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Development and Characterization of Morin Loaded Phytosomes for its Anti-Oxidant Activity

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ABSTRACT

The herbal plants contain huge number of active constituents, which shows excellent biological activity in-vitro but less in-vivo because of their poor lipid solubility, improper molecules size, degradation in gastric fluid and destruction of active components in gut by bacteria. So that poor absorption and bioavailability may occur. Phytosomal technique can be applied to herbal actives for the enhancement of bioavailability of herbal extracts and phytoconstituents. Phytosomes are prepared by patented process where standardized plant extract are bound to phospholipid. The aim of the present study was to developed and characterized the phytosomes of Morin to overcome the limitation of absorption and bioavailability. Phytosomes of Morin was formulated by thin film hydration method by using in different ratio of lipid with phytoconstituents. The prepared phytosomes were characterized for their drug content, drug entrapment, particle size, *in-vitro* release study and evaluated its in vitro antioxidant activity. From the present study, the result showed that spherical shape phytosomes having particle size 661.2nm. The poly-dispersity index and zeta potential was found to be 0.383 and -13.76 mv respectively. Result obtained from in-vitro release studies shows a sustained release of morin from formulation over a period of 12 hrs. From the present study it was concluded that morin loaded phytosmes shows better absorption and more effective antioxidants than the parent compound.

Keyword: Phytosomes, phospholipids, Morin, *in-vitro* Antioxidant activity

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INTRODUCTION

The novel drug delivery system is a new approach for plant derived drug over the last few centuries. NDDS aim to deliver drug at a rate directed by need of body during the period of treatment. A number of novel drug delivery system have emerged encompassing various routes of administration to achieve controlled and targeted drug delivery[1]. Phytochemical and phytopharmalogical sciences established the composition, biological activities and health promoting benefits of numerous plant products[2]. But many phytomedicines specially flavanoids which have numerous therapeutic potential[3] are limited in their effectiveness because they are poorly absorbed when taken by mouth either due to their large molecular size, which cannot absorb by passive diffusion or due to their poor lipid solubility limiting their ability to pass across the lipid rich biological membrane resulting in poor bioavailability[2].

To counter this problem standardized herbal drug is complexed with phospholipid molecule containing phosphatidylcholine which improve the membrane permeability, water –oil partition coefficient and thus systemic bioavailability of drug[4].

Morin (3, 5, 7, 2′, 4′-pentahydroxyflavone) a yellowish pigment is a bioflavonoid constituent of many herbs and fruits[5]. It is widely distributed in tea, coffee, cereal grains, and a variety of fruits and vegetables[6]. It shows antioxidant properties and protects cell against the free radical damage. The antioxidant properties of morin are directed to scavenging OH and the superoxide anion, highly reactive species implicated in the initiation of lipid peroxidation[7]. Pharmacological studies of morin also revealed its therapeutic potential as an antidiabeutic, hepatoprotective, antitumer and neuroprotective agent[8,9].Phytosomes are defined as " phyto" means plant and " some" means cell like, it is a novel method in which hydrophilic choline moiety (head) binds to phytoconstituents (polar) and lipophillic phosphatidyl moiety surrounds choline bound phytoconstituents[10]. Morin phytosomes consisting of microscopic vesicle that give target and selective therapeutic

response. It has better pharmacokinetic and pharmacodynamic profile which results in better pharmacological profile in comparison to free form of drug[11].

MATERIAL AND METHOD

MATERIAL

Authenticated Morin powdered drug was procured from Sigma aldirch India. The soy phosphatidyl choline (phospholipon 90G) was obtained as a gift sample from Lipoid (Switzerland), Dichloromethane and n-hexane are obtained from central laboratory of Devsthali Vidyapeeth College of Pharmacy, Rudurpur India. All the chemicals used were analytical grade.

PREPARATION OF PHYTOSOMES OF MORIN

Phytosomes of Morin were prepared by using solvent evaporation technique, where Morin and lipid were taken in 250ml of round bottom flask and dichloromethane was added .The mixture was refluxed for 2 hrs, Afterwards the solvent was removed by using rotatory evaporator rotated at 60 rpm having temperature 35±2^{0}C. Aprotic solvent n-hexane was added for precipitation of complex. The resultant morin phytosomes were dried and stored in amber colored glass bottle for further study[12].

CHARACTERIZATION OF PHYTOSOMES

Solubility study

The solubility analysis of morin, SPC and morin phytosomes were performed by using 5ml of different solvents taken in volumetric flask[12].

Particle size and zeta potential

The mean particle size of phytosomes and zeta potential of the optimized formulation was estimated with the help of Zeta-sizer nanoplus. For analysis of the sample, the sample was diluted by using double distilled water in order to reach a suitable concentration prior to the measurement[13].

Drug entrapment efficiency

20mg of the phytosomes was diluted up to 10 ml with methanol and centrifuged at 14000 rpm for 1 hour to separate the lipid and aqueous phase. Supernatant was than filtered by 0.2µ membrane filter and analyzed by UV-VIS spectroscopy at 264 nm[13].

$$\% EE = \frac{[\text{Initial drug} - \text{Free drug}]}{\text{Initial drug}} \times 100$$

Drug content evaluation

Phytosomes (10mg) was taken and dissolved in small quantity of methanol, shaken vigorously for 2 hrs after adjusting volume to 100ml using methanol there after the resultant solution was filtered and 1ml of sample was withdrawn and diluted to 10 ml in a volumetric flask and the absorbance was recorded by UV spectrophotometer at 264 nm[14].

Scanning electron microscopy (SEM)

SEM is used to observe morphological characteristics of particles. In this method phytosome was spread over a circular aluminium stub precoated with silver glue, and placed over observation area. Observation was done under SEM using various magnification and micrograph were recorded[10].

Fourier Transform Infra-Red Spectroscopy (FTIR)

Fourier transform spectra of MO, SPC and MO-SPC complex were obtained on the Perkin Elmer Spectrum Two, using wave length 500-4000 cm^{-1}.

Ex Vivo Study

In order to develop an extensive absorption profile of the MO, e*x vivo* using everted small intestine sac method was used which help in prediction of *in vivo* study. For *ex vivo* study, two small segments (2 cm in length) of goat intestine obtained from local slaughter house were isolated, washed and cleaned to free from intestinal contents and were everted using glass rod. One end of the intestine was tied tightly and thread tied canula was fitted at another end, and then it was kept in PBS solution (pH 7.4). Equimolar solutions of MO and MO-SPC (labeled as A and B, respectively) were obtained by dissolving MO and equimolar quantity of MO-SPC in phosphate buffer saline (50 ml). Mammalian Ringer's solution (2.0 ml) was injected into intestinal pieces and the pieces were then immersed separately in flasks containing MO and MO-SPC solutions. These flasks were agitated continuously at 37°C for 6 h. After the specified time period, the serosal fluid of each intestinal piece was assayed spectrophotometrically for drug content by measuring absorbance at wave length 270 nm. The cumulative absorption of MO and MO-SPC was recorded and compared.[15]

In vitro release study of Morin and MO-SPC

In vitro release of morin and MO-SPC was measured using dialysis method (diffusion) using cellophane membrane (6 kD). Activation of cellophane membrane was done in order to remove sulphur and other impurities. Activated membrane was stored in PBS pH 7.4 until used. morin, 2 mg, and 2 mg of MO-SPC was transferred to sample holder of two different diffusion cells (made up of laboratory standard glass material having total dissolution media volume of 25 mL) which receiving compartment is containing 18 mL of distilled water. The whole set is placed on a magnetic stirrer adjusted to constant speed of 150 rpm at 25 ºC. At predetermined time interval (2, 4, 6,8,10 and 12 hr), 1 mL of release medium was withdrawn for analysis and was compensated by same volume of fresh .[16]

Evaluation of antioxidant activity

a) DPPH radical scavenging activity :

The stable, DPPH, 2, 2'-diphenyl-1-picrylhydrazyl was used for the determination of free radical scavenging activity of different extract. 24mg DPPH at 20ºC was dissolved in methanol to make stoke solution .The solution used for studying was obtained by dilution of methanol and the absorbance was adjusted to 0.98±0.02 at 517nm. 3ml aliquot of this solution was mixed with 100µl of the sample at various concentration (10-500µg/ml).The mixture was then shaken and kept for incubation in dark at room temperature. absorbance was taken at 517nm .% of DPPH radical scavenging activity can be calculated using the following equation .

Scavenging effect (%) = control absorbance – sample absorbance /control absorbance ×100

b) ABTS radical scavenging assay:

free radical scavenging activity was determined by ABTS (2,2'-azinobis (3-ethyl benzthiazoline-6-sulphuric acid) cation was measured spectrophotometrically reaction of simple mixing with ABTS (7mm) and potassium persulfate

(2.4mm) to make dark green colour solution contain ABTS solution and keeping it overnight in dark .The ABTS radical cation was priory diluted with 50% methanol and adjusted to an initial absorbance of about 0.70±0.02 at 745nm and kept in temperature of 30 ºC. The radical scavenging activity was assessed next day by mixing previously prepared ABTS solution with 300µl of test solution in micropipette .The decrease in absorbance after mixing test solution with ABTS solution was measured within 1min.

Scavenging effect%= (control absorbance –sample absorbance)/control absorbance×100

RESULT AND DISCUSSION

Characterization of phytosome

Solubility study

Solubility data of MO, SPC and MO-SPC complex shows different solubility profile of MO- SPC than plain MO, which is an indication of formation of complex**(Table 1)**

Table 1.solubility profile of MO, SPC and MO-SPC Complex

S.no	Solvent	MO	SPC	MO-SPC Phytosomes
1	Distilled Water	Practically insoluble	Form miceller solution	Form miceller solution
2	Hexane	Soluble	Soluble	Sparingly soluble
3	Methanol	Soluble	Soluble	Practically insoluble
4	dichloromethane	Soluble	Soluble	Soluble

Particle size and zeta-potential

The average diameter of optimized phytosomal formulation was found to be 661.2 nm and polydispersity index was 0.383 having zeta potential -13.76 mV (**figure 1)**

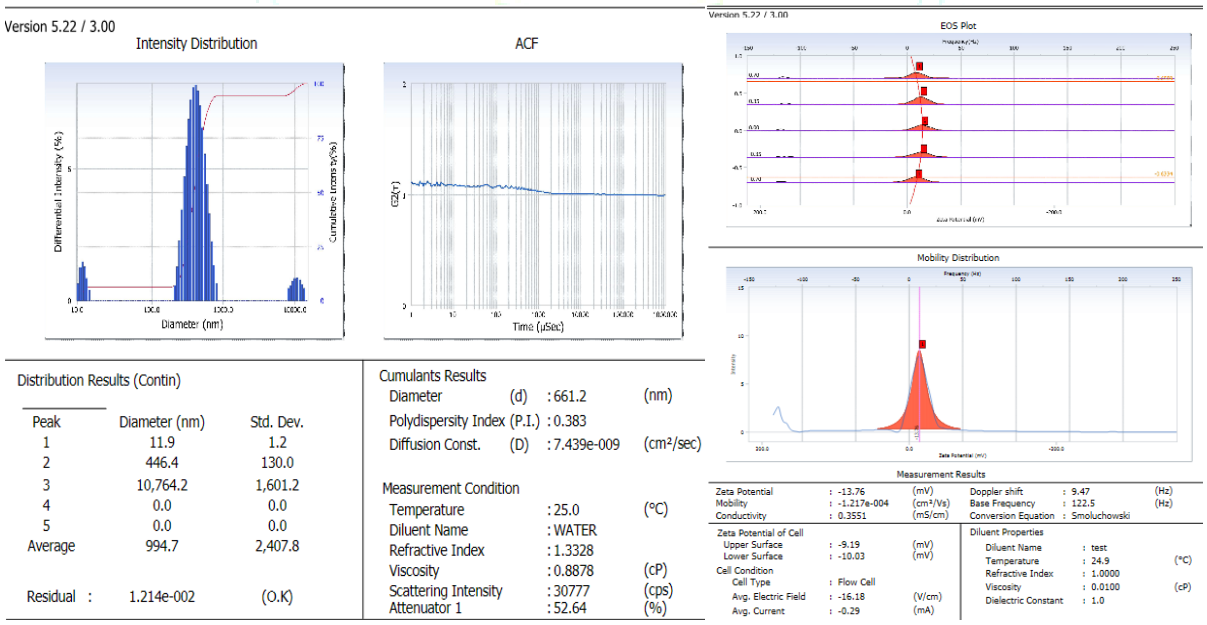

Figure 1. Particle size and zeta-potential of Phytosomes

Drug entrapment efficiency

Entrapment efficiency of different phytosomal formulation was listed in **(table 2),** the maximum entrapment efficiency was 78% and minimum was 45.46%

Table 2. Entrapment Efficiency of Different Phytosomal Formulation

Formulation	F1	F2	F3	F4	F5	F6	F7	F8
%Drug Entrapped	62.4	76.56	78	66.75	61.89	57.34	50.56	45.46

Drug content evaluation

Drug content of different formulation were shown in(**Table 3**) ,maximum drug content was 95% w/w and minimum was 65.76% w/w .

Table 3. Drug Content of Different Phytosomal Formulation

Formulation	F1	F2	F3	F4	F5	F6	F7	F8
%Drug Content	89.5	92.5	95	86.2	79.4	76.2	69.8	65.76

Scanning electron microscopy (SEM)

SEM shows that phytosomes have irregular shape having rough surface morphology. SEM images of morin phytosomes are shown in **(Figure 2).**

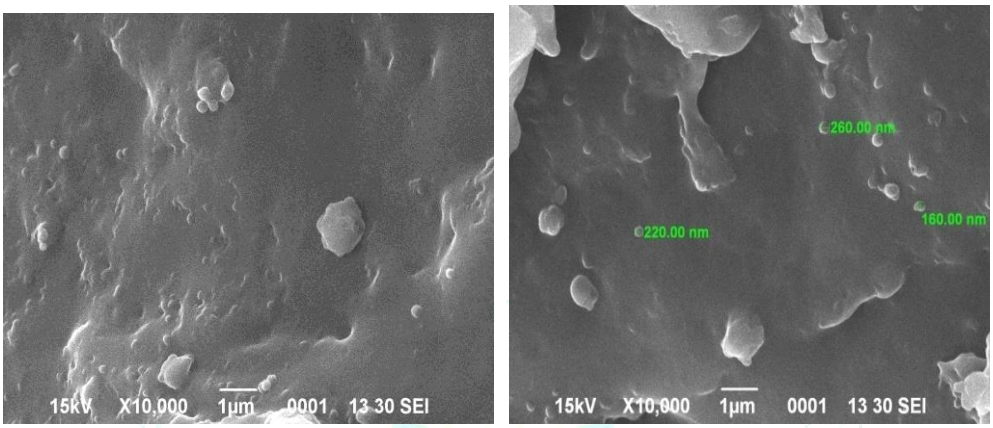

Figure 2. SEM images of phytosomes developed

FTIR Spectroscopy

The spectrum of phytosomal formulation is totally different from individual spectra of morin and SPC which conformed the reaction of OH- group of morin and coline moiety of SPC , thus formation of complex**.(Figure 3)**

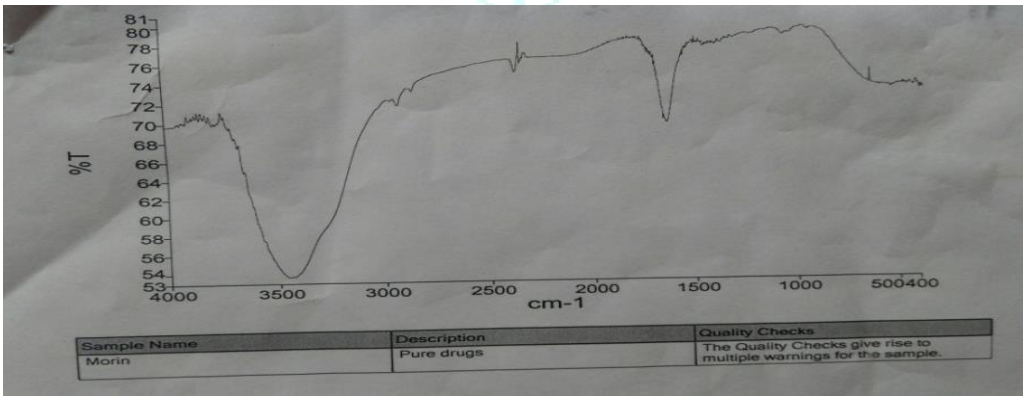

Figure 3a FTIR Spectrum of drug

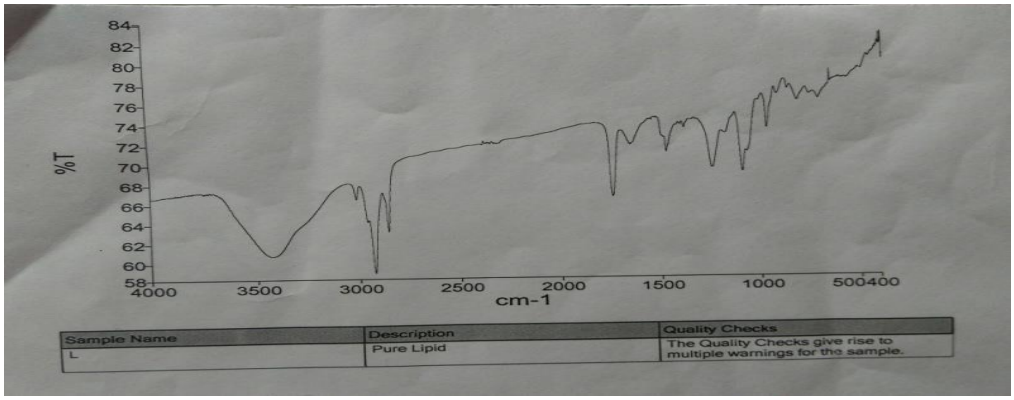

Figure 3b FTIR Spectrum of lipid

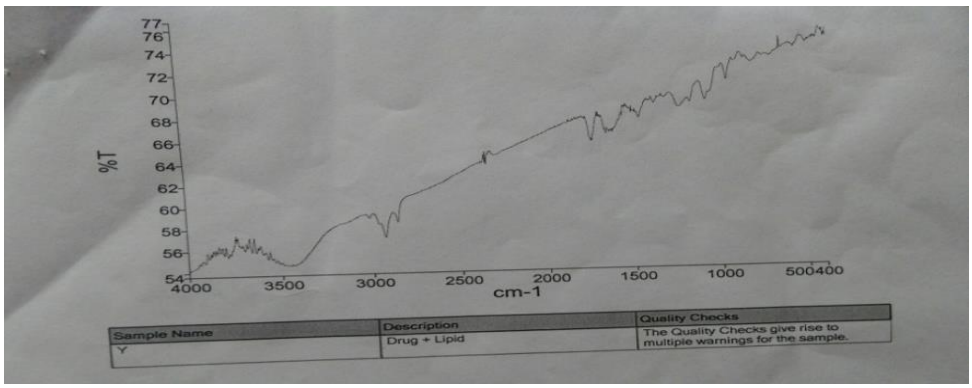

Figure 3c FTIR Spectrum of Drug+ lipid

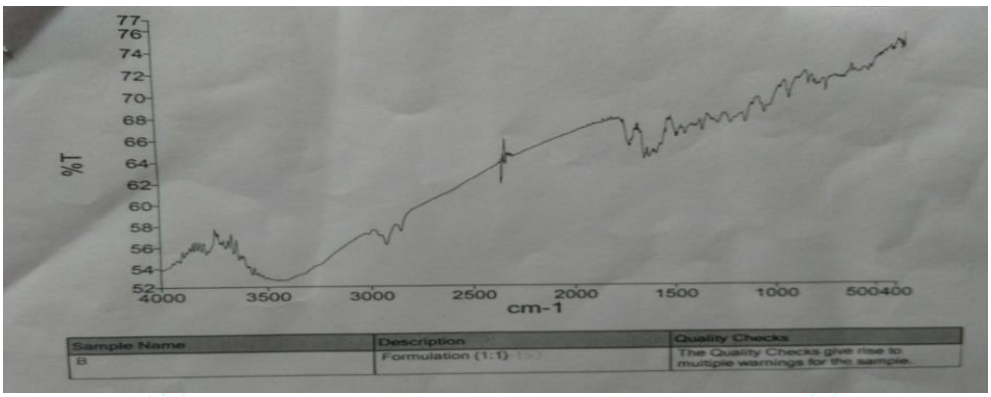

Figure 3d FTIR Spectrum of Formulation

EX VIVO STUDY

Ex vivo study shows greater absorption of MO-SPC Phytosomes than plain MO recorded at different time interval. **Figure 4** indicates greater absorption of phytosomes compared to drug alone.

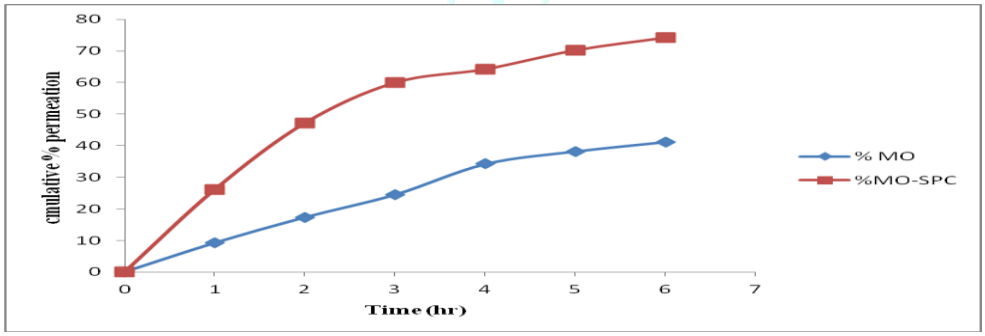

Figure 4-cumulative % drug permeation pattern of Morin and MO-SPC complex

IN VITRO DRUG RELEASE STUDY

In vitro release of morin and MO-SPC was measured by dialysis tube method result indicates that release profile of complex is more sustainable in comparison to drug alone. **Figure 5**

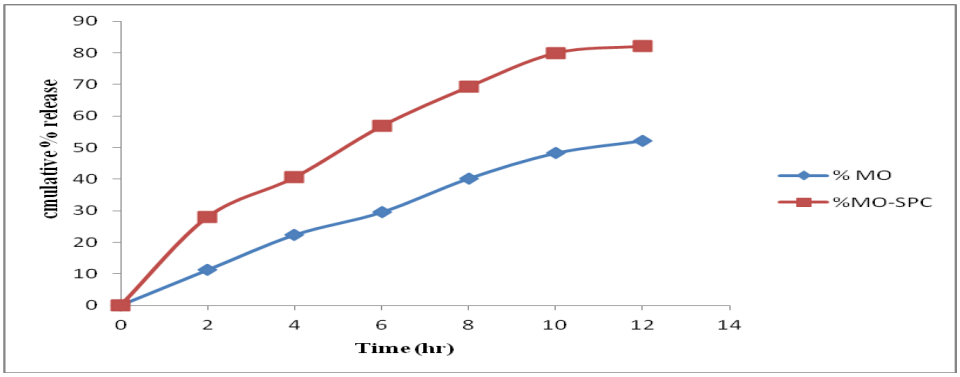

Figure 5-cumulative % drug release pattern of Morin and MO-SPC complex

Antioxidant activity

a) DPPH radical scavenging activity

DPPH radical scavenging activity of morin and its phytosomal complex is shown in **Table 3**. And it was found that percentage inhibition of drug is 83.05% having IC_{50} value 3.16 and percentage inhibition of phytosomal formulation was 88.97% having IC_{50} value3.45.

Table 3 Comparative H2O2 scavenging activity of Morin and its phytosome

Sample	Concentration (µg/ml) and % inhibition									IC50 value
	10	20	30	50	100	200	300	400	500	
Ascorbic acid	35.69	39.51	44.68	52.40	59.06	67.28	74.36	82.36	92.28	3.48
MO	40.86	45.23	49.31	55.68	53.80	61.18	69.71	75.25	83.05	3.16
Phytosomes	37.67	40.17	45.27	53.35	59.58	61.26	74.52	80.47	88.97	3.45

b) ABTS radical scavenging activity

ABTS radical scavenging activity of drug and phytosomes was shown in **Table 4**, and it was found that percentage inhibition of drug is 88.09% having IC_{50} value 3.76 and percentage inhibition of phytosomal formulation was 90.79% having IC_{50} value3.82

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Table 4 Comparative ABTS scavenging activity of Morin and its phytosome

Sample	Concentration (µg/ml) and % inhibition									IC50 value
	10	20	30	50	100	200	300	400	500	
Ascorbic acid	32.76	40.70	47.91	57.08	67.48	76.33	83.78	89.18	95.74	3.07
MO	25.90	31.04	48.59	54.81	60.08	72.61	78.67	75.94	88.09	3.76
Phytosomes	19.24	40.15	43.32	52.26	62.92	70.07	76.79	79.68	90.79	3.82

DISCUSSION

The effectiveness of any herbal drug formulation is based on delivering an effective level of the active compound. Herbal medicines obtained from plant are used from the ancient time, but many of them are poorly absorbed. The aim of the present study, is to improve therapeutic effectiveness of conventional drug by formulating them into a new drug delivery. Phospholipid plays a major in delivering drug by forming complex due to its property to enhance absorption of plant constituents. Morin has significant antioxidant property as well as other pharmacological benefits. poor absorption and solubility of morin is enhanced by complexing it with phospholipid ,Optimized phytosomes were subjected to preliminary evaluation such as solubility study, SEM, Zeta potential , IR Spestroscopy . Prepared phytosomes are also characterized for drug content and entrapment efficiency.

IR spectra of MO-SPC confirm that drug and phospholipid combined by some weak physical interaction in which there is hydrogen bonding between polyphenolic compound and choline and phosphate group of SPC. The solubility data of MO-SPC shows the formation of complex between drug and SPC. Further diffusion technique is used for determining *in-vitro* study of phytosome and drug alone which shows better bioavailability of complex when compared with plain. Moreover, antioxidant studies MO-SPC complex show better inhibitory effect as compared to plain drug when compared with ascorbic acid as standard. The prepared phytosomal complex can be formulated in the form of capsules, suspension, emulsion and other types of dosage form.

CONCLUSION

The above study concluded that phytosomes of MO are more bioavailable due to reduce degradation of morin in GIT and also have marked antioxidant property as compared to plain MO, the prepared phytosomes were more effective because of their smaller size, thus they can be given in any oral dosage form also absorption profile of MO increases which help in reducing dose of drug.

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REFERENCES

1. Singh R.P, Parpani S,Narke R,Chavan R. Phytosomes:Recent advance research for novel drug delivery system,Asian journal of pharmaceutical research and development, 2014; 2(3): 15-29.
2. Mishra N, Yadav N P, Meher JG, Sinha P. Phyto-vesicles: conduit between conventional and novel drug delivery system, Asian Pacific Journal of Tropical Biomedicine, 2012; 3(1):1728-1734.
3. Khan J , Alexander A , Ajazuddin , Saraf S , Saraf S. Luteolin–phospholipid complex preparation, characterization and biological evaluation Journal of Pharmacy And Pharmacology, 2014; 4(3):1-12.
4. Khan J, Alexander A, Ajazuddin, Saraf S, Saraf S. Recent advances and future prospects of phyto-phospholipid complexation technique for improving pharmacokinetic profile of plant actives, Journal of Controlled Release, 2013; 168: 50–60.
5. Al-Numair KS, Chandramohan G, C. Veeramani, and M.A. Alsaif. Morin, a flavonoid prevents lysosomal damage in experimental

myocardial ischemic rats, Journal of Medicinal Plants Research, 2012; 6(18): 3445–3449.
6. K`uhnau J. The flavonoids A class of semi-essential food components: their role in human nutrition, World Review of Nutrition and Dietetics, 1976; 24: 117–191.
7. Shohreh N, Mehrdad H, Mehdi R, Heidar Ali Tajmir-Riahi. DNA Adducts with Antioxidant Flavonoids:Morin, Apigenin, and Naringin, D.N.A and cell biology,2008;27:433-442.
8. Kapoor R, Kakkar P. Protective Role of Morin, a Flavonoid, against High Glucose Induced Oxidative Stress Mediated Apoptosis in Primary Rat Hepatocytes, Plos one,2012; 7(8): 1-11.
9. Caselli A, Cirri P, Santi A, Paoli P. Morin: A Promising Natural Drug,Current Medicinal Chemistry, 2016; 23:1-18.
10. Sonam and Sahu AN. Development and Characterization Of Hepatoprotective Phytosomes of Abutilon Indicum and Piper Longum, International Journal of Pharmacy and Biological Sciences, 2015; 5(4): 97-106.
11. Sikarwar M.S, Sharma.S,Jain A. Preparation characterization and evaluation of marsupsin-phospholipid complex, AAPS Pharm sci Tech,2008; 2(3) :129-137.
12. Jain PK, Khurana N, Pounikar Y, Gajbhiye A, Kharya MD. Enhancement of absorption and hepatoprotective potential through soya-phosphatidylcholine-andrographolide vesicular system, J Liposome Res, 2013 ;1(9):1532-2394.
13. Patel P, Rakesh Baria H,Ashok. Formulation and evaluation of liposomes of ketoconazole, International journal of Drug Delivery Tech , 2009;1(1):16-23.
14. Asija Sangeeta ,Jeyabalan G,Ahuja Anil, Asija Rajesh.Formulation and evaluation of prosopis cineraria (Linn.) druce phospholipid complex,Asian journal of chemical and pharmaceutical research, 2015;3(2):301-305.
15. Jain PK, Kharya murlindhar, Gajbhiye Asmita. Pharmacological evaluation of mangiferin herbosomes for antioxidant and hepatoprotective potential against ethanol induced hepatic damage,Drug development and industrial pharmacy, 2012; 4(6):1-11.
16. Parthasarathi G, Udupa N ,Umadevi P,Pillai G.K. Formulation and in vitro evaluation of vincristrine encapsulsated Niosomes,Journal of Drug Targeting, 1994; 2:173-182.