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RESEARCH PAPER

Formulation and In Vivo Evaluation of Niosome-Encapsulated Daunorubicin Hydrochloride

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ABSTRACT

The central aim of this present study was to modify the reverse evaporation process, such that an enhanced entrapment, with increased storage stability and prolonged release, could be achieved, and to translate these advantages to increased therapeutic efficacy of daunorubicin hydrochloride on Dalton's ascitic lymphoma. Niosomes prepared exhibited entrapment efficiency 20% higher than theoretically possible by the reverse evaporation process. The niosomes were found to be very stable at a storage temperature of 4°C for a duration of three months. Even the drug leakage was restricted to just 10%. The in vivo studies suggested a prolonged release of 20 hr. Niosomal daunorubicin hydrochloride exhibited an enhanced anti-tumor efficacy when compared to free drug. The niosomal formulation was able to destroy the Dalton's ascitic lymphoma cells in the peritoneum within the third day of treatment, while free drug took around six days and the process was incomplete. The hematological studies also prove that the niosomal formulation was superior to free drug treatment. An enhanced mean survival time was achieved by the niosomal formulation that finally substantiates the overall efficacy of the niosomal formulation. This study suggests that the multilamellar vesicles obtained by the presently utilized reverse evaporation process resulted in vesicles that resisted the immediate lysis in the

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Kupffer cells, whereby a prolonged drug concentration was achieved which enhanced the cell lysis. But the major factor responsible for the quicker onset of action could be the increased permeability of the niosomes into the cell membrane and the cytoplasm of the Dalton's ascitic lymphoma cells.

Key Words: *Dalton's ascitic lymphoma; Daunorubicin; Niosomes*

INTRODUCTION

The concept of delivering drugs to target organs, tissues, cells, or subcellular organelles and modified drug disposition is an accepted means to improve drug selectivity.[1] Systems proposed to date include certain macromolecules, cells, viruses, and a variety of synthetic polymer liposomes and particles. Most of these however exhibit limited utility in terms of quantitative and qualitative drug delivery.

One set of carriers that overcome these disadvantages, mainly due to their structural similarity to cell wall, and vastly fulfill a majority of perquisites of a delivery system, are the non-ionic surfactant vesicles—"niosomes." These are formed when a mixture of cholesterol and a single alkyl chain non-ionic surfactant is hydrated.[1,2] A number of non-ionic surfactants have been used to prepare vesicles, for example, polyglycerol alkyl ethers,[1,3] crown ethers,[4] ester-linked surfactants,[5] polyoxyethyelene alkyl ethers,[6] Brij,[7,8] and a series of spans and tweens.[7–9]

Niosomes improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from the biological environment,[2] minimizing drug–protein binding, increasing drug stability, and decreasing necrosis at the site of injection. Some of the bioactive agents entrapped in niosomes were sodium stibogluconate,[3,5] vincristine,[8] methotrexate,[9,10] doxorubicin,[11] diclofenac sodium,[12] pilocarpin,[13] and plumbagin.[14]

The present study is aimed at the overall improvement of therapeutic efficacy of antineoplastic drug daunorubicin hydrochloride (DHCl), through niosomal encapsulation. Daunorubicin hydrochloride is a drug of choice in the treatment of acute myelocytic leukemia and acute lymphocytic leukemia. Encapsulating this moiety in niosomes would decrease the uptake of drug by the normal tissues and enhance the drug uptake by reticulo endothelial systems (RES) more efficiently, since RES present in bone marrow, liver, spleen, and lymph are the natural targets for niosomes.[3,5,10] The overall drug dose can be minimized, which in turn reduces some serious side-effects. Since the method involved in the preparation of niosomes in this study is a modified reversed phase evaporation technique, studies have been undertaken to determine the physical stability of niosomes on storage.

MATERIALS

Daunorubicin hydrochloride was a gift sample from Rhone Poulenc, Paris. All the other reagents used were of analytical grade. Dalton's ascitic lymphoma (DAL)-bearing mouse cells were originally obtained courtesy of Cancer Research Center (CRC) Adayar, Chennai, India (originally procured from Professor Klein, Stockholm, Sweden). Swiss albino mice (25–30 g, age group 5–6 weeks) were used throughout the study. They were housed in standard microlon boxes and given a standard laboratory diet and water *ad libitum*. All the animal experiments were conducted in accordance with the Institution's Animal Ethics Committee, after their approval.

METHODS

Preparation of Span 80 Niosomes

The method followed in the preparation of niosomes was a slight modification of the reverse phase evaporation (REV) technique adopted by Parthasarathi et al.[8] Span 80, cholesterol, and dicetyl phosphate in a molar ratio of 1:1:0.15 were dissolved in a mixture of ether and chloroform (1.0:0.25). Five milliliters of aqueous phase containing DHCl drug (2 mg/mL) was added to this and the resulting two-phase system was homogenized using a homogenizer (REMI) at 4°C for 3 min at 8000 rpm. The organic phase was removed at 40°C under

reduced pressure. The resulting suspension was further homogenized for half a minute. The suspension was then heated in a water bath at 60°C for 10 min. The unentrapped drug was removed by eluting the product through Sephadex G-50 (gel chromatography) using phosphate buffer saline (PBS), pH 7.4, as the eluting fluid.[15]

Determination of Percentage of Encapsulation

One milliliter of the niosomal preparation was introduced from the top of the column of Sephadex G-50, and eluted with PBS, pH 7.4, and the amount of free DHCl determined using a UV–Visible spectrophotometer (Shimadzu UV/Vis spectrophotometer, 1601, Japan) at 234 nm after lysis of the vesicles brought about by 1 mL of 2.5% sodium lauryl sulfate, briefly homogenized and centrifuged and the supernatant assayed for drug after suitable dilution. The concentration of the drug was determined from the calibration curve of DHCl.

Estimation of Serum Concentration of Niosome Encapsulated DHCl

Male Swiss albino mice (25 to 30 g) were divided into two groups. The first group to which free DHCl was to be administered consisted of 20 mice, further divided into four groups designated A, B, C, and D, consisting of five animals in each group. A free solution of DHCl was administered in a dose equivalent to 5 mg kg^{-1},[16,17] intraperitoneally to each mouse. The serum drug concentration was determined from 1 mL of blood withdrawn by retro-orbital vein plexus puncture using heparinized capillary tubes (after anesthetizing the animal) from each animal of the groups A, B, C, and D at the end of 0.5, 1, 2, and 5 hr, respectively. The blood was allowed to stand for 5 min after collection and centrifuged to get the serum, which was then treated with 200 μL of 20% trichloroacetic acid solution and gently vortexed, and centrifuged, at 3000 rpm for 10 min. An aliquot of 50 μL from the clear supernatant was then injected into the HPLC column (intresil ODS 5μL), of a Shimadzu liquid chromatograph. The mobile phase was acetonitril (42 vol.) and water containing 2.88/L sodium lauryl sulfate and 2.25/L phosphoric acid (52 vol.). The absorption wavelength was set at 254 nm. A standard curve was obtained from 0.300–10 μg/mL of the drug prepared from the pooled serum obtained from DAL-bearing mice using high-performance liquid chromatography (HPLC). The procedure adopted was the same as described above.

Similarly, another group of 50 mice to which niosomal DHCl was to be administered were divided into 10 groups, designated A–J, consisting of five animals each. The serum drug concentration of each mouse in groups A–J was determined at the end of 1, 2, 4, 6, 8, 10, 13, 16, 20, and 25 hr, respectively, by the same procedure described for the first group.

Evaluation of Physical Stability of Niosomes at Storage and Body Temperature

The niosomal preparation was divided into three groups, stored at 4°C, room temperature, and 37°C. Each group was divided into thee parts. Two parts were transferred into ampoules and hermetically sealed, and one part was processed for size distribution study by laser diffraction on a Shimadzu Sald 1100 (Japan). The ampoules from each group were broken at predetermined intervals of 45 days and 90 days. Then the samples were processed for size distribution study, as described earlier.

Determination of Drug Leakage from Niosomes on Prolonged Storage

Leakage of the entrapped drug from the niosomes retained in 15 hermetically sealed ampoules, divided into three groups (Five ampoules in each group) and stored at three different temperatures for a period of 90 days, was determined by breaking the ampoules at predetermined intervals of 10, 20, 40, 60, and 90 days, separating the leaked drug from the noisome-retained drug by gel chromatography technique, and determining the drug content in the niosomes using the method discussed previously in the determination of entrapment efficiency.

Study of Anti-tumor Activity in Mice

Histopathological Studies

Ascities fluid from the intraperitoneal cavity of the donor animal was aseptically aspirated using a hypodermic syringe with 18-gauge needle, 7–8 days after tumor (DAL) inoculation. A small portion was tested for tumor viability by the Tryphan blue dye exclusion test. It was also tested for bacterial contamination. The viable cells were counted on a hemocytometer. The aspirate was diluted with

Dulbeco's modified Eagle's medium (DMEM) to get a concentration of 5×10^6 cells/mL. Two milliliters of this suspension (1×10^7 DAL cells) was injected intraperitoneally into a set of 24 animals divided into four groups as follows:

- Group 1—normal mice.
- Group 2—tumor control (1×10^6 DAL cells/mL).
- Group 3—tumor-bearing mice treated with free DHCl ($5\,mg\,kg^{-1}\,days^{-1}$ i.p.) on seventh day of tumor transplantation.
- Group 4—niosome-encapsulated drug, administered with drug equivalent to $5\,mg\,kg^{-1}\,days^{-1}$ i.p., on seventh day of tumor transplantation. The treatment with free and niosomal drug was continued for 6 days. About 0.5 mL of ascitic fluid was drawn aseptically using a sterile 18-gauge needle from each group. From this collected fluid smears were prepared on a glass slide and stained with maygrunwald stain.

Examination of Hematological Parameters

Another set of 24 animals were divided into four groups of six animals each, as done with the histopathological studies, and a similar dose treatment with free and niosomal formulation was carried out from the seventh day of tumor inoculation for a period of 6 days. Blood was drawn from each mouse in the conventional way and the white blood cell count, red blood cell count (using Neubaeur counting chamber), hemoglobin (using Shali's acid hematin method), proteins, differential count, and packed cellular volume determined using Wintrobe's method.[18–20]

Evaluation of Tumor Cell Lysis

The groups of animals used in the experimentation of hematological parameters were sacrificed on the 14th day after tumor inoculation. The ascitic fluid was completely drained, and the intraperitoneal cavity washed with a measured volume of normal saline, which was mixed with the drained ascitic fluid. The tumor cell lysis (evident by the inclusion of Tryphan blue dye) was determined by counting on a hemocytometer.[21]

Effect of Niosomal Formulation on Survival Time

The final set of 24 animals was divided into four groups, each consisting of six animals, and inoculated with 1×10^7 DAL cells. Group 1 animals were left untreated. Group 2 animals were administered an equal quantity of normal saline. Group 3 animals were treated for 6 days with a single dose of $5\,mg\,kg^{-1}\,day^{-1}$ of free drug administered intraperitoneally after 24 hr of inoculation of tumor cells. Group 4 animals were treated similarly with an equivalent quantity of drug as the niosomal formulation, administered intraperitoneally.

The anti-tumor efficacy of niosomal formulation was compared with that of free drug administration, and the median survival time (MST) for each group noted. The treated group (T) was compared with the control group (C) using the following calculation to give increased life span:

$$T/C\ (\%) = \frac{\text{MST of treated animals}}{\text{MST of control group}} \times 100$$

All the results were analyzed by analysis of variance[22] and Dunnet's *t*-test was performed to compare the efficiency of the niosomal drug over free drug.

RESULTS AND DISCUSSION

The niosomal DHCl used in the present study was prepared by a slight modification of a previously reported method of preparation. Since a homogenizer was used in lieu of a sonicator, it was presumed that the product would be large vesicles with a broad particle size distribution. Surprisingly, this method yielded a large quantity of niosomes with a narrow size distribution, within the range 0.7–1.5 μm, at zero storage time.

Encapsulation study performed on these niosomes also yielded a good result, since 50% or lower entrapment efficiency can be expected by the general RPE method.[8,22] A percentage encapsulation of 69% was achieved. This moderately high level of encapsulation could have been due to excess lipid over the aqueous content in the formula. This excess lipid, which forms multiple lamellae over the already formed vesicles, gets transferred to another vesicle during the gel collapsing stage without destroying their integrity, during the brief half minute homogenization, with a slight excess of aqueous phase added at this stage which aids the gel conversion without damaging any of the vesicles.[24]

Figure 1 shows the serum drug concentration study of the niosomes in comparison to free drug

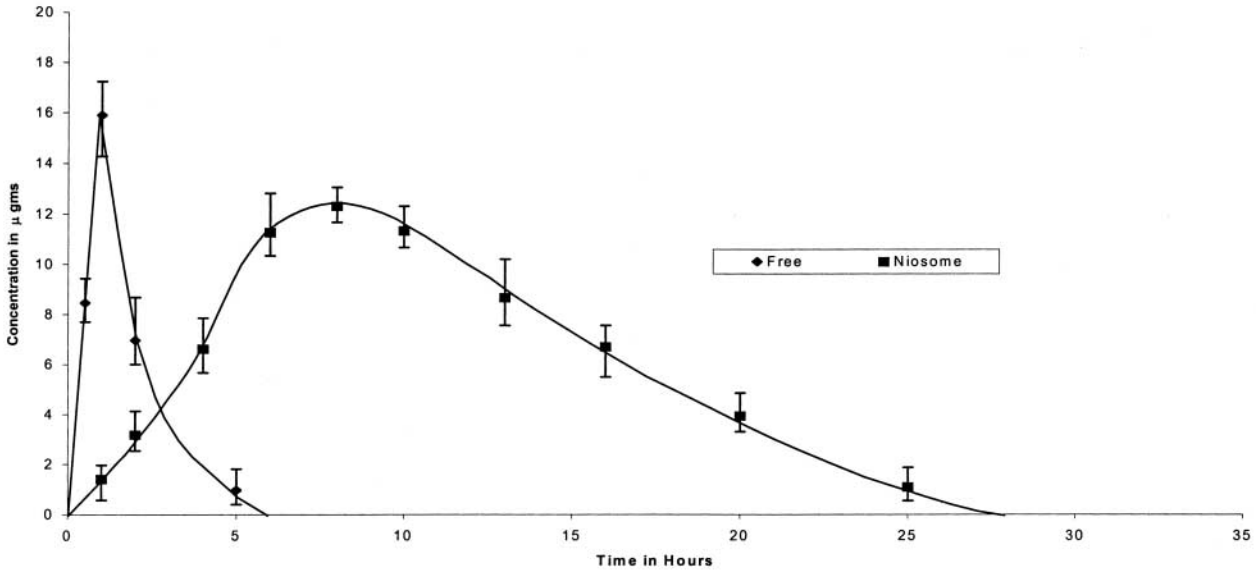

Figure 1. Serum concentration–time profile of DHCl after intraperitoneal administration of DHCl in PBS and niosome-entrapped DHCl, composed of DHCl/span 60/cholesterol/dicetyl phosphate. The points and vertical bars represent the mean and standard error mean, respectively ($n = 3$).

administered intraperitoneally, in a single dose, with peak serum concentration within 1 hr, falling to a negligible concentration by the fifth hour. With the niosomal formulation, a peak serum concentration was achieved by the eighth hour, falling to a negligible concentration by the 25th hour. The serum concentration was maintained within the range of 4 to 12 μg for a prolonged duration of 20 hr. The free drug exhibited an elimination $t_{0.5}$ of 50.4 min whereas the niosomal formulation had an elimination $t_{0.5}$ of 7 hr, and 6 min. (Data calculated from Fig. 1.) These aspects could be attributed to the fact that the liver and spleen, which are the natural targets for niosomes, act as a depot for niosomes. The niosomes of particle size achieved in this study are not capable of passing through the fenestrae of the liver. They pass slowly through the liver sinusoids and end up in the Kupffer cell, where the multilamellar vesicles are gradually broken down, layer by layer, which is responsible for the prolonged release[12,25] evident in Fig. 1.

A study of particle size characterization on prolonged storage of 90 days at three different storage temperatures (Fig. 2) showed that at 4°C, even after 90 days, there was no appreciable change in particle size distribution. This could be due to decreased motility of the bilayer at lower temperature, thus offering stability, whereas at room temperature, as seen in Fig. 3, there was a moderate shift in size distribution in the higher region. By the 90th day, the particle size ranged from 2 to 6 μm, which may be due to the fusion of smaller vesicles to form larger ones.[26] At a storage temperature of 37°C, (Fig. 4), a large proportion of vesicles fused to form vesicles in the range of 1–3 μm on the 45th day. By the 90th day, a majority of vesicles had fused to form larger vesicles in the size range of 5–12 μm. The dual size distribution observed at a lower and higher size range, noticed at 4°C storage temperature on '0' day, was not noticed, but a single large population of very big niosomes in the range of 5–12 μm was obtained. The reason could be the nearing to the transition temperature of the vesicles. However, this drastic effect was not evident on the niosomes stored at room temperature, due to the fact that there was a wide variation in the day and night temperature, with the day temperature averaging around 32°C and the night temperature averaging around 25°C. On the 45th day there was an appreciable shift in the large particle size range. This shift accelerated in due course, possibly due to large vesicles formed during the initial storage days fusing to form larger vesicles. This process tends to increase with time.

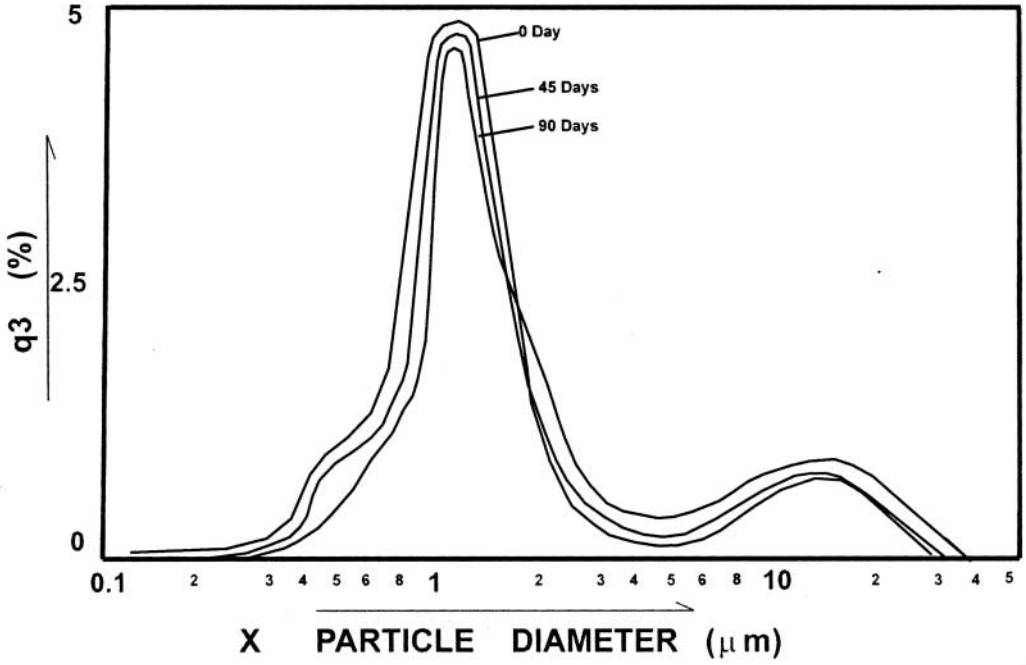

Figure 2. Particle size distribution of niosomes at a storage temperature of 4°C on 0 day, 45 days, and 90 days storage.

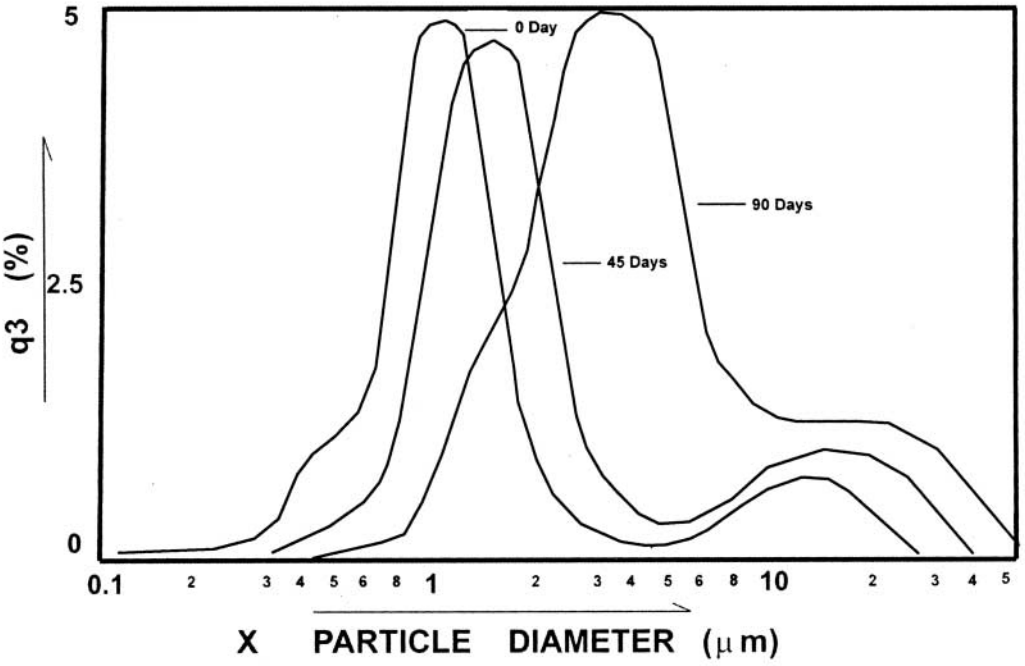

Figure 3. Particle size distribution of niosomes stored at room temperature on 0 day, 45 days, and 90 days storage.

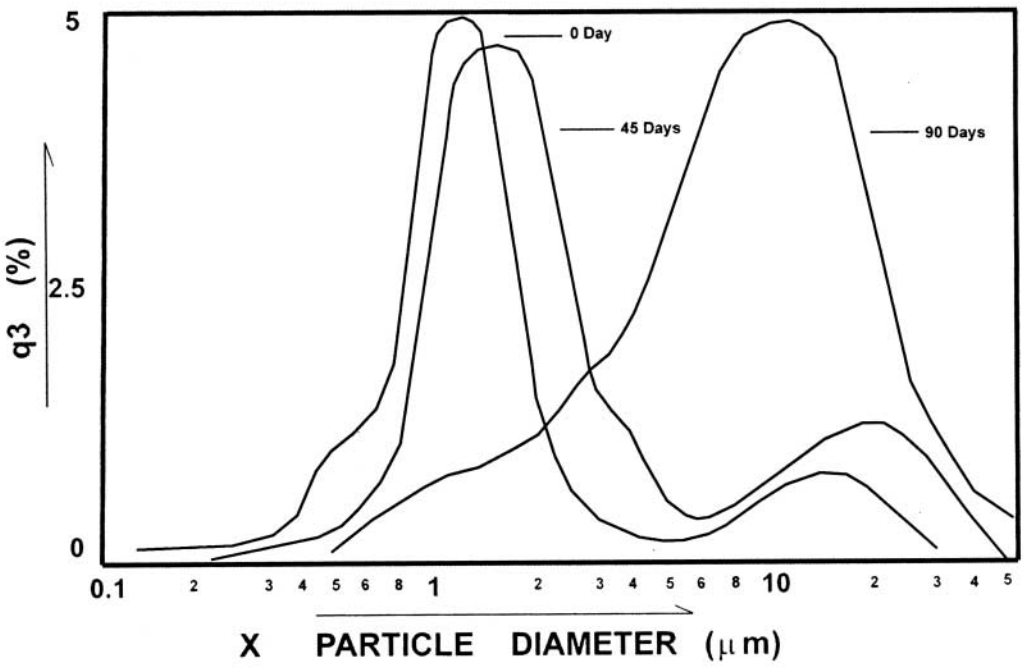

Figure 4. Particle size distribution of niosomes at a storage temperature of 37°C on 0 day, 45 days, and 90 days storage.

Simultaneously, a leak test performed on a separate set of niosomes stored at three temperatures selected in the earlier study gave a co-relatable result with respect to particle size on storage. It could be interpreted from Fig. 5 that on the 90th day drug leakage from niosomes stored at 4°C was restricted to just 10%, while the niosomes stored at room temperature and 37°C exhibited a drug leakage of 40 and 55%, respectively. The reason for this result is the same as that discussed for the size distribution variation. Lamellae at higher temperature, especially when nearing the transition temperature, achieve fluidity, therefore enhancing vesicle fusion. During fusion, it could be expected that certain large and unstable vesicles may rupture; besides, as the temperature increases, the fatty acid chain of the surfactant tends to adopt a structural conformation other than an all trans straight chain configuration. This leads to a decrease in bilayer thickness, therefore increasing the rate of diffusion across the vesicular membrane.[27] The above two conditions lead to considerable freeing of entrapped drug, which is interpreted in the study as drug leak.

The histopathology of ascitic fluid drawn from the tumor-bearing mice after the seventh day of tumor transplantation and the third day of treatment are shown in a series of photographs (1–8) at 100 × magnification (Fig. 6). In the initial stages the tumor cells appear larger in size and have irregular cytoplasmic border, a few leukocytes were also visible.

In Fig. 6, 1 and 2 correspond to the histopathology of ascitic fluid drawn from untreated mice, while 3 and 4, 5 and 6, 7 and 8 correspond to the histopathology of ascitic fluid drawn from free DHCl solution-treated mice and niosomal DHCl-treated mice drawn on the third, fourth, and sixth day, respectively. After the third day of dose administration, a number of small vacuoles appeared on the cell membrane (indicated by arrows on the photographs) for both niosomal and free drug-treated animals, as shown in 3 and 4, causing the cell membrane to exhibit a swollen appearance. This was distinctly absent in 1 and 2, photographs of untreated mice. A slight shrinkage of the ascitic cells is seen in 4, while 3 shows no perceptible change. This quicker onset of action was evident by the fourth day, where further shrinkage of ascitic cells in the niosomal drug-treated animals was evident, as observed in 6. Meanwhile the cells of free drug-treated animals, as seen in 5, still exhibited vacuoles and had undergone shrinking, although the shrinkage was not as marked as that of

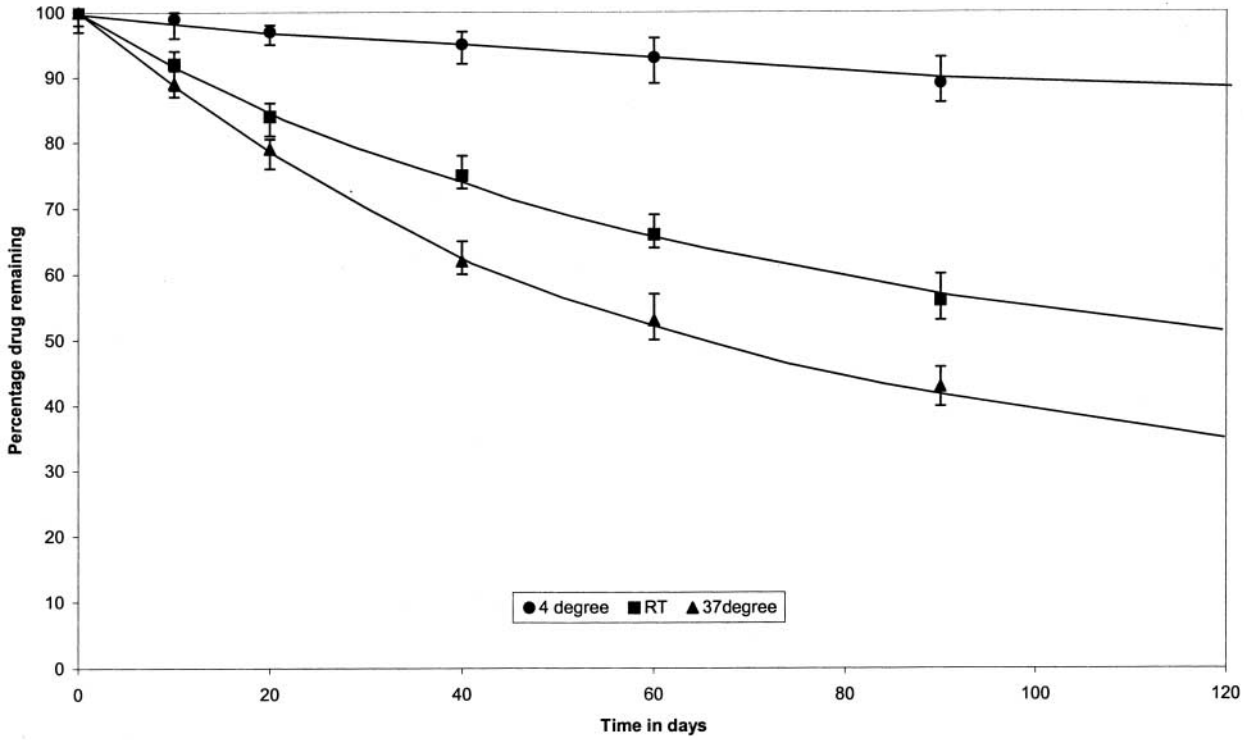

Figure 5. DHCl leakage from niosomes during prolonged storage of 90 days at three different temperatures. The points and the vertical bars represents the mean and standard error mean respectively ($n = 3$).

niosomal-treated cells. On the sixth day, photograph 8 shows a further decrease in cell size and a large number of dead cells were noticed, identified due to their increased uptake of stain. In contrast, in photograph 7, the cell size was still in the process of reduction. This enhanced efficacy of the niosomal formulation was expected, since the bilayer vesicle is able to permeate into the cell wall due to its structural similarity to the cell wall. Once within, they disrupt the cell membrane and topoisomerase II, thus bringing lysis of tumor cells.

Besides this, the majority of niosomes drain into the blood circulation via the lymphatic. Here some may disintegrate or interact with blood lipoproteins, thus releasing the drug in circulation. The rest of the niosomes gets opsonized,[28] and are taken up by the Kupffer cells and splenic macrophages, where they gradually disintegrate and release the drug in prolonged fashion. These two factors cause an increased drug concentration in the peritoneal cavity, which further enhances permeability of the drug to tumor cells, resulting in lysis.

Hematological changes are common in malignant diseases and may be the presenting feature. The commonly encountered hematological changes in malignant disorders are anemia, sideroblastic anemia, leukocytosis, eosinophillia, monocytosis, thrombophelibitis, etc. The hematological parameters of the tumor-bearing mice on the 13th day after tumor inoculation were significantly altered from the normal group.

Table 1 illustrates the details of hemoglobin content, red blood corpuscles (RBC) count, and white blood corpuscles (WBC) count, and the hemoglobin content in four groups. A percentage difference in decrease of hemoglobin (HB) content in tumor-induced mice compared to normal mice was found to be around 35.48%, while tumor-induced mice, administered with free drug, showed a difference of 10.96%, and with niosomal drug the difference was around 5.8% below the normal amount of HB. A marked improvement of 47.3% in the HB content was achieved by the niosomal formulation in comparison with free drug.

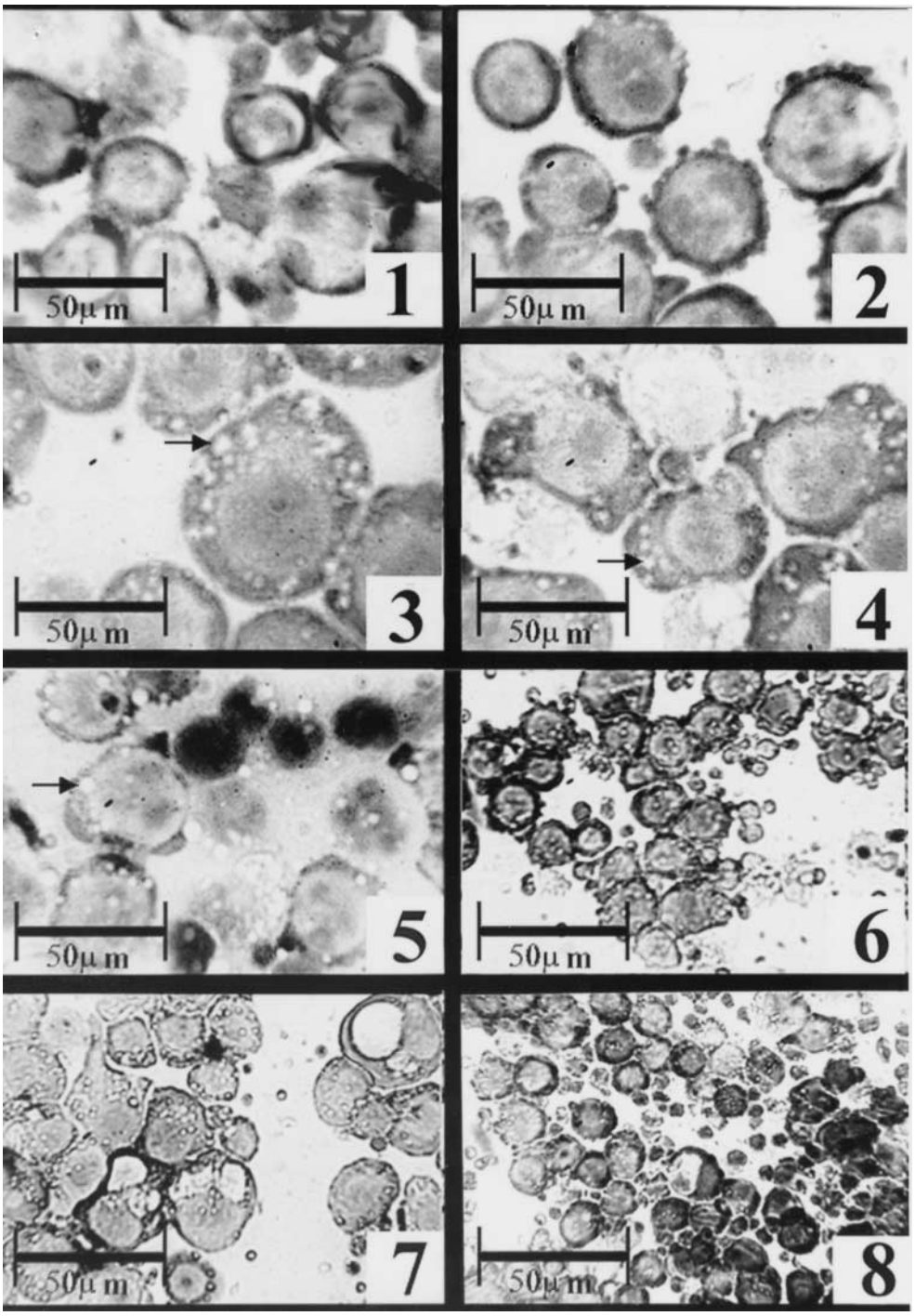

Figure 6. Photographs 1 and 2 corresponds to the histopathology of ascitic fluid drawn from untreated mice. Photographs 3 and 4, shows the histopathology of ascitic fluid drawn after the third day of treatment with free drug and niosomal formulation, respectively. Photographs 5 and 6 shows the histopathology of ascitic fluid drawn after the fourth day of treatment with free drug and niosomal formulation, respectively. Photographs 7 and 8 shows the histopathology of ascitic fluid drawn after the sixth day of treatment with free drug and niosomal formulation respectively.

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Table 1

Comparative Effect of Free and Niosomal Drug on the Hematological Parameters in Mice

Groups	Hemoglobin (g %)	Total RBC (million/mm^3)	Total WBC (million/mm^3)	Protein (g %)	PCV	Differential Count		
						Lymphocytes	Neutrophils	Monocytes
Normal mice	15.5 ± 0.82	2.31 ± 0.0042	8.068 ± 0.0031	8.8 ± 0.4	16 ± 0.44	69 ± 1.3	29 ± 2.1	2 ± 0
Tumor control	10.0 ± 0.54	1.88 ± 0.0027	36.43 ± 0.0024	13.6 ± 0.3	27 ± 0.53	31 ± 1.5	68 ± 1.9	1 ± 0
Free drug	13.8 ± 0.46^{b}	2.09 ± 0.0064^{b}	19.85 ± 0.0026^{b}	11.2 ± 0.2^{b}	22 ± 0.21^{b}	59 ± 1.4^{b}	39 ± 1.6^{b}	2 ± 0
Niosomal drug	$14.6 \pm 0.72^{a,c}$	$2.38 \pm 0.0035^{a,c}$	$12.07 \pm 0.0049^{a,c}$	$9.1 \pm 0.4^{a,c}$	$18 \pm 0.36^{a,c}$	$70 \pm 1.5^{a,c}$	$28 \pm 1.3^{a,c}$	2 ± 0
Statistical analysis	$^{a}p < 0.001$	$^{a}p < 0.001$	$^{a}p < 0.001$	$^{a}p < 0.001$	$^{a}p < 0.001$	$^{a}p < 0.001$	$^{a}p < 0.001$	
	$^{b}t = 7.32$	$^{b}t = 0.516$	$^{b}t = 31.97$	$^{b}t = 8.88$	$^{b}t = 9.68$	$^{b}t = 54.22$	$^{b}t = 56.15$	
	$^{c}t = 8.87$	$^{c}t = 1.934$	$^{c}t = 49.97$	$^{c}t = 13.69$	$^{c}t = 17.42$	$^{c}t = 75.52$	$^{c}t = 77.46$	

[a]Analysis of variance between groups.
[b]Dunnet's t value for free drug.
[c]Dunnet's t value for niosomal drug.
Numbers of animals in each group = 6.
Days of treatment = 6.

The total RBC count decreased from a normal of 2.38 million/mm^3, showing a decreased percentage of 21% in tumor-induced mice. The free drug, when administered, brought down the reduction to 12.2%, while the niosomal formulation brought it down further, by 2.94%. A marked improvement of 86.42% was noticed using niosomal drug in comparison to free drug administered.

The mechanism responsible for the fall in HB level, as well as the fall in RBC in malignant disease, is complex and multifactoral. It could be due to shortened RBC survival, impaired bone marrow response to anemia, increased synthesis of ferritin, defective transfer of iron from reticuloendothelial stores to RBC precursors, and impaired transferin production.[29]

The acceptable criterion for determining the antitumor efficacy of the formulation over the free drug is the determination of circulating WBC,[30] as well as the life span prolongation.[31] A normal WBC count of 8.068 million/mm^3 increased by around 77.9% in tumor-induced mice. Free drug administered showed an increase of 59.35%, and niosomal drug formulation showed a WBC count increase of 33.15%. An improvement of 44.06% WBC reduction was derived by the niosomal formulation over the free drug.

There was a decrease in packed cell volume after administration of niosomal formulation, which was 59.15% better than that observed with free drug. The differential count after niosomal formulation administration showed that the number of lymphocytes, neutrophils and monocytes, came back to normal.

The number of dead cells in the peritoneal fluid of mice treated with niosomal drug formulation increased fourfold when compared to untreated mice, while the number of dead cells in free drug-treated mice increased by little more than twofold, when compared to untreated mice. This result, tabulated in Table 2, substantiates the histopathological study, that by the 13th day of tumor inoculation, the niosomal formulation exhibited a significantly higher tumor cell necrosis.

The mortality rate was observed in tumor-induced mice divided into four groups. The untreated mice survived for 23 days, serving as a control for others, while mice treated with free drug survived for 31 days, showing an increased life span of 134.78%. Meanwhile those treated with niosomal formulation showed a much-enhanced MST of 42 days, indicating an increased life span of 182.6%. An enhanced life span by 47.8% over free drug administration was observed with niosomal formulation. The results are tabulated in Table 3. The effect is attributable to the increased lysis of tumor cells, which was evident in the histopathological study.

A statistical evaluation of the data from the hematological parameters, tumor lysis, and survival time, expressed as a mean in Tables 1, 2, and 3, shows that the three groups in each study were

Table 2

Comparative Evaluation of Tumor Cell Lysis

Groups	Number of Stained Dead Tumor Cells (1×10^6 cells mL^{-1})
Normal mice	—
Tumor control	1.95 ± 1.2[a]
Free drug	5.36 ± 0.95[a,b]
Niosomal drug	9.57 ± 0.83[a,c]
Statistical analysis	[a]$p<0.001$ [b]$t=14.95$ [c]$t=32.5$

[a]Analysis of variance between groups.
[b]Dunnet's t value for free drug.
[c]Dunnet's t value for niosomal formulation.
Number of animals in each group = 6.
Days of treatment = 6.

Table 3

Comparative Survival Time of Tumor-Bearing Mice After Treatment with Free Drug and Niosomal Formulation

Group	MST (days)	Increase in Life Span T/C (%)
Tumor control	23 ± 1.2[a]	
Placeo	23 ± 2	
Free drug	31 ± 1.9[a,b]	134.78
Niosomal drug	42 ± 2.3[a,c]	182.6
Statistical analysis	[a]$p<0.001$ [b]$t=6.27$ [c]$t=19.96$	

[a]Analysis of variance between groups.
[b]Dunnet's t value for free drug.
[c]Dunnet's t value for niosomal formulation.
Number of animals in each group = 6.
Days of treatment = 6.

significantly different, with a probability of error less than 0.001 in all three studies. The case was similar when Dunnet's *t*-test was performed, where the probability of error was less than 0.001 for both free drug and niosomal formulations. Alternatively, to determine the level of efficiency between the two formulations, a comparison between the *t* values of the two formulations in each individual study gives a definable level of efficiency. Accordingly, in all the studies, the niosomal formulation proved to be more efficient when compared to free drug.

CONCLUSIONS

It could be concluded from the present study that niosomes obtained by the modified reverse evaporation process, which yielded multilamellar vesicles, were sufficiently stable and uniform with good entrapment efficiency, which could be used to target diseases associated with RES. Daunorubicin hydrochloride is a narrow spectrum antibiotic, which exhibits dose-dependent toxicity. Hence there is a need to improve its acceptability by minimizing the intensity of side-effects. In the present study an attempt was made to achieve this. Niosome-encapsulated DHCl prepared by the modified method improved the therapeutic efficacy and may reduce some drug-related toxicities.

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