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STABILITY AND BIODISTRIBUTION STUDY OF TARAXACUM OFFICINALE NIOSOMES

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ABSTRACT

Recent progress in the study of such plants has resulted in isolation of about different phytoconstituents from plants. Among them 110 species of different plant families are mentioned their specific parts having protective action against certain liver disorder. Over the past several years, great advances have been made on development of novel drug delivery system (NDDS) for plant actives and extracts. The variety of novel herbal formulations like polymeric nanoparticles, microspheres, transferosomes and ethosomes has been reported using bioactive and plant extracts. The biodistribution pattern says that the dandelion loaded niosomes minimized the toxicity associated with plain dandelion solution. Vesicular systems not only help in targeting the drug the liver, but also help in providing controlled parenteral delivery by preventing metabolism of the drug from the enzymes present at the hepatic cells. Longer circulation of the niosomes showed that, incorporation of dandelion into the niosomes helped to increase the stability of dandelion by preventing it from chemical and enzymatic degradation. vesicles consisting of one or more surfactant bilayers enclosing aqueous spaces called niosomes have been considered of particular interest as they offer several advantages over liposomes with respect to chemical stability, lower cost and availability of materials. Lyophilized niosomal formulation (Proniosomes) were stable when stored at both refrigerated and room temperature.

Keywords: *Taraxacum officinale*, Niosomes, Evaluation, Proniosomes.

INTRODUCTION

Niosomes are one of the drug delivery system for targeting the specific site of the liver, brain etc. Literature revealed that the liver targeting agents like DMPC used in the vesicular formulation accumulate the maximum amount of drug in targeted cells, which would help in reducing the dose of the drug and it`s dose related toxicity. By incorporation of drug in small niosomes, the drug can be targeted directly to the site of action, thus enhancing its therapeutic efficacy. At the same time, drug entrapped in the aqueous interior can

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be protected from any destabilizing components present in the blood and, conversely the toxicity of drug was markedly reduced, when administered parenterally.

Dandelion (*Taraxacum officinale*)

The plant is weed and generally found road side, wildely grown in crops and the botanical name is *Taraxacum officinale* with asteraceae family. The roots and leaf extract have many potential activities like hepatoprotective, antioxidant, blood sugar decreasing activity, and triglyceride and cholesterol decreasing activity. The plant was traditionally used as diuretics from many years. It has high level of potassium supplement and rich in multivitamins. Dandelion also decreases blood pressure and used in treatment of hypertension. The main chief pharmaceutically bioactive constituents are beta carotene a polyphenols that can be developed as phytosome and increase the bioavailability of dandelion plant extract and its solubility that are not found in plant extract preparations.

MATERIALS AND METHODS

Experimental

Lyophilization of the final optimized batch of Primaquine phosphate loaded Niosomes was carried out to protect the drug and maintain the physicochemical properties of the vesicular system. Different concentrations of mannitol were tried in the ratio of 1:5, 1:10 and 1:15 (Drug: cryoprotectant). The niosomal dispersion, 5ml placed in 20 ml glass vials. The rapid freezing of the sample were carried by dipping the whole vial containing niosomal dispersion into liquid nitrogen. Vesicular formulation was subjected to freeze drying at -30°C for 48 hours followed by secondary drying phase of one day at 200c in VirTis Advantage, NY freeze dryer. Effects of different concentration of cryoprotectants on particle size and polydispersity index (PI) and entrapment efficiency of the formulation were studied. The vesicular system was analyzed for change in particle size, polydispersity index and % encapsulation efficiency before and after freeze drying.

Stability Testing:

Stability Testing as per ICH Guidelines: International conference on harmonization (ICH) has established a guideline for "Stability testing of new drug substances and products Q1A (R2)" [ICH, 2003]. The guideline recommends following storage conditions for drug substances intended for storage in a refrigerator.

Table 1: Stability protocol for freeze dried niosomal formulations

Condition	Storage	Sampling point points
Room temperature	Freeze dried niosomes filled in glass vials with rubber closures and sealed	0, 1, 2, 3 months
25^0C with 60% RH	Freeze dried niosomes filled in glass vials with rubber closures and sealed	0, 1, 2, 3 months
5°C ± 3°C	Freeze dried niosomes filled in glass vials with rubber closures and sealed	0, 1, 2, 3 months

Evaluation Parameters:

Taraxacum Officinale niosomes at each temperature point were analyzed in triplicate. The stability samples were evaluated for following parameters for 4 weeks.

1. Physical stability

2. pH
3. Vesicle size
4. Entrapment efficiency

Observation of physical stability of *Taraxacum officinale* niosomes

i) Physical stability:
Stability vials were observed for signs of obvious physical instability symptoms such as change in color or formation of larger aggregates.

ii) pH:
Stability of niosomal dispersion were analyzed for the changes in pH.

iii) Vesicle size and size distribution
Using a computerized inspection approach, dynamic light scattering (DLS) is performed to examine the vesicle scale, size distribution and zeta potential of an integrated Phytosomes formulation (Malvern Zeta master ZEM 5002, Malvern, UK). To assess the Phytosomes and Stern layer (zeta potential) potential diluted system was introduced into a zeta potential measuring cell.

(iv) Entrapment efficiency
Published a method for determining entrapment performance. Using cooling centrifuge (Remi) at 40°C, 12000 rpm the prepared phytosome is centrifuged for an hour. The transparent supernatant was cautiously syphoned off to isolate the non-entrapped gallic acid, and at max 273.5 nm, the absorbance of the mixture for non-entrapped gallic acid determined by UV/visible spectrophotometer (Shimadzu UV 1700). 1 mL of 0.1 percent Triton x 100 was added to the sediment to break the vesicles, Using phosphate buffer saline (7.4) it is diluted to 100 mL with and the absorbance measured at 273.5 nm. By summing the quantities of gallic acid in the supernatant and sediment, the total amount of gallic acid in 1 ml dispersion was estimated.The below formula is used to calculate entrapment percent.
Amount of Drug Percent Entrapment in sediment ---------- X 100 Total drug amount added

v) In vitro drug invasion study
Permeation study in vitro of the all formulations and market preparation was carried out utilizing a Franz diffusion cell that had been modified. This experiment is generally carried out by using skin membrane however we had replaced the skin membrane by a prepared biological membrane.

RESULT AND DISCUSSION

Table 2: Effect of ratios of mannitol on freeze drying of *Taraxacum officinale* loaded niosomes

Sr. No	Drug: Mannitol Ratio	Aqueous Dispersibility of *Taraxacum Officinale* niosomes
1	1:5	Does not go in solution
2	1:10	Buff solution obtained
3	1:15	Clear solution obtained

Table 3: Effect of freeze drying on PI of *Taraxacum officinale* niosomes

Drug : Mannitol Ratio	PI	
1 : 15	Before	After
	0.61	0.96

(n = 3; average mean value)

Table 4: Effect of freeze drying on Particle size of *Taraxacum officinale* niosomes

Time (Days)	Particle size in nm
0	322.6
1	322.67
2	323.7
3	324.9

Table 5: Effect of freeze drying on Entrapment efficiency of *Taraxacum officinale* Niosomes (*Taraxacum officinale* loaded niosomes entrapment efficiency (%)

Time (Month)	Freeze dried *Taraxacum officinale* niosomes
0	60.75
1	60.25
2	59.60
3	59.60

(n = 3; average mean value)

Table 6: Effect of storage condition on pH of niosomal dispersion

Storage In weeks Temp.		**0**	**1**	**2**	**3**
4^0C	Niosomal Dispersion	7.40	7.52	8.58	8.64
25^0C	Niosomal dispersion	7.42	7.59	8.68	8.83

n=3; Average mean value

Table 7: Effect of storage condition on vesicle size of niosomal dispersion

Storage In weeks → Temp ↓		0	1	2	3
4^0C	Niosomal formulation	219.7 nm	428.3 nm	554.5 nm	740.9 nm
25^0C	Niosomal formulation	405.9 nm	440.7 nm	650.8 nm	1.35 µm

n=3; Average mean value

Table 8: Effect of storage condition on PI of niosomal dispersion

Storage In weeks ⟶ Temp ↓		0	1	2	3
4^0C	Niosomal formulation	0.60	0.76	0.96.	0.96

Taraxacum officinale loaded niosomes were freeze dried in presence and absence of cryoprotectants to evaluate the effect of cryoprotectants on lyophilization process. Freeze drying of niosomes when carried out in absence of cryoprotectants resulted in formation of a fluffy mass with the separation of phases which did not disperse readily in aqueous solution on reconstitution and formed a precipitated mass. Presence of cryoprotectants like mannitol resulted in formation of comparatively less fluffy mass. This mass could be reconstituted with vigorous shaking. No significant change in PI was observed on evaluating the reconstituted dispersion for PI (Table no.04 The particle size before and after freeze drying was found to be 219.7 nm and 322.6 nm respectively as shown in Table no 04.

Drug: cryoprotectant ratios of 1:5 and 1:10 resulted in formation of larger aggregates. Hence freeze drying was carried out at drug: cryoprotectant ratio of 1:15. And it resulted in formation of free flowing powder was obtained which could be easily reconstituted with distilled water to yield a colloidal suspension. (Table no. 3) The reconstituted colloidal suspension showed particle size and Entrapment efficiency 322.2 nm and 60.75 % respectively. The increase in entrapment efficiency after freeze drying could be due to the adsoption of free Taraxacum officinale at the surface of niosomal vesicles as the water sublimated for cyclosporine poly (€-caprolactone) nanoparticles.

PI and Particle size of the freeze dried formulation showed significant change when compared to data of freshly prepared niosomal formulation, (Table no. 8.2 and 8.3). The particle size and entrapment efficiency of freeze dried Taraxacumofficinaleniosomes was varied from 322.6 nm to 324.9 nm and 60.75 % and 59.35 % respectively as shown in Table no 03 and table no. 4.

The reason for decease in the entrapment efficiency of the niosomal suspension over the lyophilized formulation is the hydrophilic non-ionic surfactant used in the formulation which has the low phase transition, increase the leakage of the drug from aqueous compartment and also the aggregations, but decreased in entrapment efficiency is not significant so the freeze dried niosomal formulation found to be stable for three month.

From the Table 4 it was observed that niosomal dispersion were in the range of 219-740 nm and 528 nm - 1.35 µm when stored at 4^0C and 25^0C respectively over the period of 3 weeks. The significant increased in the particle size of niosomal dispersion observed at 4^0C as well as 25^0C could be due to aggregation of vesicles to form the bigger particles, which consequently decreased the stability of niosomes. The niosomal dispersion when analyzed at both 4^0C and 25^0C showed the significant change in PI value as shown in Table 5 revealed that the niosomal membrane starts disrupting and vesicles gets aggregating, which reflected in higher PI values as shown in Table 4.

iv) Drug Entrapment

Table 9: Effect of storage condition on entrapment efficiency of niosomal dispersion

Storage In weeks ⟶ Temp ↓		0	1	2	3
4^0C	Niosomal formulation	59.7	55.3	54.1	48.45
25^0C	Niosomal formulation	50.6	48.66	42.58	30.5

n=3; Average mean value

From the Table 9, it was observed that there was decreased in entrapment efficiency of niosomal dispersion at both 4^0C and 25^0C could be due to the leakage of the drug from niosomal membrane, as membrane starts disrupting. The drug leakage at 4^0C was found to be considerable less than leakage at 25^0C. This indicates that the niosomal dispersion have better stability when stored at refrigerated condition than 25^0C.

v) *In- vitro* Permeation of LA-PHY

The permeation test was carried out in a Franz diffusion cell using a diffusion membrane made of freshly excised goat skin. Ex-vivo permeation studies were excised using skin, the control gel formulation showed a sustained and controlled delivery of the ZMT around 18 hours, and highlighted the fundamental role of the Optimized liposomal suspension and Gel enhanced up to 5.6 times the permeability rate. Permeation study results (Figure 2) revealed that the ex-vivo permeation go parallel with *In vitro* drug release studies.

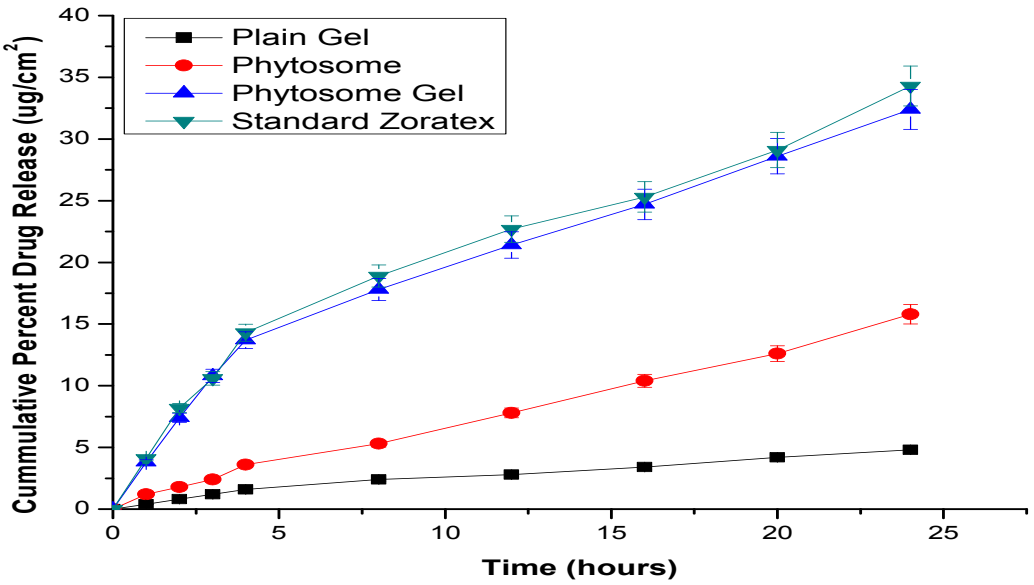

Figure 2: *In- vitro* skin permeation studies

Table 9: % Liver uptake of *Taraxacum officinale* loaded niosomes containing DCP and DMPC

Hrs	Peak area							S D	RSD	Conc. µg/ml	% Conc.
	1	2	3	4	5	6	mean				
2	122435	124395	122734	123046	123936	122551	123188	1055.929	116.6631	3.6	90
4	107575.84	105878.3	106493.7	104709	108422	106817	106649.3	559.3977	124.0977	3.12	78
6	97669	97593.61	97539	97875	96973.53	97956	97600.57	65.61938	1487.374	2.8	70

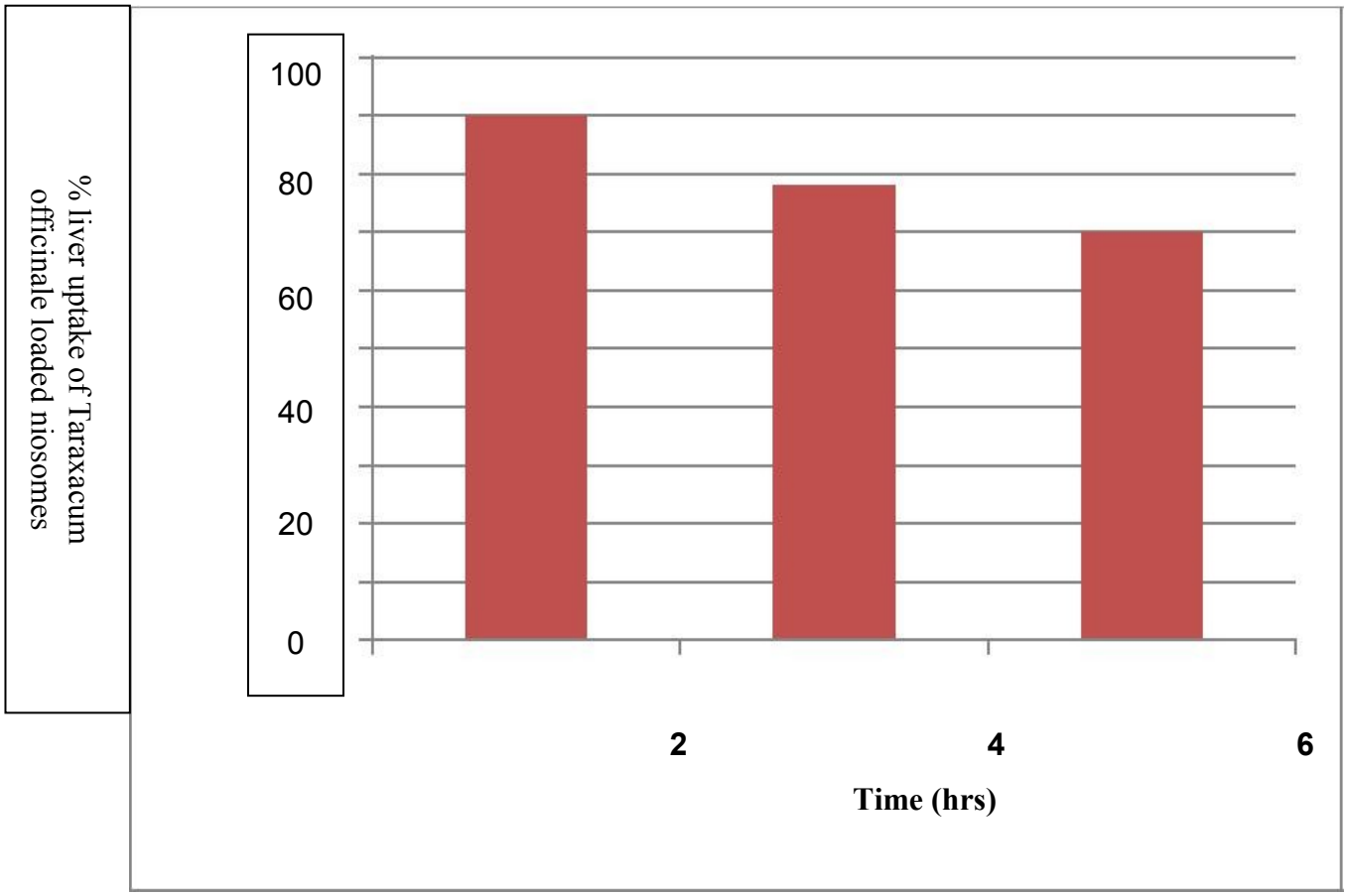

Figure 3: % Liver uptake of *Taraxacum officinale* loaded niosomes

Table 10: % Liver uptake of *Taraxacum officinale* loaded niosomes containing DCP

Hrs	Peak area							S D	RSD	Conc µg/ml	% Conc
	1	2	3	4	5	6	mean				
2	85229	85207	85329	85308	85398	85251	85286.2	71.22476	1197.421	2.4	60
4	67830	67993	68049	68013	67921	67929	67950.8	76.96341	882.8975	1.84	45
6	50646	50613	50622	50706	50514	50617	50615	62.24039	813.2189	1.28	32

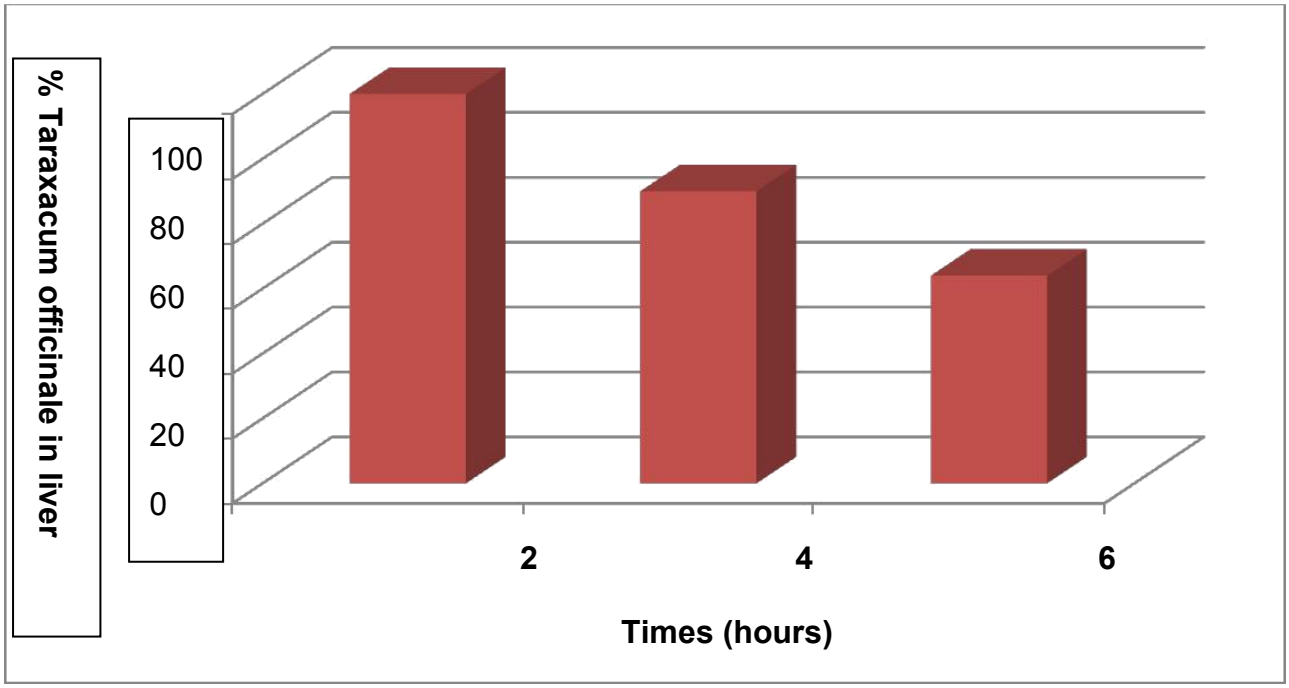

Figure 4: % Liver uptake of *Taraxacum officinale* loaded niosomes containing DCP

Table 11: % liver uptake of plain drug solution

Hrs	Peak area							S D	RSD	Conc µg/ml	% Conc
	1	2	3	4	5	6	mean				
2	81213.3	81105.2	81092.4	80897.7	81053.1	80773	81022.45	159.3057	508.5972	2.26	56.5
4	49642.35	49886.2	49583.62	49769.13	49881.8	49740.6	49750.6	123.0188	404.4142	1.96	49

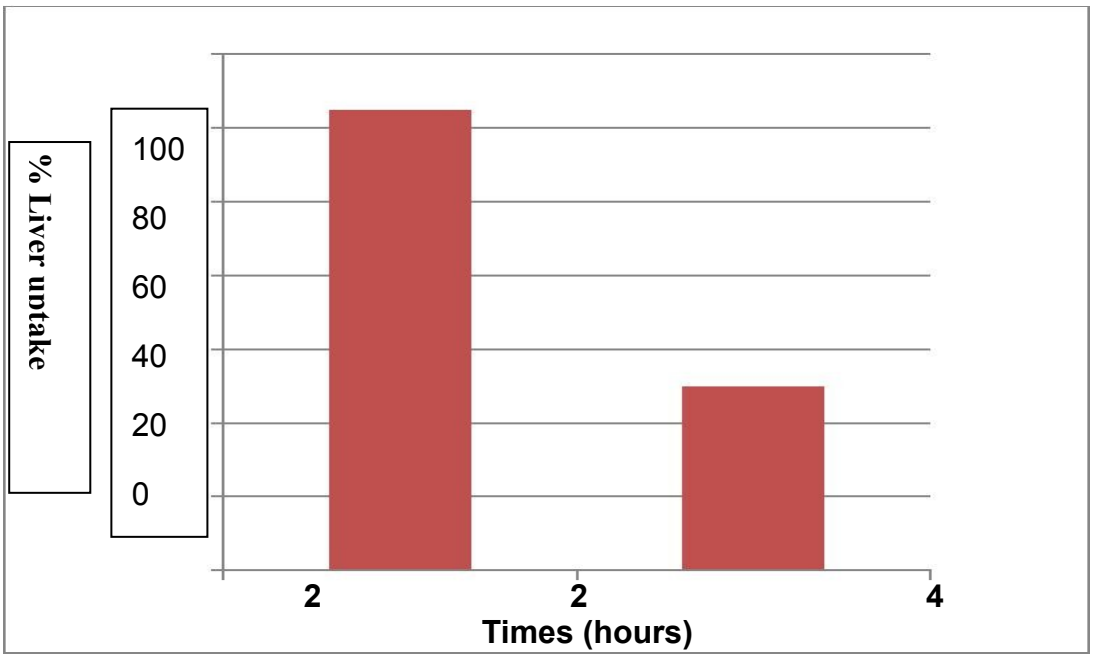

Figure 5: % Liver uptake of plain drug solution

The analysis of the drug was carried out by HPLC to measured the % of *Taraxacum officinale* accumulated in the liver with respect to time in hours. The liver uptake of the niosomal formulation containing DCP and DMPC was found to be 90 % of administered dose of *Taraxacum officinale* within the 2 hours after the initiation of study, while in case of niosomal formulation containing DCP showed 60% drug in liver. At 4^{th} and 6 th hour the accumulation of *Taraxacum officinale* niosomes containing DMPC and DCP were 78% and 70% respectively. The *Taraxacum officinale* niosomal formulation containing only DCP showed 45% and 32% of *Taraxacum officinale* in liver cells at 2^{nd} hours and 4^{th} respectively.

Scavenger receptors in the kuffer of liver were responsible for liver uptake of niosomes containing negative charge on their surface. The cells were the binding site for negatively charge niosomal formulation as were reported in literature. Incorporation of *Taraxacum officinale* into the niosomes helped to increase the stability of *Taraxacum officinale* by preventing it from chemical and enzymatic degradation which further helped to increase residence time. The percent uptake of drug from plain drug solution were 56.5% and 49% at the time point of 2^{nd} and 4^{th} hour. At the end of 6 hour the percent drug in liver from the plain drug solution was not detectable, indicating it`s elimination from liver, as the half life of drug was 3-4 hours.

The One way ANOVA (Non-parametric) applied for the significance of the results. Dunnet`s t-test applied for plain drug solution, niosomal formulation containing DCP and niosomal formulation containing DCP and DMPC. This results indicated that the DMPC containing niosomal formulation accumulate at the liver cells and thus increased the drug concentration there by relapsing the parasitic stages of infection of malaria. Due to the negative charge on the niosomal membrane the niosomes binds to the receptors of parenchymal cells and hence the combination of the *Taraxacum officinale* loaded niosomal formulation containing DCP and DMPC where found mainly in liver as compared to other organs. Statistically significant response was observed, when compared with the control group as the ***p*** value was less than 0.001 for all six hours of study as shown in table 1. In case of the niosomes containing DCP, liver uptake were found to be 60%, 45%, 32% respectively at the time interval of 2, 4 and 6 hours. Statistically significant response was observed for these results as ***p*** value was less than 0.005, at the confidence level of 95%. It was found that upon intravenous injection, drug-containing niosomes were rapidly removed from the blood and taken up to a large extent by endocytotic reticuloendothelial system-derived elements of the liver, compared to the free drug form. From the table no.1 it could be stated that *Taraxacum officinale* Entrapped within niosomes exhibited a prolonged plasmatic half-life, which was more than 6 hours whereas of free *Taraxacum officinale* it was found to be less than 3 hours, showed that the niosomes restricted the enzymatic degradation of *Taraxacum officinale* and thus increase the amount of drug at the site of action. This results revealed that the *Taraxacum officinale* had more half life when formulated in niosomal formulation and also had prolong effect on the targeted site. On the other hand the toxicity of *Taraxacum officinale* reduced drastically as compared to free drug solution.

CONCLUSION

From the above results we could conclude that, niosomal formulation found to be one of the promising approach to treat the malaria. *Taraxacum officinale* loaded niosomal formulation used for the targeting the liver stages of the parasite to treat early stage malaria. The results showed that the final niosomes were stable when stored in buffers and plasmai.e were not degraded in plasma shown by the more accumulation of the formulation in liver stages in lager concentration. From the biodistribution pattern we could conclude that the *Taraxacum officinale* loaded niosomes minimized the toxicity associated with plain *Taraxacum officinale* solution, as were at the liver for longer period of time. Longer circulation of the niosomes showed that, incorporation of *Taraxacum officinale* into the niosomes helped to increase the stability of *Taraxacum officinale* by preventing it from chemical and enzymatic degradation. DMPC and DCP containing *Taraxacum officinale* loaded niosomal formulation showed the prolong released and liver

targeting and hence ultimately increased the efficacy of the developed formulation as compared to conventional formulation.

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