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Antifertility Activity of Niosomal HPβCD - Plumbagin Complex

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Inclusion complex of drug with hydroxyl propyl betacyclodextrin (HPβCD) was prepared with a view to increase the efficacy and solubility of plumbagin. The complex was entrapped in the aqueous layer of niosomes and evaluated for antifertility activity. This in turn was compared with niosomes of the plain drug where entrapment was possible in the lipid layer. Given intraperitoneally at a dose of 5 mg/kg the niosomes of the drug complex showed promising antifertility activity when compared to the control and niosomes with lipid layer entrapment. Although complex showed lower entrapment efficiency over the plain drug the stability and antifertility activity was markedly increased.

PLUMBAGIN is a crystalline substance present in *Plumbago-zeylanica, Plumbago rosea, Plumbago europea* and *Plumbago indica,* belonging to the family plumbaginacea. *P. zeylanica* root has been reported to be a powerful poison when given internally or applied to the OS uteri causing abortion[1-2]. Plumbagin was subjected to subacute toxicity testing in albino rats for 2 weeks and 6 weeks at 4 mg/kg body weight orally and showed no histopathological or toxicity changes in any vital organs[3]. The antifertility activity of plumbagin has been evaluated and a low margin of safety has been reported[4].

HPβCD is capable of forming inclusion complexes with drugs[5]. These complexes can be used to increase the solubility and dissolution rate, decreases volatility, alter release rates, modify local irritation and increase the stability of drugs. HPβCD accommodates the water insoluble drugs within its cavities to form water soluble inclusion complexes. The present study is initiated to improve the efficacy of the drug as well as to reduce its toxicity using niosomes as carriers which have been extensively reported in literature[6-8].

EXPERIMENTAL

Materials: Hydroxy propyl Betacyclodextrin (HPβCD) was obtained from Nippo Shokuhin Ltd, Japan. Plumbagin, cholesterol, dicetyl phosphate (DCP), span 60 was supplied by Sigma chemical co., ST Louis, Mo, USA. All other chemicals and reagents were of analytical grade.

Phase solubility studies:

Phase solubility studies were performed according to the method reported by Higuchi and Connors[9]. An excess of drug was added to screw capped glass vials containing aqueous solution of various concentrations of HPβCD. Double distilled water was used as the medium and volume was made upto 10 ml. The flasks were shaken for 48 hours in a shaker bath at room temperature and protected from light. An aliquot was filtered and the concentration of the drug was determined spectrophotometrically at 268 nm after suitable dilutions. The solubility of the drug after shaking for 7 days was determined. The increase in the solubility in these systems is due to one or more molecular interactions between substrate and ligand to form distinct chemical species or complexes. Apparent stability constant ($K_{1:1}$) were calculated from the slope and the intercept of straight portion of the phase solubility diagram according to the following equation:

$$(K_{1:1}) = \frac{\text{slope}}{\text{intercept}(1\text{-slope})}$$

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Niosomes (Azmin *et al*)[6]:

Cholesterol (75 mg), span 60 (75 mg) and dicetyl phosphate (10 mg) were dissolved in 10 ml of Diethyl ether in a round bottomed flask. The ether layer was evaporated at 60° under reduced pressure in a rotary flash evaporator leaving a thin lipid layer deposited on the wall of the round bottom flask 10 ml of phosphate buffered saline pH 7.4 in which the drug was dissolved was warmed to 60° added to the flask and vortexed for 10 minutes with intermittent warming.

Entrapment efficiency:

The niosomal suspension was filled into the dialysis tube to which a sigma dialysis membrane was securely attached to one side. The dialysis tube was suspended in 100 ml of phosphate buffered saline pH 7.4 which was stirred on a magnetic stirrer. The unentrapped drug was separated from the niosomes into the medium through the semipermeable membrane. At every half hour interval 100 ml was replaced with fresh media for about 2-3 hours.

***In vitro* release study:**

The niosomes left after removal of unentrapped drug were dialyzed into fresh 100 ml of phosphate buffered saline pH 7.4. 5 ml samples were withdrawn at specific time intervals and replaced by fresh medium every hour for 6 hours and analyzed spectrometrically at 268 nm.

***In vivo* studies:**

Antifertility studies:

The method of Premakumari[4], was employed to detect the antifertility activity of different formulations containing plumbagin. Adult female Albino rats weighing between 125-150 g, showing regular 4-5 day oestrus cycle were divided into 4 groups each containing six rats. They were housed in separate cages, each cage containing three rats. Young males (body weight 200-225 g) of proven fertility were admitted to each of the cages so that the ratio between females and males is 3:1, these males were kept overnight, and were separated each morning. Vaginal smears were taken daily between 10.00 am and 12.00 noon.

The day on which thick clumps of spermatozoa were detected in the vaginal smear was termed as the day one of pregnancy. The pregnant rats were isolated and the following was carried out : Group I received no treatment and were termed as the control group. Group II was given plain plumbagin, and Group III Niosomal plumbagin each in the dose of 5 mg/kg body weight and by the intraperitoneal route. The rats were then allowed dot gestate and on the twentieth day of pregnancy, the rats were autopsied and inspected for number of implantation sites. The ovary and uterus were isolated and the histological sections were taken and observed for changes.

Stability studies:

The formulated niosomes were separated into three portions. One portion was kept at room temperature, the second portion at 37° and the third portion was kept 4° for one month. After every 15 days samples were removed and amount of the drug present in the niosomes was determined, and *in vitro* release study was carried out. Graph of drug in the niosome vs time and cumulative % of drug released vs time were plotted.

RESULTS AND DISCUSSION

Phase solubility studies:

Plumbagin is practically insoluble in water. The solubility of plumbagin increased linearly with the concentration of HPβCD (0 to 15 mM) showing A_L type of solubility behaviour as reported by Higuchi[9]. This indicated that the inclusion complex was formed at 1:1 molar ratio of drug: HPβCD. Similar observation of the complex formation at 1:1 molar ratio have been reported by Jarho[11] The higher value of solubility constant ($K = 169\ m^{-1}$) suggested the interaction of plumbagin with HPβCD (Fig. 1).

Size and Entrapment:

The size distribution analysis showed that the niosomes were mostly unilamellar and spherical in shape, although a few triangular and elongated ones were also seen. The size of the niosomes ranged from 12-25 nm, with an entrapment of 68 ± 2.45%.

***In vitro* release:**

A 40 % cumulative release for the drug complex niosomes was seen in 6 hours, with neither a burst effect nor a very slow release pattern. Due to the inclusion of drug in aqueous layer the release rats were faster compared

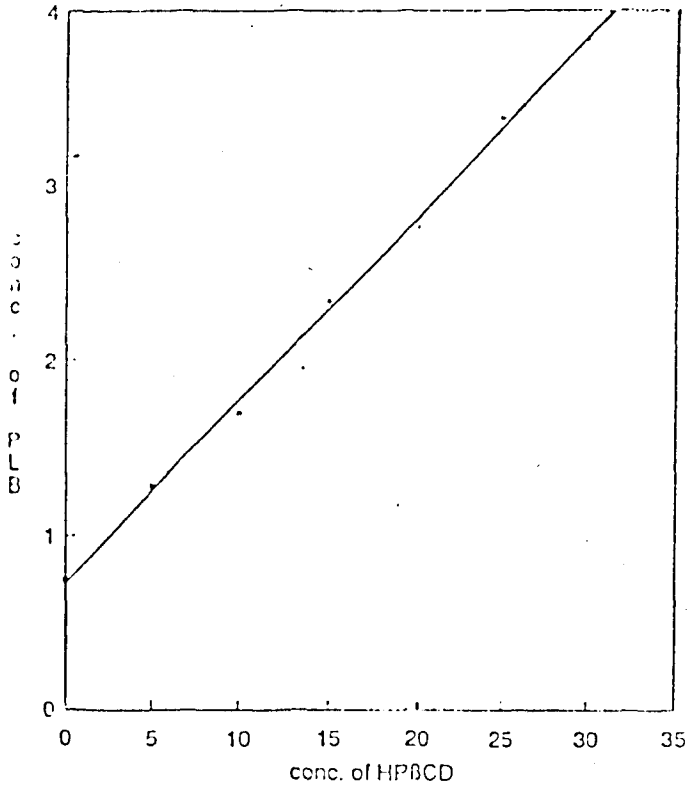

Fig.1: Phase solubilty diagram of plumbagin – HPBCD complex showing the formation of complex at 1:1 Molar Ratio

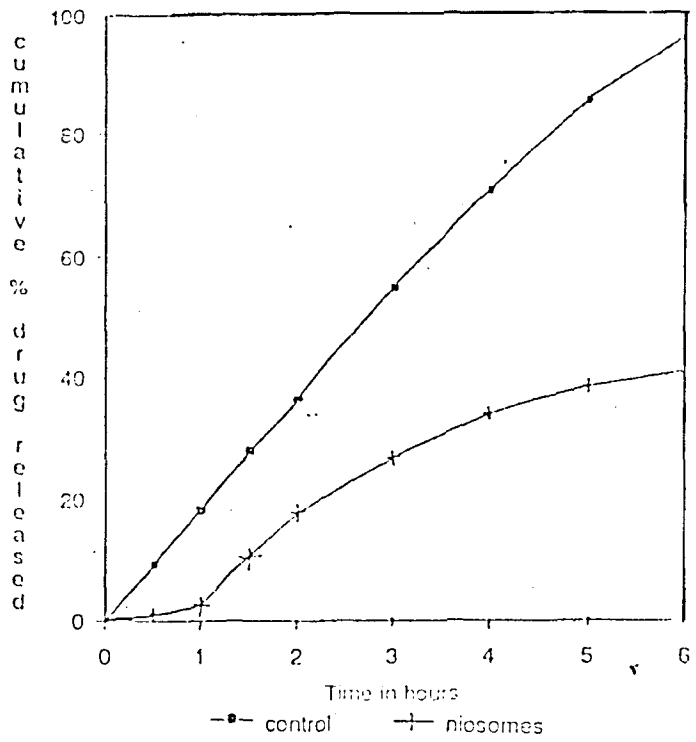

Fig. 2: *In vitro* release profile of plumbagin – HPBCD complex and Niosomes

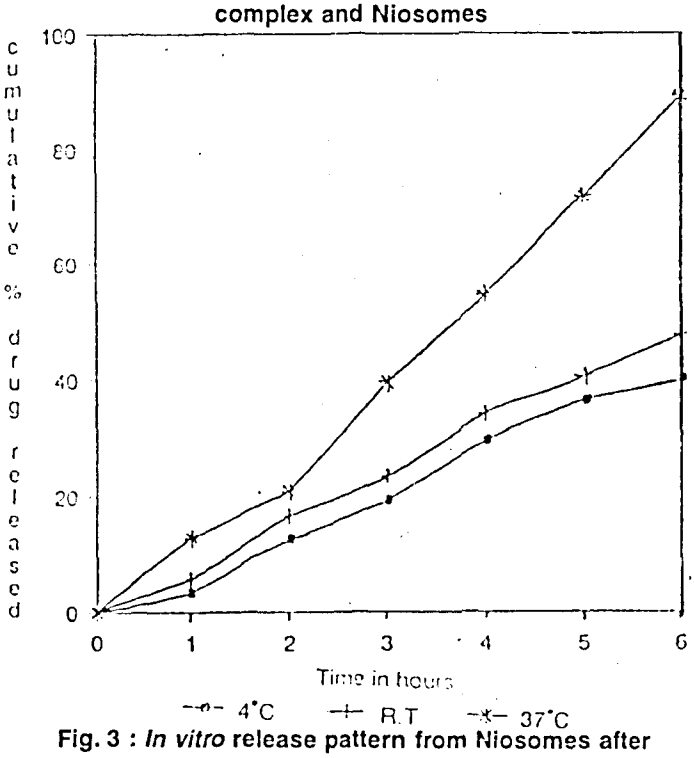

Fig. 3 : *In vitro* release pattern from Niosomes after storing at different conditions

to 27% cumulative release of plain drug as reported by Kini[12] where entrapment was in the lipid layer. (Fig 2)

The slower release of plumbagin from the lipid layer may be due to slow partitioning and diffusion of plumbagin from the lipid layer to the surrounding aqueous layer. However when the drug is incorporated in the aqueous layer, the drug may diffuse out due to concentration gradient, as well as due to the erosion and biodegradation of the lipid layer. Moreover since an inclusion complex of drug with HPbCD has been used there has been an increase in the solubilization of the drug. This complex formation and solubilization has also helped in enhancing the permeation and diffusion of the drug from the inner reservoir to the external physiological environment.

Stability studies:

Stability data of plumbagin clearly indicates that complexation of plumbagin followed by niosomes encapsulation gives good protection against degradation and there by improving the stability. It may be helpful to maintain the therapeutic efficacy of the drugs on longer duration of storage. (Table 1, Fig 3).

***In vivo* studies:**

Results of the histopathological studies may be summarized as follows: (Table 2).

Table 1 : Stability studies: Degradation rate constant (K) values of Plumbagin formulations at different storage conditions

Formulation	Degradation rate constant (K) day^{-1}		
	4°C	R.T.	37°C
Plumbagin complex	8.70×10^{-2}	9.2×10^{-2}	9.1×10^{-2}
Plain Plumbagin	8.71×10^{-2}	9.20×10^{-2}	0.1076
Plumbagin Complex dispersed	8.70×10^{-2}	9.2×10^{-2}	0.1085
Plumbagin in PBS	8.73×10^{-2}	9.2×10^{-2}	0.1094

Table 2 : Antiimplantation effect of Plumbagin in different formulation

Formulation	No. of implantation Sites on the 10th day of pregnancy		No. of rats with no implantation sites	% of anti fertility activity
	No. of rats showing implantation	No. of implantation sites in individual rats		
Control	6	8,7,10,9,12,8	-	0
Drug complex	4	11,4,7,6	2	33.33
Niosomes*	3	10,4,7	3	50

N = 6
Dose : 5 mg/kg body weight
Statistically significant Vs control at P < 0.05 (One-tailed Fischers exact test)

Uterus : There is no difference in the histopathological structures of the control and treated uterus. The epithelial cell lining the endometrium was intact and the glands were in the secretary phase in both the treated and the control groups. A slight degeneration of the mucosa was seen in the treated groups due to infiltration by eosinophils. Further no deformity like swelling was detected.

Ovary : Control : The ovary showed the graffian follicle, primordial follicle and mature follicle with or without ova. The intact corpus luteum could also be seen.

Treated : No charges in the ovary was seen when the complex was administered after dispersing in phosphate buffered saline pH 7.4.

In case of the niosomes administered group, although primordial cells were seen, mature graffian follicles were absent. Further partial degeneration of the oviductal mucosa was also seen. It is quite from the histopathological studies that the drug has some action of the ovary, as seen from the clear inhibition of the growth of the graffian follicles. Thus the drug seems to have anti-ovulatory action and thereby antifertility activity. The results indicate that the complex form of plumbagin with HPβCD has greater benefits over plain plumbagin, since there is a marked increase in the solubility, stability and therapeutic efficacy of the drug.

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