FORMULATION, OPTIMIZATION AND CHARACTERIZATION OF ELLAGIC ACID PHYTO-VESICULAR SYSTEM FOR BIOAVAILABILITY ENHANCEMENT

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ABSTRACT

Ellagic acid is a naturally occurring chemical compound found in a variety of fruits and vegetables like blackberries, raspberries, strawberries, pomegranates and cranberries. Antioxidant, antimutagenic and anticancer effects are all included in ellagic acid. Ellagic acid, on the other hand, is poorly absorbed and rapidly removed from the body, making it a challenging drug candidate. To overcome the above limitation, solvent evaporation method was used for the preparation of ellagic acid phyto-vesicle complex. Several batches were prepared for optimization at varying drug to phospholipid concentration ratios. The optimized formulation was found to have particle size in the range of 122.08 ± 9.66 nm, zeta potential -36.2mV, entrapment efficiency 95.65 ± 0.33 % and a drug loading capacity of 22.9 %. The *in vitro* release profile of the optimized batch shows maximum release behaviour of up to 69 % at 24 h. The *ex vivo* intestinal permeation, however shows 85.38 % release within 140 minutes.

INTRODUCTION

Herbal medicines are in high demand at present, owing to the fact that they have fewer side effects and are not harmful to the environment. Indigenous systems of medicine in India and southeast Asia use herbal medicines (Ayurveda, Unani, Siddha, Chinese and others)[1]. In some countries, a herbal is used as a supplement or alternative medicine. Nutraceutical, or food supplement, is the preferred term for a herbal product[2]. It is well known that phytoconstituents can be used for a variety of health-related purposes[3]. Polar or water-soluble molecules make up most of the plant's bioactive constituents (e.g. phenolics, glycosides, tannins and flavonoids)[4]. Due to their high molecular size and poor lipid solubility when taken orally or administered topically, water-soluble phytoconstituents have limited efficacy[5]. As a result, the bioavailability of phytoconstituents is negatively impacted. Poor absorption is due to high molecular weight and low lipid solubility.

The issue of poor drug absorption is a major one, but there are numerous ways to address it[6]. The idea of forming phyto-vesicles for enhanced medicinal efficacy has been frequently studied and documented in recent years. An appropriate solvent is used to generate a hydrogen bond between stoichiometric concentrations of drug functionality and the polar heads of phospholipids[7]. The stability of phyto-vesicles is better than that of liposomes because of hydrogen bonding. Fruits and vegetables contain phenolic phytochemicals like ellagic acid, which are significant for their health benefits against oxidation-related chronic diseases like cancer and cardiovascular disease. Either ellagic acid directly counteracts the deleterious effects of oxidative stress or it activates/induces cellular antioxidant enzyme systems, according to several studies. These models can't explain how phytochemicals maintain unique cellular homeostasis and how that leads to their preventive mode of action and therapeutic effects in various biological systems and cell types, which are more comprehensive antioxidant-related functions[8].

Nanoscale medication delivery has received a lot of attention recently because of the benefits they offer in terms of both pharmacological efficacy and toxicity[9]. It is worthwhile to investigate a nanoscale drug delivery technology for ellagic acid which can overcome the problem of poor intestinal permeability and increase the bioavailability for future therapeutic treatments. Woody plants, berries, grapes and nuts are all sources of ellagic

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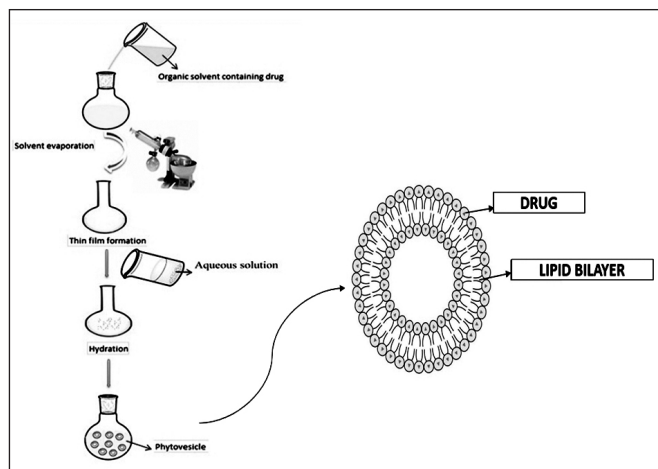*For Correspondence: E-mail: varsharawat1707@gmail.com

Fig. 1: Formulation process of phyto-vesicles via solvent evaporation technique

acid (Fig. 1), a dimeric gallic acid derivative. Ellagic acid has been linked to a number of important biological functions, including radical scavenging, chemopreventive and antiviral properties[10]. It is possible to increase the water-oil partition coefficient, enhance membrane permeability and so improve the bioavailability of medications by using phyto-vesicles, a soft nanoscale drug delivery system that incorporates phospholipid molecules in its inner structure. Improved lipophilicity and hydrophilicity, reduced side effects and higher bioavailability and therapeutic efficacy have been achieved by the phospholipid-drug molecular complexation approach[11]. To increase the bioavailability and medication delivery of ellagic acid, it is possible to use phyto-vesicles filled with phospholipids and ellagic acid. The study's goal was to create an ellagic acid phyto-vesicle complex that would boost the drug's bioavailability.

MATERIALS AND METHODS

Materials

Ellagic acid and soya phospholipid were procured from Hi Media Chemicals, Mumbai, India. Intestinal gut mucosa was purchased from the local market, Raipur, Chhattisgarh. All the chemicals used in the study were of analytical grade.

Preparation of ellagic acid phospholipid complex (EAPC)

EAPC was developed by refluxing accompanied by a solvent evaporation method[12]. EAPC was made in varied ratios of ellagic acid to phosphatidylcholine (Table I). Ellagic acid and phosphatidylcholine were dissolved in dimethylsulphoxide (DMSO) and dichloromethane respectively (Fig. 1). Both the solutions were mixed,

and placed in a 200 mL round-bottom flask. The mixture was refluxed for 3-4 h and at varied temperatures of 45-65 °C. After 3 h, the liquid was chilled and then placed into a petri plate. The dish was held open overnight at room temperature for evaporation of the solvent. Then, the product was placed in a hot air oven at 60°C for 2 h. The dried EAPC was collected and stored at room temperature in an ambered colour glass bottle for further studies.

Characterization of EAPC complex

Vesicle size and zeta potential

The vesicle size and zeta potential of the prepared EAPC complex were determined by laser light diffractometry using Malvern zeta sizer ZS 90 (Malvern Instrument Inc. USA). The scattered light was detected at an angle of 173°. Intensity distribution was used to determine the hydrodynamic vesicle size of EAPC. The results were validated by performing all measurements in accordance with industry standards, including sample dilution and while maintaining a constant temperature of 25.4 °C[13]. The mean and standard deviation were computed for each of the samples after they were examined in triplicate.

Entrapment efficiency

UV–visible spectrophotometer was used to assess the entrapment efficiency (EE) (UV-1800, Shimadzu). Weighed quantity of EAPC, corresponding to 10 mg EA was added to 50 mL of methanol in a 100 mL beaker. For 4 h, the mixture was agitated using a magnetic stirrer was allowed and is let to stand for an additional h[14]. It was centrifuged for 30 minutes at 5000 rpm to form a clear supernatant liquid. Whatman 0.45μ filter paper was used to filter the supernatant after centrifugation and the absorbance of the medication was measured in UV at 260 nm. To ensure accuracy, all the analyses were performed in triplicate.

% EE was determined by the following formula:

$$\%EE = \frac{\text{Initial drug} - \text{free drug}}{\text{Initial drug}} \text{ X } 100$$

Drug content

Drug content of the optimized batch was evaluated by dissolving correctly weighed quantity of phytosome dispersion in 10 mL ethanol[15]. After adequate dilution absorbance was estimated by UV spectrophotometer (SHIMADZU 1800) at 260 nm and drug content was estimated by using the formula

$$\text{Drug content (\%)} = \frac{\text{Amount of drug in ellagic acid}}{\text{Amount of ellagic acid}} \text{ X } 100$$

Fourier transform infrared (FTIR) spectroscopy

For FTIR, it was necessary to prepare the sample as a pellet in order to get accurate infrared absorption spectra. A milligram of the material was mashed in a mortar with around 100 times the amount of potassium bromide needed to make the pellets. The finely ground powder was introduced into a stainless-steel die. A 2 ton pressure was applied to anvils made of polished stainless steel to crush the powder into the die. The drug's infrared spectrum was captured and evaluated[16].

Differential scanning calorimetry (DSC)

DSC experiments of pure medicine and optimized formulation were conducted separately. Heating curves were obtained in the temperature range of 25-500 °C at a heating rate of 10 °C min^{-1} under a steady flow of dry nitrogen using accurately weighed samples, neatly put in DSC boats[17]. The experiment was carried out on a differential scanning colorimeter and a differential scanning colorimeter STARe, SW 13.00, METTLER Temperature Range: -25 °C to 600 °C and TA-50WS/PC computerised data station.

Transmission electron microscopy (TEM)

To explore the shape and aggregation of optimized EAPC, transmission electron microscope JEOL-JEM-1011 (JEOL-Tokyo, Tokyo, Japan) was used. One drop of the phyto-vesicle sample was put on a carbon-coated grid in distilled water. The material was also stained negatively using 1% phosphotungstic acid[18]. The material was then dried for 15 minutes at room temperature before being examined.

Scanning electron microscopy (SEM)

The morphology of the EAPC was visualized by scanning electron microscopy (SEM, LEO 1530VP, Elektro nenmikroskopie GmbH, Oberkochen, Germany). In order to produce the SEM samples, the dried samples were first placed on a Si substrate and then an Au coating of 2 nm was applied. Then, pictures were taken using a secondary electron detector and scanned with an acceleration voltage of 20 keV[15].

In vitro drug release study

Process of preparing the egg membrane

From a neighbouring retail outlet, egg was purchased. The egg yolk was extracted precisely by virtue of the hole on the shell of the egg. After that, the egg shell was soaked in HCl for 2 h with constant stirring, followed by the separation of the entire egg membrane.

The membrane was washed with phosphate buffer pH 7.4 and afterwards employed for the experimental activity.

Release of drug from the egg membrane

In vitro drug release investigations for EAPC were conducted in an open diffusion tube that was open at both the top and bottom. All of the EAPC were evenly distributed and then attached to one end of a tube, ensuring that they occupied every inch of its diameter. Separately taken 100 mL beaker containing 50 mL of pH 7.4 phosphate buffer in a water bath was used as a diffusion medium, and the lower end of the tube containing EAPC was just touching (1-2 mm deep) the surface of the bath[20]. 2 mL of phosphate buffer pH 7.4 was taken at time interval of (1, 2, 3, 4, 5, 6, 7, 8, 24 h) from the receptor fluid and 2 mL was restored at each time interval. Spectrophotometric measurements of the released medication were at the wavelength of 255 nm.

Ex vivo drug absorption study

Everted small intestine sac techniques

Ex vivo permeation studies of ellagic acid and its phyto-vesicles were carried out using noneverted gut sac technique namely by isolating a tiny segment of intestine from a laboratory animal, often a rat or hamster, everting the segment, and filling the sac with a small amount of drug-free physiologic media, such as mammalian Ringer's solution. Both ends of the segments were tied off, and the sac is immersed in a higher amount of buffer solution containing the drug in an Erlenmeyer flask[21]. The medicinal solution washing the luminal surface of the gut is known as mucosal fluid, while the solution inside the sac is known as serosal fluid. The flask and its contents were then constantly oxygenated and swirled at 37°C for a given period of time. The drug content of the serosal fluid was evaluated following incubation. The epithelial cells of the mucosal surface are exposed directly to the oxygenated mucosal fluid when the gut is everted. As a result, it is recommended as a technique of extending the viability and, maybe, the integrity of the preparation after it has been withdrawn from the intestinal.

RESULTS AND DISCUSSION

Preparation and optimization of EAPC

To assist in making phyto vesicles, organic solvents, drug and soy lecithin were combined using solvent evaporation. The lecithin content, drug and the speed of rotation were selected (Table I). All the experimental runs were performed in triplicate.

Table I: Experimental design

Sr. No	Formulation code	Drug/PC ratio (mg)	Stirring speed (rpm)	Vesicle size	Entrapment efficiency (%EE)
1	PF_1	0.5:1	30	125.95 ± 1.3	95.65 ± 0.33
2	PF_2	0.75:1	45	137.15 ± 2.22	58.65 ± 2.55
3	PF_3	1:75	45	138.86 ± 4.09	21.35 ± 3.65
4	PF_4	1:0.5	60	141.03 ± 5.46	38.68 ± 8.9
5	PF_5	1:1	90	130.18 ± 8.87	59.61 ± 4.2
6	PF_6	1:2	120	139.92 ± 6.01	84.79 ± 5.2
7	PF_7	1:3	250	130.87 ± 3.63	41.93 ± 6.1
8	PF_8	2:1	260	138.65 ± 7.5	96.48 ± 4.2
9	PF_9	2.6:1	280	122.08 ± 9.66	22.24 ± 9.1
10	PF_{10}	3:1	300	122.45 ± 2.47	86.13 ± 6.4

All the experiments were performed in triplicate (n=3)
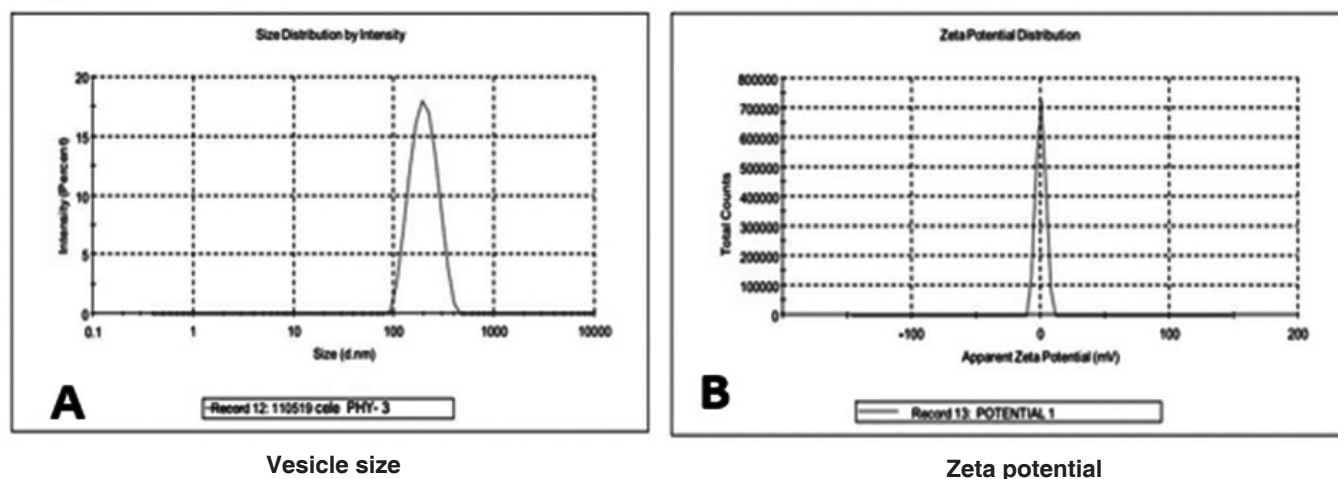

Vesicle size

Zeta potential

Fig. 2: Results of determination of A) Vesicle size and B) Zeta potential

Vesicle size and zeta potential

When drugs are released from lipid complexes, their release rate and extent are heavily influenced by the size of the vesicles that contain them. Vesicle size affects vesicle stability. The vesicle size was determined using dynamic light scattering (DLS). The 2 most important factors, ie, vesicle size and surface charge are to be considered while designing a medication delivery system. According to zeta sizer study (Fig. 2A), the EAPC complex has an average distribution of average zeta sizes. It was discovered that the Z average was 122.08 ± 9.66 nm in size. The medication is released from the complex through diffusion and surface erosion because of the reduced vesicle size. Drug phospholipid complexes can also cross and penetrate physiological drug barriers attributable to these lipids. The average size of EA nanogels manufactured by Gautam et al. was 217 nm, which validates the findings of our study[22]. The zeta potential of a finished product is a crucial factor in evaluating its long-term viability. Because the drug-loaded phospholipid complex is highly charged, substantial repellent forces can be generated among the vesicles to keep it from aggregating. Colloidal dispersions with

zeta potentials of more than ± 30 mV, can be considered physically stable. Since the EAPC zeta potential of the complex in solution form was found to be -36.2mV form. The stability of the optimized formulation was confirmed (Fig. 2B). The selected batch zeta potential value showed a stable formulation. Because strongly charged particles inhibit particle aggregation due to electrostatic repulsion, high ZP (negative or positive) indicates steady phytovesicle dispersion. Yu et al developed a berberine phospholipid complex in which the average vesicle size were found to be 165.2 ± 5.1 nm and zeta potential was found to be -24.6 mV. Therefore, based on our results our formulation is well suited in terms of size and zeta potential as compared to the other findings[12].

Entrapment efficiency and drug loading

Several batches were prepared using soya lecithin, drug and solvent to determine the optimal concentration of each ingredient. Based on the data collected from measurements of vesicle size and zeta potential as well as tests to determine entrapment efficiency, an ideal formulation was chosen. The optimized formulation had a 95.65 ± 0.33 % EE and showed 22.9 % drug loading. Entrapment might be explained by the medication's lipophilic nature, which has greater affinity for the lipid matrix it was designed for. The ellagic acid-loaded phyto-vesicles entrapment efficiency (EE) was modulated, as shown in Table I. Increasing the quantity of phosphatidylcholine in the formula may have increased the number of phosphatidylcholine molecules that may interact with ellagic acid. As a result, more phytoconstituents can be trapped by phosphatidylcholine since it has several binding sites for phytoconstituents. The amount of soya lecithin, the amount of the drug

and the volume of solvent were all modified to achieve optimal encapsulation efficiency. Sasongko et al developed *Momordica charantia* loaded phytosome for transdermal delivery and they found 90.06 ± 1.07 % entrapment efficiency in their optimized batch[23]. Sahu et al developed *Boswellia serrata* loaded phytosome for the enhancement of bioavailability of extract and the entrapment efficiency of the optimized formulation ranged from 39.8 ± 3.7% to 74.2 ± 4.3 %[24]. Thus, this result strongly supports our findings.

Fourier transform infrared (FTIR) spectroscopy

Guest and host molecules can be studied using the FTIR technique by analysing the shape and location of their absorbance bands. The FTIR spectrophotometric data clearly shows that the spectra of ellagic acid and excipients are slightly altered when they are physically mixed together. Spectra derived from pure ellagic acid were discovered 3600.48 cm^{-1} and 1700.25 cm^{-1} for O-H stretching and C=O respectively. The results obtained by analysing FTIR spectra of both the ellagic acid and excipients physical mixture were found to be as 3241 cm^{-1}, 3260.32 cm^{-1}, and 3410.91 cm^{-1} for O-H stretching group. Spectrography of C=O stretching reveals peaks at wave numbers 1680.64 cm^{-1} and 1721.75 cm^{-1} for ellagic acid and phospholipid, respectively. The findings reveal no interference between ellagic acid and excipient. There were no substantial variations in wave numbers of ketone (C=O) and hydroxyl (O-H) in comparison to the observed value of ellagic acid. Thus, the findings reveal that the ellagic acid and phyto-vesicles complex have no incompatibility issue (Figs. 3A and 3B).
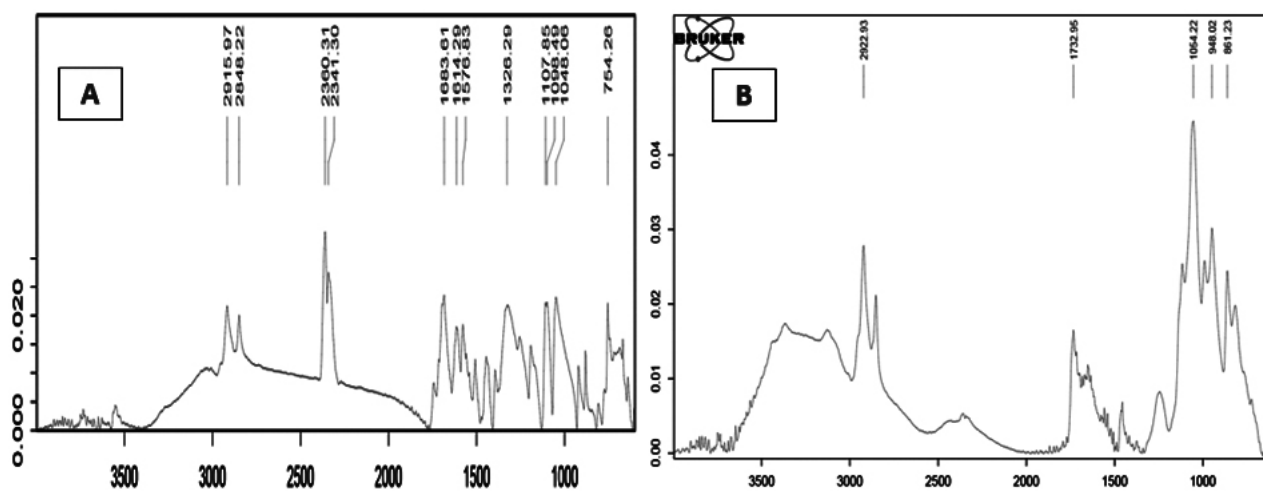
Differential scanning calorimetry (DSC)

Fig. 3: FTIR spectra of A) Ellagic acid and B) Optimized formulation

Ellagic acid showed a sharp endothermic peak at 140.73 °C, which corresponds to its melting point. The ellagic acid phytovesicle showed two different peaks, the first of which was a sharp endothermic peak at 196 °C and the second was at is 85 °C, and the complex's thermograph showed its endothermic peak, indicating that the phytovesicle formulation are in an amorphous state. As a result, it was possible to assume that the medicine was homogeneously spread inside the amorphous phytovesicle (Figs. 4A and 4B).

Transmission electron microscopy (TEM)

The optimised EAPC size and shape were studied using TEM. The DLS approach yielded data that matched the TEM micrograph's measurements of vesicle size.

However, the EAPC phyto-vesicles revealed some degree of aggregate formation on further examination of their morphology, suggesting that the phyto-vesicles were spherical in shape and that the drug precipitate was not present (Fig. 5).

Scanning electron microscopy (SEM)

SEM study indicates the presence of spherical structures in the complex. Phospholipid complex was made up ellagic acid and phosphatidylcholine (EAPC) and SEM micrograph showed spherical shape. It is proposed that ellagic acid seems to be uniformly dispersed in phospholipids and forms the smooth spherical structure (Fig. 6).
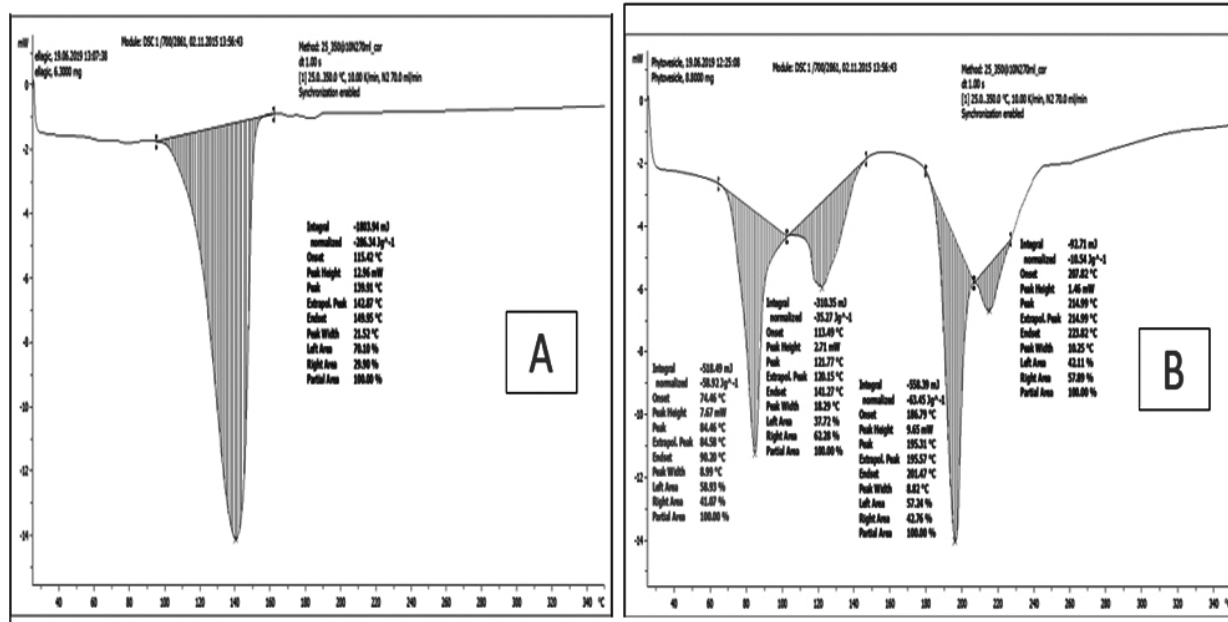

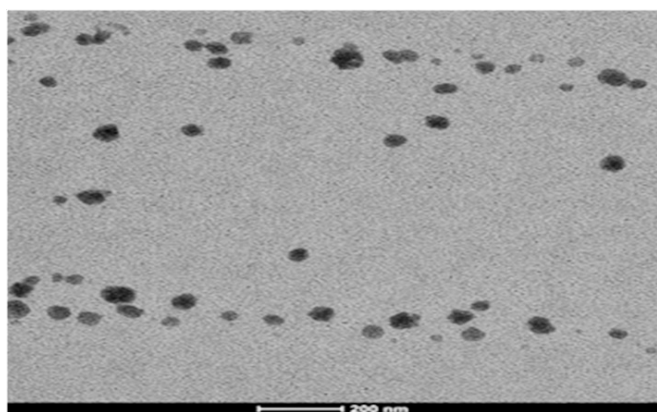**Fig. 4: DSC thermogram of a) Pure Ellagic acid and b) Physical mixture**

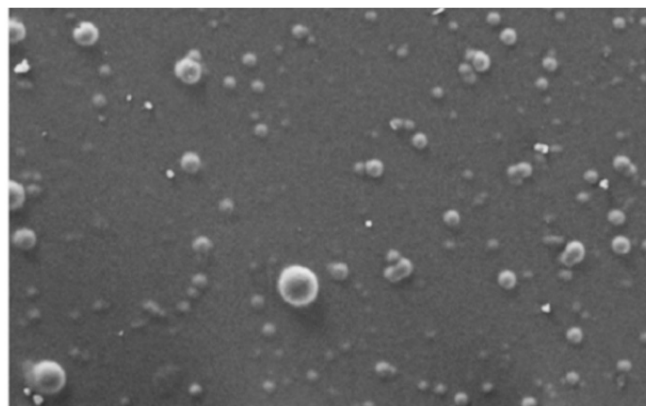**Fig. 5: TEM micrograph of EAPC phyto-vesicle**
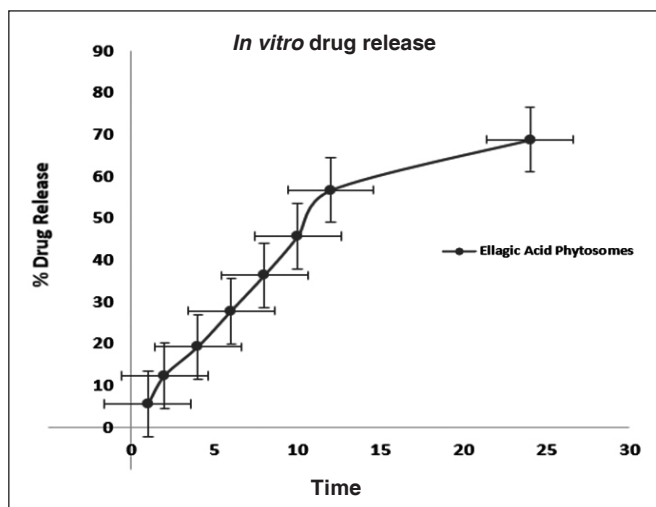

Fig. 6: SEM micrograph of EAPC phyto-vesicle

Fig. 7: *In vitro* drug release profile of ellagic acid phyto-vesicle complex

Fig. 8: Cumulative permeation of EAPC and crude ellagic acid through noneverted rat intestine in Ringer's buffer medium (pH 9) at 37 °C

In vitro drug release studies

The drug release study was carried out using egg membrane approach. Fig. 7 shows the results of *in vitro* drug release studies using ellagic acid release from the EAPC complex at various time intervals (1,2, 3, 4, 5, 6, 7, 8, 24 h) to see if EAPC may be used as an effective drug delivery system. Phosphate buffer (pH 7.4) was chosen as the *in vitro* releasing media for EAPC release since it stimulates our body fluid. After a fast release of EAPC for up to 12 h, it shows 58% release while the steady and sustained release lasted for 24 h (69 %). When EAPC is adsorbing onto soy lecithin, it may be released quickly, but when it is released from polymer matrix, it can be released over a long time. Anthrayose et. al prepared nano chrysin phyto-vesicles to enhance the *in vitro* release profile of the drug. The outcome of the study revealed that at 6 h, the release profile of chrysinphyto-vesicles shows 65.51 %[20]. Thus, the results of our experimental data indicate that our formulation shows better *in vitro* profile.

Ex vivo drug permeation studies

The intestinal mucosa was used for the *ex vivo* permeation tests. It is common practice to employ *in vitro* absorption models to study transport pathways, classify permeability, and predict *in vivo* medication absorption in humans for lower labour costs and experimental expenses[25]. There are different rat intestinal sac models that researchers use to investigate drug absorption[26]. There are numerous advantages of using a non-everted sac model instead of a non-everted saline sac model, such as the reduced number of test samples needed and the ability to collect serosal samples over time with
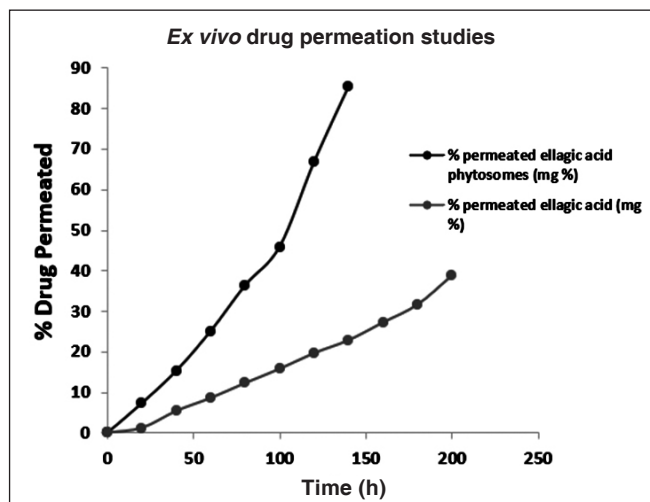
less intestinal morphological changes[27]. Intestinal tissue can be harmed during the everting process, and the existence of muscularis mucosa and a small, confined serosal compartment are all documented drawbacks of the everted gut sac model. Tissue viability in intestinal sacs was preserved for up to 2 h using oxygenated tissue culture media (TC 199) and appropriate preparation techniques[28]. Permeation of EAPC formula and crude ellagic acid suspension in the intestines are shown in Fig. 8. Near-sink conditions were achieved by employing 0.1 N NaOH in Ringer's solution, with a pH of 9. Ellagic acid was estimated in the crude drug permeation flask at all times during the trial, as described. In contrast, after 140 minutes, the phyto-vesicles formula showed an ellagic acid permeation of 85.38 percent. After 200 minutes, crude ellagic acid showed a 38.9% penetration rate. The phospholipid molecule structure, which has a water-soluble head and two fat-soluble tails, may be to blame for such a significant increase in penetration. The phospholipid is a good emulsifier because of its dual solubility[19]. The bioavailability of lipid-soluble medications can in general be increased by combining the soy phosphatidylcholine emulsifying action with phyto-vesicles nanometric size, resulting in faster and improved intestinal absorption.

CONCLUSION

To improve ellagic acid absorption property, phospholipid complexation was attempted in this study. It was determined that the EAPC has both physical and functional properties. Vesicular drug-phospholipids complex formation was confirmed by FTIR, DSC, TEM and SEM analyses. EAPC's *in vitro* dissolution experiments showed a considerable improvement in the water solubility of ellagic acid, drug release and membrane permeability.

The phyto-vesicles were spherical and distinct in shape when they were prepared. Vesicles were observed to have a particle size range of 122.08 ± 9.66 nm. Entrapment efficiency was determined to be 95.65 ± 0.33 percent. Stability of phytosome was confirmed by the zeta potential value of 36.2mV the optimised batch. Drugs with a high percentage of drug release indicate that they are rapidly entering the small intestine, which may be due to the nanosized vesicular size. Drugs that are weakly permeable may benefit from this observation. We can therefore deduce from the information presented above that ellagic acid-containing phytosome can serve as a convenient and stable substitute for traditional dosing forms.

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