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Review Article

Phospholipid-encapsulated herbal extract to enhance anti-angiogenic activity: Phytosomes in angiotherapy

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ABSTRACT

Angiogenesis is a process in which pre-existing vessels develop new blood vessels. It is a key factor to nourish embryos as well to develop the growth of most tumors. The absorption and bioavailability of water soluble antiangiogenic compounds from several acclaimed traditional medicinal plants varies remarkably because of their deferential solubility pattern in gastrointestinal tract. Phytosome is a complex formed between an herbal constituents and a natural phospholipid to bioavailability and bioefficacy. Basically, the choline heads of the phosphatidylcholine molecules bind to the herbal constituents; and then, the lipid-soluble phosphatidyl portions, which compose the body as well as the tails of these molecules, envelope the choline-bound components. So many products in the market are available which have been designed using phytosome technology including popular herbal extracts such as Ginkgo biloba, grape seed, olive oil flavonoids etc. In this review, a comprehensive discussion with respect to the advantages and potential uses of phytosomes to enhance the bioavailability and antiangiogenisity of herbal extracts is presented.

Keywords: Phytosome, Angiogenesis, Angiotherapy, Bioavailability, Anti-cancer, Traditional herbal medicines

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INTRODUCTION

Angiogenesis is the process in which pre-existing vessels develop new blood vessels. It is key to embryology and the development of most tumors [1]. It also plays a definite role in normal physiological functions, such as reproduction, repair and development [2]. This process can be regulated by some stimulators like chemokines, growth factors, some special angiogenic enzymes, some endothelial receptors, and adhesion molecules; and also by some inhibitors like angiostatin and endostatin [3]. Imbalance between inhibitors and inducers levels can cause a variety of health problems, such as cancer, arthritis, and heart and brain ischemias; and many other diseases [4]. The vascular endothelial growth factor (VEGF) is particularly incites angiogenesis [5]. As VEGF plays a critical role in some malignant and benign cancers, it can be a suitable target for cancer therapy [6]. In order for new blood vessels to be formed, a series of physiological events need to occur. These processes include the dispersion of the extracellular matrix; the migration, adhesion and proliferation of endothelial cells; and tube formation [7]. Therefore, the inhibitors of the pathological angiogenesis have promising potentials in curing the angiogenesis-diseases that establishes the new class of therapeutic agents in the field of "angiotherapy".

Studies have shown that many nutraceutical agents, including those derived from natural products, demonstrate poor bioavailability when consumed orally [7][8]. So far, most of the new potential therapeutics have demonstrated poor pharmacokinetics and biopharmaceutical properties. Therefore, enhanced drug delivery systems are warranted to allow the distribution of therapeutically active drug molecules to the site of action, while minimizing the exposure of healthy organs and tissues to these molecules as much as possible. Nanotechnology may serve this purpose by playing a key role in the development of future therapeutic agent "nanomedicines". These new therapeutic agents are sought to necessitate lower doses and offer better therapeutic effectiveness and safety profiles. To be considered efficient delivery systems, nanoparticles are supposed to be in the nanometer-size range, preferably between 1 and 200 nm, and must be able to contain encapsulated, dispersed, adsorbed, or conjugated drugs and imaging agents [8].

Phospholipids are useful carrier molecules utilized as a drug delivery technology. They are essential vehicles for the drug molecules which need to be administered in controlled release forms [9][10]. The preparation of drugs in lipid complexes may lead to improved solubility and decreased GI toxicity [10].Phytosome is phospholipid complex of drug possessing amphiphilic properties and hydrogen atoms capable of binding to phospholipids [11].

The objectives of the present review is to present the information about development, optimization, and characterization of phytophospholipids-complex (phytosome) using standardized extract of traditional herbs. In addition, the present review also focuses on the importance of the bioabsorption and bioavailability of phytsomal formulation. Finally, the emphasis of the current review is to use the technology of phytosome formulation to enhance the therapeutic efficacy of antiangiogenic agents derived from herbal extracts.

Angiogenesis pathways: Transcription factors like the hypoxia-inducible factors (HIFs) regulate more than 200 genes via hypoxic signaling, hence regulating cell invasion, angiogenesis and mitogenesis [12]. Such HIF factors also regulate some proteins, such as Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF) and Human Enhancer of Filamentation [13]. VEGF is expressed in both primary and metastatic human colon cancers; and its expression is markedly high in metastatic cells [14]. Thus, one of the serum markers used to detect colon cancer in the early stages is VEGF. In hypoxic conditions, the β and α subunits of HIF make dimers and translocate to the nucleus, where they regulate the expression of genes such as those of the vascular endothelial growth factor, Transforming growth factor alpha and Platelet-derived growth factor beta [14]. When the amount of oxygen is normal, prolin and asparagine hydroxylase hydroxylate HIF-1α, setting it to bind to VHL and later be degraded by proteasome [15][7].

Regulation of angiogenesis: Angiogenesis is regulated by a strict balance between mediatory activators and inhibitors. There are three different regulatory stages, which involve the destruction of the extracellular matrix (ECM); adjustments to the levels of angiogenic mediators like cytokines, growth factors and some other enzymes; and interactions on two different levels (cell-cell and cell-matrix interactions), which make for the final stage of regulation of angiogenesis [16].

Matrix metalloproteinases (MMPs) play the main role in the degradation of ECM [17]. They break down the protein constituents of ECM and create suitable conditions for cell migration by destroying collagen and other cellular barriers. Hence, MMPs play a key role in tissue turn-over, which resembles

a reaction to the environment due to the presence of physiological factors, like the normal growth factors, or pathological conditions, like inflammation and cancer [17]. MMPs activity can be regulated by two different ways, either by influencing its expression and activation level by manipulating proteolytic enzymes, or by adjusting its level of inhibition by manipulating its inhibitors [18]. Degradation of ECM due to the effects of different growth factors promotes endothelial cell migration and proliferation, which leads to the formation of new blood vessels [19]. Adhesion to other cells or to their matrices represents another class of regulatory events in angiogenesis, which are strictly regulated in normal cell lines; as errors in the regulation of these events lead to different kinds of human cancers. The process of cell adhesion occurs with the involvement of certain adhesion molecules, ECM components, some endogenous inhibitors and proteolytic enzymes. Unfortunately, decrease in the rate of adhesion promote cancer progression [20]. Adhesion molecules are categorized into 5 major protein groups: the selectins, the integrins, the immuneglubin superfamily, the cadherins and the hyaluronans groups. These proteins represent surface receptors which can be targeted to control cancer progression in different cancer types, and offer a variety of therapeutic techniques [20].

Tumor angiogenesis: Angiogenesis plays a central role in tumor development. It encompasses a series of interactions between a wide range of intracellular mediators, leading to enhanced endothelial cell proliferation and invasion [21]. One of the most important modulators of pathological angiogenesis is VEGF. Loss of one allele of the VEGF gene can illicit major deficiencies in the vascular system, and is lethal in the embryonic stage [22]. A certain percentage of somatic mutations can cause normal cells to proliferate continuously, transforming them into cancerous cells [23], whose gradual growth leads to the formation of cancerous tumors. Exaggerated proliferation rates cause cells to need a greater blood supply in order for them to obtain more nutrients. This results in a lack of oxygen and nutrients in normal cells, leading to their death [24]. The spreading of tumor cells via the circulatory system, a process called metastasis, enables those cells to reach other organs in the body, causing secondary tumors (Figure 1).

When a tumor grows more than its blood supply can allow, HIF-1 is produced, causing VEGF to be transcribed more [25]. VEGF increases the permeability of blood vessels, and causes endothelial cells to migrate and proliferate more than usual. A lack of oxygen can also cause other pro-angiogenic molecules, like nitric oxide synthase and PDGF, to be produced more. It can also affect the levels of growth factors alpha and beta, angiopoietins and the basic fibroblastic growth factors (bFGF) [26]. VEGF is one of the most influential molecules which stimulate angiogenesis. Other regulatory molecules include: the basic fibroblast growth factor, the platelet-derived growth factor, and metalloproteinases (MMPs). Moreover, the interferon family (α, β and γ), thrombospondin-1 and -2, angiostatin, and endostatin make a group of endogenous regulators which inhibit angiogenesis [27, 28].

Mechanisms of angiogenesis therapy: Angiogenesis is an essential process in tumor growth and can be a suitable target for treatment. Hence, the purpose of using anti-angiogenic factors in cancer therapy is to interrupt critical stages of angiogenesis. Anti-angiogenic therapy can lead to reduced vessel permeability and blood perfusion, and vascular shrinkage which decreases the possibility of a tumor receiving oxygen and nutrients [29]. Theoretically, anti-angiogenic therapy may revert tumor blood vessels into the normal state, and improve the quality and delivery of cytotoxic therapeutic agents [30]. Hence, anti-angiogenic agents function by reducing the permeability of blood vessels in tumors, and the dispersion of interstitial fluids. This leads to a decline in interstitial pressure, and, eventually, reduces tumor hypoxia [6]. If anti-angiogenic therapy is used along with cytotoxic agents, the effect of these agents increases, while tumor vessels are simultaneously normalized [31].

A number of anti-angiogenic therapeutic agents are undergoing clinical trials. These agents can be classified into 3 categories: The first comprises the endothelial cell growth inhibitors, the preeminent of which is endostatin. This class of angiogenesis inhibitors induces apoptosis and inhibits endothelial cell growth [32]. The second category includes drugs which act as angiogenesis-signaling blockers, an example of which is Avastin®. They are inhibitors of the basic fibroblast growth factor (bFGF) and VEGF [33]. The third category is composed of the receptor blockers which inhibit ECM destruction, like the inhibitors of MMPs, which act by inhibiting the receptor activities of multiple growth factors [34].

MEDICINAL PLANTS AS A SOURCE OF CANCER THERAPEUTICS

During the previous century, phytochemical research and pharmacological studies have been carried out on numerous herbal extracts to elucidate their chemical compositions and to investigate clinical manifestations associated with the use of

herbal remedies. Traditional approaches to treat cancer employ medicines derived from natural sources, such as animals, minerals and herbal products.

Plants can be used as herbal beverage, or can be extracted and their crude extracts may be formulated in the form of capsules or pills [12]. Herbal products can be used to manage a variety of health problems, including cancer. There is plenty of evidence indicating that phytochemical products can prevent the progression of cancer [35]. Phytochemicals can be used by patients who have undergone surgery, those at risk of cancer due to family history, and those undergoing chemotherapy and suffering its side effects [36].

HERBAL PRODUCTS AS CANCER TREATMENTS

Natural drugs have been used for decades to cure several diseases. Like many kinds of supplementary or, sometimes, alternative treatments, most people on earth have for long used herbs to help themselves feel better or to gain more control of medical conditions like hay fever, painful bowel syndrome, menstrual (period) difficulties and skin problems such as eczema [37, 38]. A number of studies estimate that approximately 6 out of each 10 people with cancer (60%) use herbal products combined with conventional cancer therapeutic agents [39]. There are many types of therapeutic herbal products, several of which overlap with food items. Plants like echinacea, St John's wort, green tea and ginger [40] are frequently used as remedies.

There is an increasing trend worldwide for the utilization of supplementary natural products and complementary alternative medicine (CAM) to aid or substitute conventional drugs in the management of various health problems, like the generalized body weakness resulting from continuous use of standard chemotherapeutic agents [41]. Phytochemical products from plant roots, bulbs, barks, leaves, and stems as well as other herbal components have demonstrated potential beneficial qualities, like anti-cancer activities. They have even been shown to provide ingredients useful for the synthesis of modern drugs [42]. A number of studies on herbal drugs have resulted in the identification of compounds with promising biological activities. Thus, investigations directed towards the determination of the proper dosage regimens of these herbs/compounds are highly sought to improve the treatment outcomes.

BIOAVAILIBILITY OF PHYTONUTRIENTS

Oral bioavailabilities of many compounds from natural products such as *Eurycoma longifolia*J. (Tongkat Ali), *Andrographis paniculate* (Hempedu Bumi), and *Orthosiphon stamineus* (Misai Kucing) have been found to be less than 1% [21]. The major bioactive compounds in *O. stamineus* are polyphenols and flavonoids (e.g. rosmarinic acid, 3',4',5,7-tetramethoxyflavone, etc.) [2]. It was reported that the poor bioavailability of flavonoids was due to their large particle sizes and their poor miscibility with oils and other lipids, which is ought to hinder the absorption of flavonoids by the membranes of the small intestines [43, 44]. Lipid solubility and particle size are the major factors preventing certain molecules from passing through biological membranes and being systematically absorbed following oral administration [45]. The effectiveness of an herbal product or medication is dependent upon delivering an effective level of its active compounds to the blood stream [44]. Most of the bioactive phytoconstituents, like flavonoids, terpenoids, tannins, xanthones, polyphenols, etc. are water souble, [46] and, hence, have bad absorption prfiles [47].

Methods to improve bioavailability: Low bioavailability is the major shortcoming in terms of the therapeutic potential of plant-based drugs. To address this issue the development of good formulations is required to improve the bioavailability and enhance the therapeutic effectiveness of such drugs. A liposome is produced by the addition of a water soluble compound to phosphatidylcholine [48]. No chemical bonds are created, but it is rather that the molecules of phosphatidylcholine act as a group to enclose the water-soluble compound. Lots of phosphatidylcholine molecules are needed to surround a compound fully.Phytophospholipid conjugated herbal extracts (phytosome extracts) are prepared by mixing water-soluble herbal extracts with phosphatidylcholine, which results in the generation of chemical bonds between certain plant constituents and phosphatidylcholine. 1:1 or 2:1 stoichiometric complexes are usually what makes up phytosomes. They result from interactions between an extract or a phytoconstituent and a phospholipidic molecule, leading to enhanced absorption of bioactive constituents as compared with liposomes in which no interactions takes place between the herbal extract and phosphatydilcholine [49]. Compared to phytosomes, among the few drawbacks of liposomes is that they are more deficult to formulate, pose higher production costs, and have a greater tendency to leak the encapsulated drugs. Free phospholipids are sometimes prone to oxidation, hydrolysis and other reactions, causing them to have short half lives, poor solubility, stability issues, immunogenic effects, and limited target availability. They may

also be rapidly cleared from the circulation as a result of their uptake by the reticuloendothelial system (RES), which primarily exists in the liver [1]. On the other hand, formulations involving phytosomes have been shown to be safe; and their components have all been approved for pharmaceutical use [50].

Phytophospholipid complexes as drug delivery systems: Amphiphilic drug–phospholipid complexes are stable and possess good bioavailability, which is why they are widely used in today´s drug delivery systems [51]. Decreased interfacial tension between a delivery system and GI fluids facilitates drug movement through the membranes, tissues, and cell walls in the organism [5]. Phytosome is a phospholipid complex of drug compound which imparts better pharmaceutical and biological properties than the drug alone, leading to increased bioavailability [38]. Those complexes have been prepared for different non-steroidal anti-inflammatory drugs, antineoplastic drugs and some proteins. Formulating a drug as a phytosome complex increases its absorption, and minimizes its gastrointestinal toxicity [52]. However, these novel carriers should have two important properties: First of all, they should deliver the drug at a specified rate subject to the patient's body needs during treatment. Second, they should direct the active compounds in herbal drugs to the targeted site of action. Standard dosage forms allowing prolonged-release of a drug do not normally possess such properties.

In phyto-formulation research, nano dosage forms, such as polymeric nanoparticles, nanocapsules, solid-lipid nanoparticles, nanoemulsions and phytosomes, offer some advantages, like enhanced solubility and bioavailability, improved pharmacological activities, and decreased toxicity. Hence, research is currently focused on finding ways to protect phytosomes from degradation and achieve better stability profiles. Herbal nano drug delivery systems are expected to overcome most of the problems related to herbal medicine use in the future [38], as phytosomes, which are made by binding particular components of herbal extracts with phosphatidylcholine, represent a dosage form with superior absorption rates and better levels of effectivity compared to unformulated traditional herbal extracts.

DEFINITION OF A PHYTOPHOSPHOLIPID COMPLEX

The Greek word "phyto" signifies a plant; and "some" is often used to mean cell-like. Hence, a "phytosome" is an herbal cell-like construction. It refers to an advanced natural formula consisting of bioactive constituents of plant extracts encapsulated in lipid [20].

Rationale of phytosome formulations: Many of the bioactive components of phytomedicines are water-soluble in nature. This includes flavonoids, glycosides, tannins and terpenoids. As flavonoids make for a large category of bioactive compounds, they have been shown to possess a wide range of curative activities [20, 26]. However, they are improperly absorbed. This is in part due to their large molecular sizes, which cannot really allow them to be absorbed by passive diffusion. It is also possibly due to their weak lipid solubility, which significantly restricts their capability to go across the lipid-rich biological membranes in the human body.

Phytosomes offer superior pharmacokinetic and pharmacodynamic profiles compared with traditional natural extracts. Phytosome technological innovations have been appropriately employed to promote the bioavailability of several therapeutic herbal extracts, including the extracts of milk thistle, *ginkgo biloba*, grape seeds, green tea, hawthorn, and ginseng, allowing them to be marketed for different medicinal applications and as nutritional supplements [53]. Phytosomes can also be possibly utilized in pharmaceutical preparations to achieve better anti-inflammatory actions and allow phytosome extract cosmetic combinations.

PHYTOSOME TECHNOLOGY

A phytosome forms as a micro sphere structure around a plant extract and its active components, and provides a protective layer against gastric secretions and gut bacteria alike [54]. Phytosomes are obtained via a reaction between soy phospholipids and particular botanical derivatives in an appropriate solvent. On account of their physiochemical and spectroscopic characteristics, some complexes of the sort might possibly be considered novel system of drug delivery [55].

Characteristics of a phytosome

Chemical characteristics: However, there is complexity regarding the outcome of adding different stoichiometric quantities of a phospholipidic material to a chosen polyphenolic compound, which might be a simple flavonoid, in a non-polar solvent [56].

Biological properties: Pharmacokinetic and pharmacodynamic analyses in experimental animals and human subjects have been made to reveal the natural tendencies of phytosomes [53]. The enhanced bioavailability of phytosomes in contrast with non-complexed botanical derivatives

continues to be the focus of researches worldwide [37].

Advantages of phytosomes over conventional dosage forms: Great improvement in the bioavailability of botanical extracts can occur upon being formed into complexes with phospholipids[57]. This process yields better absorption in the intestinal tract. It allows non-lipophillic botanical extracts to readily permeate the intestinal lumen, something which is, in any other case, impossible [38]. Phosphatidylcholine, an extremely important part of a cell's membrane, has been utilized in phytosome technology to act as a carrier, and to aid in skin nourishment [49]. Phytosomes are more stable than liposomes as chemical bonds are formed not only between the phosphatidylcholine molecules themselves but also between them and the phytoconstituents present [39]. Phytosomes are not to be mistaken for liposomes. They are structurally quite different. As opposed to a phytosome, a liposome is formed by combining a water-soluble compound with phosphatidylcholine. Not a single chemical covalent bond is created. It is rather that the phosphatidylcholine molecules enclose the water-soluble compound [41]. A great number of phosphatidylcholine molecules might be necessary to enclosing a water-soluble product. Conversely, with the application of a phytosome procedure, phosphatidylcholine molecules and a particular herbal extract basically form 1:1, or perhaps 2:1, complexes, based on the contents of the extract. Medicinal liposomal complexes are produced in the presence of water or a buffer solution. This allows the phytosomes to react with the solvent and obtain lower dielectric potentials [20]. With regards to phytosome supplements, many studies have shown that they can be significantly better absorbed and present significantly enhanced biological effectiveness [38].

Phytophospholipid complexes in cancer chemotherapy: Numerous dietetic vegetables, therapeutic herbs and other plants have been studied and their cancer chemopreventative efficacies evaluated. Consumption of herbal supplements may have a considerable therapeutic value in terms of reducing the risk of cancer and the side effects of chemotherapy [7][8]. Herbal compounds can aid in reducing the inflammatory episodes which play a critical role in carcinogenesis [45]. It has been estimated that almost one-third of all cancer-related deaths in America could possibly be avoided by better dietetic choices [46]. Emerging data implies that numerous dietetic agents and medicinal herbs can be utilized solely or in combinations alongside conventional chemotherapeutic agents to halt cancer progress; avoid the adverse incidence of cancer and metastasis; and, possibly, cure cancer [8, 58].

CONCLUSION AND FUTURE RESEARCH DIRECTION

The main aim of this review was using phytosome formulation technology in order to improve bioavailability and antiangiogenic activity of herbal constituents. HPLC, FT-IR, scanning electron microscope, transmission electron microscope, and similar physic-chemical spectral analyses can confirm the characteristics of the active compound-phospholipid complexes (phytosome). Such formulation has the potentials to increase the solubility, GIT absorption, bioavailability and bioeffeciacy of active phytoconstituents. Overall, phytosome formulations have the potentials to be used as therapeutic agents to enhance the antiangiogenic activity of herbal extracts. However, clinical trials need to be done to confirm the pharmacokinetic profiles and the bioefficacy of the phytosomes derived from the herbal active compounds.

Conflict of Interest: All the authors of the manuscript declare that there is no conflict of interest

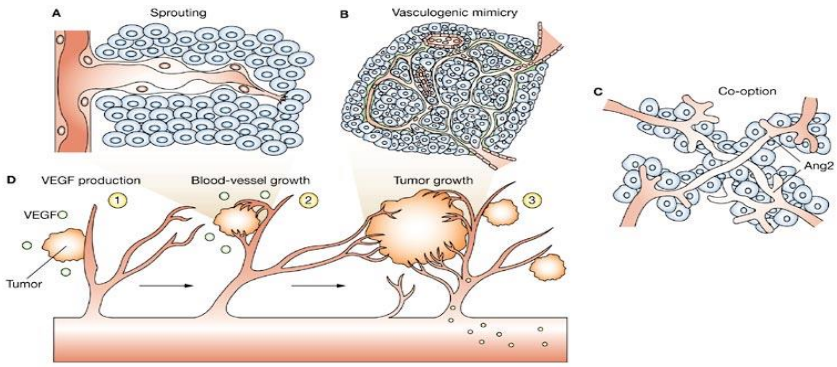

Figure 1: Mechanisms of tumor neovascularization [56]

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