SHORT COMMUNICATIONS

Niosomal withaferin A with better antitumor efficacy

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Withaferin A was entrapped in niosomes. The release of the drug from the niosome was slower compared to plain withaferin A dispersed in phosphate buffered saline. The mean survival time (MST) of the animals treated with withaferin A entrapped in the niosome was enhanced compared to the plain drug.

THE concept of carriers to deliver drugs to target organs and modify drug disposition has been widely discussed[1]. Niosomes are one such carriers. Niosomes, the nonionic surfactant vesicles are microscopic lamellar structures formed on admixture of nonionic surfactant cholesterol and dicetyl phosphate with subsequent hydration in aqueous media. Niosomal entrapment helps to prolong the circulation of entrapped drug and alters its organ distribution and metabolic stability. Niosomes are found to improve therapeutic efficacy of drugs in cancer therapy, parasitic, viral and microbial diseases[2].

Withania somnifera ('Ashwagandha' in Sanskrit) plant is reported to have wide range of therapeutic applications including anticancer activity[3,4]. Withaferin A is the most important alkaloid isolated from the leaves of *Withania somnifera*. It has been receiving a good deal of attention because of its antibiotic and antitumor activities.[5,6,7]

Withaferin A was isolated and characterized as per the procedure[8] previously reported. Cholesterol, directly phosphate, span 60 and dialysis membrane were procured from Sigma Chemical Co., St Louis, MO, USA.

Niosomes were prepared by lipid layer hydration method[9]. Withaferin A was dissolved in 0.05 ml of chloroform. Cholesterol, dicetyl phosphate and span 60 were dissolved in 10 ml of anaesthetic ether in a round bottomed flask. The flak was rotated at 1.5 cm above water bath under reduced pressure at 60° until all the organic phase evaporated and a white slimy layer formed on the wall of the flask. Ten ml of aqueous phase was warmed to 60° and added to the dried film with gentle agitation. The mixture was intermittently mixed in a vortex mixer.

Separation of unentrapped drug was done by suspending the niosomes into a dialysis tube to which a Sigma dialysis membrane was securely attached to one side. The dialysis tube was suspended in 100 ml methanol which was stirred on a magnetic stirrer. The unentrapped drug was separated from the niosome suspension into the medium through semipermeable membrane. At every half an hour interval 100 ml of the whole medium was replaced with fresh medium (for about 2-3 h) till the absorbance reached zero.

For *in vitro* drug release the niosomes left after removal of unentrapped drug were dialysed into 100 ml of methanol. Five ml samples were withdrawn at pre determined intervals and replaced by fresh medium. The absorbance of samples were measured at 217 nm spectrophotometrically. The *in vitro* release of the plain drug drug was done by dispersing the drug in phosphate buffered saline and dialysed into 100 ml of methanol. Stability of the niosomes were studied at different temperatures 4°, 28° and 37°. At predetermined time intervales cumulative percentage of drug released was determined.

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Table 1 :Antitumor efficacy of plain withaferin A and niosome entrapped withaferin A

	MST	%ILS	Survivors at the end of 120 days
Control (PBS)	19.00		0
W A 10 mg/kg	27.00	42.10	10
W A 20 mg/kg	28.00	47.38	30
Empty Niosome	19.00		0
Niosomal W A 10 mg/kg	30.00	57.89	30
Niosomal W A 20 mg/kg	36.00	89.47	50

No of animals - 10, Schedule day - 1
W A - withaferin A

In vivo studies were carried out by using six to eight weeks old Balb/c mice (20-25 g) of either sex from an inbred colony maintained in our animal house under controlled conditions of temperature (23 ± 2°), humidity (50 ± 5%) and light (10 and 14 h of light and dark respectively). The animals were given sterile food prepared in the laboratory as per the standard formulation (wheat 70%, Bengal gram 20%, fish meal 5%, yeast powder 4%, sesame oil 0.75% and shark liver oil 0.25%) and filtered water *ad libitum*. Throughout the experiment 5 to 6 animals were kept in propylene cage containing sterile husk as bedding material.

Ehrlich ascites tumor cells, obtained from Amala Cancer Research Institute, Thrissur, Kerala, India., were maintained and propagated by serial transplantation intraperitoneally in female Balb/c mice. Experimental animals were prepared by injecting 10^6 cells into the intraperitoneal cavity[8]. Experiments were commenced 24 h after tumor inoculation. All the mice were weighed on the day of tumor inoculation and at different time intervals. Plain drug/withaferin A entrapped niosomes were administered intraperitoneally (i.p.) at the dose of 10 mg/Kg and 20 mg/Kg body weight. Animal survival was recorded upto 120 days which was approximately comparable with 5 years survival in man[10]. The tumor response was assessed on the basis of increase in life span (% ILS).

The formulated niosomes had size in the range of 12-34 microns. The niosomes were mostly spherical in shape. The drug entrapment efficiency was 55%. *In vitro* release profile showed 34% drug release occurred from niosomes at the end of 6 hours whereas for plain drug release was 50%. This indicates that niosomes provide a good carrier system for controlling the release of the drug in a slow and sustained manner. (Fig 1).

The niosomes showed better stability at lower temperatures. As the temperature increased the release from the niosomes also increased and amount of remaining drug decreased (Fig 2 and 3).

The mean survival time of the control animals was 19 days. The lower dose of 10 mg/kg plain as well as niosome entrapped withaferin A treated produced significant increase in mean survival time compared to control. Higher dose (20 mg/kg) also showed further increase in mean survival time.

Niosomal drug also showed significant ($p < 0.05$) increase in life span as compared to the plain drug as evidence by increase in survival rate. At 10 mg/kg plain withaferin A showed 42.10% increase in life span whereas niosome entrapped withaferin A showed 57.89% increase in life span. Still higher dose of 20 mg/kg showed further increase in life span upto 89.47%. Niosome treated animals showed 50% of survival for 120 days with the dose of 20 mg/kg whereas only 30% survived in the case of plain drug.

The antitumor activity of niosomal withaferin A was compared with plain withaferin A. The maximum percentage increase in life span (89.47%) was achieved with niosomal withaferin A while with the plain withaferin A was only 47.38%. This is obtained as a result of enhanced

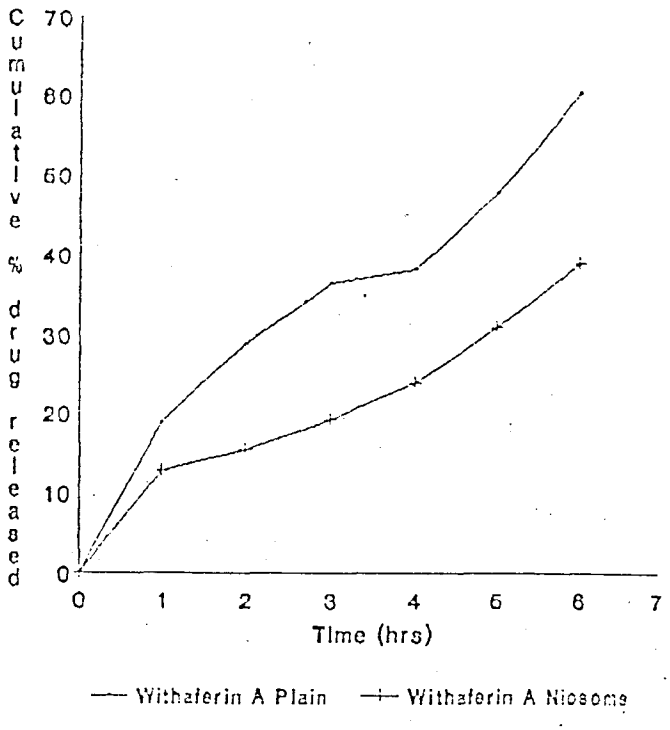

Fig.1 : *In vitro* release profile of plain withaferin A and niosome entrapped withaferin A in methanol

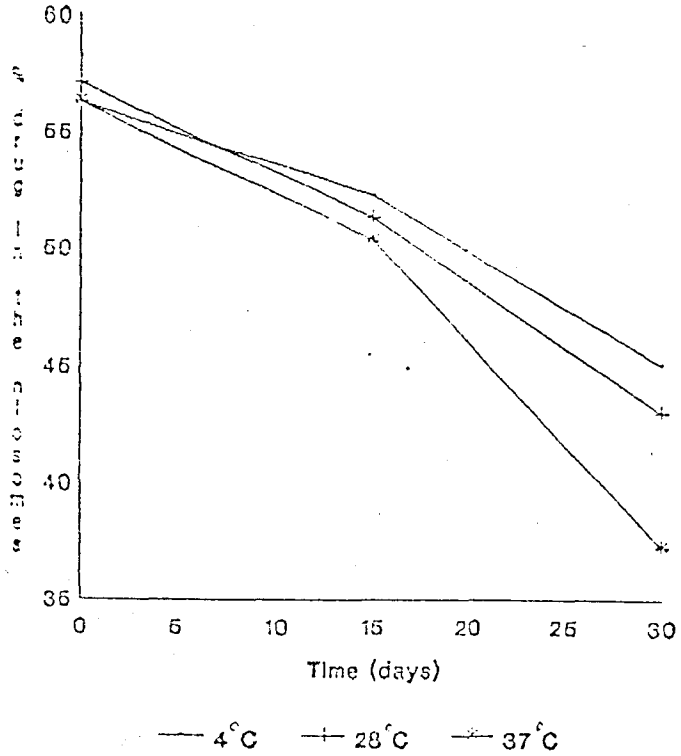

Fig.2 : Stability studies of withaferin A in niosomes

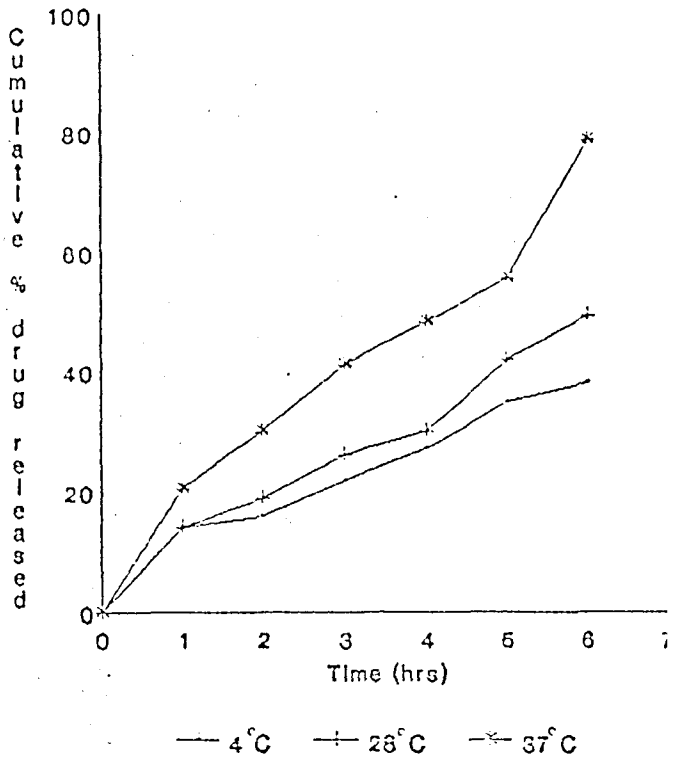

Fig.3 : *In vitro* release profile of withaferin A from niosomes after 30 days

antitumor activity over plain withaferin A at equivalent doses (20 mg/kg). An overall view of these results suggests better antitumor activity of niosomal Withaferin A.

The hypothesis proposed for increased antitumor efficacy of vesicle entrapped antineoplastics is slow release from the vesicles and the resulting increased duration of circulation of the drug.[11,12] The same hypothesis can also be extended to niosome entrapped withaferin A. The therapeutic activity of the drug in the present study may have been improved due to increased duration of the sojourn of the vesicle bound drug in the circulation and the likelyhood of extended release. The *in vitro* and *in vivo* studies indicate that increased withaferin A efficacy correlates well with increased duration and concentration of the drug in the circulation[13]. It can also be argued that niosomal withaferin A is delivered preferentially to the tumor site there by enhancing cell kill and decreasing the toxicity in non cancerous tissues. Similar effects have been reported for antineoplastics entrapped in niosomes earlier[14,15].

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Assay Methods for a new Analgesic Enkephalin Analogue CDRI compound No. 82/205

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HPLC andTLC-densitometric methods for the estimation of L-tyrosyl-D-alanyl-glycyl-L-N-methyl phenyl alanyl glycinyl-N-isopropyl amide [compound 82/205] in bulk samples and formulations are described. The calibration curves were linear in the range of 4-40 µg/ml for HPLC and in the range of 0.5 - 20 µg for TLC-densitometric method.

STANDARD compound 82/205[1-5] is a pale yellow powder with molecular weight 569. It was obtained from this institute. Methanol and chloroform used were of AR grade. Dual wavelength tlc-scanner (Shimadzu model CS-910) fitted with Shimadzu U-235 data recorder, precoated silicagel plates 60F254 with a layer thickness 0.25 mm [E. Merck] and micro syringe (50 µl, Top) were used for TLC densitometric analysis. The hplc system consists of a Perkin Elmer 250 solvent delivery pump, Perkin-Elmer LC 235 diode array detector, Rheodyne 7125 injector fitted with a 20 µl loop, a C18 column Lichrospher 100 RP-18, 5 µm, 250 x 4 mm (E. Merck) and GP 100 printer plotter (Perkin Elmer).

Five mg of 82/205 was dissolved in 10 ml methanol to get a standard solution with concentration of 0.5 ug/ul. Stability of 82/205 in this solution was also checked. It was observed that not more than 5% of 82/205 decomposed when kept in its solution form for 24 h, at room temperature.

Bulk drug sample or formulation equivalent to 5 mg of

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