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Preparation and characterization of phytosomes containing extracted catechin for hepatoprotective activity

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Abstract

Catechin, a bioflavonoid with anti-inflammatory, antioxidant, antitumor, and hepatoprotective properties, shows limited clinical potential due to poor gastrointestinal absorption, rapid metabolism, and low bioavailability. This study aims to enhance catechin's therapeutic efficacy by formulating and characterizing catechin-loaded phytosomes. Phytosomes, formed by complexing bioactive compounds with phospholipids, improve membrane permeability, cellular uptake, and sustained drug release, making them suitable for hepatoprotective delivery. Catechin will be extracted from natural sources and encapsulated into phytosomes, which will be evaluated using analytical methods such as particle size analysis to confirm complex formation and optimize formulation for stability and high entrapment efficiency. The hepatoprotective potential of catechin-phytosomes will be assessed in vivo using a carbon tetrachloride (CCl_4)-induced liver injury model by analyzing oxidative stress markers, histopathology, and liver enzyme levels (ALT, AST, ALP). Comparisons with pure catechin will determine improvement in therapeutic performance.

Key words : Catechin, Phytosomes, Bioavailability Enhancement, Hepatoprotective Activity, CCl_4 -Induced Liver Injury Model.

Novel herbal drug delivery system :

Over the past few decades, there has been a lot of interest in creating a novel drug delivery system (NDDS) for herbal treatments. Conventional dose formulations, such as those with extended release, cannot guide the phytoconstituents to reach their designated target location in order to achieve the maximum therapeutic response, nor can they hold the drug component at a specific rate as required by the body during the course of treatment[6,11].

Since the dawn of time, physicians and pharmacists have endeavored to provide patients with the best medications possible to aid in their speedy and full recovery from illnesses. The preparation is sometimes referred to as a

dosage form or drug delivery system, and the drugs are prepared in a suitable formulation that considers the acceptability, safety, and efficacy of different substances. Dosage forms have changed from simple mixtures and pills to incredibly sophisticated NDDSs as all fields of study and engineering have progressed[5,12,13].

Over the past few decades, there has been a lot of interest in creating a novel drug delivery system for herbal remedies. Herbal medicines are gaining popularity in the modern world because they can be used to treat a variety of illnesses with better therapeutic outcomes and fewer negative side effects. However, a variety of issues with plant actives and herbal extracts, including their instability in extremely acidic pH and liver metabolism, have caused the drug levels to drop below the therapeutic concentration in the blood, which has reduced or eliminated the healing effect [2,9].

Pharmaceutical approach of Herbosomal Technology :

The stoichiometric reaction of phospholipids (phosphatidylcholine, phosphatidylserine, etc.) produces the cell-like structures known as phytosomes. Conventional herbal extracts perform better in terms of absorption than standard extracts or polyphenolic components of a non-polar solvent. Phospholipids are among the most important components of life and one of the most important components of cellular membranes. For both polar and non-polar active compounds, they are generally believed to be natural transporters and digestive aids. Herbosomal preparations' amphiphilic properties boost bioavailability[3,4,15].

Mechanism of working of Phytosome :

Extracts from plants that include polyphenolic components are known to bind

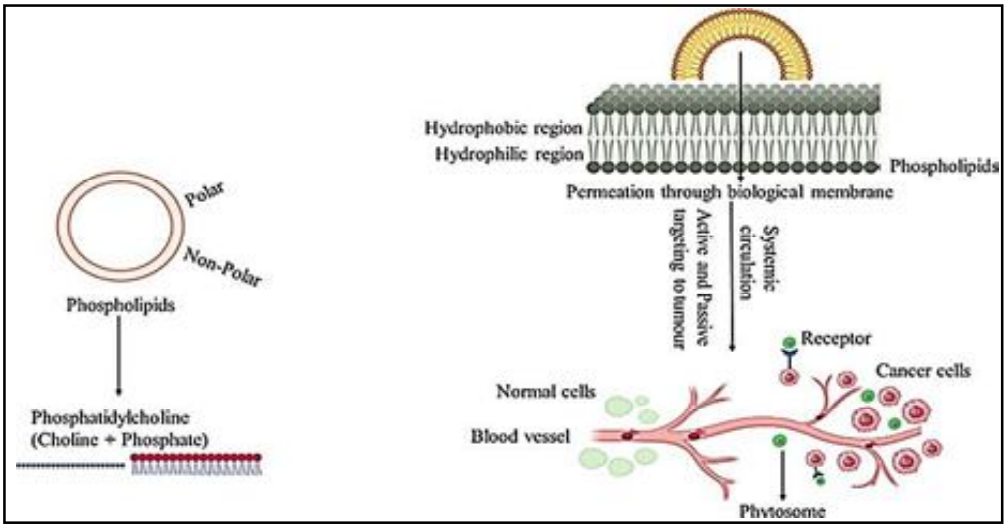

Figure 1. Mechanism of Phytosome loaded complex (Joshi *et al*., 2024)

to phosphatidylcholine right away. When combined with polyphenolic components, such as simple flavonoids, or typical solvent-based extracts that are aprotic, phospholipids, like soy phosphatidylcholine, can create phytosomes. The lipid-soluble phosphatidyl section, which consists of a body and a tail, envelops the choline head of phosphatidylcholine, which primarily binds these molecules. Phytomolecules are chemically connected to the polar head of phospholipids, according to spectroscopic methods. However, according to the chemical study, the Phytosome unit usually contains at least one phosphatidylcholine molecule and one or more flavonoid molecules[1,8,14].

Phosphotidylcholine and herbal extract :

One important class of lipids that make up every cell membrane are phospholipids. Phospholipids are also used by higher animals, including humans, as a natural digestive aid and as a form of fat-soluble and water-soluble nutrients. They are essential for biological membranes and can be extracted mechanically or chemically using hexane from soy beans or egg yolks. Because of the choline moiety's hydrophilic properties and the phosphatidyl moiety's lipophilic tendencies, phosphatidylcholine serves two purposes. The elements of the herbal extract are specifically bonded A tiny microsphere or cell is created when the lipid-soluble phosphatidyl part envelops the choline head of the phosphatidylcholine molecule[10].

Phytosomes are made from a variety of widely used standardized plant extracts, such as flavanoids, polyphenolics, terpenes, alkaloids, and volatile oils. A few years ago, it was shown that triterpenic acids, flavonoids, and saponins could combine to form real complexes based on phospholipids and that these substances could be chemically standardized. The complexity of flavanoids and polyphenolics with phospholipid molecules is the main process that creates phytosomes. Approximately 4,000 flavonoids have been identified in nature, each with a unique chemical composition and three-dimensional shape. After dispersion into the water phase, flavonoids, which are distinguished by their triple-ring topologies, are part of a broader family of antioxidants called polyphenols that are present in food below 40 °C [7].

Pharmaceutical approach of herbosomal Technology :

Hepatotoxicity :

Hepatotoxicity is the term used to describe liver damage or malfunction brought on by an excess of medications or xenobiotics. Hepatotoxins, also known as hepatotoxicants, are the substances that harm the liver. Overdoses of specific pharmaceutical medications, industrial chemicals, and natural substances like microcystins, herbal treatments, and nutritional supplements are examples of exogenous compounds of clinical significance that are known as hepatotoxicants. Even when taken within therapeutic ranges, several medications have the potential to harm the liver. Hepatotoxicity may result not only from direct toxicity of the primary chemical but also from a reactive metabolite or from immunologically-mediated reaction affecting hepatocytes, biliary epithelial cells and/or liver vasculature[15].

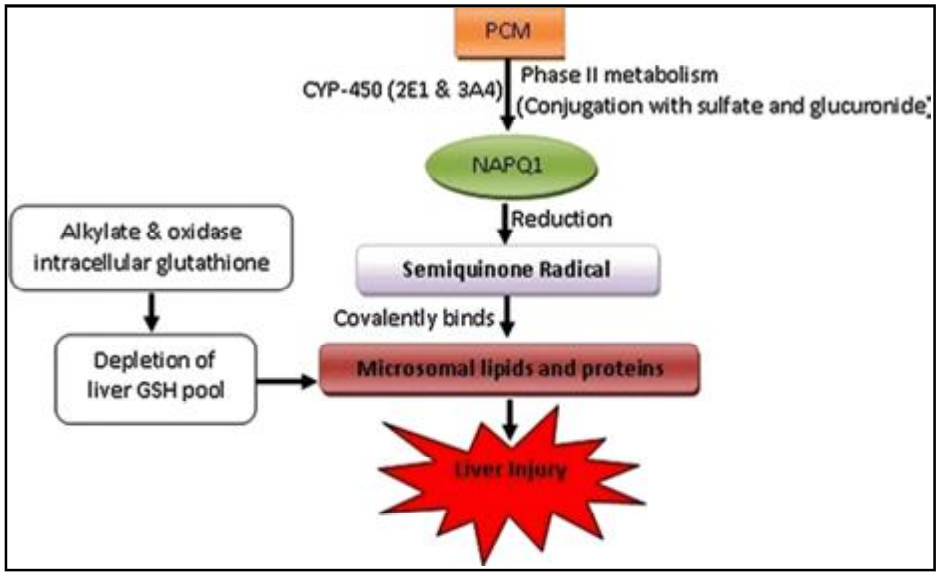

Figure 2. Mechanism of Hepatotoxicity (Chidiac *et al.,* 2023).

Solubility study :

Table-1. Solubility study of Catechin

Drug	Solvents	Observation/ Inference
Catechin	Water	Freely soluble (+++)
	Ethanol	Sparingly soluble (+)
	Methanol	Freely soluble (+++)
	Acetone	Soluble (+)
	DMSO	Freely Soluble (+++)

Determination of pH :

Table-2. pH determination

S. No.	Drug	Observed
1	Catechin	4.5

The digital pH meter used to determine the pH of a substance. The pH of the Catechin was found to be 4.5 which are well within the limits of the drug specification.

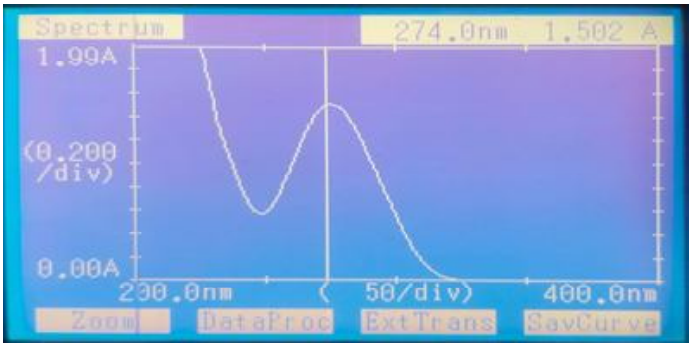

Figure 3. UV graph of Catechin (274.0 nm)

Melting Point :

Table-3. Melting Point of Catechin

Drugs	Observed	Reference
Catechin	176ºC	175 to 177ºC

Determination of λ max by UV spectroscopy:

Standard calibration curve :

Table-4. Calibration Curve of Catechin in Methanol

S. No.	Concentration (µg/ml)	Mean Absorbance
1	**10**	**0.128**
2	**20**	0.288
3	**30**	0.394
4	**40**	0.445
5	**50**	0.597
Mean		0.479857143
SD		0.239163423
% RSD		49.895615866388

Characterization of optimized Phytosomes formulation particle size

Table-5. Result of Particle size of all formulations

S. No	Formulations	Particle size (nm)	PDI Value
1.	Phytosomes (F1)	154.3 nm	0.010
2.	Phytosomes (F2)	154.7 nm	0.312
3.	**Phytosomes (F3)**	**149.1 nm**	**0.374**
4.	Phytosomes (F4)	551.2 nm	3.449
5.	Phytosomes (F5)	165.2 nm	0.082

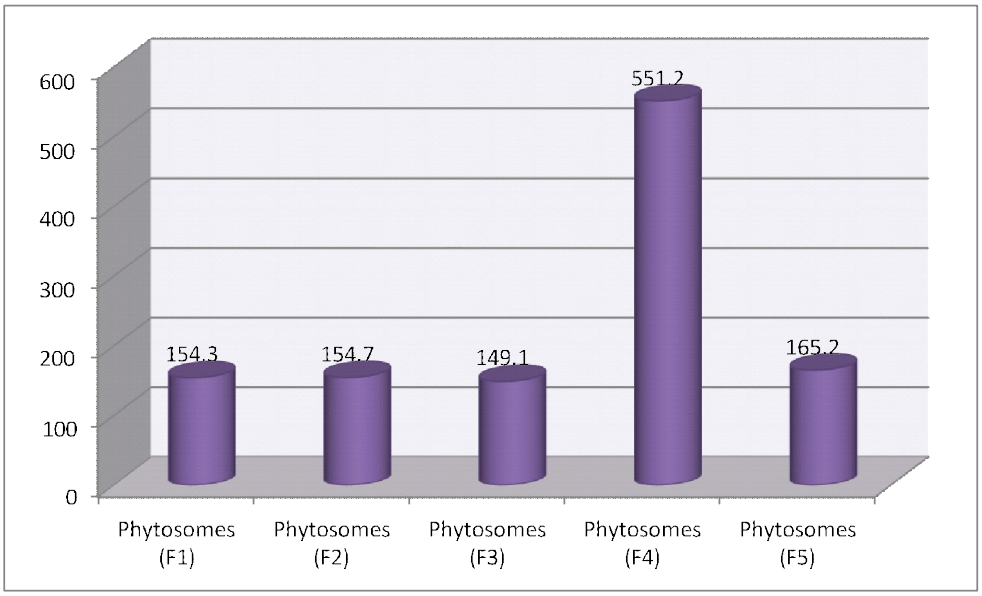

Figure 4. Particle size (F1 to F5)

Particle size is a key factor in determining the effectiveness, stability, and bioavailability of phytosome formulations. In this study, the particle size of catechin-loaded phytosomes was measured using a Malvern Zetasizer. The results showed that the average particle sizes ranged from 149.1 nm to 551.2 nm, which falls within the expected range for phytosomal systems.

These nanoscale sizes are ideal for improving drug absorption and stability. Among all formulations, Formulation F3 had the smallest particle size at 149.1 nm, suggesting it may offer enhanced bioavailability and better performance.

Entrapment efficacy :

Table-6. Entrapment efficacy

S. No.	Formulations	Entrapment efficacy (%)
1.	Phytosomes (F1)	80.12
2.	Phytosomes (F2)	82.25
3.	**Phytosomes (F3)**	**94.54**
4.	Phytosomes (F4)	55.34
5.	Phytosomes (F5)	75.51

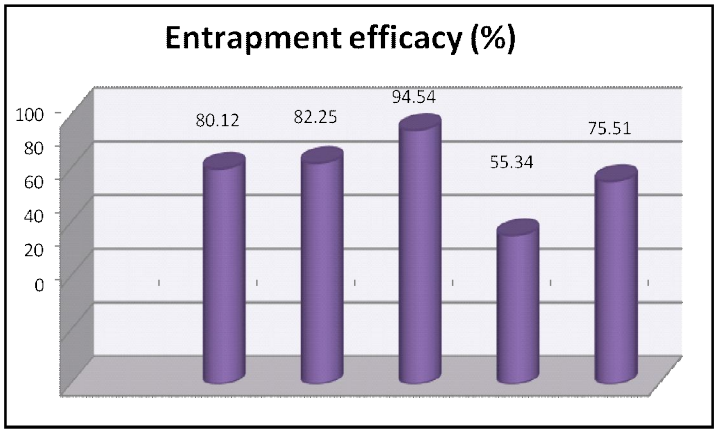

Figure 5. Graphical representation of Entrapment efficiency

Table-7. Result of Zeta potential of all formulations

S. No	Formulation	Zeta potential
1	Phytosomes (F1)	-69.7 mV
2	Phytosomes (F2)	-69.2 mV
3	**Phytosomes (F3)**	**-97.3** mV
4	Phytosomes (F4)	-62.0 mV
5	Phytosomes (F5)	-92.2 mV

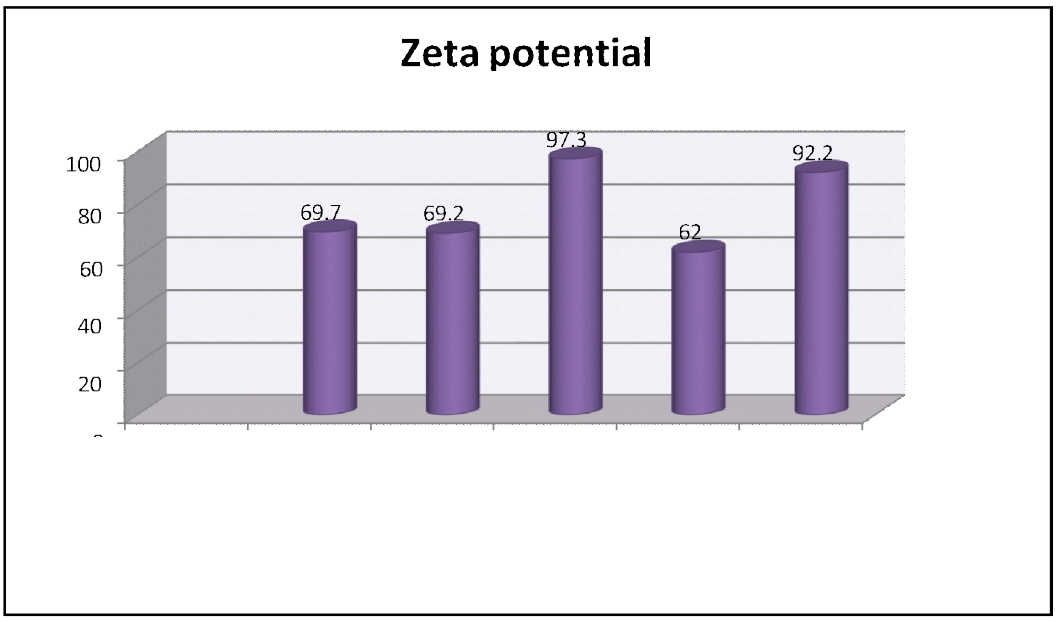

Figure 6. Graphical representation of Zeta potential

Zeta potential analysis is carried out to find the surface charge of the particles. The magnitude of zeta potential is predictive of the colloidal stability. Zeta potential was found to be all formulation range -69.2 to -97.3 mV with peak area of 100% intensity. These values indicate that the all formulated phytosome is stable. Results are shown in table-7.

The study was aimed to prepare and characterize phytosomes containing extracted catechin and evaluate their hepatoprotective activity. Initially, pre-formulation studies of catechin were carried out. Organoleptic evaluation showed that catechin was White to light brown in color, had a characteristic odor, a Solid powder appearance, and existed in a solid state. The solubility study revealed catechin was soluble in Acetone and sparingly soluble in Ethanol Freely soluble in Water, DMSO and Methanol. The melting point was determined to be 176ºC, confirming its purity. The pH of the catechin solution was recorded at 4.5, indicating slight acidity. Identification of the pure drug was confirmed through FTIR spectroscopy, which showed characteristic peaks corresponding to functional groups of catechin.Following pre-formulation, catechin-loaded phytosomes were successfully formulated using the thin film hydration (TFH) method. The phytosomes were characterized for their physical properties, showing a spherical shape with a smooth surface. The average particle size was found to be 149.1 nm, and the zeta potential was -97.3 mV, indicating good stability. SEM analysis further confirmed the uniform shape and surface morphology of the particles. In conclusion, catechin-loaded phytosomes were successfully formulated and exhibited promising hepatoprotective activity. The phytosomal formulation improved the bioavailability and stability of catechin, as

demonstrated by the favorable particle size, surface charge, and enhanced pharmacological results. These findings suggest that phytosomes are an effective drug delivery system for natural compounds like catechin in the treatment of liver disorders.

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