Summary and Conclusion
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“Investigation of pharmacological activities, formulation of liposomes and pharmacokinetic studies of lignans from aerial parts of *Phyllanthus amarus* Schum & Thonn.”, embodied with the work on an important medicinal plant i.e., *Phyllanthus amarus* which was mentioned in the Indian Ayurvedic system of medicine used for the treatment of various ailments like gastropathy, diarrhoea, dysentery, intermittent fevers, ophthalmopathy, scabies, ulcers and wounds. It is widely spread throughout the tropical and subtropical countries of the world including India.

As systematic scientific investigations have not been carried out on the aerial parts of this plant, this study was aimed to ascertain whether the aerial parts of the selected plant possess antioxidant and anti-inflammatory activity as reported in other parts of the selected plant; *in vitro* and *in vivo* anticancer activities as reported in other species of this genus.

On the basis of information and literature survey, the author examines the nature of chemical constituents, evaluates pharmacological activities for different solvent extracts and isolated lignans of the selected plant and formulates the conventional and pegylated liposomes for enhancing the oral bioavailability of isolated bioactive lignans.

For a systematic representation of the work carried out, the thesis was presented in nine chapters.

**Chapter I** deals with introduction. The introductory chapter explains about the importance of the medicinal plants, traditional medicine and novel drug delivery systems related to herbal medicine.

**Chapter II** deals with selection of plant followed by the stepwise plan of the work undertaken in the present study. A detailed review of literature of the selected medicinal plant (*Phyllanthus amarus*) which is used even today by the people in the treatment of various diseases was given. Extensive literature survey on the selected plant was made by the author from all available scientific sources. The survey of literature has presented in the thesis under the headings of taxonomical features, uses, phytochemical and pharmacological information.
Chapter III deals with the preliminary phytochemical screening for the presence of various phytochemical constituents in selected plant extracts. Qualitative phytochemical screening of different extracts of *Phyllanthus amarus* aerial parts revealed the presence of different phytochemical constituents like sterols, lignans, terpenoids, carbohydrates, glycosides, alkaloids, tannins, flavonoids and saponins (Table 3.02).

Chapter IV describes the isolation and characterization of lignans from the hexane extract. Column chromatography using silica gel for CC as adsorbent and gradient elution technique was used for isolation of the phytoconstituents and characterization of isolated compounds was done by using I.R, $^1$H & $^{13}$C N.M.R, COSY, HMBC and HSQC spectroscopy’s. The hexane extract of the aerial parts of *P. amarus* when subjected to column chromatography, yielded β-sitosterol, phyllanthin and hypophyllanthin.

Chapter V deals with the acute oral toxicity studies for hexane (PAHE), ethylacetate (PAEA) and methanolic (PAME) extracts of *Phyllanthus amarus* as per OECD guidelines 420 using oral route on albino mice. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Regd. No. 516/PO/c/01/CPCSEA, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

Chapter VI deals with the introduction to free radicals, antioxidants and list of plants with antioxidant activity. The experimental procedures for *in vitro* anti-oxidant activity of the extracts of the selected plant and known antioxidant ascorbic acid as standard were tested on superoxide, hydroxyl and DPPH radicals followed by their results and discussion.

The superoxide radical scavenging activity was determined by the IC$_{50}$ values of the three tested extracts, lignans of selected plant and ascorbic acid. The lower the IC$_{50}$ value, then the free radical scavenging activity will be higher. The mean IC$_{50}$ values for superoxide radical with hexane extract(PAHE), ethyl acetate extract(PAEA), methanolic extract(PAME), phyllanthin(PAPH) and hypophyllanthin(PAHP) of *Phyllanthus amarus* aerial parts were found to be 1147.2, 550.8, 395.6, 36.9 and 46.25µg; with ascorbic acid was found to be 30.42µg respectively.
The hydroxyl radical scavenging activity was determined by the IC$_{50}$ values of the three tested extracts, lignans of selected plant and ascorbic acid. The mean IC$_{50}$ values for hydroxyl radical with hexane extract(PAHE), ethyl acetate extract(PAEA), methanolic extract(PAME), phyllanthin(PAPH) and hypophyllanthin(PAHP) of *Phyllanthus amarus* aerial parts were found to be 1124.5, 514.27, 241.6, 31.59 and 39.85µg; with ascorbic acid was found to be 27.61µg respectively.

The DPPH radical scavenging activity was determined by the IC$_{50}$ values of the three tested extracts, lignans of selected plant and ascorbic acid. The mean IC$_{50}$ values for DPPH radical with hexane extract(PAHE), ethyl acetate extract(PAEA), methanolic extract(PAME), phyllanthin(PAPH) and hypophyllanthin(PAHP) of *Phyllanthus amarus* aerial parts were found to be 729.26, 388.56, 214.4, 26.56 and 28.14µg; with ascorbic acid was found to be 20.88µg respectively. Among the test results, phyllanthin showed better free radical scavenging activity than hypophyllanthin (PAHP) and other extracts (PAHE, PAEA & PAME).

**Chapter VII** discusses in detail the different models for screening anti-inflammatory activity and lists out the plants having anti-inflammatory activity in the first part. In the second part it describes the principle, procedure followed and the results of screening the three extracts (PAHE, PAEA & PAME) and lignans (PAPH & PAHP) of *Phyllanthus amarus* for anti-inflammatory activity.

Carrageenan-induced rat paw oedema model and Zeitlin’s apparatus was used for screening the extracts and isolated lignans for anti-inflammatory activity. The results were expressed as maximal paw oedema (Maximal peak during the 6hours) and as total paw oedema (area under curve) and presented as mean±S.E.M, n=6.

Carrageenan produced significant oedema in the left hind paw of the vehicle treated group and the paw oedema was significantly reduced (P<0.001) in the standard drug, indomethacin (1.3x10$^{-5}$ moles/kg b.w.) treated group at all hours when compared to control group.

Phyllanthin (PAPH) showed more potent anti-inflammatory activity than the other treatment groups (Table 7.2 to Table 7.5). The methanol extract (PAME) of aerial parts
of *P. amarus* exhibited significant reduction in paw oedema when compared to hexane extract (PAHE) and ethylacetate extract (PAEA) treated groups. The significant anti-inflammatory activity of the methanol extract (PAME) compared to the hexane (PAHE) and ethyl acetate (PAEA) indicated that the more polar constituents present in the methanol extract (PAME) may be responsible for its anti-inflammatory activity.

**Chapter VIII** gives an introduction about breast cancer; a list of cytotoxic drugs developed from plants and the proposed mechanisms by which phytochemicals may prevent cancer has been given. This chapter further elaborates on the materials and methods, experimental procedures for *in vitro* and *in vivo* breast cancer studies followed by results and discussion. The *in vitro* anticancer activity was performed against two cancer cell lines, namely, human estrogen-receptor positive breast cancer (MCF-7) cell lines and Human estrogen-receptor negative breast cancer (MDA-MB-231) cell lines. The percentage cell growth inhibition was calculated at 80µg/mL. At different concentrations the extracts and isolated lignans of *P. amarus* aerial parts were tested against the two breast cancer cell lines and a concentration dependant inhibitory activity was observed. By comparing the IC₅₀ values of the tested extracts and isolated compounds the most promising activity was observed for phyllanthin against both the cancer cell lines. The order of activity of tested extracts and isolated compounds against the cancer cell lines was phyllanthin (PAPH)>hypophyllanthin (PAHP)>methanolic extract (PAME)>ethylacetate extract (PAEA) >hexane extract (PAHE).

The *in vivo* anticancer activity was done by using MNU induced mammary cancer model in Sprague-Dawley rats. The methanolic extract (PAME) showed better cytotoxic activity than hexane (PAHE) and ethylacetate (PAEA) extracts against two breast cancer cell lines (MCF-7 & MDA-MB-231) in *in vitro* studies. So the methanolic extract (PAME) is further subjected to *in vivo* anticancer evaluation along with phyllanthin (PAPH) and hypophyllanthin (PAHP) by using MNU induced mammary cancer model.

Mammary carcinoma was induced by injecting 50mg/kg of N-methyl N-nitrosourea (MNU) to Sprague-Dawley rats and after 12 weeks of induction of mammary cancer the rats were given different treatments i.e., Tamoxifen (2mg/kg), methanolic extract (PAME-500mg/kg), phyllanthin (PAPH-5mg/kg & 10mg/kg) and
hypophyllanthin (PAHP-5mg/kg & 10mg/kg) daily for four weeks. After treatment, different tumor parameters like percentage tumor incidence, tumor multiplicity per rat and tumor weight per group were measured. After the completion of treatment, blood was collected from retro-orbital puncture and the hematological parameters like red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), and platelets (PLTs) were estimated (Perse et al., 2009; Jagatheesh et al., 2010).

The incidence rate in the MNU control group was found to be 85.7%. The tumor incidence in the Tamoxifen (2mg/kg) treated group was 25% where as the tumor incidence rates in the methanolic extract (PAME), phyllanthin (PAPH) and hypophyllanthin (PAHP) treated groups were found to be 75% (PAME), 50% (PAPH-5mg/kg), 31.5% (PAPH-10mg/kg), 62.5% (PAHP-5mg/kg) and 50% (PAHP-10mg/kg) (Table 8.5). The mean tumor multiplicity per rat of MNU control group was 2.14±0.58. The Tamoxifen treated group showed significant (P<0.05) decrease in the tumor multiplicity and its mean tumor multiplicity per rat is found to be 0.5±0.26. The phyllanthin treated groups [1.12±0.47 (PAPH-5mg/kg) and 0.87±0.27 (PAPH-10mg/kg)] showed dose dependent prominent reduction in tumor multiplicity when compared to hypophyllanthin [1.38±0.32 (PAHP-5mg/kg) and 1.25±0.41(PAHP-10mg/kg)] and methanolic extract (PAME-1.5±0.33) treated groups.

The weight of grossly detectable mammary tumors in MNU control group was 35.85g ranged from 0.06 g to 4.9 g per tumor. The weight of total mammary tumors per group (total tumor mass) ranged from 4.67g to 35.85g. Tamoxifen treated group showed significant (P<0.01) decrease of total tumor weight to 4.67g. The phyllanthin and hypophyllanthin treated groups showed significant (P<0.001) decrease in tumor weight when compared to MNU control group. The total tumor weight of phyllanthin treated group was 11.95g (PAPH-5mg/kg) and 8.87g (PAPH-10mg/kg). No significant decrease in tumor weight [12.82g (PAHP-5mg/kg) & 12.06g (PAPH-10mg/kg)] was observed with the increase of hypophyllanthin dose.

The number of red blood cells (6.18±2.21×10^6/µL) and haemoglobin (11.22±2.35g/L) were comparatively lower in the MNU treated group than in the control group, indicating a tendency to anemia. On the other hand the number of white blood
cells (WBC) considerably increased in MNU control group indicating the diseased state. In the Tamoxifen treated group there was significant (P<0.05) decrease in WBC’s to 7.51±1.31x10^3/µL when compared to MNU group (12.62±2.65 x10^3/µL). The number of platelets (PLT) was found to be 660.07±31.61 x10^3/µL for normal control group and they decreased to 448.24±28.11 x10^3/µL in the MNU group.

The phyllanthin treated rats showed small sized tumors with significant decrease in tumor multiplicity and tumor weight. From the histology studies it was observed that phyllanthin treated rat tumors showed very few necrotic cells (Fig.8.14), this may be due to potent inhibition of mammary tumor growth by phyllanthin treatment whereas the hypophyllanthin treated rat mammary tumors showed moderate number of necrotic cells (Fig.8.15) and total cancer tissue necrosis was observed in MNU control group (Fig.8.10), this necrosis was due to increased tumor size followed by the congestion of blood vessels and insufficient blood supply to the tumor tissue. The order of in vivo anticancer activity in MNU induced breast cancer model in rats was Tamoxifen>phyllanthin (PAPH)>hypophyllanthin (PAHP)>methanolic extract (PAME).

**Chapter IX** deals with the various types of liposomes and their different methods of preparation along with their intestinal permeability pathways. The role of surface modification of liposomes by pegylation and functionalization by binding ligands and their role in cancer therapy were briefly discussed.

Chemoresistance of cancer cells to anticancer drugs, normal tissue toxicity accompanied by undesirable side effects are the major complications associated with the anticancer drugs. The chemoresistance of cancer cells to anticancer drugs resulted in high mortality of the breast cancer patients. To control the side effects and mortality of the breast cancer patients and as alternative to conventional chemotherapeutic agents, highly potent new anticancer molecules should be isolated from natural origin. In the present study anticancer molecules like phyllanthin and hypophyllanthin have been isolated from the aerial parts of the plant *Phyllanthus amarus*. The anticancer potential of phyllanthin and hypophyllanthin was also evaluated in the chapter 8. When administered orally being lipophilic these molecules are rapidly transferred from the intestinal lumen to systemic circulation through the GIT membrane and being poorly water soluble these molecules
are incompletely absorbed. The enhancement of oral absorption of these drug molecules was then tried by particle size reduction and also by increasing the hydrophilicity of the by formulating them into liposomes.

Formulation of an anticancer dosage form as an oral nanosized liposomal drug delivery system would provide many biopharmaceutical advantages when compared with solid single-unit dosage forms in terms of a more even and predictable distribution and transportation in the gastrointestinal tract that is fairly independent of the nutritional state, predictable gastrointestinal transit time, less localized gastrointestinal disturbances and greater product safety; as well as having an application in the improvement of patient compliance. In view of the many benefits offered by multiple unit dosage forms, it is felt that such systems are particularly useful for site-specific targeting as the small unilamellar liposomes after reaching the blood circulation from the GIT specifically enters the cancer tissue due to the presence of large interstitial space in the tumor tissue and due to improper drainage system the liposomal vesicles get concentrated in the tumor thus releasing the enclosed drug specifically in the tumor tissue (Saetern et al., 2004).

The primary objective of the present investigation is to study the applicability of phospholipids and cholesterol for the incorporation of two anti-cancer compounds isolated from Phyllanthus amarus i.e., phyllanthin and hypophyllanthin for the formation of liposomes. It is also desired to study the different ratios of distearoyl phosphotidylcholine (DSPC) and cholesterol with respect to their capacity to control the release of these drugs. The plan of work then, involves the pegylation of the liposomes using DSPE-MPEG 2000 at different concentrations and its effect is to be investigated.

The plan of work was to prepare conventional liposomes of phyllanthin and hypophyllanthin separately with different ratios of DSPC and cholesterol and to select the optimum ratio of (DSPC) and cholesterol and to pegylate the liposomes of the optimized formula with DSPE-MPEG2000 and to evaluate the prepared liposomes by determination of the encapsulation efficiency, particle size analysis, polydispersity index (PDI), zeta potential, TEM analysis, IR studies, DSC-TGA studies, powder X-RD analysis and in vitro drug release studies. It was further planned to study the stability and in vivo performance in rats for the optimized formulations.

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Calibration curves were constructed for phyllanthin and hypophyllanthin by the developed HPLC method. New HPLC methods for the estimation of phyllanthin and hypophyllanthin in methanol and plasma samples were developed. The mobile phase consists of methanol and water in the ratio of 66:34, v/v.

For analyzing the spiked samples, 125µL of spiked plasma was taken and to this 25µL of internal standard (carbamazepine stock solution 10µg/mL in methanol) was added and then vortexed for 60 seconds. Then 500µL of methanol was added to precipitate proteins and vortexed for 5 min and centrifuged at 5000 rpm in a microcentrifuge for 10 min. Supernatant was taken and dried in vacuum oven at 40 °C. Dried samples were then redispersed in 100µL methanol and vortexed. The supernatant was transferred in to a microcentrifuge tube and from this 20µL was injected for HPLC analysis. The eluent was detected by UV detector at 225nm for phyllanthin and hypophyllanthin. The retention time for phyllanthin was 27.65mins and for hypophyllanthin was 24.16mins. The method was validated and was found to be accurate and precise.

Film hydration technique was used for the preparation of liposomes containing phyllanthin and hypophyllanthin separately using DSPC, cholesterol, stearic acid and DSPE-MPEG2000. Different ratios of drug (phyllanthin or hypophyllanthin), DSPC, cholesterol, stearic acid and DSPE-MPEG2000 were weighed and are dissolved in mixture of chloroform/methanol (9:1, v/v) in a round-bottomed flask. The flask was then connected to a rotary evaporator (Buchi Rotavapor, Switzerland) and was rotated about 40 rpm at 65 °C for 25 minutes before starting the vacuum pump. To minimize the oxidation of the lipid film, oxygen inside the flask was replaced with nitrogen.

The lipid film was then hydrated at 65 ºC (approximately 10 ºC above the phase transition temperature (Tg) of DSPC) with 10mL of phosphate buffered saline (PBS; pH-7.4) and vortexed (Vortex mixer, Genei Mumbai) for one hour. The resulted large unilamellar vesicles (LUVs) were extruded 10 times through extruder having 200nm polycarbonate filter papers (Whatmann, Denmark) at 65°C above Tg of DSPC to obtain small unilamellar vesicles (SUVs). The extruded SUV suspension was then freeze-dried to obtain fine powder of liposomes for the storage stability purpose.
By taking different ratios of drug, DSPC, cholesterol and stearic acid (Table 9.4.1&9.4.2), five formulations for each drug were prepared each drug i.e., PHL1, PHL2, PHL3, PHL4 & PHL5 for phyllanthin and HPL1, HPL2, HPL3, HPL4 & HPL5 for hypophyllanthin by the above procedure. When these formulations were evaluated for different characteristics PHL2 and HPL2 were found to be best. PHL2 and HPL2 were considered as the best formulations depending on their encapsulation efficiency, particle size, PDI and in vitro drug release studies.

For enhancing the stearic stability, hydrophilicity and drug release further it was planned to pegylate the liposomes of formula PHL2 and HPL2 with different concentrations of DSPE-MPEG2000. Three pegylated formulations were prepared for each drug by taking three concentrations of DSPE-MPEG2000 i.e., PHL6, PHL7 and PHL8 for phyllanthin and HPL6, HPL7 and HPL8 for hypophyllanthin. When these formulations were evaluated for different characteristics PHL7 and HPL7 were found to be best formulations based on their high encapsulation efficiency, particle size, PDI and in vitro drug release studies.

Formulations F1 –F10 were analyzed by different tests.

- The encapsulation efficiencies of the phyllanthin loaded conventional liposomes was in the range of 66.43%-86.47% and the encapsulation efficiencies of the hypophyllanthin loaded conventional liposomes was in the range of 61.88%-84.83% respectively. The encapsulation efficiencies of phyllanthin loaded pegylated liposomes was in the range of 82.14%-83.68% and for hypophyllanthin loaded pegylated liposomes the encapsulation efficiencies was in the range of 81.52%-82.62% respectively.

- The particle size analysis for the different liposomal formulations showed the following results. The particle size of different liposomal formulations loaded with phyllanthin (PHL1-PHL8) was in the range of 129-198nm and the particle size range of different hypophyllanthin loaded liposomes were found to be 126-205nm.
• FT-IR studies revealed that phyllanthin and hypophyllanthin are compatible with DSPC, cholesterol, stearic acid and DSPE-MPEG2000 and are free from chemical or physical interactions with them.

• DSC-TGA studies revealed that the two drugs phyllanthin and hypophyllanthin exhibited their transition in the thermograms at 97.7°C and 130.49°C respectively. As the melting points of the drugs (PAPH & PAHP) have not shown any significant shift in the thermograms of their respective formulations (PHL2, PHL7, HPL2 & HPL7) it was concluded that the drugs have not formed any inclusive complex with excipients and the drugs may be considered to be free from any physical or chemical interaction.

• XRD studies revealed that there was no significant change in the 2θ values in any of the phyllanthin and hypophyllanthin formulations. But there was a minimization of intensity of XRD peak in all the formulations. Thus it can be concluded that the drug is free from interaction and is present in an amorphous form in the formulations.

• TEM studies indicated that the conventional and pegylated liposomes containing anticancer compounds were found to be almost spherical, smooth and non-aggregated.

**Drug release studies indicated the following points**

• Drug release was slow and extended over 24 hrs for all the liposomal formulations PHL1-PHL8 and HPL1-HPL8.

• In all the liposomal formulations the drug released at pH 1.2 in the first two hours was very less. When the pH of the dissolution medium was changed from 1.2 to 7.4, there was a sudden rise in drug release rate; this is due to higher solubility of phyllanthin and hypophyllanthin at pH 7.4 than at pH 1.2.

• In case of PHL3, PHL5, HPL3 and HPL5 the increase in loaded drug concentration (10%-w/w) decreased the stability of conventional liposome formulations. It caused precipitation of the liposomes within 4 to 4.5 hours leading to burst release of drug.
• The conventional liposomal formulations PHL5 and HPL5 are less stable than PHL3 and HPL3 and this may be due to the decrease in the concentration of cholesterol from 40% to 30%.

• PHL1 and PHL4 showed drug release of 62.39% and 59.84% in 24 hours but PHL2 showed drug release of 66.48% in 24 hours. Since PHL2 releases most of its drug in a controlled manner in 24 hrs, it is considered as the best formulation among PHL1, PHL2, PHL3, PHL4 and PHL5.

• HPL1 and HPL4 showed drug release of 64.57% and 60.73% in 24 hours but HPL2 showed drug release of 67.46% in 24 hours. Since HPL2 releases most of its drug in a controlled manner in 24 hrs, it is considered as the best formulation among HPL1, HPL2, HPL3, HPL4 and HPL5.

• For both of the drugs phyllanthin and hypophyllanthin, the conventional liposomal formulations PHL2 and HPL2 were considered as the best because of their high entrapment efficiency and maximum drug release up to 24 hours. So the formulations PHL2 and HPL2 were taken for further processing and were pegylated with DSPE-MPEG2000.

• In the pegylated liposome formulations PHL6 and HPL6 showed drug release of 71.68% and 73.19% in 24 hours whereas PHL7 and HPL7 showed drug release of 84.62% and 86.09% in 24 hours.

• The increase in drug release rate of PHL7 and HPL7 may be because of the increase in DSPE-MPEG2000 concentration from 5% to 10%.

• PHL7 and HPL7 showed drug release of 84.62% and 86.09% in 24 hours whereas PHL8 and HPL8 showed drug release of 84.85% and 86.50% in 24 hours.

• Further increase of DSPE-MPEG2000 concentration from 10% to 15% does not showed any significant increase in the drug release rate. So that PHL7 and HPL7 formulations are giving almost the same drug release as that of PHL8 and HPL8.

• Since PHL7 and HPL7 formulations are giving almost the same drug release as that of PHL8 and HPL8, for a lesser concentration of DSPE-MPEG2000, PHL7 and
HPL7 were considered as the best formulation among the three pegylated formulations.

- PHL2 and HPL2 showed drug release of 66.48% and 67.46% in 24 hours whereas PHL7 and HPL7 showed drug release of 84.62% and 86.09% in 24 hours. It indicates that the rate of drug release from the pegylated liposomes was more than the conventional liposomes; this was because of the enhanced hydrophilicity occurred due to pegylation of conventional liposomes with DSPE-MPEG2000.

- The results of the dissolution studies indicated that the first order kinetics is a better fit to explain the release data in all the conventional and pegylated liposome formulations of phyllanthin and hypophyllanthin.

- The mechanism of drug release appears to be diffusion as indicated by high correlation coefficients for Huguchi equation (0.8408-0.9849) in the case of all formulations.

- The ‘n’ values in Peppas equations indicated that all the liposome formulations drug release followed non-Fickian diffusion.

These in vitro drug release studies may be concluded in the following manner. The incorporation of cholesterol into conventional liposomes caused increased stability of liposome formulations and extended the release in first order manner up to 24 hrs. The decrease in the concentration of cholesterol decreased the stability of conventional liposomes. The increase in drug concentration from 5%w/w to 10%w/w caused precipitation of the liposomes. The increase in lipid to DSPE-MPEG2000 concentration from 5% to 10% increased in drug release rate. Further increase of DSPE-MPEG2000 concentration from 10% to 15% does not showed any significant increase in the drug release rate. The pegylation of conventional liposomes with DSPE-MPEG2000 increased the release rate of both phyllanthin and hypophyllanthin from liposomal formulations.

- Toxicity studies revealed that, the administration of drug loaded or drug free liposomes did not produced any significant increase in hepatic (AST & ALT) and nephrotoxic (BUN, creatinine & plasma urea) parameters. There was no evidence of
any biochemical hepato and nephrotoxicity with respect to the control group animals. It was concluded that formulations PHL2, PHL7, HPL2 and HPL7 are not having hepato and nephrotoxicity.

- Pharmacokinetic studies done in rats revealed that conventional and pegylated liposomes containing phyllanthin and hypophyllanthin gave significantly sustained release pattern when compared with that of pure drugs. This study showed that all the pharmacokinetic parameters from liposomes were significantly different from the pure drug. The *in vivo* data demonstrated that the experimental formulations (PHL2, PHL7, HPL2 and HPL7) exhibited sustained release pattern when compared with their respective pure drugs. The $t_{1/2}$ and AUC values of experimental formulations are significantly higher when compared to the pure drugs indicating the extended drug release and higher bioavailability of developed liposomal formulations prepared with DSPC, cholesterol and DSPE-MPEG2000. A single oral administration of conventional liposomes and pegylated liposomes of phyllanthin and hypophyllanthin resulted in sustained plasma drug levels for 24 hrs. However, drugs were not detected in plasma beyond 12hrs after oral dosing with pure drugs and the drugs were not detected in plasma beyond 24hrs after oral dosing with conventional (PHL2 & HPL2) and pegylated liposomes (PHL7 & HPL7) of phyllanthin and hypophyllanthin. Therefore, indicating the efficiency of the developed formulation in sustaining the drug release. Thus the results of the present study clearly indicated the applicability of DSPC, cholesterol and DSPE-MPEG2000 in the design of oral controlled release liposomal drug delivery systems of bio-active lignans with higher bioavailability.

- The stability of the PHL2, PHL7, HPL2 and HPL7 liposomal formulations was evaluated in simulated gastric (SGF-pH 1.2) and intestinal fluids (SIF-pH 6.8). The pegylated liposomes (PHL7 & HPL7) were found to be more stable in gastrointestinal fluids than conventional liposomes (PHL2 & HPL2) loaded separately with phyllanthin and hypophyllanthin. The storage stability of the lyophilized formulations of PHL2, PHL7, HPL2 and HPL7 were evaluated by filling separately in glass vials and storing them at $25 \pm 2^\circ C$, $60\%$ RH $\pm 5\%$ RH for 6 months. The lyophilized formulations were found to be more stable without any
significant changes (p>0.05) in their particle size, PDI, zeta potential and encapsulation efficiency when stored at 25 ± 2°C. In vitro release studies were conducted for the set of selected formulations stored at 25 ± 2°C in order to compare their release kinetics before and after storage. The results indicated that the drug release characteristics of all the liposomes tested remained unaltered during the storage period.

In the last decades, there has been growing interest in using herbal medicine for the treatment of various diseases because of the side effects of modern allopathic medicine. The reasons for this interest are varied, and include low cost, medicinal value, drug resistance, limitations of medicine, cultural exchange and commercial value. Phyllanthus amarus Schum. & Thonn. belonging to family Euphorbiaceae is an important medicinal plant mentioned in the Indian Ayurvedic system for the treatment of various disease related to stomach, wounds, liver, genitor-urinal system and spleen.

On the basis of the results in the present study, it is concluded that the selected plant i.e., Phyllanthus amarus is endowed with potential pharmacological activities (Antioxidant, Anti-inflammatory and Anticancer) and the results of the present study scientifically justifies their use in the folklore remedies since ancient times. Formulation of conventional and pegylated liposomes loaded with phyllanthin and hypophyllanthin was done along with their isolation, characterization and pharmacological evaluation. From the pharmacokinetics study of pure lignans and their respective liposomes, it was observed that the oral bioavailability of the phyllanthin and hypophyllanthin was enhanced by loading them in to conventional and pegylated liposomes.
**SCOOPE FOR FURTHER WORK**

The developed liposomal formulations of both the bioactive lignans, phyllanthin and hypophyllanthin are showing extended drug release over 24 hrs in *in vitro* drug release studies and also showing enhanced oral bioavailability as indicated by AUC values of 5265.30±275.52 ng.h/mL (phyllanthin), 15217.60±987.96 ng.h/mL (PHL2), 30810.23±2587.96 ng.h/mL (PHL7) and 7354.42±578.25 ng.h/mL (hypophyllanthin), 29222.4 ±1951.84 ng.h/mL (HPL2), 58631.87±2515.46 ng.h/mL (HPL7) and their presence in the plasma was observed up to 24 hrs for phyllanthin and hypophyllanthin loaded liposomal formulations in comparison to their free drugs (phyllanthin and hypophyllanthin) which were cleared from plasma within 12 hrs.

The enhanced bioavailability of lignan loaded liposomes may be helpful for the production of desired pharmacological activity relatively at a lower dose when compared to their respective free drugs. Hence, it may be worthwhile to proceed these lignan loaded liposomal formulations for further pharmacological evaluation.