Journal on New Biological Reports **3**(3): 212 – 220 (2014) **ISSN 2319 – 1104 (Online)**

Phytosomes: A Noval Drug Delivery System for Phytoconstituents

Pallav Kaushik Deshpande *[1], Anupam Kumar Pathak [2], Ragini Gothalwal [1]

[1]*Department of Biotechnology,Barkatullah University,Bhopal.*
[2] *Department of Pharmacy,Barkatullah University,Bhopal.*

(Received on: 02 October, 2014; accepted on: 31 October, 2014)

ABSTRACT

Advanced biochemical and pre-clinical studies have proved the potential of plant flavonoids and other hydrophilic natural compounds for the treatment of skin disorders, different types of carcinoma, anti-aging and many other areas of therapeutics and preventive medicine. The hydrophilic nature and unique chemical structure of these compounds pose major challenge because of their poor bioavailability through the skin or gut. The use of phytosomes is a novel formulation technology which helps to overcome these problems. The effectiveness of any herbal medication is dependent on the delivery of effective level of the therapeutically active compound. But a severe limitation exists in their bioavailability when administered orally or by topical applications. Phytosomes are recently introduced herbal formulations that are better absorbed and as a result produced better bioavailability and actions than the conventional phytoconstetuients or botanical extracts. Phytosomes are produced by a process whereby the standardized plant extract or its constituents are bound to phospholipids, mainly phosphatidylcholine producing a lipid compatible molecular complex. Phytosome exhibit better pharmacokinetic and pharmacodynamic profile than conventional herbal extracts.

Key words: Phytosomes, Phosphatidyl choline, Phytoconstituent, Flavonoid.

INTRODUCTION

The advancement in the field of herbal drug delivery started recently with the aim to manage human diseases efficiently. Every nation is seeking health care beyond the traditional boundaries of modern medicine; turning to self medication in the form of herbal remedies (Gold et al. 2000; Mukherjie 2001). Nowadays expensive research in novel drug delivery systems is going on to improve the therapeutic efficacy of the existing natural molecules. Toxicity and limited absorption of different phytoconstituents obtained from herbs are major problems in exploring their real potentials against different diseases. So, extensive research in the field of herbal drug delivery systems as a means of improving the therapeutic indices of drugs is inevitable. During the last century, chemical and pharmacological studies have been performed on a lot of plant extracts in order to know their chemical composition and confirm the indications of traditional medicine. The Phytosome process produces a little cell because of that the valuable components of the herbal extract are protected from destruction by digestive secretions and gut bacteria. Phytosomes are better able to transition from a hydrophilic environment into the lipid-friendly environment of the enterocyte cell membrane and from there into the cell, finally reaching the blood (Dang 2000). Most of the bioactive constituents of phytomedicines are flavonoids (e.g., anthocyanidins from bilberry, catechins from green tea, silymarin from milk thistle). However, many flavonoids are poorly absorbed; the poor absorption of flavonoid nutrients is likely due to two factors. First, they are having multiple-ring molecules that are too large to be absorbed by simple diffusion. Secondly, flavonoid molecules typically have poor miscibility with oils and other lipids, which limited their ability to pass across the lipid-rich outer membranes of the enterocytes of the small intestine. Watersoluble flavonoid molecules can be converted into lipid-compatible molecular complexes; aptly called phytosomes. The term

Corresponding author:
kaushik.pallav@gmail.com

"phyto" means plant while "some" means cell like(Mukherjee et al. 2006). Phytosome is a newly introduced patented technology developed to incorporate standardized plant extracts or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes, called as phytosomes (also often referred as herbosome in certain literature) and so vastly improve their absorption and bioavailability (Bombardelli et al. 1993). The lipid-phase substances employed to make flavonoids lipid-compatible are phospholipids from soy, mainly phosphatidylcholine. Phosphatidylcholine is the principal molecular building block of cell membranes miscible both in water and in oil environments, and is well absorbed when taken by mouth. Chemical analysis indicates that the phytosome is usually a flavonoid molecule linked with at least one phosphatidylcholine molecule. A bond is formed between these two molecules, creating a hybrid molecule. This highly lipid-miscible hybrid bond is better suited to merge into the lipid phase of the enterocyte's outer cell membrane. Phosphatidylcholine is not merely a passive "carrier" for the bioactive flavonoids of the phytosomes, but is itself a bioactive nutrient with documented clinical efficacy for liver disease, including alcoholic hepatic steatosis, drug-induced liver damage, and hepatitis. The phytosome process has been applied to many popular herbal extracts including *Ginkgo biloba, grape seed, hawthorn, milk thistle, green tea,* and *ginseng.* The flavonoid and terpenoid components of these herbal extracts lend themselves quite well for the direct binding to phosphatidylcholine. Specifically, the choline head of the phosphatidylcholine molecule binds to these compounds while the fat-soluble phosphatidyl portion comprising the body and tail then envelopes the choline-bound material. The result is a little microsphere or cell like structure (Mukherjee et al. 2001). Phytosomes have improved pharmacokinetic and pharmacological parameters, which in result can advantageously be used in the treatment of acute and chronic liver disease of toxic metabolic or infective origin or of degenerative nature. It can also be used in anti-inflammatory activity as well as in pharmaceutical and cosmetic compositions (Mascarella et al. 1989). PC is miscible both in the water phase and in oil/lipid phases, and is excellently absorbed when taken by mouth. PC is the principal molecular building block for cell membranes (Fig. 1), and the molecular properties that suit PC for this role also render it close to ideal for its phytosome role.

Preparation of phytosome

Phytosomes are novel complexes which are prepared by reacting from 2-3 moles but preferably with one mole of a natural or synthetic phospholipid, such as phosphatidylcholine, phosphatidylethanolamine or phosphatidylserine with one mole of component for example-flavolignanans, either alone or in the natural mixture in aprotic solvent such as dioxane or acetone from which complex can be isolated by precipitation with non solvent such as aliphatic hydrocarbons or lyophilization or by spray drying. In the complex formation of phytosomes the ratio between thesetwo moieties is in the range from 0.5-2.0 moles. The most preferable ratio of phospholipids to flavonoids is 1:1(Magistretti et al. 1987). In the phytosome preparations, phospholipids are selected from the group consisting of soy lecithin, from bovine or swine brain or dermis, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine in which acyl group may be same or different and mostly derived from palmitic, stearic, oleic and linoleic acid. Selection of flavonoids is done from the group consisting of quercetin, kaempferol, quercretin-3, rhamnoglucoside, quercetin-3-rhamnoside, hyperoside, vitexine, diosmine, 3-rhamnoside, (+) catechin, (-) epicatechin, apigenin-7-glucoside, luteolin, luteolinglucoside, ginkgonetine, isoginkgonetine and bilobetine. Some liposomal drugs complex operate in the presence of the water or buffer solution where as phytosomes operate with the solvent having a reduced dielectric constant. Starting material of component like flavonoids are insoluble in chloroform, ethyl ether or benzene. They become extremely soluble in these solvents after forming phytosomes. This chemical and physical property change is due to the formation of a true stable complex (Sharma et al. 2005).

PROPERTIES OF PHYTOSOMES

1) Physico Chemical properties:

Phytosomes is a complex between a natural product and natural phospholipids, like soy phospholipids. Such a complex is obtained by reaction of stoichiometric amounts of phospholipids and the substrate in an appropriate solvent. On the basis of spectroscopic data it has been shown that the main phospholipids-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 functionalities of the substrate. When treated with water, phytosomes assumes a micellar shape forming liposomal-like structures. In liposomes the active principle is

dissolved in the internal pocket or it is floating 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. For example in the case of the catechindistearoylphosphatidylcholine complex, there is the formation of H-bonds between the phenolic hydroxyl ends of the flavones moiety and the phosphate ion on the phosphatidylcholine moiety. Phosphatidyl choline can be deduced from the comparison of 1H-NMR and 13C-NMR spectra of the complex with those of the pure precursors. The signals of fatty chain remain almost unchanged. Such evidence inferred that the too long aliphatic chains are wrapped around the active principle, producing a lipophilic envelope, which shields the polar head of the phospholipid and flavanoid molecule and enables the complex to dissolve in low polarity solvents (www.indena.com, Bombardelli et al. 1991).

2) Biological properties

Phytosomes are advanced forms of herbal products that are better absorbed, utilized and as a result produce better results than conventional herbal extracts. The increased bioavailability of the phytosome over the non complexed botanical derivatives has been demonstrated by pharmacokinetic studies or by pharmacodynamic tests in experimental animals and in human subjects (Franco et al. 1998).

CHARACTERIZATION OF PHYTOSOMES

The behaviour of phytosomes in both physical and biological system is governed by the factors such as physical size, membrane permeability, percentage of entrapped solutes, chemical composition as well as the quantity and purity of the starting materials. Therefore, the phytosomes are characterized for physical attributes i.e. shape, size, its distribution, percentage drug capture, entrapped volume, percentage drug release and chemical composition (Jain 2005). Liposomes are used primarily in cosmetics to deliver water-soluble substances to the skin. Mixing a water-soluble substance with phosphatidylcholine forms a liposome. No chemical bond is formed and there may be hundreds or even thousands of phosphatidylcholine molecules surrounding the water-soluble compound. In contrast, with the Phytosome process the phosphatidylcholine and the individual plant components actually from a 1:1 or a 2:1 complex depending on the substance (Bombardelli 1994). Phytosomes are not liposomes; structurally the two are distinctly different as shown in fig. no.3. The phytosome is a unit of few molecules this makes difference so the phytosomes being much better absorbed than liposomes. Not surprisingly, Phytosomes are also superior to liposomes in skin care products while the liposome is an aggregate of many phospholipid molecules that can enclose other phytoactive molecules but without specifically bonding to them. Liposomes are touted delivery vehicles, but for dietary supplements their promise has not been fulfilled. But for phytosome products numerous studies prove they are markedly better absorbed and have substantially greater clinical efficacy. Companies have successfully applied this technology to a number of standardized flavonoid preparations. The phytosomes technology is a breakthrough model for (Kidd et al. 2005).

- Marked enhancement of bioavailability
- Significantly greater clinical benefit
- Assured delivery to the tissues
- No compromise of nutrient safety

ADVANTAGES OF PHYTOSOMES

Phytosomes have the following advantages (Bombardelli et al. 1991 Bombardelli 1994, Kidd et al. 2004).

1) It enhances the absorption of lipid insoluble polar phytoconstituents through oral as well as topical route showing better bioavailability, hence significantly greater therapeutic benefit.
2) Appreciable drug entrapment.
3) As the absorption of active constituent(s) is improved, its dose requirement is also reduced.
4) Phosphatidylcholine used in preparation of phytosomes, besides acting as a carrier also acts as a hepatoprotective, hence giving the synergistic effect when hepatoprotective substances are employed.
5) Chemical bonds are formed between phosphatidylcholine molecule and phytoconstituent, so the phytosomes show better stability profile.
6) Application of phytoconstituents in form of phytosome improves their percutaneous absorption and act as functional cosmetics. Recent research shows improved absorption and bioavailability with phytosomes as compared to the conventional means.

Most of the phytosomal studies are focused to *Silybum marianum* (milk thistle) which contains premier liver protectant flavonoids. The fruit of the milk thistle plant contains flavonoids known for hepatoprotective effects (Hikino et al. 1984;

Wellington et al. 2001). Silybin is the chief and most potent constituent of silymarin, the flavonoid complex from milk thistle. A standardized extract from *Silybum marianum* is an excellent liver protectant but very poorly absorbed orally. Yanyu et al. (2006) prepared the silymarin phytosome and studied its pharmacokinetics in rats. In the study the bioavailability of silybin in rats was increased remarkably after oral administration of prepared silybin-phospholipid complex due to an impressive improvement of the lipophilic property of silybin-phospholipid complex and improvement of the biological effect of silybin. Tedesco et al. reported silymarin phytosome show better anti hepatotoxic activity than silymarin alone and can provide protection against the toxic effects of aflatoxin B1 on performance of broiler chicks.[21] Busby *et. al.,* reported that the use of a silymarin phytosome showed a better foetoprotectant activity from ethanol-induced behavioural deficits than uncomplexed silymarin (Busby et al. 2002). Grange et al. (1999) conducted a series of studies on silymarin phytosome, containing a standardized extract from the seeds of *S. marianum*, administered orally and found that it could protect the foetus from maternally ingested ethanol. Silymarin phytosomes, in which silymarin (a standardized mixture of flavanolignans extracted from the fruits of *S. marianum*) was complexed with phospholipids. Phytosomes showed much higher specific activity and a longer lasting action than the single constituents, with respect to percent reduction of edema, inhibition of myeloperoxidase activity,antioxidant and free radical scavenging properties (Bombardelli et al. 1994). In the human subjects silybin from phytosomes effectively reaches the intended target organ, the liver. This was proven by Schandalik et al. (1992) using nine volunteer patients who had earlier undergone surgical gall bladder removal necessitated by gallstones. They received single oral doses of 120 mg silybin as silybin phytosome and bile was assessed for silybin levels. Silybin appeared in the bile and peaked after 4 hours. In the case of phytosomal silybin, the total amount recovered in the bile after 48 hours accounted for 11 per cent of the total dose. In the case of silymarin, approximately 3 per cent of the silybin was recovered. These data demonstrate a four times greater passage through the liver for phytosomal silybin (Schandalik et al.1994; Mukherjie et al. 2001) one human study designed to assess the absorption of silybin when directly bound to phosphatadylcholine. Plasma silybin levels were determined after administration of single oral doses of silybin phytosome and a similar amount of silybin from milk thistle in healthy volunteers. The results indicated that the absorption of silybin from silybin phytosome is approximately seven times greater compared to the absorption of silybin from regular milk thistle extract (70-80 % silymarin content) (Barzaghi et al. 1990) .

Moscarella et al. (2006) investigated in one study of 232 patients with chronic hepatitis (viral, alcohol or drug induced) treated with silybin phytosome at a dose of 120 mg either twice daily or thrice daily for up to 120 days, liver function returned to normal, faster in patients taking silybin phytosome compared to a group of controls.[27] Studies have shown ginkgo phytosome (prepared from the standardized extract of *Ginkgo biloba* leaves) produced better results compared to the conventional standardized extract from the plant (GBE, 24 % ginkgo flavone glycoside and 6 % terpene lactones). In a bioavailability study conducted with healthy human volunteers the levels of GBE constituents (flavonoids and terpenes) from the phytosomal form peaked after 3 hours and persisted longer for at least 5 hours after oral administration. It was found that the phytosomal GBE produced a 2-4 times greater plasma concentration of terpenes than did the non-phytosomal GBE. Its major indications are cerebral insufficiency and peripheral vascular disorders, and it also can ameliorate reduced cerebral circulation. Its improved oral bioavailability and good tolerability makes it the ideal ginkgo product even for long term treatment.

Studies with ginkgo phytosome in patients with peripheral vascular disease (e.g. Raynaud's disease and intermittent circulation) have shown to produce a 30-60 % greater improvement compared to regular standardized GBE (Vitamedics 1999). Grape seed phytosome is composed of oligomeric polyphenols (grape proanthocyanidins or procyanidins from grape seed extract, *Vitis vinifera*) of varying molecular size, complexed with phospholipids. The main properties of procyanidin flavonoids of grape seed are an increase in total antioxidant capacity and stimulation of physiological antioxidant defenses of plasma, protection against ischemia/reperfusion induced damages in the heart, protective effects against atherosclerosis thereby offering marked protection for the cardiovascular system and other organs through a network of mechanisms that extend beyond their great antioxidant potency (Schwitters et al. 1994).

In another study, rabbits were fed with a high cholesterol diet for 6 weeks, to markedly elevate their blood cholesterol and induce atherosclerotic lesions in their aortas and carotid arteries. One group of rabbits received grape seed phytosome in their feed for the first 6 weeks, then 4 weeks of the high-cholesterol diet. These developed significantly less aortic plaque than did the control groups which

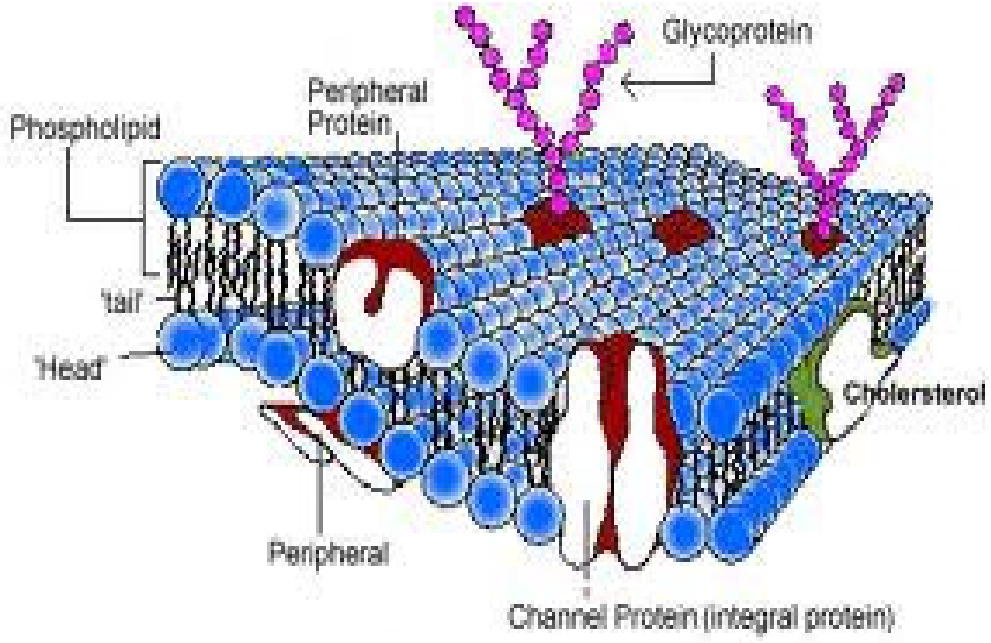

Fig 1: Cell membranes are largely lipid phase. A double molecular layer consisting of PC and other phospholipids provides a continuous matrix into which the proteins insert.

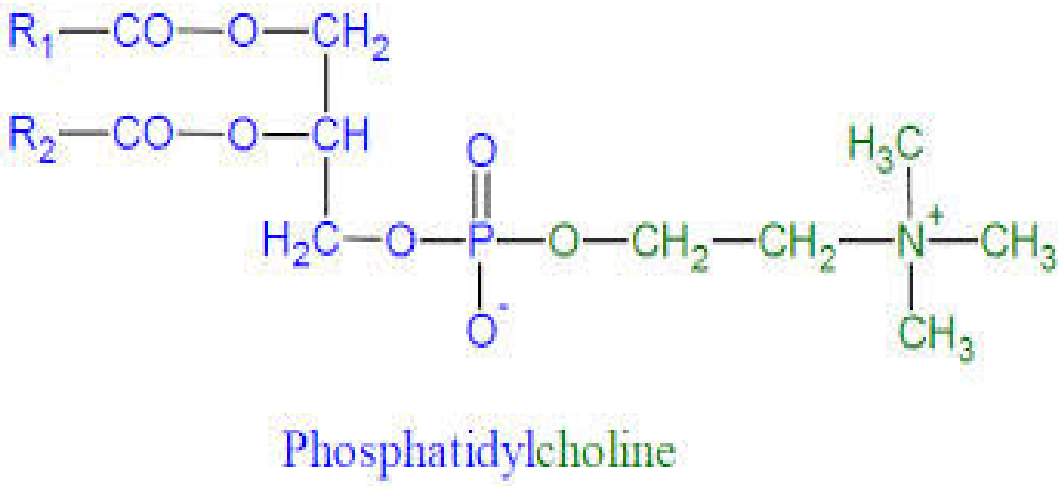

Fig 2. Structure of phosphatidyl choline

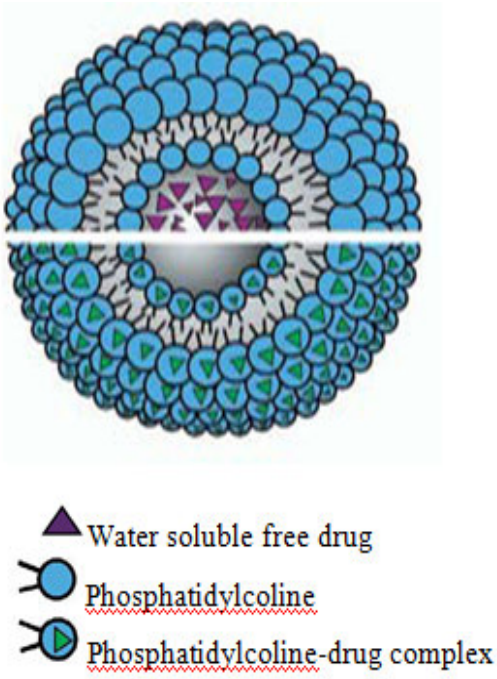

Fig. 3: Major difference between liposome (upper section) and phytosome (lower section). The molecular organization of the liposome (upper segment) versus many individual phytosomes (lower segment)

Table 1. Commercially available phytosome products

Product	Company	Indications	Dosage form
Ginkoselect phytosome	Indena S.p.A.,Italy	Improves memory, brain functions, cerebral and peripheral circulation, oxygenation and blood flow	Capsule
Greentea phytosome	Natural factors,Canada	Powerful antioxidant effects, preserving cell health to improved longevity, health and well being.	Capsule
Grapeseed phytosome	Natural factors,Canada	Natural antioxidant protection	Capsule
Panax gingseng phytosome	Natural factors,Canada	Promotes adaptogenic functions and resistance to stress	Capsule
Hawthorn phytosome	Natures herbs,USA	Helps to strengthen heart and cardiovascular system	Softgel capsule
Milk thistle phytosome	Indena S.p.A,Italy	It guards against free radical damage by acting as an anti oxidant,and stimulates the formation of new liver cells	Capsule

received conventional standardized grape seed extract in similar regimen. In a randomized human Trial, young healthy volunteers received grape seed phytosome once daily for 5 days. The blood TRAP (Total Radical-trapping Antioxidant Parameter) was measured at several time intervals during 1st day, then also on 5th day. Already by 30 minutes after administration on 1st day, blood TRAP levels were significantly elevated over the control which received conventional standardized grape seed extract (Facina et al. 1994). Green tea extract generally contains a totally standardized polyphenolic fraction (not less than 66.5 %, containing epigallocatechin and its derivatives) obtained from green tea leaves (*Thea sinensis*) and mainly characterized by the presence of epigallocatechin 3-O-gallate, the key compound.
These compounds are potent modulators of several biochemical processes linked to the breakdown of homeostasis in major chronic-degenerative diseases such as cancer and atherosclerosis. Green tea has got several long term beneficial activities such as antioxidant, anticarcinogenic, antimutagenic, antiatherosclerotic, hypocholesterolemic, cardioprotective, antibacterial and anticariogenic effects. Despite such potential actions green tea polyphenols have very poor oral bioavailability from conventional extracts. The complexation of green tea polyphenols with phospholipids strongly improves their poor oral bioavailability. A study on absorption of phytosomal preparations was performed in healthy human volunteers along with non complexed green tea extract following oral administration. Over the study period of 6 hours the plasma concentration of total flavonoids was more than doubled when coming from the phytosomal versus the non-phytosomal extract. Antioxidant capacity was measured as TRAP (Total Radical-trapping Antioxidant Parameter). The peak antioxidant effect was a 20% enhancement and it showed that the phytosome formulation had about double the total antioxidant effect (www.phospholipidsonline.com).

Quercetin-phospholipid phytosomal complex were developed by a simple and reproducible method and also showed that the formulation exerted better therapeutic efficacy than the molecule in rat liver injury induced by carbon tetrachloride (Maiti et al. 2007). They developed the phytosomes of curcumin (flavonoid from turmeric, *Curcuma longa*) and naringenin (a flavonoid from grape fruit, *Vitis vinifera*) in two different studies (Maiti et al. 2006; 2007). The antioxidant activity of the complex was significantly higher than pure curcumin in all dose levels tested. In the other study the developed phytosome of naringenin produced better antioxidant activity than the free compound with a prolonged duration of action, which may be due to decrease in the rapid elimination of the molecule from body. Hesperetin is a potent phytomolecule abundant in citrus fruits, such as grapefruit and oranges. In spite of several therapeutic benefits viz. antioxidant, lipid-lowering, anti-carcinogenic activities their shorter half life and lower clearance from the body restricts its use. To overcome this limitation, recently Mukerjee et al. (2008) developed a novel hesperetin phytosome by complexing hesperetin with hydrogenated phosphatidyl choline. This complex was then evaluated for antioxidant activity in CCl4 intoxicated rats along with pharmacokinetic studies. It was found that the phytosome had a sustained release property for over 24 hr and enhanced antioxidant activity.

Pharmacokinetic study revealed that the phytosome had higher relative bioavailability than that of parent molecule at the same dose level (Mukherjee et al. 2008). In this way different phytosome products have demonstrated significant therapeutic or health giving effects when compared with the conventional plant extracts. Some commercially available phytosome products are summarized in the Table 1.

CONCLUSION

Phytosomes are novel compounds comprising of lipophilic complexes of components of various plants like *Silybum Marianum, Ginkgo Biloba, ginseng* etc with phospholipids. Preparation of phytosomes is usually carried out by non conventional method. Absorption of phytosome in gastro intestinal tract is appreciably greater resulting in increased plasma level than the individual component. Complex formation ratio of component and phospholipids is 1:1 and 2:1. Phytosomes are used as a medicament and have wide scope in cosmeticology. Many areas of phytosome are to be revealed in future in the prospect of pharmaceutical application. Phytosomes forms a bridge between the conventional delivery system and novel delivery system.

REFERENCES

Barzaghi N, Crema F, Gatti G, Pifferi G, Perucca E. 1990. Pharmacokinetic studies on IdB 1016, a silybin phosphatidylcholine complex in healthy human subjects. Eur. J Drug Metab Pharmacokinet 15:333-38.

Bombardelli E, Curri S.B, Loggia Della R, Del N.P, Tubaro A, Gariboldi P. 1989. Complexes between phospholipids and vegetal derivatives of biological interest. Fitoterapia 60:1-9.

Bombardelli E, Spelta M, Loggia Della R, Sosa S, Tubaro A. 1991. Aging Skin: Protective effect of silymarin- phytosome. Fitoterapia: 62(2): 115-22.

Bombardelli E. 1991. Phytosomes in functional cosmetics. Fitoterapia: 65 (5): 320-27.1994.

Bombardelli E., Mustich G. Bilobalide phospholipid comlex, their uses and formulation containing them, U.S. Patent No.EPO-275005.

Bombardelli Ezio. 1994. Phytosome in functional cosmetics, Fitoterapia LXV (5): 387-401.

Busby A, La Grange L, Edwards J, Kings J. 2002. The use of a silymarin/phospholipids compound as a fetoprotectant from ethanol-induced behavioral deficits. J Herb Pharmacother 2 (1):39-47.

Dang Yi 2000. New product concept, UPC code-0300540111783.

Facina RM, et al. 1994. Free radicals scavenging action and antienzyme activities of procyanidins from Vitis vinifera. A mechanism for their capillary protective action. Arzneim Forsch 44: 592-601.

Franco PG, 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.

Gold JL, Laxer DA, Rochon PA, 2000. Herbal remedies; a critical perspective. Ann R Coll Physician Surg Can; 33:497-498.

Hikino H, Kiso Y, Wagner H, Fiebig M. Antihepatotoxic actions of flavonolignans from Silybum marianum fruits. Planta Med: 50:248-250.1984.

Jain NK. 2005. Controlled and novel drug delivery, 1st edition, CBS publisher, 321-326.

Kidd P, Head K.2005. A review of the bioavailability and clinical efficacy of milk thistle Phytosome: a silybinphosphatidylcholine complex. Altern Med Rev: 10 (3):193-203.

La Grange L, Wang M, Watkins R, Ortiz D, Sanchez ME, Konst J, Lee C, Reyes E. 1999. Protective effects of the flavonoids mixture, silymarin, on fetal rat brain and liver. J. Ethnopharmacol 65: 53-61.

Magistretti MJ. 1987. Bombardelli E. Pharmaceutical compositions containing flavanolignans and phospholipida active principles, U.S. Patent No-EPO209037.

Maiti K, Mukherjee K, Gantait A, Ahamed H.N, Saha B.P, Mukherjee P.K. 2005. Enhanced therapeutic benefit of quercetinphospholipid complex in carbon tetrachloride induced acute liver injury in rats: a comparative study. Iran J Pharmacol Ther: 4: 84–90.

Maiti K, Mukherjee K, Gantait A, Saha BP, Mukherjee PK. 2007. Curcumin-phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats. Int J Pharm 330 (1-2): 155-163.

Maiti K, Mukherjee K, Gantait A, Saha B.P, Mukherjee PK. 2006. Enhanced therapeutic potential of naringenin-phospholipid complex in rats. J Pharm Pharmacol 58 (9):1227-33.

Mascarella S. 1993. Therapeutic and antilipoperoxidant effects of silybin-phosphatidylcholine complex in chronic liver disease, Preliminary results. Curr Ther Res 53(1): 98-102.

Moscarella S, Giusti A, Marra F, Marena C, Lampertico M, Relli P, Gentilini P, Buzzelli G. 1993. Therapeutic and antilipoperoxidant effects of silybin phosphatidylcholine complex in chronic liver disease: preliminary results, Curr Ther Res 53:98-102.2006.

Mukherjee K, Maiti K, Venkatesh M, Mukherjee PK. 2008. Phytosome of hesperetin, a value added formulation with phytomolecules. 60th Indian Pharmaceutical Congress; New Delhi, 2008 Dec 12- 14; p. 287.

Mukherjee P.K, Wahile A. 2006. Integrated Approaches towards drug development from Ayurveda and other Indian System of Medicine.J. Ethanopharmacol 103:25-35.

Mukherjie PK 2001. Evaluation of Indian Traditional Medicine. Drug Information J.; 35(2):623-631.

Parris Kidd, Kathleen H 2005. A review of the bioavailability and clinical efficacy of milk thistle phytosome: a silybinphosphatidylcholine complex, Altern Med Rev 10(3): 193-203.

Phospholipids: The vital lipids.2014. Available at: www.phospholipidsonline.com

Phytosomes: a technical revolution in phytomedicine. 2014. Available at: http:IIwww.indena.com

Schandalik R, Perucca E. 1994. Pharmacokinetics of silybin following oral administration of silipide in patients with extrahepatic biliary obstruction. Drugs Exp Clin Res 20:37-42.

Schandalik R, Gatti G and Perucca E. 1992. Pharmacokinetics of silybin in bile following administration of silipide and

silymarin in cholecystectomy patients. Arzneimittelforschung. 42:964-68.

Schwitters B, Masquelier J. 1993. OPC in practice: Biflavanals and their application. Alfa Omega, Rome, Italy.

Sharma S, Sikarwar M. 2005. Phytosome: a review, Planta Indica, Vol.1, No. 2, 1-3.2005.

Tedesco D, Steidler S, Galletti S, Tameni M, Sonzogni O, Ravarotto L. 2004. Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B1 in broiler chicks. Poult Sci 83 (11):1839-43.

Vitamedics, Phytosome Products, Available at http://www.vitamedics.com. Accessed – Sept. 19, 2008.

Wellington K, Jarvis B. 2001. Silymarin: a review of its clinical properties in the management of hepatic disorders. Bio Drugs :15: 465-89.

Yanyu X, Yunmei S, Zhipeng C, Quineng P. 2006. The preparation of silybin-phospholipid complex and the study on its pharmacokinetics in rats. Int J Pharm: 307 (1):77-82.