Herbosomes in the Delivery of Phytotherapeutics and Nutraceuticals: Concepts, Applications and Future Perspective

Anwana U. Ubong-Isaac[*1], Eghianruwa A. Queensley[1], Akpan J. Enomfon[1]

[1]Department of Biochemistry,
Faculty of Basic Medical Sciences,
University of Uyo, Uyo, Akwa Ibom State
*ubongisaacanwana@uniuyo.edu.ng

***Abstract**:* Recent advances in natural products chemistry and phytomedicine research has been aimed at novel lipid based drug delivery systems. Herbosome technology is one of such systems that incorporate phospholipids into standardized active ingredients of herbal extracts, thus effectively enhancing the bioavailability of water-soluble bioactive constituents of phytomedicines such as flavonoids, phenolics and hydrophilic compounds. These phytoconstituents have been established to exhibit a variety of biological activities that have pharmacological benefits. However, poor absorption of these phytoconstituents limits their bioavailability. The poor absorption is principally due to the failure of these constituents to reach their site of action before being degraded as well as their inability to pass through the small intestine due to their multi ring structures and the lipid nature of the intestinal wall. This review chronicles the recent advances made in herbosome technology, highlighting the concepts, applications and future perspective of herbosome use.

***Key words**:* Herbosomes, Phytomedicine, Lipid based delivery systems, Phytosomes, Phyto-phospholipid complex.

Introduction

Phytomedicines have been used for the treatment of various ailments since ancient times. In recent years there has been an increase in research output relating to natural products chemistry especially in Nigeria and Africa in general. Various plant materials have been observed to exhibit a variety of biological activity such as antilipidemic activity, hepatoprotective activity, immunomodulatory activity etc. Currently, as many as one-third to approximately one-half of all the drugs available are derived from plants or other natural sources [1].

The drug formulations of traditional systems of medicine like the African, Chinese and Indian systems usually contain crude extracts of different herbs which incorporate in them undesirable and many times, toxic

principles along with the active principles. With the developments in the field of phyto- and analytical chemistry, specific ingredients or a group of similar ingredients from plants are being extracted, isolated and tested for their different therapeutic applications [2]. The bioactive components of these herbs have been identified as mostly flavonoids, tannins, glycosides, phenolics and other hydrophilic molecules. Nevertheless, isolation and purification of individual components from whole herbal extracts often lead to partial or total loss of therapeutic activity. The chemical complexity of the crude or partially purified extract appears to be crucial for the bioavailability of the active constituents; hence standardization of herbal extracts has become imperative [3].

Although having excellent bioactivity *in vitro*, plant extracts often exhibit poor effectiveness *in vivo* or in animal models. The basic reasons for the low bioavailability of herbal extracts are that the bioactive components of these herbs possess multi- ring molecular structures which cannot be absorbed into the blood by simple passive diffusion and the bioactive phytoconstituents are mostly water soluble, hence, their poor lipid solubility limits their ability to pass across lipid biomembranes. This has restricted the use of pharmacologically effective polyphenolic plant actives for treating different disorders. Moreover, when taken orally, bioactive phytoconstituents are destroyed by or lost to the gastric environment or they may be rendered less effective by interaction with other drugs or nutraceuticals [4].

To counter these problems pharmaceutical research has been geared towards the development of novel lipid-based drug delivery systems to improve the bioavailability of drugs while maintaining the therapeutic activity of the drug. One delivery system designed to improve the in vivo solubility and hence bioavailability of poorly soluble herbal drugs involves the incorporation of standardized herbal extracts into phospholipids to form a ―lipid-friendly‖ complex called a herbosome or phytosome [5][6]. In view of their ampiphilic properties, herbosomes are more bioavailable (as demonstrated by pharmacokinetics and activity studies in animals), when applied topically or orally, as compared with simple herbal extracts owing to their enhanced capacity to cross into the blood through the lipid-rich biomembranes [3][6]. The active components of herbal formulation are also well protected from destruction by the gastric environment [7].

Lipid drug delivery systems have advantages over polymer based systems. The advantages include: heightened drug absorption, reduced side effects, controlled drug release and site specific targeting. Also,

most lipid formulations have high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration [7].

Properties and Morphology of Herbosomes

The term ―phyto/herb‖ refers to plants while ―some‖ means cell-like. Herbosomes are lipid compatible molecular complexes. They are lipophilic substances with a clear melting point. They are freely soluble in nonpolar solvents (in which the hydrophilic drug moiety are not), and moderately soluble in fats. When treated with water, herbosomes assume a micellar shape forming liposomal-like structures. The size of the phyto- phospholipid complex molecules customarily varies from 50 nm to about 500 μm.

In herbosomes, phytomolecules are anchored through chemical bonds to the polar head of the phospholipids. Molecular imaging and NMR studies of phyto-phospholipid complexes show one or more phosphatidylcholine molecules effectively embedded with a polyphenol molecule and it has been shown that the main phospholipid-substrate interaction is due to the formation of hydrogen bonds between the polar head of phospholipids (i.e. phosphate and ammonium groups) and the polar functional groups of the substrate [6]. Some researchers had also suggested the formation of Van der Waals forces between the two moieties. It has been proposed that the aqueous head of phosphatidylcholine molecule i.e. the choline binds to the water-soluble compounds and the phosphatidyl portion being lipophilic encloses the choline bound structure. [2].

Pharmacokinetic and pharmacodynamic studies in experimental animals and in human subjects have been used to demonstrate the biological behavior of herbosomes [8]. The increased bioavailability of the phytosomes over the non complexed botanical derivatives has been evaluated from these studies. The studies suggest that most of the drugs having crystal structure when complexed with phospholipids get transformed into molecularly dispersed or amorphous form. The X-ray diffraction pattern of drug and drug–phospholipid physical mixture in almost all the studies has shown crystalline peaks, which disappear in the diffraction pattern of the drug–phospholipid complexes. The improved lipid solubility of the herbosome complexes has been attributed to this change of crystalline state. The herbosome complexes of different drug molecules have been shown to be slightly spherical in shape with a rough surface morphology and good flow properties [2]. Herbosomes are different from liposomes, and are not to be confused with them. In liposomes the active principle is dissolved in the medium in the cavity or internal pocket or floats in the layer membrane, while in

phytosomes the active principle is anchored to the polar head of phospholipids, becoming an integral part of the membrane [3].

Merits of the Herbosome System

1. Enhanced Bioavailabilty, Systematic Absorption and Assured Delivery to the Target Site: herbosomes provide an actual means of improving the use of herbal formulations as herbosomes are liable to get transported across the lipophilic environment of cell membranes, into the cell and eventually into the blood. There is improved intestinal lumen absorption of the extracts as well as better permeation of the extracts through the skin for dermal and transdermal delivery.

Fig. 1. Lipid Based Drug Delivery Systems [7].

Due to enhanced bioavailability, the dose of phytoconstituents required to produce a desired effect is reduced. The duration of action is also increased. Herbosomes are more stable in the gastric environment and resist the action of gut microbes. Furthermore, drug entrapment is not a problem with herbosomes, as the complex is biodegradable [3][9].

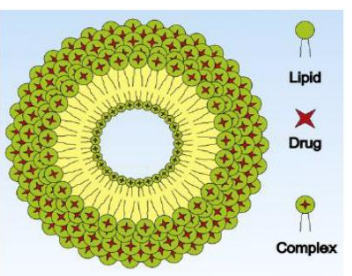

Fig. 2. The Herbosome complex [2]

2. Safety: the herbosome system is passive and non-invasive. The components of the herbosome are non-toxic and non-mutagenic and they have been approved for pharmaceutical and cosmetic use [2][6]. The conventional approaches for preparing the herbosome complexes using harmful organic solvents like tetrahydrofuran and dichloromethane have now been widely replaced by the hydrophilic solvents liethanol, thus increasing their safety and potential for use in clinical applications.

3. Other additional benefits: The process of manufacturing herbosomes is relatively simple, with no complicated technical investments required. Herbosome technology has no large drug development risk since the toxicological profiles of its component are well documented in scientific literature [6].

The phospholipids employed in herbosome formulation, apart from a physiologically compatible pharmacokinetic and toxicological profile, also possess some medicinally significant properties in human. Phospholipids add to the nutritional value of the plant extract like phosphatidylserine, which acts as a brain cell nutrient.

Phospholipids are a good source of phosphatidylcholine and choline, both of which liquefy the fat deposited in the liver as in case of hepatic steatosis or fatty liver. Soya phospholipids have been shown to be hepatoprotective in nature, preventing liver damage by alcohol drugs and other toxins thus providing a synergistic effect for liver protection. They have also been reported to
aid in clearance of serum cholesterol and increase circulating HDL levels in plasma. [2][10][11]. Phospholipids such as phosphatidyl choline show unique compatibility with biological membranes. phosphatidylcholine have shown to be incorporated in the cell membrane to replace cellular phospholipids and thus affect the fluidity of the membrane hence they maintain and nourish the skin.

Furthermore, the low solubility of herbosomes in aqueous media allows for the formulation of stable emulsions and creams.

The Phospholipid Complexation Technique (Herbosome Formulation)

Herbosome is a patented process, developed in the year 1989 by Indena, an Italian pharmaceutical and neutraceutical company. They patented the technology as PHYTOSOME®.This phytosome is a cell like structure, which is a combination of soy lecithin with standardized extracts containing polyphenolic compounds, which had vastly improved their absorption and utilization [2]. Herbosomes result from the chemical reaction of a stoichiometric amount of the phospholipid to the standardized herb extract or specific active phytoconstituents and are generally prepared by solvent evaporation or anti solvent precipitation techniques using alcoholic or organic solvents as reaction media. The supercritical fluid technique has also been incorporated into herbosome technology (for preparing puerarin–phospholipid complex) by researchers such as Li and coworkers [12]. In the more frequently used solvent evaporation technique the drug (standardized extract or isolated bioactive phytoconstituents) and the phospholipids are placed in the same flask containing a suitable solvent system. The reaction is carried out at suitable fixed temperature for a fixed duration of time to get maximum possible yield and drug entrapment [2]. The optimum ratio of phospholipid to drug is 1:1 although different molar ratios ranging from 0.5:1 to 3:1 have also been employed with success [13][14]. The herbosome complex thus formed can be isolated by precipitation with an aliphatic hydrocarbon or lyophilization or spray drying [15].

The common stages for the preparation of herbosomes are shown in Fig. 3. Usually, Aprotic solvents like acetone, methylene chloride, ethyl acetate, dioxane etc. are used as reaction media for formulating herbosomes, however they have been largely replaced by protic solvents

like ethanol. Other Solvents such as tetrahydrofuran, dichloromethane and n-hexane have also been used by researchers [13][16][17]. Most of the recent works have been carried out using absolute ethanol as the reaction medium.

The common criterion for selection of phospholipids for herbosome formulation was the ratio of phosphatidyl group present in them .The most commonly used phospholipids are those derived from soya bean containing higher proportions (that is about 76%) of phosphatidylcholine with a high content of polyunsaturated fatty acids like linoleic acid about 70%, linolenic acid and oleic acid. The phospholipids of soya bean have been the phospholipid of choice because of the higher content of phosphatidylcholine in them offers compatibility and similarity with the mammalian plasma membrane [2]. Soy lecithin, phosphatidylserine, and 1,2-distearoyl-sn-glycero-3-phosphocholine have also been used.

Evaluation and Characterization of Herbosomes

Factors such as size, membrane permeability, the amount and purity of preparatory materials, the percentage of entrapped phytochemicals and chemical composition, determine how a herbosome would behave in a biological system. A variety of techniques have been employed for the study and characterization of herbosomes.

Transmission Electron Microscopy and Scanning Electron Microscopy have been used to visualize the herbosome after formation to assess its size and shape. The formation of the phyto-phospholipid complex can be confirmed by FTIR spectroscopy, X-ray diffraction, NMR and Molecular imaging techniques while the drug content of the of the complex can be quantified used HPLC. Other techniques used include Dynamic light scattering (DLS) coupled with a computerized inspection system and Photon correlation spectroscopy, to determine the particle size and Zeta potential of the complex and Ultracentrifugation to determine the entrapment efficiency of an extract by a herbosome.

Applications of Herbosomes

Herbosomes formulations in solutions, emulsions, creams, lotions, gels etc., have gained importance in various fields like the pharmaceutical, veterinary, cosmetic and nutraceutical fields.

Companies involved in production and marketing of herbosomal products include, Indena in Milan, Italy; Jamieson Natural Sources in Ontario, Canada; Thorne Research in Dover, England and Natural Factors in Canada.

The herbosome process has been applied to many popular herbal extracts including Gingko bilboa, grape seed, hawthorn, olive fruits and leaves, green tea, ginseng, kushenin, marsupsin and curcumin [7]. These phytosomes are significantly more bioavailable and

hence therapeutically more effective than the standardized extracts or their conventional forms and are useful in various disorders.

Maiti *et al.* [18], have demonstrated the improvement in pharmacokinetic profile of curcumin on carbon tetrachloride-induced acute liver damage in rats by preparing its complexation with phospholipids. The antioxidant activity of the herbosome was significantly higher than that of pure Curcumin at all dose levels tested [2][6][18]

Maiti *et al.* [19], also demonstrated the improvement in pharmacokinetic profile of naringenin herbosome. The developed naringenin herbosome exhibited better antioxidant activity than the free compound with a prolonged duration of action. [6][19].

Yanyu *et al*. have evaluated the bioavailability of silybin–phospholipid complex against silybin-N- methylglucamine. The phospholipid complex showed prolonged plasma therapeutic level and increased bioavailability [20].

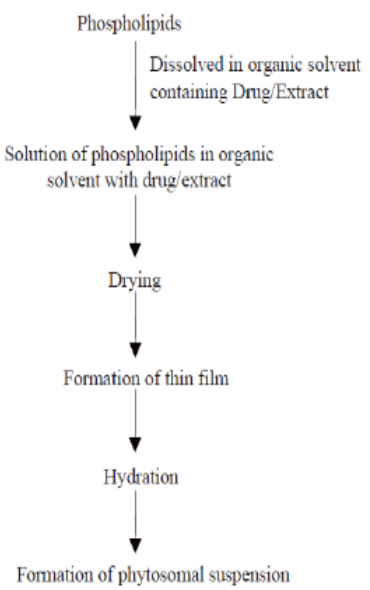

Fig. 3. Common stages for preparation of Herbosomes [6].

Chen *et al*. [21], have demonstrated the pharmacokinetic profile of quercetin,kaempferol and isorhamnetin present in *Ginkgo biloba* extract after oraladministration in rats by formulating its phospholipid complex. The results demonstrated an immense increase in bioavailability of the extract in its phospholipid complexed form [2][21].

Other therapeutically efficient phytosome complexes from different plant extracts/ active compounds developed in recent years are summarized in Table 1 along with the improvement in pharmacodynamic and pharmacokinetic profiles of the crude drug and their clinical utility.

Herbosome Technology in Nigeria

One of the most prevalent fields of research in Nigeria is ethnopharmacology and Natural Products Chemistry. There is a wealth of literature and research findings on the pharmacology of a wide variety of medicinal and lesser known plants found in Nigeria. Bioactive phytoconstituents are being discovered yearly, not only in new medicinal plants but in those that have already been investigated. Bioactive phytochemicals that have been identified include: flavonoids, glycosides, terpenes, lectins, alkaloids, tannins and saponins. Crude extracts of plant materials as

well as isolated bioactive derivatives have been studied and observed to have such exploitable biological properties including antihyperlipidemic, allelopathic, antisickling and hepatoprotective effect.

Still, in order for these plant products to be useful clinically, it is necessary to have appropriate formulations and delivery systems, which provide optimum delivery of the active ingredients. Unfortunately, in spite of the wealth of scientific data and literature available on the medical significance of a vast number of Nigerian plants, there is little or no data or research carried on ideal delivery systems for these herbal products. There is no data available on the use of herbosomes for herbal drug formulations in Nigeria, in spite of the recorded improvement in pharmacodynamic and pharmacokinetic profiles of herbal drugs and isolated bioactive components when complexed with phospholipids. Hence, plant extracts are still being administered in extremely large doses and the possibility exists that some herbal plants which have great clinical significance been judged ineffective due to their low bioavailability.

Future Perspectives and Conclusion

Herbosomes improve the *in vivo* bioavailability of herbal drugs, which in spite of positive *in vitro* results fail to deliver a similar response in vivo. The hydrophilic constituents of plants like flavonoid and others polyphenolic constituents have immense therapeutic potential but because of their inability to cross lipoidal barriers their application in treatment of several disorders and disease conditions. The incorporation of these phytoconstituents with dietary phospholipids has successfully solved this issue and has offered a preparation of herbal drugs with sufficient lipid penetrability, higher concentration and sustained therapeutic levels in plasma with a slower rate of elimination.

TABLE I. LIST OF PHYTO-PHOSPHOLIPID COMPLEXES PREPARED USING PLANT ACTIVE COMPOUNDS AND EXTRACTS ALONG WITH THE ADVANCEMENT IN THEIR PHARMACOLOGICAL PROFILE [2].

Phytoconstituent	Source	Method of preparation	Improvements in Pharmacological Profile		Biological Activity
			Pharmacokinetics	Pharmacodynamics	
Evodiamine	Plants of Tetradium family	Solvent evaporation	Enhanced solubility in water, relative bioavailability of complex increased to 218.82% compared to evodiamine	Expected improvement in biological activity	Stimulant and lipid lowering
Gallic acid	Amla, grapes	Solvent evaporation	Apparent solubility drug increased in water from 10.86 to 18.12 and from 6.63 to 11.66 in n-octanol.	Improved free radical scavenging activity	Anti-oxidant
Emodin	Rhubarb	Solvent evaporation	The water and n-octanol solubility of emodin was improved from 2.25 to 9.97 and from53.45 to 77.62 µg/ml, respectively	Expected improvement in biological activity	Anti-oxidant, anti-cancer
Curcumin	*Curcuma longa*	Anti-solvent precipitation	Micellar solubility in water, Cmax increased from 258.64 to 803.86	Increased therapeutic efficacy	Hepatoprotective
Embelin	*Embelia ribes*	Solvent evaporation	Solubility in n-octanol increased from 4 µg/ml (pure embelin) to 38 µg/ml (phytosome) and from1 µg/ml to 40 µg/ml in water	Expected improvement in biological activity	Anti-tumor, anti-inflammatory and anti-diabetic
Glycyrrhizic Acid	*Glycyrrhiza glabra*	Solvent evaporation	Cmax of phospholipid complex 2.14 times higher than free glycyrrhizic acid, the AUC of GL-PLC found 1.74 times higher than that of free GL	Improved anti-inflammatory action	Anti-inflammatory
Paeonia emodi root extract	*Paeonia emodi*	Solvent evaporation	–	Enhanced antianxiety, antioxidant and Antidepressant actions	Antianxiety, antioxidant antidepressant
Ellagic acid	*Quercus alba* and others	Solvent evaporation	Improved Cmax of 0.21 µg/ml to 0.54 µg/mL with increase in duration of action in rats	Improved hepatoprotective action	Hepatoprotective, anti-oxidant
Salvianolic acid	*Salvia miltiorrhiza*	Solvent evaporation	Improved water and lipid solubility	Expected improvement in biological response	Antioxidative agent and free radical scavenger
Quercetin	Different fruits and vegetables	Precipitation	–	Enhanced therapeutic efficacy to almost 2 times the extract	Hepatoprotective

To further justify herbosomes as a productive novel lipid-based drug delivery system, the scope of the research needs to be broadened to solve the issues of the preparation technique, complex stability after preparation, mechanism of absorption and actual clinical advantage of these drug delivery systems. The solvent evaporation technique, which is the frequently used technique for formulating herbosomes, involves a number of processing steps, which are time consuming. Furthermore, the quality of the end product in terms of particle size, morphology and hygroscopicity many times depends upon the method adopted for drying of the residue, which has not been optimized in any of the studies. To counter these problems, techniques such as the supercritical fluid technique, which has emerged as an effective tool for preparing particle sizes ranging from 5 to 2000nm and which has been utilized for improving the solubility profile of poor soluble drug molecules can be incorporated to overcome the drawbacks of conventional methods as the particle size and its distribution can be more precisely controlled at very mild temperature conditions. The uniformity in particle size further improves the systemic bioavailability [2]. The CO2 supercritical fluid is

nonhazardous and provides stable inert conditions for processing of sensitive drug molecules. Hence, further research work for formulation optimization using the supercritical fluid technique and its impact on in vivo parameters of herbal drugs need to be carried out.

The yield of the herbosome complexes obtained from various studies varied significantly ranging from about 25% to more than 90%. These variations have been attributed to different formulation factors like drug to phospholipid ratio, temperature and duration of processing. These aspects of the formulation have to be optimized in future research works to get the formulation of best quality. Furthermore, statistical tools can be used for optimizing the molar ratios of drug candidates with phospholipids, along with the temperature and other variables to get maximum entrapment efficiency and a superior drug release profile [2].

Emphasis has been given on the characterization and evaluation of pharmacokinetic parameters of phyto-phospholipid complexes without going in to the clinical aspects of prepared formulations. More exhaustive studies establishing correlation between improvement of in vivo and in vitro pharmacokinetic parameters with the pharmacological efficacy of drug molecules in their phospholipid complexed forms are required to fill this gap and to correlate the improvement in bioavailability with clinical efficacy [2].

TABLE II. THERAPEUTIC APPLICATIONS OF DIFFERENT PHYTOSOMES WITH THEIR DOSE [6].

Phytosomes	Phytoconstituent (complexed with Phosphatidyl Choline)	Daily dosage	Indication
Leucoselect® Phytosome	Procyanidolic oligomers (PCOs) from grape seeds	50–100 mg	Systemic antioxidant.
Greenselect® Phytosome	Epigallocatechin 3-O-gallate from *Camelia sinensis* (Green tea)	50–100 mg	Systemic antioxidant.
Ginkgoselect® Phytosome	24 % ginkgo flavono glycosides from *Ginkgo biloba*	120 mg	Protects brain and vascular lining
Silybin phytosome Siliphos™ milk thistle phytosome	Silybin from silymarin (milk thistle)	120 mg	Additional antioxidant protection for skin and liver.
Hawthorn phytosome	Flavonoids	100 mg	Used in heart disease
Panax ginseng Phytosome	37.5% ginsenosides from roots of *Panax ginseng*	150 mg	As a Food Product
Mirtoselect® Phytosome	Anthocyanosides from an extract of Bilberry	—	Improves capillary tone, reduce abnormal blood vessel permeability & are potent antioxidants. They hold great potential for the management of retinal blood vessel problems and venous insufficiency.
Sabalselect® Phytosome	An extract of saw palmetto berries through supercritical CO_2 extraction	—	Prostate health.
Polinacea™ phytosome	Echinacosides and a unique high-molecular weight Polysaccharide from *Echinacea angustifolia*	—	It enhances immune function in response to a toxic challenge.
Oleaselect™ Phytosome	Polyphenols from olive oil	—	As potent antioxidants, inhibit harmful oxidation of LDL cholesterol, and also have anti-inflammatory activity.

The stability of the herbosomal complexes are another area of which needs more attention and exploration. The data in support of the stability of the phyto-phospholipid complexes on storage are insufficient in terms of their market utility and survival. Such preparations are at risk of aggregate formation and chemical degradation on storage [2]. Preparations with more than 90% purity of phospholipids are considered to be more susceptible for oxidative changes, which may be a decisive factor in terms of stability of the final product. Zeta potential plays a key role in determining stability of solid dispersions and inter-particle interactions and so is temperature related hygroscopicity of the formulations [2]. Experimental work has revealed strong moisture absorbing potential of the phytophospholipid complexes when compared with pure drug and the formulation has also exhibited to become more viscous when kept in free air [14]. These parameters have remained more or less untouched in most of the studies and require more emphasis in the future to establish and improve the stability of the phyto-phospholipid complexes.

The exact mechanism of absorption of the drug– phospholipid complexes from the gastrointestinal tract should be an area of focus in future research. In conclusion, in order to be clinically useful, the vast number of plant products being isolated and studied in countries such as Nigeria, need to have appropriate formulations and optimum drug delivery systems for the polar bioactive ingredients. Herbosome technology aids to explore maximum potential of these polar phytoconstituents. The formulation technology is simple and can be easily upgraded to a commercial scale and the components of the herbosome are relatively safe. Hence, several plant extracts which have been reported to possess various pharmacological and health promoting properties but which show poor bioavailability can be standardized and formulated into herbosomes for further systematic studies and clinical application.

Acknowledgments

We thank the Management of the University of Uyo, Uyo, Akwa Ibom State.

References

[1] Barnes, P.M., Powell-Griner, E., McFann, K. and Nahin, R.L. (2004). Complementary and alternative medicine use among adults: United States. Adv. Data, Vol. 27(343) pp. 1–19.

[2] Khan, J., Alexander, A., Ajazuddin, A., Saraf, S. and Saraf, S. (2013). Recent advances and future prospects of phyto-phospholipid complexation technique for improving pharmacokinetic profile of

plant actives. J. controlled release, Vol. 168, pp. 50–60.

[3] More, M.S., Shende, M.A., Kolhe, D.B. and Jaiswal, N.M. (2012). Herbosomes: Herbo-phospholipid complex an approach for absorption enhancement. Int. J. Bio. Pharm Res., Vol. 3(8), pp. 946-955.

[4] Bhattacharya, S. (2009). Phytosomes: the new technology for enhancement of bioavailability of botanicals and nutraceuticals. Int. J. Health Res., vol. 2, pp. 225–232.

[5] Bombardelli, E., Curri, B., Loggia Della R. and Sosa, S. (1989). Complexes between phospholipids and vegetal derivatives of biological interest. Fitoterapia, vol. 60 pp. 1-9.

[6] Patel, J., Patel, R., Khambholja, K. and Patel, N. (2009). An overview of phytosomes as an advanced herbal drug delivery system. Asian J. Pharm. Sci., Vol. 4(6), pp. 363-371.

[7] Chime, S.A. and Onyishi, I.V. (2013). Lipid-based drug delivery systems (LDDS): Recent advances and applications of lipids in drug delivery. African J. Pharmacy and Pharmacol., Vol. 7(48), pp. 3034-3059.

[8] Franco, P.G. and Bombardelli, E. (1998). Complex coppouns of bioflavonoids with phospholipids, their preparation and uses and pharmaceutical and cosmetic compositions containing them. U.S. Patent No: EPO 275005.

[9] Kidd, P.M. (2009). Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea, and grape seed extracts. Alt. Med. Rev, Vol. 14, pp. 226–246.

[10] Kapoor Silki, D., Malviya, S., Talwar, V. and Prakash, K.O. (2012). ―Potential and promises of phospholipid structured novel formulations for hepatoprotection. Int. J. Drug Dev. Res., Vol. 4, pp. 51–58.

[11] Gundermann, K.J., Kuenker, A., Kuntz, E. and Drozdzik, M. (2011). ―Activity of essential phospholipids (EPL) from soybean in liver diseases. Pharmacol. Rep., Vol. 63, pp. 643–659.

[12] Li, Y., Yang, D.J., Chen, S.L., Chen, S.B. and Chan, A.S. (2008). Process parameters and morphology in puerarin, phospholipids and their complex microparticles generation by supercritical antisolvent precipitation. Int. J. Pharm., Vol. 359, pp. 35–45.

[13] Yue, P.F., Yuan, H.L., Li, X.Y., Yang, M. and Zhu, W.F. (2009). Preparation,

characterization and pharmacokinetics in vivo of oxymatrine– phospholipid complex. J. Bioequiv. Availab., Vol. 1, pp. 099–102.

[14] Qin, X., Yang, Y., Fan, T.T., Gong, T., Zhang, X.N. and Huang, Y. (2010). Preparation, characterization and in vivo evaluation of bergenin– phospholipid complex. Acta Pharmacol. Sin., Vol. 31, pp. 127–136.

[15] Mascarella, S. (1993). Therapeutic and antilipoperoxidant effects of Silybin- phosphatidylcholine complex in chronic liver disease. Curr. Ther. Res., Vol. 53, pp. 98-102.

[16] Maiti, K., Mukherjee, K., Gantait, A., Saha, B.P. and Mukherjee, P.K. (2010). Enhancing bioavailability and hepatoprotective activity of andrographolide from Andrographis paniculata, a well-known medicinal food, through its herbosome. J. Sci. Food Agric., Vol. 90, pp. 43–51.

[17] Ajazuddin, A. Alexander, B. Amarji, B. and Kanaujia, P. (2013). ―Synthesis, characterization and in vitro studies of pegylated melphalan conjugates. Drug Dev. Ind. Pharm., vol. 39(7), pp. 1053 – 1062.

[18] Maiti, K., Mukherjee, K., Gantait, A., Saha, B.P. and Mukherjee, P.K. (2007). Curcumin–phospholipid complex: preparation, therapeutic evaluation and pharmacokinetic study in rats,‖ Int. J. Pharm, Vol. 330, pp. 155– 163.

[19] Maiti, K., Mukherjee, K., Gantait, A., Saha, B.P. and Mukherjee, P.K. (2006). Enhanced therapeutic potential of naringenin-phospholipid complex in rats. J. Pharm. Pharmacol, Vol.58, pp. 1227-1233.

[20] Yanyu, X., Yunmei, S., Zhipeng, C. and Qineng, P. (2006). The preparation of silybin–phospholipid complex and the study on its pharmacokinetics in rats. Int. J. Pharm., Vol. 307, pp. 77–82.

[21] Chen, Z.P., Sun, J., Chen, H.X., Xiao, Y.Y., Liu, D., Chen, J., Cai, H., and Cai, B.C. (2010). ―Comparative pharmacokinetics and bioavailability studies of quercetin, kaempferol and isorhamnetin after oral administration of Ginkgo biloba extracts, Ginkgo biloba extract phospholipid complexes and Ginkgo biloba extract solid dispersions in rats. Fitoterapia, Vol. 81, pp. 1045–1052.