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Research Article

DEVELOPMENT, CHARACTERISATION AND/ TOXICITY EVALUATION OF NANOPARTICLES OF ANDROGRAPHOLIDE

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ABSTRACT

The objective of the present study is to prepare nanoparticles of Andrographolide and investigate the physiochemical characteristics of the prepare nanoparticles. The nanoparticles of nanoparticles of Andrographolide with eudragit S 100 were formulated using nanoprecipitation technique. Formulation variables were optimized to prepare nanoparticles of Andrographolide such as polymer concentration and stabilizer concentration. The effects of these variables on the particle size and entrapment efficiency were studied. The physiochemical characteristics of nanoparticles were studied applying particles size analysis, zeta potential and Scanning Electron Microscopy analysis. All the prepared formulation resulted in desire size and relative spherical morphology. The zeta potential was remaining in the range of negative value. It represented an index of particle stability. The *in vivo* toxicity study in swiss albino mice were carried out, it shown no significant change in biochemical and haematological examination. Hence the designed system could be safe and it will improve the patient compliance.

Keywords: Andrographolide, Eudragit, Zeta potential, *In vivo* toxicity

INTRODUCTION

The development of drug delivery systems as improved the therapeutic and toxicological properties of existing chemotherapies and facilitated the implementation of few ones, by including the drug in technologically optimized drug delivery systems or conjugating the drugs with different polymers, it is possible to modify the pharmacokinetics and bio distribution of drugs improving the efficiency and security of the therapy [1]. In the recent decades nanoparticles containing therapeutic agents have gained much interest in scientific and commercial fields owing to their potential for site specific drug delivery and accordingly the optimization of drug delivery. Specifically polymers take part of an important role as drug carrier devices. Pharmacologically active agent possible will be incorporated into a polymeric matrix[2]. Nanoparticlulate carriers have always been attractive on account of their size and capacity of spatial and temporal controlled delivery of bioactives[3].

Andrographolide, a labdane diterpenoid extracted from the leaves of the Indian medicinal plant *Andrographis paniculata*. It is sparingly soluble in water, limiting its bio distribution and localization. It is unstable in extremes of gastrointestinal alkaline and acidic conditions and has a very short biological half life (2 Hours) [4]. Andrographolide has several pharmacological activities included analgesic, anti inflammatory, hepatoprotectant, anti viral anticancer and hypoglycaemic[5]. Aim of the present investigation is to develop a nanoparticles carrier of andrographolide for controlled delivery. Formulated andrographolide nanoparticles were characterized and study the toxicity in animal model.

MATERIALS AND METHODS

Andrographolide was isolated form *Andrographis paniculata* extract according to the method reported by Rajana et al., 2000[6]. Eudragit S100 were purchased from (Rohm pharma) tween 80 was purchased from Sd fine chemical and other chemical and solvents were used analytical grade.

Fourier transforms infrared studies

FT-IR spectra for Andrographolide, Eudragit S100, and physical mixture of both at the ratio of 1:1 were revealed by means of FT-IR spectrophotometer using the instrument Perkin Elmer Spectrum RXI. Samples were prepared in KBr disks technique. The scanning range was 400-4000 cm^{-1} [2].

Preparation of nanoparticles of andrographolide

Nanoparticles, placebo or loaded with andrographolide were prepared by nanoprecipitation method[7]. Briefly a 20mg andrographolide and various proportions of eudragit S 100 were dissolved in acetone (5ml). The organic phase was poured drop wise into 10ml of aqueous phase containing 1% of tween 80 under magnetic stirring at room temperature. Nanoparticles were spontaneously formed and turned the solution into milky colloidal suspension. Then acetone was removed by continuing stirring for overnight at room temperature. Formulation optimization was pursued to obtain nanoparticles of desired physical properties. Effect of various polymer drug rations from 1:1, 1:2 and 1:4 and the stabilizer concentration 0.25,0.5,1%w/v were assessed on drug encapsulation efficiency and particle size and poly dispersibility index.

Particle size and Zeta potential measurement

Measurement of the mean particles diameter of the nanoparticles dispersions was conducted with the use of a dynamic light scattering particles size analyzer (Zetasizer Ver.6.2 Malvern instruments Ltd., UK). The final particle diameter was calculated from the average of at least three measurements. The zeta potential values of the nanodispersion were measured, which measured the distribution of the electrophoretic mobility of particles[8].

Scanning Electron Microscopy observation

Scanning Electron Microscopy (SEM) was performed to evaluate the surface morphology of nanoparticles (JOEL JSM 5610, Japan). Nanoparticles were lyophilized before analysis[9]. A small amount of nanoparticles was stuck on a double sided tap attached on a metallic sample stand then coat under argon atmosphere with a thin layer of gold. A scanning electron microscopy photograph was taken at the acceleration voltage of 20KV.[10]

Encapsulation efficiency determination

The amount of andrographolide entrapped within nanoparticles was determined by measuring the amount of nonentrapped drug in the supernatant recovered after centrifugation[11]. Andrographolide content was analyzed spectrometrically at 232nm. Each experiment was repeated in triplicate. Percentage drug entrapment was determine by the following formula

$$\frac{\text{Amount of andrographolide actually percent in nanoparticles}}{\text{Amount of andrographolide actually used}} \times 100$$

Acute toxicity studies

The acute toxicity studies of the placebo and Andrographolide loaded nanoparticles were carried out in Swiss albino mice. Control animals received placebo nanoparticles made by Eudragit S 100 and treated animals received Andrographolide nanoparticles (1:2 drug polymer ratios). All the mice were daily observed for 14 days for biological changes and mortality. Sufficient food and water was given to the animals. Body weight was recorded at the beginning and after 14 days study period. The following general behaviours studies were observed. The haematological parameters and biochemical parameters were analysis routine laboratory by procedures. These estimations were carried after collection and transfer of blood into the heparinised tubes at the end of the study.

RESULT AND DISCUSSION

Interaction between the drug and polymers commonly lead to identifiable change in the FT-IR pattern. Andrographolide, Eudragit S100 and physical mixture of Andrographolide and Eudragit S 100 are demonstrated in Figure 1. Matching up to FT-IR spectrum of Andrographolide with physical mixture revealed no distinctive changes in the pattern of FTIR spectrum. Hence the polymer was compatible with drug.

Colloidal drug delivery system offers a number of advantages over conventional dosage form, due to their particle size[12]. Eudragit S100 Nanoparticles were successfully prepared by the nanoprecipitation technique. The method is simple, reproducible, fast, economic and one of the easiest procedures for the preparation of nanoparticles. Nanoparticles were spontaneously formed when the organic phase (acetone) was added drop wise into stirred aqueous surfactant solution (1%w/v tween 80).

Instantaneous formation of a colloidal suspension occurred as a result of the polymer deposition on the interface between the organic phase and water when partially water miscible organic solvent (acetone) diffused out quickly into the aqueous phase from each transient particle intermediate. According to the marangoni effect, the transient particle intermediate causes a size reduction to the nano range[13]. Nanoparticle size is affected by processing parameter such as drug polymer ratio, concentration of surfactant and phase ratio.

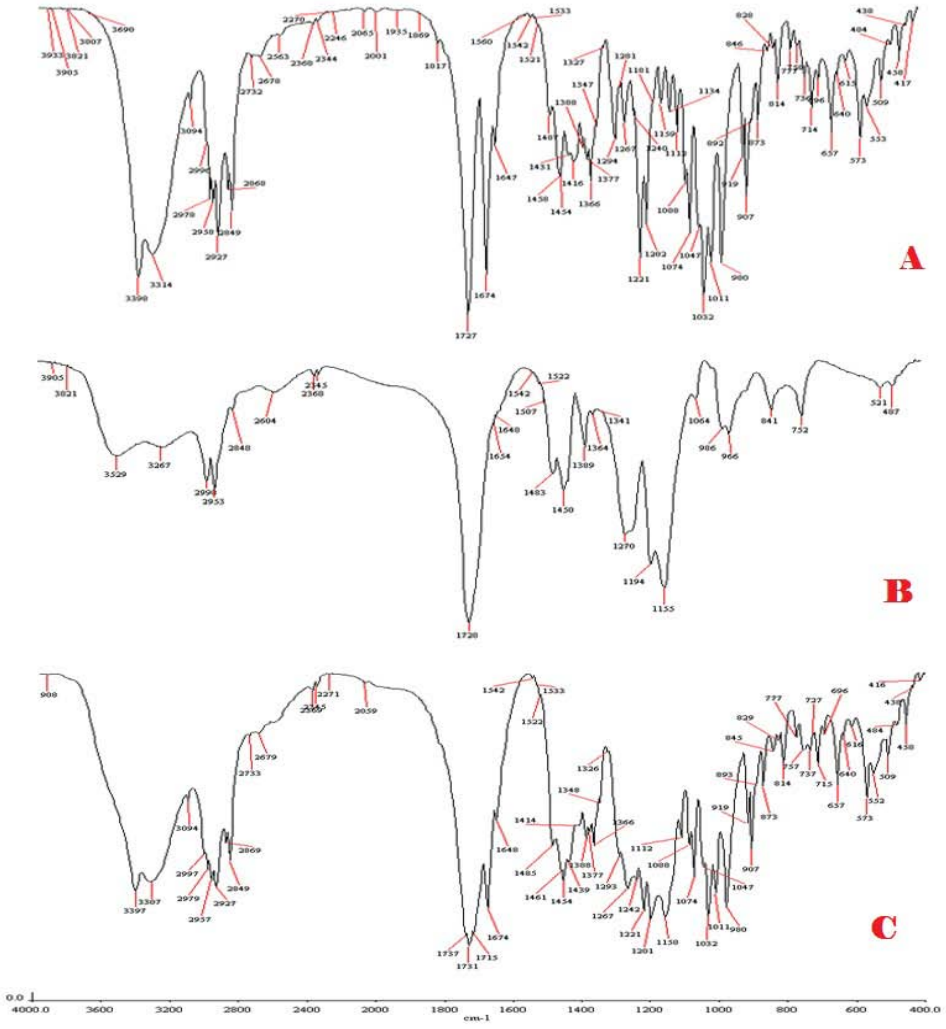

Fig. 1: FT-IR spectrum of Andrographolide (A), Eudragit S 100 (B), physical mixture of Andrographolide and Eudragit S 100 (C)

Hence, drug polymer ratio was used to optimize desire size and encapsulation of andrographolide in nanoparticles made by eudragit. The effects of the drug to polymer ratio on size of the nanoparticles were studied namely 1:1, 1:2 and 1:4. The batch

placebo and nanoparticles in which no drug was added showed a mean particle size of 299 nm and mean polydispersity index of 0.216. The mean particle size (Z-average diameter) for drug loaded batches varied in the narrow range from 158 nm to 246 nm. All batches of the nanoparticles showed mean sizes which were below 500 nm, therefore suitable for pharmaceutical drug delivery of colloidal carrier. The results are tabulated in table 1.

Table 1: Effect of drug polymer ratio on nanoparticles of Andrographolide

Drug Polymer ratio	Conc. of tween 80 (%)	Z average diameter (nm)	Polydispersity Index	Zeta Potential (mV)	Encapsulation Efficacy (%)
Placebo	1	299.0	0.216	-21.2	-
1:1	1	158.7	0.300	-33.4	66.34±1.92
1:2	1	189.2	0.530	-28.5	76.13±1.68
1:4	1	246.0	0.145	-32.8	60.74±1.57

The presence of an anionic surfactant is important to reduce the dynamic interfacial tension and to stabilize the nanosuspension. The surfactant is adsorbed on the nanosphere surface, increasing the steric repulsion between particles. The concentration of the surfactant was optimized in order to obtain small particles with maximum encapsulation. A concentration 1% W/V of tween 80 a maximum average size 185nm and entrapment was 66.34±1.92% were recorded. The various concentration used in the preparation of nanoparticles were 0.25, 0.5 (Results not shown) and 1% of tween 80.

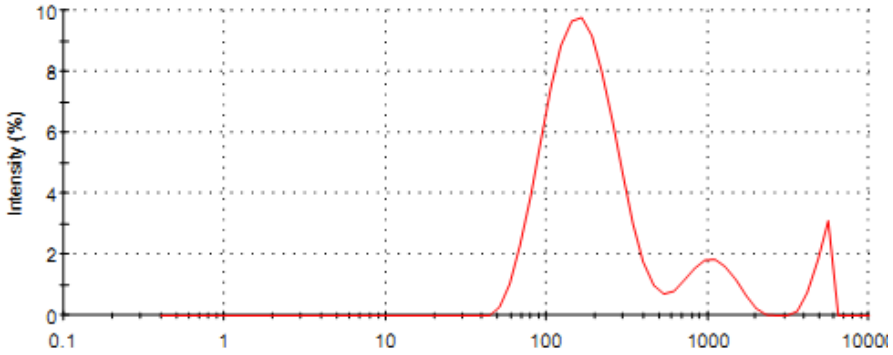

Fig. 2: Zeta average particle distribution of drug polymer ratio (1:2)

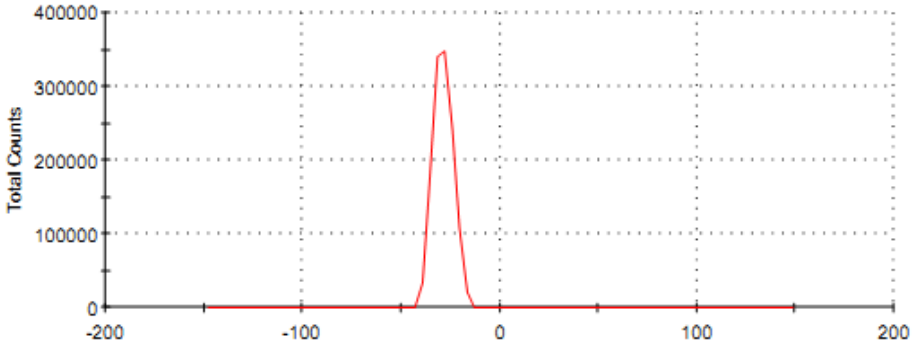

Fig. 3: Zeta potential distribution of drug polymer ratio (1:2)

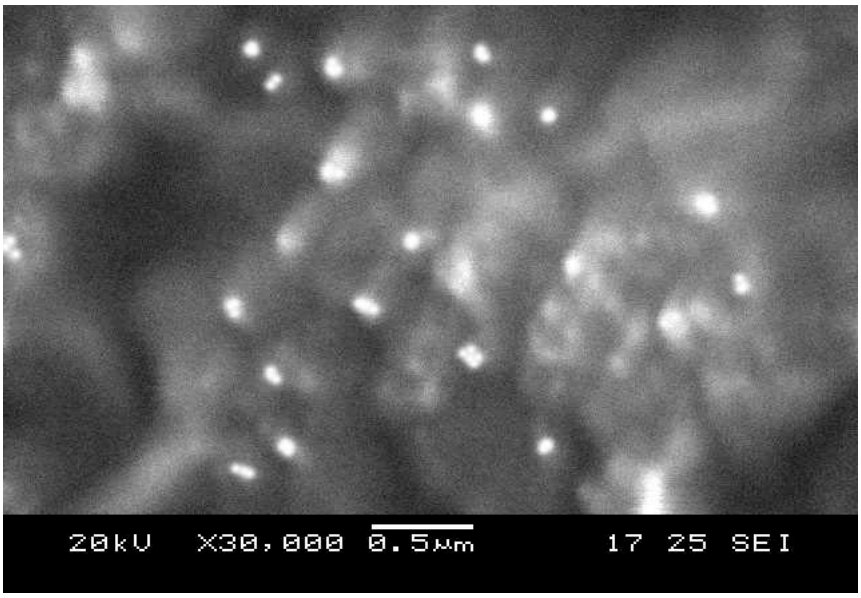

Fig. 4: SEM photograph of drug polymer ratio (1:2)

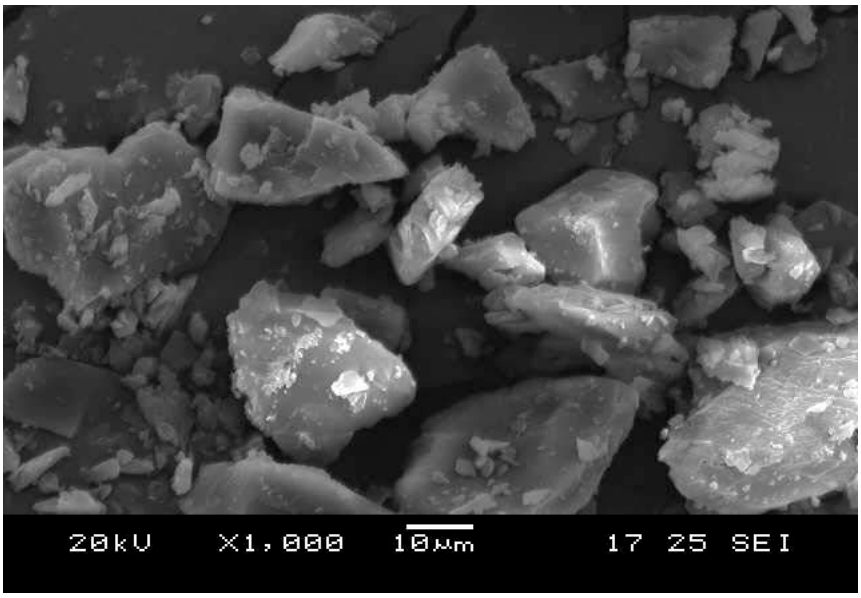

Fig. 4: SEM photograph of Andrographolide

Particle size increased with increasing drug polymer ration, smaller particle size was observed with the formulation having drug polymer ration 1:1 due to the surfactant concentration enough to maintain the stability of the nanoparticles and equal molar ration of drug polymer and coalescence of droplet did not occur. Maximum drug encapsulation was formed in the formulation having drug polymer ration 1:2. The SEM characterisation revealed that the nanoparticles were spherical in shape with a relative smooth surface compared with pure andrographolide. Based on the particle size and entrapment efficiency the nanoparticles of andrographolide prepared by drug polymer ratio 1:2 was selected for *in vivo* toxicity studies using male albino mice. The toxicity study was carried out using dose of 2g/kg body weight of nanoparticles formulation for behavioural changes, haematology and biochemical parameters. The results are tabulated in table 2 and table 3 respectively. The study period of 14 days, there was no mortality or morbidity observed in the experimental animals followed by single administration of nanoparticles of Andrographolide at dose of 2g/kg body weight. The results of current study revealed no adverse change in cage side observation.

All animals in the control and the treated group were found healthy as well as active. Similarly no significant difference was observed in the body weight between the groups. Morphological observation of abdominal content and organs like heart, liver and kidney were studied and found that there were no sign of inflammation or toxicity in both groups. All haematological parameters of nanoparticles of Andrographolide treated group was compared to that control group, there were no significant changes in haematological parameters like Hb, WBC, RBC, PCV and platelet counts. Evaluation of hepatic and renal function is of prime important to assess the inherent toxic properties of drugs. However the results of assay of these enzymes in plasma (Serum Glutamic Oxaloacetic Transaminase, Serum Glutamic Pyruvic Transaminase) revealed no difference between control and treated group. There were no significant changes in the total cholesterol, triglyceride, sugar level, protein and creatinine level in treated group when compared with control group.

Table 2: Effect of nanoparticles of Andrographolide on the haematological parameter

Parameter	Control Group	Treated Group
Hb (gm %)	15.20±0.6	14.44±0.47
WBC (10^3/mm)	09.06±0.15	08.84±0.25
RBC(10^6/mm)	09.48±0.21	09.71±0.14
PCV (%)	42.67±3.05	40.67±2.08
Platlets(10^3/mm)	173.30±6.11	179.00±5.50

Table 3: Effect of nanoparticles Andrographolide on the biochemical parameter

Parameter	Control Group	Treated Group
Total cholesterol (mg/dl)	60.67±3.05	57.00±3.60
Triglycerides (mg/dl)	57.34±4.16	55.34±4.16
Sugar (mg/dl)	58.86±1.41	59.22±2.66
SGOT (IU/L)	32.50±1.65	30.90±1.41
SGPT (IU/L)	71.45±2.84	67.82±3.06
Protein (mg/dl)	04.38±0.11	04.51±0.19
Creatinine (mg/dl)	00.34±0.05	00.28±0.12

CONCLUSION

Nanoparticles of andrographolide were prepared by nanoprecipitation technique. Drug polymer ratio and surfactant concentration found to be important for obtaining desire size and encapsulation, the development of nanoparticles could be reduce frequency dose, decrease side effect and improve patient compliance.

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