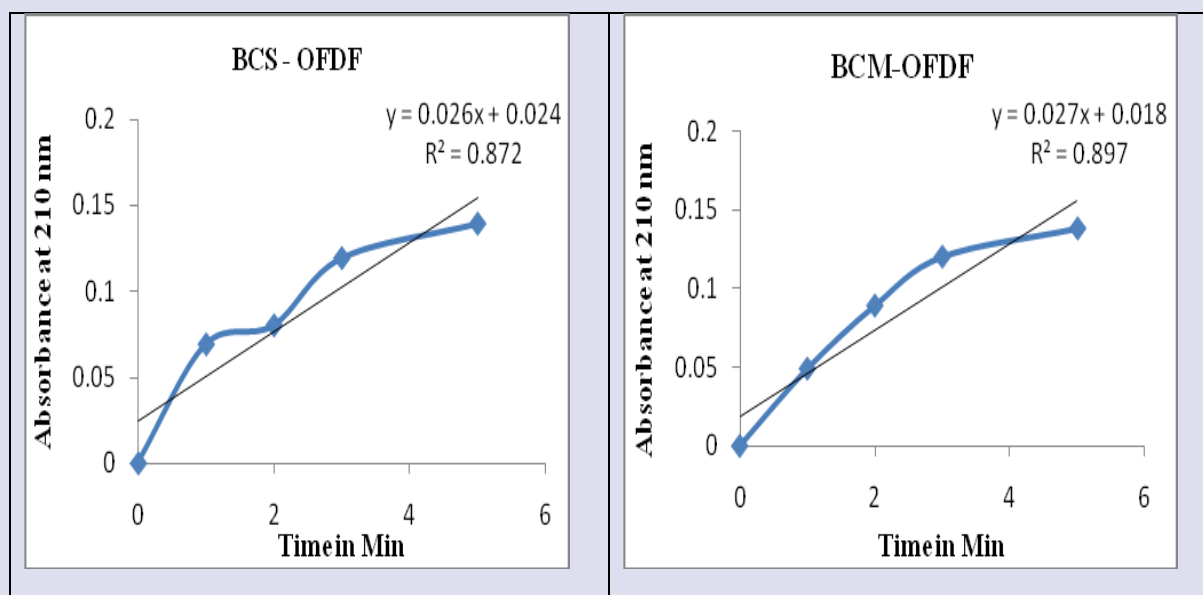


Figure 3. Drug release pattern of different films where a: Drug release pattern of BCWS –OFDF. b: Drug release pattern of BCWM –OFDF

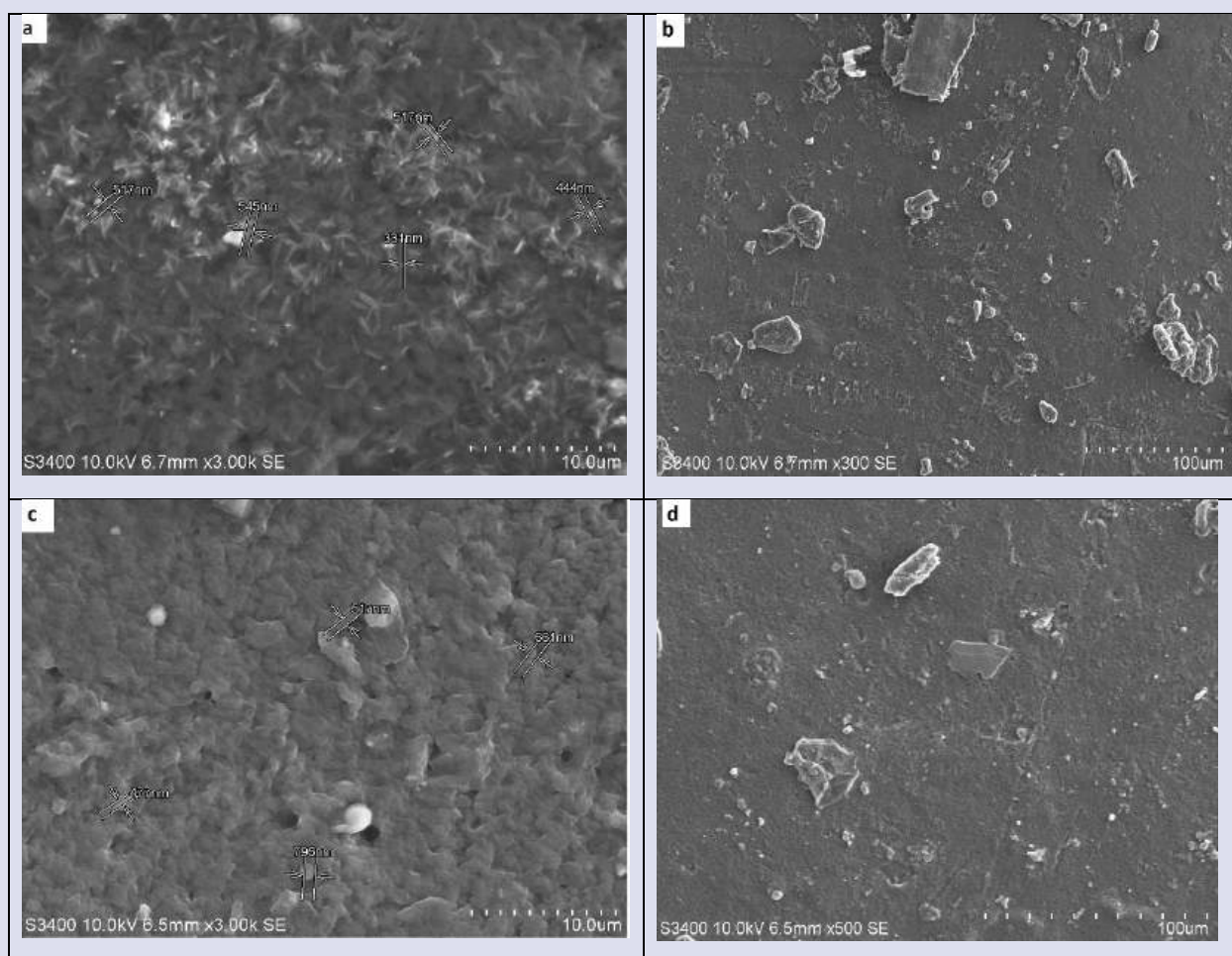


Figure 4: SEM images of different films where **a:** SEM image of BCWM-OFDF showing particle size 300 – 450 nm of extract embedded in HPMC matrix. **b.** SEM image of BCWM-OFDF showing roughness of film due to aggregates of extract particles on surface. **c:** SEM image of BCWS-OFDF showing particle size 400 – 800 nm nm of extract embedded in HPMC matrix. **d:** SEM image of BCWS-OFDF showing roughness of film due to aggregates of extract particles on surface

However, no significant abnormal complex formations were observed in the IR spectra of OFDF.

Surface Morphology Studies

The surface morphology and extent of extract blending with polymer in BCWS-OFDF and BCWM-OFDF was analyzed using scanning electron microscopy (SEM). SEM analysis of both films revealed a slightly rough film surface due to the presence of a few aggregates of excess extract particles during film drying (as shown in the figure 4 a, b, c, d). The porous matrix structure of the HPMC polymer was observed in both films. The extracts were found to be uniformly embedded in the HPMC polymer porous matrix with maximum efficiency. The particle size of the embedded BCWM extract combination was found to be 300 – 450 nm while the particle size of the embedded BCWS extract combination was between 400 – 800 nm.

Thermal behaviour and Physical Stability studies

Differential Scanning Calorimeter (DSC) graph of the blank film (as shown in Figure 5) reveals that melting occurs at 90.68°C with a heat flow of 11.58 W/g. The film retains the lost energy during melting, and at 130°C, slight crystallization occurs at 11.58 W/g. The film then undergoes melting at 216°C with a heat flow of 13.28 W/g, and again retains the lost energy before undergoing another melting process at 355°C with a heat flow of 14.67 W/g.

In the DSC graph of BCM-OFDF, crystallization begins at 99.85°C with a heat flow of 9.04 W/g. The film retains the lost energy during crystallization, and at 144°C, slight melting occurs at 10.44 W/g. The film then undergoes crystallization at 236.61°C with a heat flow of 13.58 W/g, and retains the lost energy once again (as shown in Figure 5).

In the DSC graph of BCWS-OFDF, melting starts at 91.86°C with a heat flow of 8.077 W/g. The film retains the lost energy during melting, and at 159.69°C, slight crystallization occurs at 10.44 W/g. The film then undergoes slight melting at 264.14°C with a heat flow of 13.08 W/g (as shown in figure 5).

From these three graphs, it can be observed that the melting and crystallization pattern of BCWS-OFDF and the placebo polymeric film are similar. The huge changes in melting and crystallization patterns due to the polymer and loaded bioactive fraction mixture are not significantly observed. However, a somewhat distinct pattern of crystallization and melting was detected in the case of BCWM - OFDF. This may be attributed to complex formation with other phytoconstituent functional groups present in the BCWM extract.

Stability Studies

Upon completion of the 3-month stability study, BCWS-OFDF and BCWM-OFDF oral films were subjected to physical parameter analysis, including appearance, taste, folding endurance, and weight variation. The results are presented in the table 3. No significant alterations were observed in appearance, folding endurance, weight, and taste, indicating that both films have successfully passed the stability test.

In Vivo Comparative Neuroprotective Evaluation

Acute toxicity Studies

During the 24-hour study, there were no instances of mortality observed. Saliva secretion was observed for 30 minutes immediately after oral film administration, as expected, since the formulation contains a saliva stimulating agent to enhance film degradation. Restlessness was observed in a few rats during the first 30 minutes, but after a 4-hour period, all rats were observed to be normal. Gross pathological examination performed after 24 hours did not reveal any unusual stains or scars on tissues, nor was any noticeable changes observed in internal tissue appearance. Over the 14-day observation period, animals exhibited normal eating and drinking habits, and no abnormal behavior was observed. Thus, the oral film formulations containing Bacopa-Centella-Withania standardized extracts were determined to be safe for consumption, with a lethal dose value (LD₅₀) greater than 2000 mg/kg body weight in animals.

Behavioural Studies

The neuroprotective effectiveness of fast-dissolving oral films, extracts, and the standard drug Donepezil HCl, were compared to the Control group using the Y-maze and Morris Water Maze behavioral study models. The Y-maze study assessed spatial working memory by measuring the spontaneous alterations of rats after administering the oral film formulations, extracts, and standard drug with the anticholinergic drug scopolamine. According to the findings, BCWS -OFDF at a dosage of 200 mg/kg was found to be just as effective in preserving memory as the blend of Convolvulus-Bacopa-Withania standardized extract at a dose of 500 mg/kg. Similar observations noted for BCWM- OFDF and BCWM extract. In the Morris Water Maze study, the escape latency and retention time parameters were measured. The rats administered with fast-dissolving oral film formulations showed a significant improvement in both parameters compared to the extract combinations. (Figure 6) This study confirmed that the oral film formulation enhances the neuroprotective efficiency of Convolvulus-Bacopa-Withania extracts by increasing the

Table 2. Outcomes of physical characterization of BCS-OFDF and BCM-OFDF

Sr. No	Physical Parameters	BCWS-OFDF	BCWM-OFDF
1	Appearance		
		Partially transparent, opaque and partially smooth in texture	Non transparent, opaque and partially smooth in texture.
2	Thickness (mm)	0.21±0.01	0.21 ±0.02
3	Folding Endurance	109.47±3.26	113.73±2.65
4	PH	6.78±0.14	6.74±0.14
5	Disintegration Time (Min)	2.19±0.03	2.29±0.01
6	Weight Variation (gm)	0.1236±0.001	0.1238±0.000

Physical evaluation was performed in triplicate; Data expressed in mean ± SD; data found significant with p < 0.05

Table 3. 90-days stability study outcomes of BCWS-OFDF and BCWM-OFDF

Evaluation Parameters	BCWS-OFDF		BCWM-OFDF	
	0 Months	3 Months	0 Months	3 Months
Appearance	 Colour: dark brown Odor: characteristic Taste: Tangy after bitter	 Colour: dark brown Odor: characteristic Taste: Tangy after bitter	 Colour: dark brown Odor: characteristic Taste: Tangy after bitter	 Colour: dark brown Odor: characteristic Taste: Tangy after bitter
Weight Variation (mg)	0.122 ± 0.34	0.120 ± 0.34	0.122 ± 0.34	0.120 ± 0.34
Time to dissolve the film (min)	2.29 ± 0.69	2.27 ± 0.62	2.29 ± 0.69	2.27 ± 0.62
Folding endurance	112.6 ± 4.70	110.6 ± 4.20	112.6 ± 4.70	110.6 ± 4.20
PH of films	6 to 7 [slight acidic to Neutral]	6 to 7 [slight acidic to Neutral]	6 to 7 [slight acidic to Neutral]	6 to 7 [slight acidic to Neutral]
Thickness (mm)	0.21±0.05	0.21±0.05	0.21±0.05	0.21±0.05

Experiment performed in triplicate; Data expressed in mean ± SD; data found significant with p < 0.05

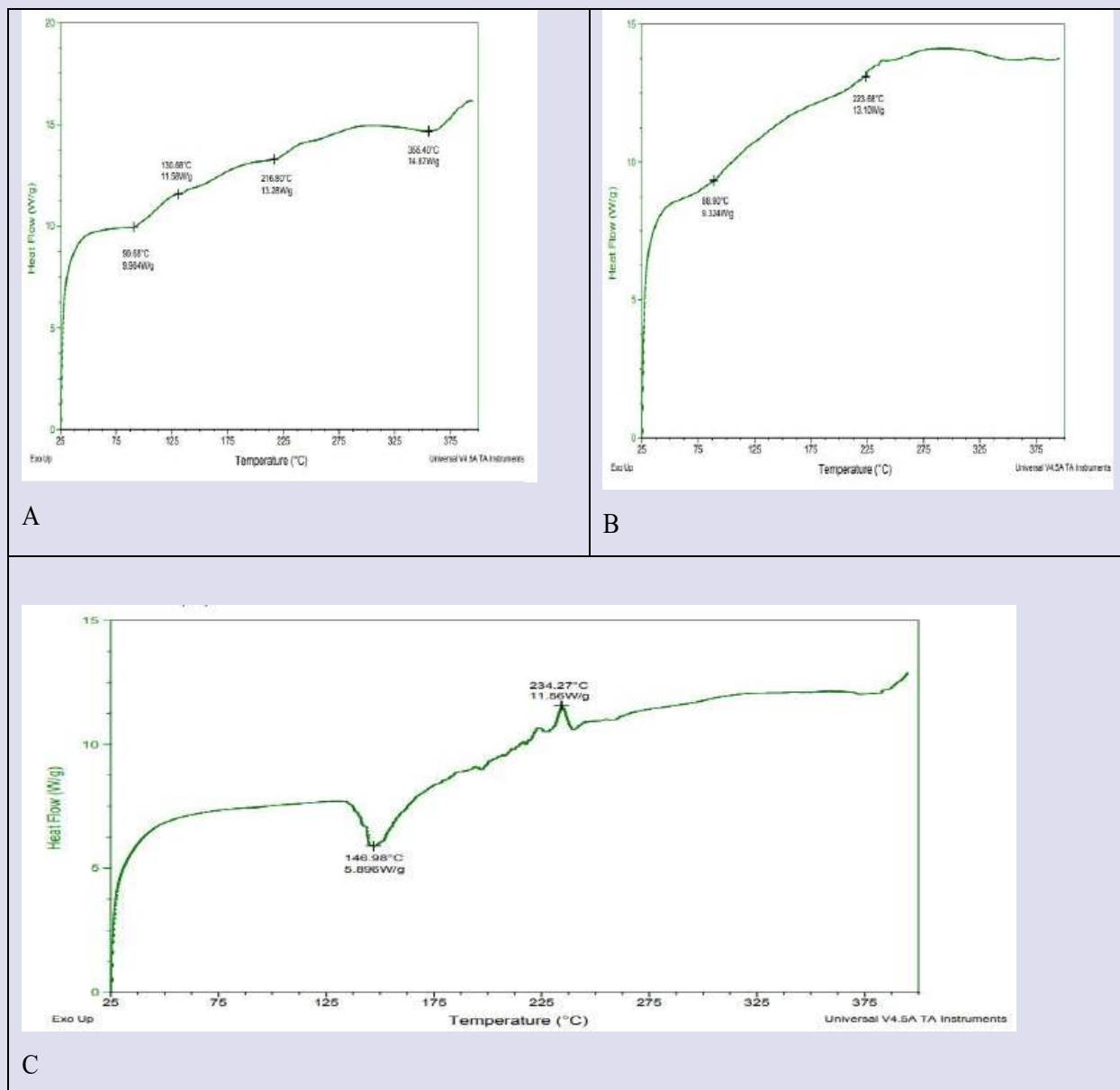


Figure 5. A: DSC graph of placebo OFDF. **B:** DSC graph of formulation BCWM – OFDF. **C:** DSC graph of formulation BCWS – OFDF

Table 4. Behavioural study outcomes of the film formulations and extracts

Sr. No	Group names	Dose	Y - Maze	Morris Water Maze (MWM)	
			% Spontaneous Alterations	Escape Latency	Retention Time
1	BCWS - OFDF	100	51.11±31.68	24.67±28.99	37.33±22.05
		200	69.67±20.82	18.67±18.72	46.33±12.66
2	BCWM-OFDF	100	40±40	25.67±22.14	32.67±21.03
		200	49.44±30.37	20.33±21.73	35±23.07
3	BCWS	200	63.33±23.76	20.00±7.00	21.33±14.29
		500	68.22±12.33	15.00±10.15	34.33±23.35
4	BCWM	200	43.33±40.41	30.33±22.01	26.00±19.70
		500	51.22±45.51	24.00±24.43	29.33±15.95
5	Donepezil HCl	10	44.44±13.88	31.66±21.03	42.33±13.05
		20	55.55±31.50	25.33±17.66	44±13.75
6	Scopolamine	10 mg/ml	36±40.60	45±13.23	26.33±16.29
7	Control		53.33±30.55	25.33±21.46	43.67±16.44

Values are expressed as mean ± SD. The data was analyzed by Single factor ANOVA and data found statistically significant with p < 0.05

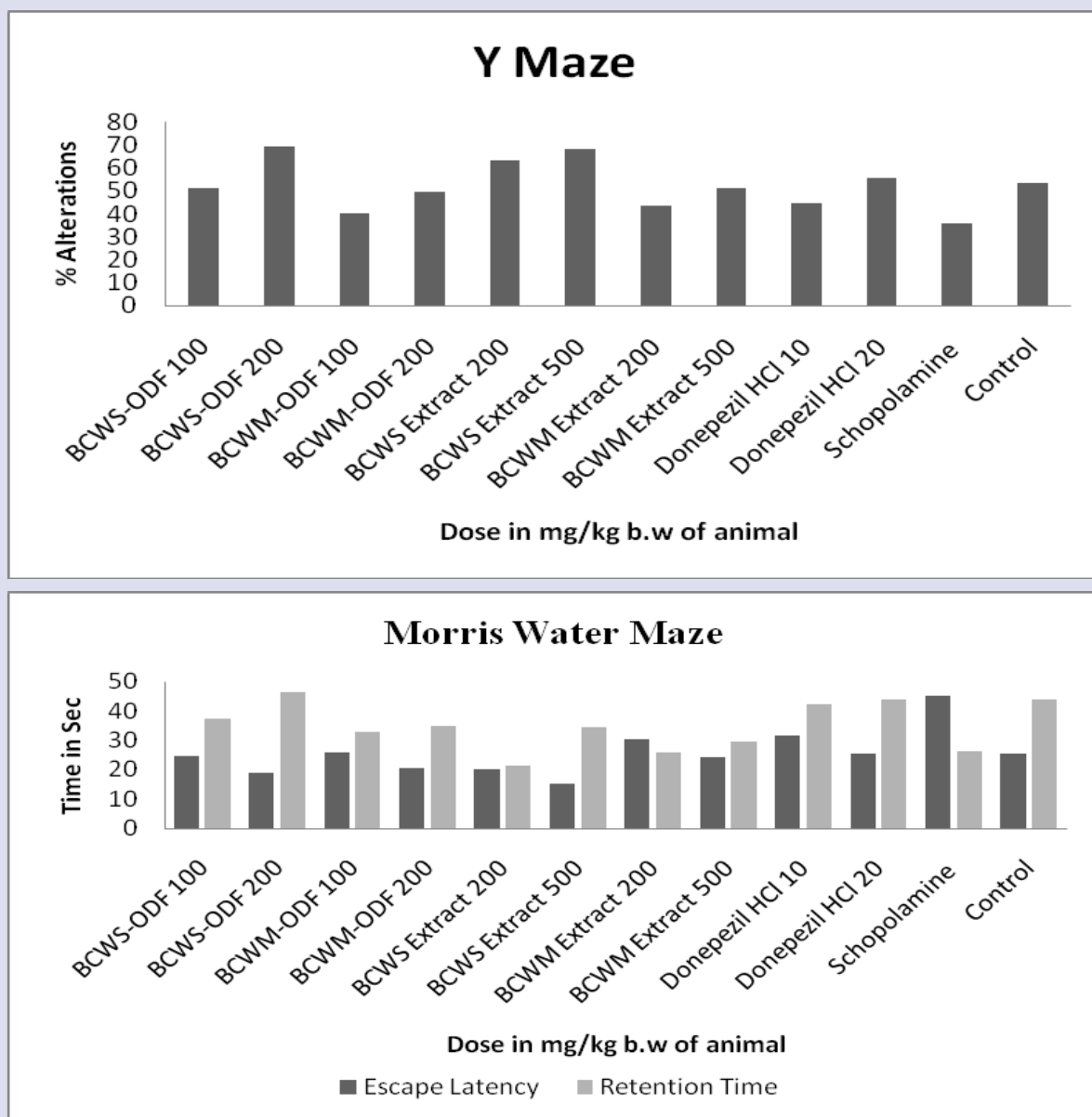


Figure 6. Y maze and Morris Water Maze study outcomes of Convolvulus-Bacopa-Withania Standardized extract & Mother extract fast dissolving oral films and Convolvulus- Bacopa-Withania standardized, mother extracts

bioavailability of neuroprotective phytoconstituents. The enrichment of bioactive in plant extracts is an added advantage to achieve the maximum therapeutic effect at a lower dose, and it also reduces the drug loading amount, which is highly favorable for ODF formulation (Table 4).

CONCLUSION

The developed fast-dissolving oral films incorporating bioactive-rich fractions of *Convolvulus*, *Withania*, and *Bacopa* represent a scientifically advanced transformation of traditionally acclaimed Asian medicinal herbs into a modern, patient-friendly neurotherapeutic system. By integrating Indian ancient herbal wisdom with contemporary drug delivery technology, the study offers a promising approach toward safer, purer, and more effective management or adjunct therapy for neurodegenerative disorders, particularly Alzheimer's disease and associated cognitive decline. These formulations may emerge as an innovative and impactful alternative for the multitargeted management of Alzheimer's-associated memory impairment and neurodegenerative dysfunction, offering patients a more acceptable, effective, and holistic therapeutic option.

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