Chapter

Phospholipid Based Nano Drug Delivery Systems of Phytoconstituents

Mohammad Hossain Shariare and Mohsin Kazi

Abstract

The development of phytochemistry and phyto-pharmacology has enabled elucidation of composition and biological activities of several medicinal plant constituents. However phytoconstituents are poorly absorbed due to their low aqueous solubility, large molecular size and poor membrane permeability when taken orally. Nanotechnology based drug delivery systems can be used to improve the dissolution rate, permeability and stability of these phytoconstituents. The current chapter aims to present the extraction of phytoconstituents, their identifications, and development/utilization of phospholipid based nano drug delivery systems (PBNDDS). The content of the chapter also provides characteristic features, *in-vitro, in-vivo* evaluations and stability performance of PBNDDS. The results from the UHPLC and GC-MS showed different phytoconstituents in the extracted samples with quantitative value. Dynamic light scattering (DLS) data showed PBNDDS of different phytoconstituents in the range of 50–250 nm with PDI value of 0.02–0.5, which was also confirmed by the electron microscopic data. Phytoconstituents loading or entrapment for PBNDDS was in the range of 60–95%. PBNDDS exhibited better *in-vitro* and *in-vivo* performance with improved Physico-chemical stability.

Keywords: phospholipid, liposome, phytosome, epigallocatechin gallate (EGCG), phytol, *Aphanamixis polystachia*, thymoquinone

1. Introduction

Phospholipid based nano drug delivery systems (PBNDDS) are becoming more promising due to its biocompatibility, amphiphilic characteristics, Physico-chemical stability and can be prepared for different diseases with sustain release and targeted delivery of different drugs [1]. PBNDDS can protect the drug from biodegradation, transformation and reduce cell toxicity by altering the bio-distribution. PBNDDS are easy to scale-up, sterilize in product development and cost effective. PBNDDS performance depends on size, morphology of particles and possesses some unique properties like surface area to mass ratio which is larger than other colloidal systems. Controlled release and targeted drug delivery depend on the rate and mechanism of drug release from the carrier based drug delivery systems like PBNDDS, which can vary depending on the formulation, processing and routes of administration [1–6].

Phospholipids are heterolipids which can be extracted from both animal and plant origin, have been shown to generate lipid matrices of low crystallinity.

Figure 1.
Phospholipid based nano drug delivery systems (PBNDDS) [1–3].

Different types of phospholipid based nano drug delivery systems (**Figure 1**) are used for both synthetic and natural source of drugs [7, 8].

Natural source of medicines have been used from ancient time [9]. Phytoconstituents present in plants having different pharmacological properties are useful substitutes to synthetic drugs. There are over 100 active ingredients derived from natural plants for use as drugs and medicines. Chronic inflammatory (stroke, chronic respiratory diseases and heart disorders), and central nervous system (CNS) diseases are major cause of global mortality. Different synthetic drugs used to treat these diseases results in severe adverse effects. Research is going on for the development of new drugs from natural medicinal plants [10].

Phytoconstituents showed strong anti-inflammatory activities due to their strong free radical scavenging action [11] and have shown beneficial effects on cancers, diabetes, cardiovascular diseases, stroke and obesity etc. Phytoconstituents also exhibit activity against neurodegenerative diseases (Alzheimer's disease, and Parkinson's disease) through different pathways [12–21].

2. Natural plant extract and phytoconstituents

In this chapter four different plant extracts and its phytoconstituents (Black seed oil containing thymoquinone, Jute leaf extract containing phytol, *Aphanamixis polystachia* leaf extract, green tea extract containing EGCG) are discussed (**Figure 2**), which were formulated as phospholipid based nano drug delivery systems (PBNDDS).

Thymoquinone a natural component of Black seed oil, which can be obtained from the seeds of *Nigella sativa*, found to have different pharmacological activity for the treatment of various diseases [22–26]. However, despite the various pharmacological properties of thymoquinone, its administration *in-vivo* remains crucial due to its poor water solubility and stability issues. Therefore an advanced drug

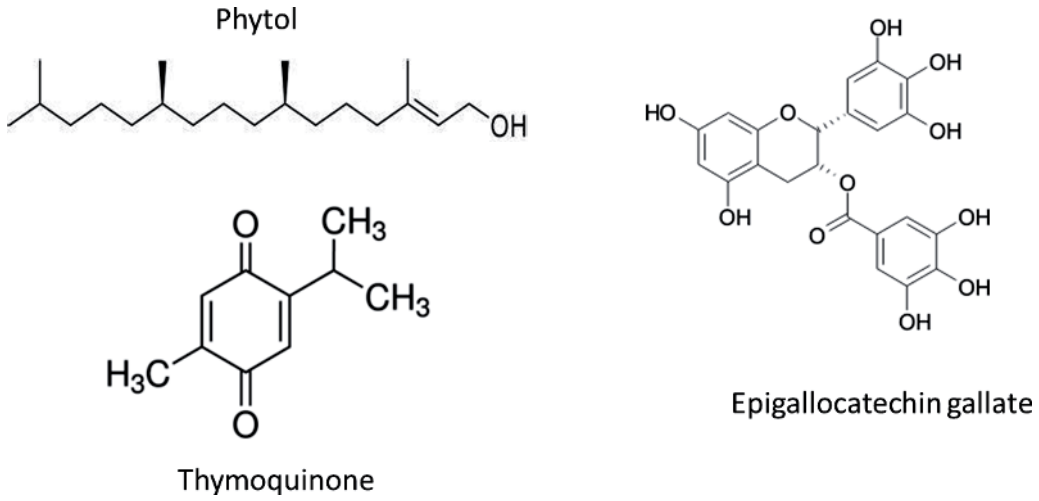

Figure 2.
Chemical structure of phytol, thymoquinone and epigallocatechin gallate.

delivery system is required to improve the therapeutic outcome of thymoquinone by enhancing the solubility and stability in water [27].

Jute leaf obtained from *Corchorus olitorius L.* [28, 29] has been used as traditional medicine. Jute leaf extract contains different phytoconstituents which are medicinally active and exhibits pharmacological effects against different diseases [30–33]. Phytol is one of the main phytoconstituents found in jute leaf extract demonstrates pharmacological activity against different diseases and in different *in-vitro* cell line studies [29, 34]. Phytol due to its multiple ring structure shows poor water solubility and absorption through the intestinal wall.

Aphanamixis polystachya a natural plant which contains phytoconstituents found to have different medicinal activities [35–38]. Leaf extract of *A. polystachya* plant showed CNS activities [39], therefore in this chapter *A. polystachya* leaf extracts and its phospholipid based nano drug delivery system (PBNDDS) activity against animal model of dementia is discussed.

Epigallocatechin-3-gallate (EGCG) is a main potent constituent of green tea extract (*Camellia sinensis)*, which is one of the major catechins [40]. EGCG exhibit pharmacological activity against different diseases [41, 42] and also showed activity against carcinogenic effects in different animal models with different cancers [43–46]. EGCG has high water solubility however it exhibits low permeability across the gastrointestinal tract (GIT) leading to poor bioavailability [47, 48].

3. Issues with natural phytoconstituents

Phytoconstituents showed a range of pharmacological activity and less side effects compared to synthetic drugs; however phytoconstituents exhibit low water solubility, poor permeability through gastrointestinal tract and impede fast systemic clearance [49]. Physical and chemical stability of phytochemicals is another issue [50–54]. Treatment of CNS and cancer diseases require targeted drug delivery for better therapeutic outcome. Nano drug delivery systems may be a promising platform for the improvement of aforementioned issues of natural plant extracts and their phytoconstituents. Therefore phospholipid based nano drug delivery systems of natural phytoconstituents could be the potential delivery system with better performance and stability [55].

4. Nanoparticle based drug delivery of phytoconstituents

Novel nano drug delivery systems can improve the solubility, permeability, physicochemical stability and reduce toxicity of drugs [52]. Previous studies showed that the phospholipid based nano drug delivery systems can improve the oral delivery of thymoquinone [56, 57], and effective against breast cancer cell line. Mesoporous silica and chitosan nanoparticles are developed for delivery of thymoquinone to the brain [58]. In other study self nanoemulsifying and alginate beads delivery system were developed to improve the bioavailability, stability and targeted delivery of black seed oil [59].

Nanoparticulate based drug delivery system of phytol was used for Alzheimer's disease [60]. Previous research also showed strong cytotoxic, anti-phytopathogenic and hepatoprotective effect of phytol loaded nano drug delivery systems [61, 62]. Phospholipid based nano formulation of EGCG are developed to enhance the release characteristics, bioavailability, and stability [63–66]. Previous study data suggest that nanoparticulate based delivery of EGCG showed better cytotoxic and *in-vivo* performance compared to pure EGCG [67–69].

5. Phospholipid based nano drug delivery systems (PBNDDS) of plant extracts and phytoconstituents

Two types of phospholipid based nano drug delivery systems (PBNDDS) have been discussed in this chapter for four different natural extracts and its phytoconstituents, which are liposomes and phytosomes.

Liposome is a phospholipid based lipid bilayer vesicles where both hydrophilic and lipophilic drugs can be entrapped. Liposomal drug delivery system has become a budding technology for delivering drugs to improve the bioavailability, efficacy, safety and stability of both synthetic and natural source of medicines [70, 71]. Liposomal drug delivery system can be used to deliver drugs for neurodegenerative diseases through blood brain barrier (BBB) [72–74].

Phytosomes are structures prepared using natural plant extract with phospholipid matrix. Phytosomal delivery system can improve the absorption and bioavailability of phytoconstituents. In phytosome drug form complex with phospholipid like matrix formation while in liposomes, drug is entrapped in the core or lipid bilayer of phospholipids. Phytoconstituents of plant extracts showed better biological activity when delivered through phytosomes [75–79].

This chapter is mainly focused on the development, preparation and solid state characterization of liposomal drug delivery systems of black seed oil, *A. polystachya* leaf extracts and *Corchorus olitorius* leaf extracts and their main phytoconstituents. Phytosomal delivery system development of green tea extract and EGCG is also discussed with different solid state characterizations. Finally stability, *in-vitro* and *in-vivo* studies were discussed for phospholipids based nano preparations of all extracts and their phytoconstituents.

Phospholipid can be extracted from both plant and animal source of origin. Phospholipid used in these studies was extracted from egg yolk, which is known as lecithin or egg lecithin. Results (UHPLC data) showed the presence of phosphatidylcholine (PC) peak (the main phospholipid component for liposome) and suggest that per gram of egg lecithin contain 100–200 mg of PC, where filtrate of egg phospholipid contain the most of the PC content compared to solid residue (**Table 1**) [80]. Phosphatidylcholine (PC) content was also quantified for peanut using UHPLC analysis and results demonstrate that less amount of PC is present in per gram of peanut (**Table 1**).

Sample no/name	Mass (mg/g)	%
Egg sample 1 (filtrate)	212.80 ± 3.22	21.38
Egg sample 2 (solid residue)	9.71 ± 1.19	0.97
Peanut	5.56 ± 0.27	0.56

Table 1.
Amount of PC present in egg yolk. Sample 1 represents extracted PC as filtrate and sample 2 represents PC as solid residue. Data are mean ± SD (N = 3).

6. Plant extraction, identification and quantification by UHPLC and GC-MS

All four plants described in this chapter was extracted using maceration method (**Figure 3**). Plant extraction, its phytoconstituents identification and quantification were performed using UHPLC and GC-MS analytical methods [80–83]. Results from UHPLC data showed that the concentration of thymoquinone was 2.28 ± 0.68 mg/g of black seed oil [80].

Table 2 shows main phytoconstituents determined for *Aphanamixis polystachya* leaf extracts using GC-MS including Octadec-9-enoic acid, hexadecanoic acid, 2-Pentanone, 2-hydrazino-2-imidazoline and beta-elemene etc. [81]. Previous researches in this area suggest that these phytoconstituents exhibit strong antioxidant, anticancer and anti-inflammatory property [84–88], which found to have impact in neurodegenerative disorders including stroke [89–92]. However few of these phytoconstituents have poor solubility in water.

Major phytoconstituents present in the methanolic extract of *Corchorus olitorius* leaf are mentioned in **Table 3**, which are oleic acid, hexadecanoic acid, and

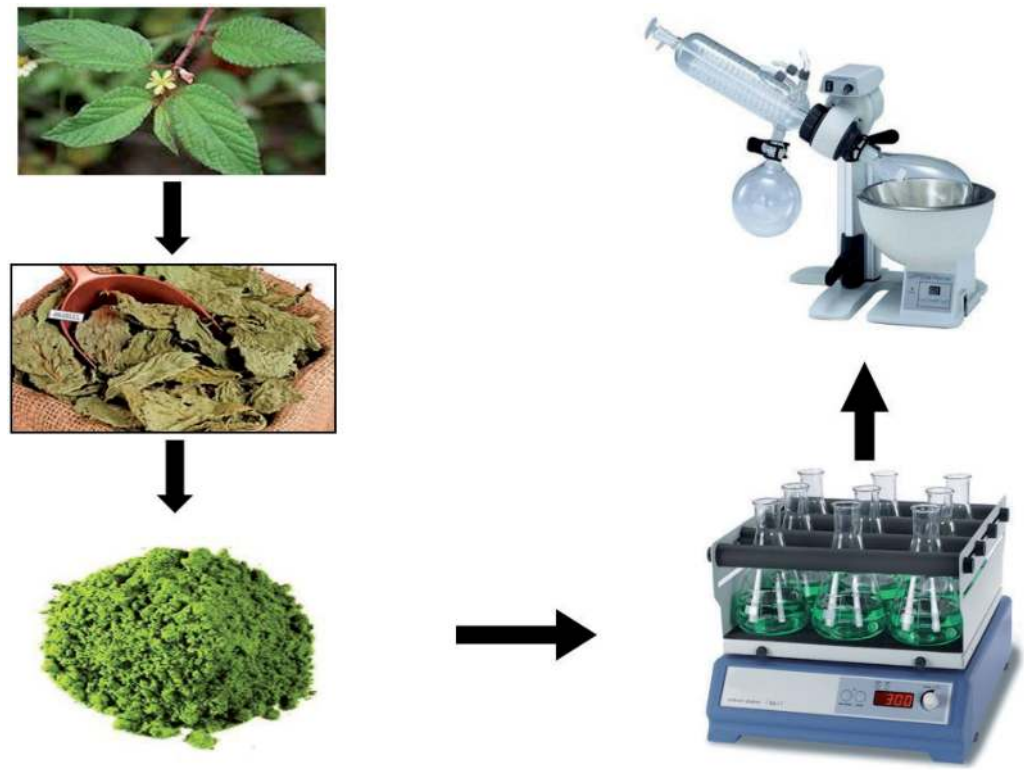

Figure 3.
Plant extraction process using maceration method.

No.	Name	RT	Area %	N area %
1	4-hexen-2-one	4.68	1.72	6
2	Acetic acid, butyl ester	5.06	2.36	8.24
3	3-acetoxydodecane	5.68	1.83	6.39
4	2-pentanone	5.88	14.62	51.08
5	4,4-dimethyl-1-hydroxy-2-cyclo	8.94	2.55	8.92
6	Acetic acid, hexyl ester	10.78	4.73	16.53
7	1,2-cyclohexanediol	13.76	4.12	14.39
8	Acetic acid	15.4	4.05	14.14
9	2-hydrazino-2-imidazoline	17.6	3.41	11.91
10	Beta-elemene	22.4	0.4	1.39
11	5-hydroxypipecolic acid	29	1.88	6.56
12	2-hexadecen-1-ol, 3,7,11,15-TE	33.36	0.63	2.19
13	Octadecanal	33.48	0.67	2.36
14	9-hexadecenoic acid	35.88	5.66	19.77
15	Hexadecanoic acid	36.32	8.83	30.84
16	4-hydroxytetradec-2-ynal	38.96	1.69	5.89
17	Octadec-9-enoic acid	39.72	28.63	100
18	1.beta.-allylperhydro-2.alpha.	44.32	1.32	4.6
19	Cyclopentadecanone	44.86	4.02	14.02
20	1-tetradecene	45.82	3.18	11.09
21	Tridec-4-en-2-ynal	50.06	3.71	12.96

Table 2.
List of major components present in the ethanolic leaf extract of Aphanamixis polystachya (adapted from [81]).

No.	Name	RT	Area %	N area %
1	2,3-dihydro-3,5-dihydroxy-6-me	15.1	4.58	8.08
2	D-neoisomenthol	15.9	1.21	2.14
3	Neophytadiene	33.36	1.15	2.02
4	Tetradecanoic acid	35.3	3.25	5.72
5	14-pentadecenoic acid	35.9	4.31	7.6
6	Hexadecanoic acid	36.36	16.16	28.48
7	Hexadecanoic acid	37.28	1.54	2.72
8	Caryophyllene diepoxide	38.7	1.76	3.1
9	2-hexadecen-1-ol	38.96	6.48	11.41
10	Oleic acid	39.76	56.75	100
11	9-tricosene	41.84	2.81	4.94

Table 3.
List of major components present in the methanolic leaf extract of Corchorus olitorius (adapted from [82]).

2-hexadecan-1-ol (phytol) etc [82]. Chromatographic results also suggest that 500 μg EGCG was present in one milliliter of green tea leaf, which was extracted using water as solvent at different temperatures [83]. Results also suggest that

Figure 4.
Green tea leaf extracted at different temperature using water as solvent.

extraction process performed at high temperature (80°C) exhibited high content of EGCG, which was also observed by other research study (**Figure 4**) [83].

7. Preparation of PBNDDS of plant extracts and its phytoconstituents

Phospholipid based nano drug delivery systems using liposomes & phytosomes were prepared for plant extracts and phytoconstituents. Phospholipid based nano drug delivery systems (PBNDDS) batches of plant extracts and its phytoconstituents showed average particle size of 50–250 nm, PDI value of 0.02–0.5 and entrapment efficiency up to 90% (**Figures 5** and **6**). It was observed that the average size, polydispersity and entrapment efficiency of PBNDDS were markedly affected by the process and formulation factors used in different studies.

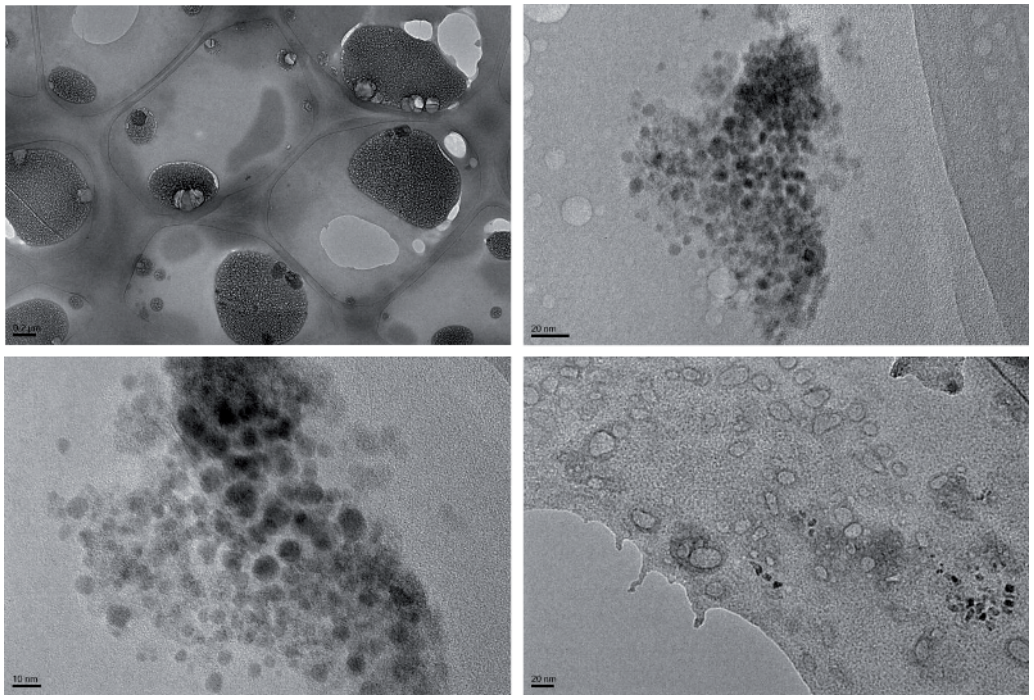

Figure 5.
TEM images for PBNDDS of phytoconstituents.

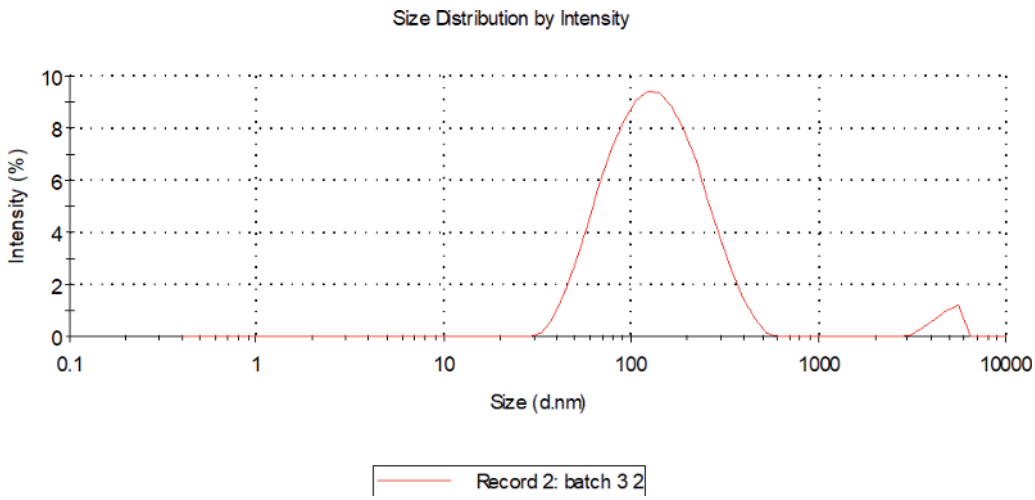

Figure 6.
DLS data of PBNDDS of phytoconstituents.

Entrapment efficiency of black seed oil loaded liposomes was increased markedly while cryoprotectant (sugar) and cholesterol were used in the preparation of liposomes. It was also observed that entrapment efficiency of liposomes was high for larger sized liposomes compared to small average size of liposomes.

8. Effect of process parameters and formulation attributes on development of PBNDDS of plant extracts and its phytoconstituents

Process parameters perspective injection rate, stirring speed, stirring time and processing temperature (solvent-antisolvent mixing) found to have marked impact on the average particle size, polydispersity and entrapment efficiency of PBNDDS [80–83, 93, 94]. It was observed that high injection rate and processing temperature found to have major impact leading to low average size of phospholipid based nano drug delivery systems (PBNDDS). Low stirring speed (<1000 rpm) and stirring time exhibit low average size of PBNDDS. Interactions between process parameter also have marked impact on average size of PBNDDS, where batches prepared using high injection rate and slow stirring speed demonstrate low average size (**Figure 7a** and **b**).

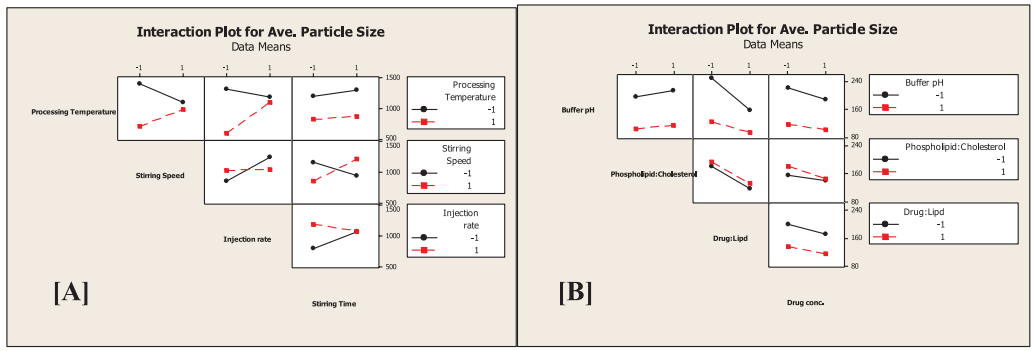

Figure 7.
Two way interaction plots of [A] process parameters and [B] formulation attributes for average particle size of PBNDDS.

Processing temperature found to be the most important process parameters which have significant impact on entrapment efficiency or loading of phytoconstituents on PBNDDS [83]. PBNDDS prepared using high temperature and injection rate leading to low entrapment efficiency. This phenomenon also probably related to low average size of PBNDDS developed at these conditions, while PBNDDS with high average particle size having high entrapment or loading efficiency. Polydispersity of PBNDDS was markedly affected by processing temperature and stirring speed and suggesting that batches processed at low temperature and high stirring speed found to be lessly polydispersed [81, 82].

Formulation attributes - ratio of drug: phospholipid and phospholipid: cholesterol, solvent system and its properties (phytoconstituents solubility, pH of the solvent), drug concentration found to have major impact on average particle size, polydispersity and entrapment efficiency of PBNDDS of phytoconstituents [80–83].

It is very imperative to find out the optimum level and amount of each of these process parameters and formulation attributes to achieve low average size with high entrapment or loading of phytoconstituents for PBNDDS. It was evident that not only the impact of individual parameters but its interactions also exhibited marked impact on the average size and loading of phytoconstituents for PBNDDS (**Figure 7**) [81–83].

9. Stability study of PBNDDS of phytoconstituents

Stability study data suggest that PBNDDS of phytoconstituents prepared using egg phospholipid were stable at 25°C and 65% RH for three months compared to accelerated conditions (10°C/45% RH and 40°C/75% RH) [80]. However previous research study suggested that PBNDDS developed using DPPC was more stable at 10°C/45% RH compared to other storage conditions [95]. This phenomenon probably related to egg phospholipid composition which is different from DPPC. DPPC is only one type of phosphatidylcholine, while egg phospholipid (lecithin) contains multiple types of phosphatidylcholine and phospholipids. PBNDDS blank and phytoconstituents loaded PBNDDS were studied using gastric media (pH 1.2) to evaluate the physical stability of PBNDDS. It was observed that PBNDDS batches of phytoconstituents were stable in gastric medium after 4 hours (maximum transit time in the stomach) and also suggests that no physical changes (precipitation or degradation) were observed for PBNDDS prepared using egg phospholipid even after 24 hrs [81].

10. *In-vitro* and *in-vivo* study of PBNDDS of phytoconstituents

Sustain release of phytoconstituents was observed when delivered through PBNDDS, which can be utilized for better therapeutic outcome against certain diseases. PBNDDS of phytoconstituents demonstrate better performance during *in-vitro* cancer cell line study performed on different cell lines. *In-vitro* cell line study data suggest that PBNDDS of phytoconstituents showed better activity in terms of % cell viability against AML and leukemia cell line compared to pure

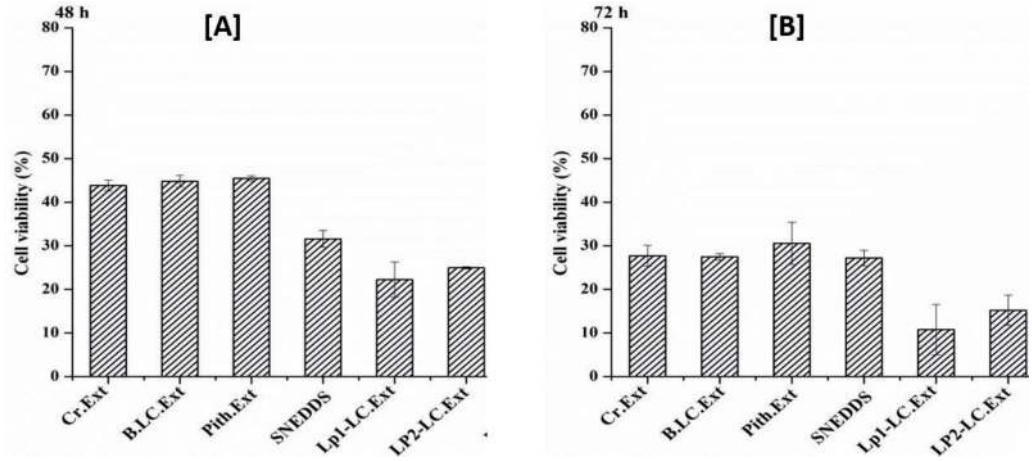

Figure 8.
In-vitro cell line study of plant extracts and PBNDDS of different plant extracts after [A] 48 h and [B] 72 h.

phytoconstituents only. It was also observed that PBNDDS of phytoconstituents showed better activity against specific AML and leukemia cell lines compared to all cell lines used in the study. This is possibly due to more permeability of PBNDDS occurred through those specific cell lines, which suggest that PBNDDS may be selective for specific cancer cell lines which may be related to the phospholipid composition, type and drug delivered through PBNDDS [82]. PBNDDS of different phytoconstituents also exhibited better activity against breast cancer cell line (MCF7) study compared to phytoconstituents in isolation (**Figure 8**).

Significant improvement was observed for *in-vivo* analgesic activity for the PBNDDS of black seed oil containing thymoquinone compared to black seed oil only and control groups. This phenomenon probably related to the improve bioavailability of thymoquinone when delivered through PBNDDS. Previous research study also showed analgesic and anti-inflammatory activity for black seed oil containing thymoquinone [80, 96].

Strong anti-inflammatory activity was observed for different plant extracts and its phytoconstituents against carrageenan induced paw edema (**Table 4**). It was also observed that plant extract and phytoconstituents delivered through PBNDDS exhibit better anti-inflammatory activity, which is possibly due to enhancement of dissolution, bioavailability and stability of phytoconstituents when delivered through PBNDDS [81–83].

Neurobehavioral study of PBNDDS of phytoconstituents was performed using open field, arm maze and water maze studies (**Figure 9**). Marked improvement in locomotor activity, ambulatory performance and memory function of dementia induced mice model was observed for PBNDDS of phytoconstituents compare to disease and plant extract groups [81]. This phenomenon probably related to strong anti-inflammatory along with antioxidant activities observed for the plant extract in different research studies. CNS inflammation is one of the pathway for developing neurodegenerative disorders, therefore by reducing inflammation significantly through PBNDDS of plant phytoconstituents in dementia induced mice model might be an option to treat neurodegenerative disease. Natural phytoconstituents may contain some ingredients which also can be effective against neurodegenerative disease through another mechanism of action which need to be confirmed in future study.

Time	Positive control	*Corchorus olitorius* leaf extract		Extract PBNDDS		Standard	
	Paw volume (ml)	Paw volume (ml)	(%) Reduction	Paw volume (ml)	(%) Reduction	Paw volume (ml)	(%) Reduction
5 h (1 day)	1.95 ± 0.15	1.31 ± 0.24	30.98	1.31 ± 0.35	33.5	0.75 ± 0.08	59.32
2 days	1.74 ± 0.32	1.20 ± 0.30	29.6	0.88 ± 0.34	50	0.20 ± 0.04	88.04
4 days	1.08 ± 0.20	0.45 ± 0.22	55.57	0.30 ± 0.27	79.06	0.14 ± 0.05	89.1
Time	**Positive control**	**Green tea leaf extract**		**Extract PBNDDS**		**Standard**	
	Paw volume (ml)	**Paw volume (ml)**	**(%) Reduction**	**Paw volume (ml)**	**(%) Reduction**	**Paw volume (ml)**	**(%) Reduction**
5 h (1 day)	1.92 ± 0.14	1.40 ± 0.12	25.8	1.80 ± 0.11	7.5	0.78 ± 0.08	59.02
2 days	1.70 ± 0.30	1.05 ± 0.10	37.52	1.35 ± 0.12	21.93	0.34 ± 0.09	80.67
4 days	1.05 ± 0.20	0.48 ± 0.13	52.53	0.51 ± 0.15	67.64	0.12 ± 0.05	88.78
Time	**Positive control**	*A. polystachya* **leaf extract**		**Extract PBNDDS**		**Standard**	
	Paw volume (ml)	**Paw volume (ml)**	**(%) Reduction**	**Paw volume (ml)**	**(%) Reduction**	**Paw volume (ml)**	**(%) Reduction**
5 h (1 day)	1.90 ± 0.15	1.12 ± 0.18	39.8	0.62 ± 0.17	68.54	0.76 ± 0.08	61.02
2 days	1.71 ± 0.32	0.90 ± 0.07	46.56	0.34 ± 0.13	80.68	0.37 ± 0.09	79.05
4 days	1.06 ± 0.20	0.63 ± 0.06	40.21	0.08 ± 0.05	92.73	0.12 ± 0.05	88.79

Table 4.
Anti-inflammatory studies of plant extracts, and its PBNDDS (adapted from [81–83]).

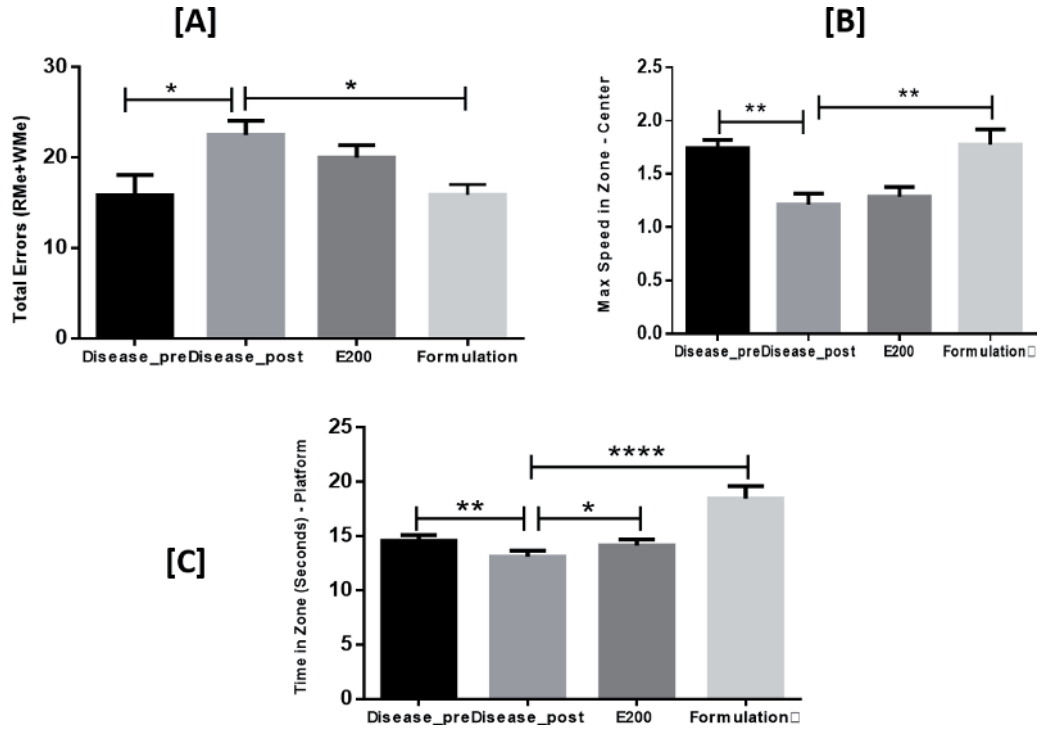

Figure 9.
*[A] Number of total errors for different mouse groups in arm maze study [B] maximum speed into central zone for different mouse groups in open field study [C] time spent on platform for different mice groups in water maze study (where **** means $p \leq 0.0001$, **means $p \leq 0.01$ and * mean $p \leq 0.05$) [four different groups—1. Pre disease group 2. Post disease group 3. E 200 - extract group and 4. Formulation—PBNDDS of extracts] (adapted from [81]).*

11. Conclusion

Plant extract found to have a range of major phytoconstituents which were identified and quantified by UHPLC and GC-MS. Major phytoconstituents emonstrate marked pharmacological activities which were evident by different *in-vitro* and *in-vivo* studies. Phytoconstituents delivered through PBNDDS exhibit better performance compared to phytoconstituents in isolation. It was observed that process parameters and formulation attributes showed significant impact on average size, polydispersity and entrapment or loading of phytoconstituents for PBNDDS. Processing temperature, injection rate, solvent system properties (pH, solubility level), phospholipd concentration related to drug and cholesterol are major factors affecting the quality output of PBNDDS. PBNDDS prepared using egg phospholipid was physico-chemically stable even at ambient conditions (25°C, 60% RH). This phenomenon might be a great advantage for developing PBNDDS of different phytoconstituents for improving the bioavialabilty, stability and targeted drug delivery. PBNDDS also exhibit better selective activity against cancer cell lines which is an indication for treating different types of cancer by developing PBNDDS using different formulation attributes. PBNDDS also showed better analgesic, anti-inflammatory and neurobehavioral activities compared to phytoconstituents only. Therefore PBNDDS can be a promising platform for delivering phytoconstituents with better therapeutic outcome. PBNDDS having average size of <150 nm with ≥85% entrapment or loading might exhibit desirable performance to treat chronic inflammatory disease, cancer, and CNS diseases.

Acknowledgements

This work was funded by National plan of Science, Technology and Innovation (MAARIFAH), King Abdul Aziz city for Science and Technology, Kingdom of Saudi Arabia, Award Number (13NAN929-02).

Conflict of interest

The authors declare no conflict of interest.

Author details

Mohammad Hossain Shariare[1*] and Mohsin Kazi[2]

1 Department of Pharmaceutical Sciences, North South University, Dhaka, Bangladesh

2 Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia

*Address all correspondence to: mohammad.shariare@northsouth.edu

References

[1] Singh RP, Gangadharappa HV, Mruthunjaya K. Phospholipids: Unique carriers for drug delivery systems. Journal of Drug Delivery Science and Technology. 2017;**39**:166-179. DOI: 10.1016/j.jddst.2017.03.027

[2] Attama A, A, Momoh MA, Builders PF. Chapter 5: Lipid nanoparticulate drug delivery systems: A revolution in dosage form design and development. In: Sezer AD, editor. Recent Advances in Novel Drug Carrier Systems. InTech; 2012. pp. 107-140. DOI: 10.5772/50486

[3] Rahman HS, Othman HH, Hammadi NI, Yeap SK, Amin KM, Abdul Samad N, et al. Novel drug delivery systems for loading of natural plant extracts and their biomedical applications. International Journal of Nanomedicine. 2020;**15**:2439-2483. DOI: 10.2147/IJN.S227805

[4] Lipid Based Drug Formulation. Northern Lipids Inc; 2008. Available from: http://www.northernlipids.com/ourfacilities.htm [Accessed: April 17, 2012]

[5] Muller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Advanced Drug Delivery Reviews. 2002;**54**(Suppl. 1):S131-S155

[6] Muller RH. Medicament Vehicle for the Controlled Administration of an Active Agent, Produced from Lipid Matrix-Medicament Conjugates. 2000; WO0067800

[7] Stuchlík M, Žák S. Lipid-based vehicle for oral drug delivery. Biomedical Papers. 2001;**145**(2):17-26

[8] Attama AA, Müller-Goymann CC. Investigation of surface-modified solid lipid nanocontainers formulated with a heterolipid-templated homolipid. International Journal of Pharmaceutics. 2007;**334**(1):179-189

[9] Atmakuri LR. Current trends in herbal medicines. Journal of Pharmacy Research. 2010;**3**:109-113

[10] Bjarnason I, Hayllar J, MacPherson AJ, Russell AS. Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. Gastroenterology. 1993;**104**:1832-1847. DOI: 10.1016/0016-5085(93)90667-2

[11] Zhang YJ, Gan RY, Li S, et al. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules. 2015;**20**(12):21138-21156. DOI: 10.3390/molecules201219753

[12] Atanasov AG et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology Advances. 2015;**33**:1582-1614. DOI: 10.1016/j.biotechadv.2015.08.001

[13] Yan JJ et al. Protection against beta-amyloid peptide toxicity in vivo with long-term administration of ferulic acid. British Journal of Pharmacology. 2001;**133**:89-96. DOI: 10.1038/sj.bjp.0704047

[14] de la Puerta R, Forder RA, Hoult JR. Inhibition of leukocyte eicosanoid generation and radical scavenging activity by gnaphalin, a lipophilic flavonol isolated from Helichrysum picardii. Planta Medica. 1999;**65**:507-511. DOI: 10.1055/s-1999-14005

[15] Harborne JB, Baxter H, Webster FX. Phytochemical dictionary: A handbook of bioactive compounds from plants. Journal of Chemical Ecology. 1994;**20**:815-818

[16] Howes MJR, Perry NS, Houghton PJ. Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other

cognitive disorders. Phytotherapy Research. 2003;**17**:1-18

[17] Perry EK et al. Neocortical cholinergic activities differentiate Lewy body dementia from classical Alzheimer's disease. Neuroreport. 1994;**5**:747-749

[18] Loizzo MR, Tundis R, Menichini F, Menichini F. Natural products and their derivatives as cholinesterase inhibitors in the treatment of neurodegenerative disorders: an update. Current Medicinal Chemistry. 2008;**15**:1209-1228

[19] Ahmad S et al. Chemical composition, antioxidant and anticholinesterase potentials of essential oil of D. Don collected from Rumex hastatus the North West of Pakistan. BMC Complementary & Alternative Medicine. 2016;**16**:29

[20] Rajakrishnan V, Viswanathan P, Rajasekharan K, Menon VP. Neuroprotective role of curcumin from *Curcuma longa* on ethanol-induced brain damage. Phytotherapy Research. 1999; **13**:571-574

[21] Perry N, Court G, Bidet N, Court J, Perry E. European herbs with cholinergic activities: Potential in dementia therapy. International Journal of Geriatric Psychiatry. 1996;**11**:1063-1069

[22] Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: Formulation, process and storage considerations. Advanced Drug Delivery Reviews. 2006;**58**(15):1688-1713

[23] Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pacific Journal of Tropical Biomedicine. 2013;**3**(5):337-352

[24] Al-Awadi F, Fatania H, Shamte U. The effect of a plant mixture extract on liver gluconeogenesis in streptozotocin-induced diabetic rats. Diabetes Research. 1991;**18**(4):163-168

[25] Al-Ghamdi MS. Anti-inflammatory, analgesic and anti-pyretic activity of Nigella sativa. Journal of Ethnopharmacology. 2001;**76**(1):45-48

[26] Alijabre SHM, Alakloby OM, Randhawa MA. Dermatological effect of nagellasative. Journal of Dermatology and Dermatologic Surgery. 2015;**19**(2):92-98

[27] Salmani JMM, Asghar S, Lv H, Zhou J. Aqueous solubility and degradation kinetics of the phytochemical anticancer thymoquinone; probing the effects of solvents, pH and light. Molecules. 2014;**19**(5):5925-5939

[28] Hand R. Supplementary notes to the flora of Cyprus III. Willdenowia. 2003;**33**:305-325

[29] Işeri OD, Yurtcu E, Sahin FI, Haberal M. *Corchorus olitorius* (jute) extract induced cytotoxicity and genotoxicity on human multiple myeloma cells (ARH-77). Pharmaceutical Biology. 2013;**51**(6):766-770

[30] Das AK, Bag S, Sahu R, Dua TK, Sinha MK, Gangopadhyay M, et al. Protective effect of *Corchorus olitorius* leaves on sodium arsenite-induced toxicity in experimental rats. Food and Chemical Toxicology. 2010;**48**(1):326-335

[31] Al Batran R, Al-Bayaty F, Abdulla MA, Al-Obaidi MM, Hajrezaei M, Hassandarvish P, et al. Gastroprotective effects of *Corchorus olitorius* leaf extract against ethanol-induced gastric mucosal hemorrhagic lesions in rats. Journal of Gastroenterology and Hepatology. 2013;**28**(8): 1321-1329

[32] Patil DK, Jain AP. In-vivo antidiabetic activity of methanolic extract of *Corchorus olitorius* for the management of type 2 diabetes. Journal of Pharmacognosy and Phytochemistry. 2019;**8**(3):3213-3218

[33] Airaodion AI, Akinmolayan JD, Ogbuagu EO, Airaodion EO, Ogbuagu U,

Awosanya OO. Effect of methanolic extract of corchorus olitorius leaves on hypoglycemic and hypolipidaemic activities in albino rats. Asian Plant Research Journal. 2019;**2**(4):1-13

[34] Soykut G, Becer E, Calis I, Yucecan S, Vatansever H. Apoptotic effects of *Corchorus olitorius* L. leaf extracts in colon adenocarcinoma cell lines. Progress in Food & Nutrition Science. 2018;**20**:689-698

[35] Snigdha HSH, Ali R, Das DK, Wadud MA. Biological evaluation of ethanolic extract of Aphanamixis polystachya (Wall.) Parker leaf. International Journal of Recent Advances in Multidisciplinary Research. 2016;**3**: 13-21

[36] Mishra AP et al. Aphanamixis polystachya (wall.) Parker, phytochemistry, pharmacological properties and medicinal uses: An overview. World Journal of Pharmaceutical Sciences. 2014;**3**: 2242-2252

[37] Apu AS et al. Phytochemical analysis and bioactivities of Aphanamixis polystachya (Wall.) R. Parker leaves from Bangladesh. Journal of Biological Sciences. 2013;**13**:393-399

[38] Krishnaraju AV, Rao CV, Rao TV, Reddy K, Trimurtulu G. In vitro and in vivo antioxidant activity of Aphanamixis polystachya bark. American Journal of Infectious Diseases. 2009;**5**:60-67

[39] Hossain MM, Biva IJ, Jahangir R, Vhuiyan MMI. Central nervous system depressant and analgesic activity of Aphanamixis polystachya (Wall.) parker leaf extract in mice. African Journal of Pharmacy and Pharmacology. 2009;**3**: 282-286

[40] Singh BN, Shankar S, Srivastava RK. Green tea catechin, epigallocatechin-3-gallate (EGCG): Mechanisms, perspectives and clinical applications. Biochemical Pharmacology. 2011;**82**:1807-1821. DOI: 10.1016/j.bcp.2011.07.093

[41] Prasanth MI, Sivamaruthi BS, Chaiyasut C, Tencomnao T. A review of the role of green tea (*Camellia sinensis*) in antiphotoaging, stress resistance, neuroprotection, and autophagy. Nutrients. 2019;**11**:474. DOI: 10.3390/nu11020474

[42] Crespy V, Williamson G. A review of the health effects of green tea catechins in in vivo animal models. The Journal of Nutrition. 2004;**134**:3431S-3440S. DOI: 10.1093/jn/134.12.3431s

[43] Conney AH, Lu YP, Lou YR, Xie JG, Huang MT. Inhibitory effect of green and black tea on tumor growth. Experimental Biology and Medicine. 1999;**220**:229-233. DOI: 10.3181/00379727-220-44371

[44] Mantena SK, Meeran SM, Elmets CA, Katiyar SK. Orally administered green tea polyphenols prevent ultraviolet radiation-induced skin cancer in mice through activation of cytotoxic T cells and inhibition of angiogenesis in tumors. The Journal of Nutrition. 2005;**135**:2871-2877. DOI: 10.1093/jn/135.12.2871

[45] Yokozawa T, Nakagawa T, Kitani K. Antioxidative activity of green tea polyphenol in cholesterol-fed rats. Journal of Agricultural and Food Chemistry. 2002;**50**:3549-3552. DOI: 10.1021/jf020029h

[46] Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I. Beneficial effects of tea catechins on dietinduced obesity: Stimulation of lipid catabolism in the liver. International Journal of Obesity and Related Metabolic Disorders. 2002; **26**:1459-1464

[47] Patel AR, Seijen-ten-Hoorn J, Velikov KP. Colloidal complexes from associated water soluble cellulose

derivative (methylcellulose) and green tea polyphenol (*Epigallocatechin gallate*). Journal of Colloid and Interface Science. 2011;**364**(2):317-323

[48] Cai ZY, Li XM, Liang JP, Xiang LP, Wang KR, Shi YL, et al. Bioavailability of tea catechins and its improvement. Molecules. 2018;**23**:2346. DOI: 10.3390/molecules23092346

[49] Amin T, Bhat SV. A review on phytosome technology as a novel approach to improve the bioavailability of nutraceuticals. International Journal of Advanced Research in Science and Technology. 2012;**1**:1-5

[50] Musthaba SM, Baboota S, Ahmed S, Ahuja A, Ali J. Status of novel drug delivery technology for phyto-therapeutics. Expert Opinion in Drug Delivery. 2009;**6**:625-637

[51] Bonifacio BV et al. Nanotechnology-based drug delivery systems and herbal medicines: A review. International Journal of Nanomedicine. 2014;**9**:1

[52] Saraf AS. Applications of novel drug delivery system for herbal formulations. Fitoterapia. 2010;**81**:680-689

[53] Li S et al. Catanionic lipid nanosystems improve pharmacokinetics and anti-lung cancer activity of curcumin. Nanomedicine. 2016;**12**: 1567-1579

[54] Bansal SS, Goel M, Aqil F, Vadhanam MV, Gupta RC. Advanced drug delivery systems of curcumin for cancer chemoprevention. Cancer Prevention Research. 2011;**4**:1158-1171

[55] Fonseca-Santos B, MPD G, Chorilli M. Nanotechnology-based drug delivery systems for the treatment of Alzheimer's disease. International Journal of Nanomedicine. 2015;**10**:4981-5003

[56] Odeh F, Ismail SI, Abu-Dahab R, Mahmoud IS, Al BA. Thymoquinone in liposomes: A study of loading efficiency and biological activity towards breast cancer. Drug Delivery. 2012;**19**(8):371-377. DOI: 10.3109/10717544.2012.727500

[57] Ravindran J, Nair HB, Sung B, Prasad S, Tekmal RR, Aggarwal BB. Thymoquinone poly (lactide-co-glycolide) nanoparticles exhibit enhanced anti-proliferative, anti-inflammatory, and chemosensitization potential. Biochemical Pharmacology. 2010;**79**(11):1640-1647. DOI: 10.1016/j.bcp.2010.01.023

[58] Fahmy HM, Fathy MM, Abd-elbadia RA, Elshemey WM. Targeting of Thymoquinone-loaded mesoporous silica nanoparticles to different brain areas: In vivo study. Life Sciences. 2019;**222**: 94-102

[59] Azad AK, Al-Mahmood SMA, Chatterjee B, Wan Sulaiman WMA, Elsayed TM, Doolaanea AA. Encapsulation of black seed oil in alginate beads as a pH-sensitive carrier for intestine-targeted drug delivery: In vitro, in vivo and ex vivo study. Pharmaceutics. 2020;**12**(3):219

[60] Sathya S, Shanmuganathan B, Saranya S, Vaidevi S, Ruckmani K, Devi KP. Phytol-loaded PLGA nanoparticle as a modulator of Alzheimer's toxic Aβ peptide aggregation and fibrillation associated with impaired neuronal cell function. Artificial Cells Nanomedicine and Biotechnology. 2018;**46**(8):1719-1730

[61] Ismail EH, Saqer AMA, Assirey E, Naqvi A, Okasha RM. Successful green synthesis of gold nanoparticles using a *Corchorus olitorius* extract and their antiproliferative effect in cancer cells. International Journal of Molecular Sciences. 2018;**19**(9)

[62] Azeez L, Lateef A, Wahab AA, Rufai MA, Salau AK, Ajayi EIO, et al. Phytomodulatory effects of silver nanoparticles on Corchorus olitorius: Its

antiphytopathogenic and hepato-protective potentials. Plant Physiology and Biochemistry. 2019;**136**:109-117

[63] Zhang J, Nie S, Wang S. Nanoencapsulation enhances epigallocatechin-3-gallate stability and its antiatherogenic bioactivities in macrophages. Journal of Agricultural and Food Chemistry. 2013;**61**:9200-9209. DOI: 10.1021/jf4023004

[64] Dube A, Nicolazzo JA, Larson I. Chitosan nanoparticles enhance the intestinal absorption of the green tea catechins (+)-catechin and (−)-epigallocatechin gallate. European Journal of Pharmaceutical Sciences. 2010;**41**:219-225. DOI: 10.1016/j.ejps.2010.06.010

[65] Hu B, Ting Y, Yang X, Tang W, Zeng X, Huang Q. Nanochemoprevention by encapsulation of (−)-epigallocatechin-3-gallate with bioactive peptides/chitosan nanoparticles for enhancement of its bioavailability. Chemical Communications. 2012;**48**:2421-2423. DOI: 10.1039/c2cc17295j

[66] Smith A, Giunta B, Bickford PC, Fountain M, Tan J, Shytle RD. Nanolipidic particles improve the bioavailability and α-secretase inducing ability of epigallocatechin-3-gallate (EGCG) for the treatment of Alzheimer's disease. International Journal of Pharmaceutics. 2010;**389**:207-212. DOI: 10.1016/j.ijpharm.2010.01.012

[67] Song Q, Li D, Zhou Y, Yang J, Yang W, Zhou G, et al. Enhanced uptake and transport of (+)-catechin and (−)-epigallocatechin gallate in niosomal formulation by human intestinal Caco-2 cells. International Journal of Nanomedicine. 2014;**9**:2157-2165. DOI: 10.2147/ijn.s59331

[68] Yeh MK, Chen CC, Hsieh DS, Huang KJ, Chan YL, Hong PD, et al. Improving anticancer efficacy of (−)-epigallocatechin-3-gallate gold nanoparticles in murine B16F10 melanoma cells. Drug Design, Development and Therapy. 2014;**8**:459-474. DOI: 10.2147/dddt.s58414

[69] Lambert JD, Sang S, Hong J, Kwon SJ, Lee MJ, Ho CT, et al. Peracetylation as a means of enhancing in vitro bioactivity and bioavailability of epigallocatechin-3-gallate. Drug Metabolism and Disposition. 2006;**34**:2111-2116. DOI: 10.1124/dmd.106.011460

[70] Schnyder A, Huwyler J. Drug transport to brain with targeted liposomes. NeuroRx. 2005;**2**(1):99-107. DOI: 10.1602/neurorx.2.1.99

[71] Li C, Zhang Y, Su T, Feng L, Long Y, Chen Z. Silica coated flexible liposomes as a nanohybrid delivery system for enhanced oral bioavailability of curcumin. International Journal of Nanomedicine. 2012;**7**:5995-6002. DOI: 10.2147/IJN.S38043

[72] Kesarwani K, Gupta R. Bioavailability enhancers of herbal origin: An overview. Asian Pacific Journal of Tropical Biomedicine. 2013;**3**:253-266

[73] Akbarzadeh A et al. Liposome: classification, preparation, and applications. Nanoscale Research Letters. 2013;**8**:102

[74] Sułkowski W, Pentak D, Nowak K, Sułkowska A. The influence of temperature, cholesterol content and pH on liposome stability. Journal of Molecular Structure. 2005;**744**:737-747

[75] Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: Food sources and bioavailability. The American Journal of Clinical Nutrition. 2004;**79**:727-747. DOI: 10.1093/ajcn/79.5.727

[76] Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. The Journal of Nutrition. 2000;**130**:2073S-2085S. DOI: 10.1093/jn/130.8.2073s

[77] Kidd P, Head K. A review of the bioavailability and clinical efficacy of milk thistle phytosome: A silybin-phosphatidylcholine complex (Siliphos). Alternative Medicine Review. 2005;**10**:193-203

[78] Semalty A, Semalty M, Rawat MSM, Franceschi F. Supramolecular phospholipids–polyphenolics interactions: The PHYTOSOME® strategy to improve the bioavailability of phytochemicals. Fitoterapia. 2010;**81**:306-314. DOI: 10.1016/j.fitote.2009.11.001

[79] Pietta PG, Simonetti P, Gardana C, Brusamolino A, Morazzoni P, Bombardelli E. Relationshipbetween rate and extent of catechin absorption and plasma antioxidant status. Biochemistry and Molecular Biology International. 1998;**46**:895-903. DOI: 10.1080/15216549800204442

[80] Rushmi ZT, Akter N, Mow RJ, Afroz M, Kazi M, de Matas M, et al. The impact of formulation attributes and process parameters on black seed oil loaded liposomes and their performance in animal models of analgesia. Saudi Pharm Journal. 2017;**25**(3):404-412. DOI: 10.1016/j.jsps.2016.09.011

[81] Shariare MH, Rahman M, Lubna SR, et al. Liposomal drug delivery of Aphanamixis polystachya leaf extracts and its neurobehavioral activity in mice model. Scientific Reports. 2020;**10**:6938. DOI: 10.1038/s41598-020-63894-9

[82] Shariare MH, Noor HB, Khan JH, Uddin J, Ahamad SR, Altamimi MA, et al. Liposomal drug delivery of Corchorus olitorius leaf extract containing phytol using design of experiment (DoE): In-vitro anticancer and in-vivo anti-inflammatory studies. Colloids and Surfaces B: Biointerfaces. 2021;**199**: 111543

[83] Shariare MH, Afnan K, Iqbal F, Altamimi MA, Ahamad SR, Aldughaim MS, et al. Development and optimization of epigallocatechin-3-gallate (egcg) nano phytosome using design of experiment (DoE) and their in vivo anti-inflammatory studies. Molecules. 2020;**25**(22):5453. DOI: 10.3390/molecules25225453

[84] Jiang Z, Jacob JA, Loganathachetti DS, Nainangu P, Chen B. β-Elemene: Mechanistic studies on cancer cell interaction and its chemosensitization effect. Frontiers in Pharmacology. 2017;**8**:105

[85] Liu J et al. β-Elemene-induced autophagy protects human gastric cancer cells from undergoing apoptosis. BMC Cancer. 2011;**11**:183

[86] Zhang Y et al. The role of E3 ubiquitin ligase Cbl proteins in β-elemene reversing multi-drug resistance of human gastric adenocarcinoma cells. International Journal of Molecular Sciences. 2013;**14**:10075-10089

[87] Zhong Y et al. β-Elemene reduces the progression of atherosclerosis in rabbits. Chinese Journal of Natural Medicines. 2015;**13**:415-420

[88] Meng X et al. Beneficial effect of β-elemene alone and in combination with hyperbaric oxygen in traumatic brain injury by inflammatory pathway. Translational Neuroscience. 2018;**9**:33-37

[89] Kinney JW et al. Inflammation as a central mechanism in Alzheimer's disease. Alzheimer's Dementia. 2018;**4**:575-590

[90] Hertz L, Chen Y, Waagepetersen HS. Effects of ketone bodies in Alzheimer's disease in relation to neural hypo-metabolism, β-amyloid toxicity, and astrocyte function. Journal of Neurochemistry. 2015;**134**:7-20

[91] Chen H et al. Hydroxycarboxylic acid receptor 2 mediates dimethyl fumarate's protective effect in EAE. Journal of Clinical Investigation. 2014;**124**:2188-2192

[92] Rahman M et al. The β-hydroxy-butyrate receptor HCA 2 activates a neuroprotective subset of macrophages. Nature Communications. 2014;**5**:1-11

[93] Song J, Shi F, Zhang Z, Zhu F, Xue J, Tan X, et al. Formulation and evaluation of celastrol-loaded liposomes. Molecules. 2011;**16**:7880-7892. DOI: 10.3390/molecules16097880

[94] Laouini A, Charcosset C, Holdich RG, Vladisavljevic GT. Preparation of liposomes: A novel application of microengineered membranes-investigation of the process parameters and application to the encapsulation of vitamin E. RSC Advances. 2013;**3**(15):4985-4994

[95] Khan D, Rezler E, Lauer J, Fields G. Effects of drug hydrophobicity on liposomal stability. Chemical Biology & Drug Design. 2008;**71**:3-7. DOI: 10.1111/j.1747-0285.2007.00610.x

[96] Hajhashemi V, Ghannadi A, Jafarabadi H. Black cumin seed essential oil, as a potent analgesic and anti-inflammatory drug. Phytotherapy Research: PTR. 2004;**18**:195-199