Research Article

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EFFECT OF PLGA-PEG NANOENCAPSULATION OF ACTIVE CONSTITUENTS FROM *CARALLUMA ADSCENDENS* (ROXB.) ON HT-29 COLON CANCER CELL

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ABSTRACT

Colon cancer is the most commonly diagnosed cancer disease in westernized countries its management remains a challenge in everyday oncology practice. Thus, advanced therapeutic strategies are required to treat colon cancer patients. *Caralluma adscendens* (Roxb.) (CAR) is a promising anticancer agent for various cancer types. The main objective of this study was to develop a polymeric drug delivery system for active components from *Caralluma adscendens* (Roxb.) intended to be intravenously administered, capable of improving the therapeutic index of the extract. To achieve this goal *Caralluma adscendens* (Roxb) loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles (PEG-PLGA-Nps) were prepared by single-emulsion solvent-evaporation technique. The nanoparticles were characterized by SEM and TEM analysis. The *in vitro* cytotoxic activity of CAR-PLGA-Nps developed was assessed using a human colon cancer cell line (HT-29) in MTT assay and DNA fragmentation assay and compared to the *in vitro* anti-tumoral activity of the *Caralluma adscendens* (Roxb.) extract.

Keywords: Colon cancer, *Caralluma adscendens* (Roxb.), poly (lactic-co-glycolic acid), Poly ethylene Glycol, DNA fragmentation assay, MTT assay, HT-29 colon cancer cell lines

INTRODUCTION

Nanoparticulate drug delivery systems using liposomes and biodegradable polymers have attracted increasing attention in recent years. The most noticeable nanotechnology applications in medicine have been related to oncology¹.

Nanoparticles are able to absorb and / or encapsulate a drug, thus protecting it against chemical and enzymatic degradation. In recent years, biodegradable polymeric nanoparticles have attracted considerable attention as potential drug delivery devices in view of their applications in the controlled release of drugs, in targeting particular organs/tissues, as carriers of DNA in gene therapy, and in their ability to deliver proteins, peptides and genes through peroral route².

A 2006 European Technological Observatory survey showed that more than 150 pharmaceutical companies were developing nanoscale therapeutics. The idea of controlled drug delivery has been shown to improve the therapeutic index of drugs by increasing their localization to specific tissues, organs, or cells³.

In about 80 % of cases, colorectal cancer is due to improper diet and hence may be prevented by dietary modifications. Risk reduction by nutritional intervention may provide an alternate approach in secondary prevention of cancer⁴.

To deliver therapeutic agents to tumor cells *in vivo*, one must overcome the following problems: (i) drug resistance at the tumor level due to physiological barriers (non cellular based mechanisms), (ii) drug resistance at the cellular level (cellular mechanisms), and (iii) distribution, biotransformation and clearance of anti-cancer drugs in the body. Approach involving polymer-based nanoparticles for oral delivery of the drug is

being actively investigated for treatment of cancer and various other diseases⁵.

The natural products are valuable sources of bioactive compounds, and have been considered the single most successful discovery of modern medicine. In recent years, natural dietary agents have drawn a great deal of attention both from researchers and the general public because of their potential ability to suppress cancers as well as reduce the risk of cancer development^{6,7,8}.

Also because of their low solubility, many phytochemicals are poorly absorbed by human body, thus one of the most important and interesting applications for encapsulation of phytochemicals is to enhance the bioavailability of phytochemicals by changing the pharmacokinetics and biodistribution⁹.

This approach tends to decrease potential side effects by leaving the normal sensitive cells unharmed. Contemporary systemically administered chemotherapeutic agents are extremely toxic to cancer cells, but can also harm normal cells leading to serious side effects. Biocompatible nanoparticles have been developed as inert systemic carriers for therapeutic compounds deliver to target cells and tissues¹⁰.

Plant phytochemicals exhibit high potent antioxidant activity. Incorporation of antioxidant compounds in manufactured foods, nutraceuticals or cosmetic preparations is a growing area of research. The genus *Caralluma adscendens* (Roxb.) is a very variable herb, up to 1 m. in height, with fleshy, almost leafless stems, deep purple-brown or yellowish white flowers, and have 10-12 cm slender follicles. It distributed in peninsular India from Andhra Pradesh and Maharashtra to Kerala up to 600 m. *Caralluma* species commonly used in treatment of rheumatism,

diabetes, leprosy, fever, tumor, fungal diseases, snake and scorpion bite and stomach pain¹¹.

So the study was designed with the objectives namely extraction of active constituents from *Caralluma adscendens* (Roxb.) using suitable solvent and lyophilization of extract, encapsulation natural antioxidants isolated from *Caralluma adscendens* (Roxb.) using biodegradable poly (lactide-co-glycolide) (PLGA) and the stabilizer polyethylene glycol (PEG)-5000, confirmation of diameter and morphology of nanoparticles using Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) and comparative analysis of anti-proliferative activity of nanoparticles and crude extract by MTT and DNA fragmentation assay.

MATERIALS AND METHODS

Sample Collection

The plant *Caralluma adscendens* (Roxb.) was collected from the local fields of Coimbatore and authenticated at Botanical Survey of India Coimbatore (No. BSI/SRC/5/23/10-11/tech-312). After collection of plant, it was washed thoroughly with double distilled water and shade dried at room temperature for 15 days and it was ground to fine powder and used for further studies.

Preparation of Plant Extract

The plant extracts was obtained by weighing out 20g of the ground powder and it was soaked in 100ml of 70% ethanol. The obtained extract was then concentrated to dryness under reduced pressure at 45°, using rotary evaporator. The filtrate was allowed to dry at room temperature until dry ethanol extract was obtained. The crude extract was weighed to calculate the yield and stored in a refrigerator (-4°C), until used for further work¹².

Collection of Cell Lines

HT29 (Human, colon cancer) cell line was procured from National Centre for Cell Sciences (NCCS) Pune, India and maintained in suitable laboratory condition.

Culturing of HT29 Colon Cancer Cell Lines

Stock cells of HT29 were cultured in DMEM supplemented with 10% inactivated fetal Bovine serum (FBS), penicillin (100 IU/ml), Streptomycin (100 µg/ml) and amphotericin B (5µg/ml) in a humidified atmosphere of 5 % CO₂ at 37°C until confluent. The cells were dissolved with TPVG (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The stock cultures were grown in 25 cm² culture flasks and all experiments were carried out in 96 microtitre plate¹³.

Reagents Required

PLGA (50:50), Poly ethylene glycol (PEG), Ethyl acetate Methylene chloride, polyvinyl alcohol (PVA)

Synthesis of *Caralluma adscendens* (Roxb.) Loaded PLGA-PEG blend nanoparticles

The nanoparticles were obtained by the single-emulsion solvent-evaporation technique. Briefly, *Caralluma adscendens* (Roxb.) extract (5mg) and PLGA(50mg) were dissolved in a mixture of ethyl acetate (1.5ml) and methylene chloride (0.5ml) with or without PEG (10mg) at room temperature. This organic phase was rapidly poured into 10ml of PVA aqueous solution

(0.5% w/v) and emulsified by sonication for 5 min, resulting in an oil-in-water (O/W) emulsion. Next, the organic solvent was rapidly eliminated by evaporation under vacuum (20 min) at 37°C. The particles were then recovered by centrifugation (19,975 x g, 30 min, 4°C) and washed twice with water to remove the surfactant. The resulting nanosuspension was cooled to -18°C and freeze-dried¹⁴.

Scanning Electron Microscopy (SEM)

SEM analysis was done using Hitachi S-4500 SEM machine. Thin film of sample were prepared on a carbon coated grid by just dropping a very small amount of sample on the grid, extra solution was removed using a blotting paper and the film on the SEM grid allow to dry by putting it under a mercury lamp for 5 minutes¹⁵.

Transmission Electron Microscopy (TEM)

The morphology of the nanospheres was ascertained by Transmission Electronic Microscopy (TEM) (CM-12, Philips). A drop of the nanoparticles suspension was placed on copper electron microscopy grids and stained with a 2% (w/v) phosphotungstic acid solution (Sigma). After 30 seconds the sample was washed with ultra-purified water and the excess fluid removed with a piece of filter paper. The dried drop removed with a piece of filter paper¹⁶.

MTT Assay

The toxicity of *Caralluma adscendens* (Roxb.) loaded PLGA-PEG nanoparticles and free *Caralluma adscendens* (Roxb) extract against HT29 cells was investigated by the MTT assay¹⁷. HT 29 cells were seeded in 24-well plates at a density of 10,000 cells per well supplemented with 10% fetal bovine serum. Twenty four hours after plating, different amounts of a *Caralluma adscendens* (Roxb.) crude extract in water and extract loaded nanoparticles (suspended in water) were added in the wells. After 24 h of incubation at 37°C, 50 µl of MTT solution (5 mg/ml in PBS pH 7.4) was added into each well and the plates were incubated at 37°C for 3 h. The medium was withdrawn and 200 µl of acidified Isopropanol (0.33 ml HCl in 100 ml Isopropanol) was added in each well and agitated thoroughly to dissolve the formazan crystals. The solution was transferred to 96-well plates and immediately read on a microplate reader at a wavelength of 490 nm. Cell viability was calculated from the ratio between the absorbance provided by the cells treated with the extract and the absorbance provided by non-treated cells (control)

DNA Fragmentation Assay

HT-29 colon cancer cell line (3x 10⁶ /ml) were seeded into 6 well plates and incubated at 37°C with 5% CO₂ atmosphere for 24 h. The cells were washed with medium and were treated with different doses of the extract, standard drug and incubated at 37°C, 5% CO₂ for 24 hrs. At the incubation time ended, the chromosomal DNA of cancer cells was prepared with Roche apoptotic DNA ladder kit. Briefly, cells were harvested and lysed with lysis buffer for 10 min. Then the samples were mixed with Isopropanol before passing through the filter and washed. The DNA was eluted from the filter and treated with RNase at 37°C for 30 min before loading onto 2% Agarose gel electrophoresis and run 50 V/cm for 3 hrs. The gel was visualized under UV transilluminator and photographed¹⁸.

Table 1: Effect of PLGA-PEG encapsulation of *Caralluma adscendens* (Roxb.) extract on cell viability of HT-29 cell LINES by MTT assay

Sl. No	Name of Test Compound	Test Conc. ($\mu\text{g/ml}$)	% Cytotoxicity	CTC ₅₀ ($\mu\text{g/ml}$)
1	<i>Caralluma adscendens</i> (Roxb.) sample	1000	36.69 $\pm$ 0.5	>1000
		500	31.85 $\pm$ 0.8	
		250	14.17 $\pm$ 2.8	
		125	7.01 $\pm$ 2.4	
		62.5	3.05 $\pm$ 0.5	
2	PLGA-PEG loaded <i>Caralluma adscendens</i> (Roxb.) sample	1000	29.22 $\pm$ 0.8	>1000
		500	16.11 $\pm$ 0.4	
		250	13.72 $\pm$ 0.3	
		125	9.00 $\pm$ 0.3	
		62.5	4.59 $\pm$ 1.8	

The value of the control (unexposed) cells was taken as 100% and the percentage of cell growth inhibition of *Caralluma adscendens* (Roxb.) exposed cells was calculated. *Significant difference ($p < 0.05$) compared to control (0 $\mu\text{g/ml}$).

Figure 1: SEM analysis of PEG-PLGA encapsulated *Caralluma adscendens* (Roxb.) **Figure 2: TEM micrograph of PLGA – PEG encapsulated *Caralluma adscendens* (Roxb.)** **Figure 3: Effect of PLGA-PEG encapsulation of *Caralluma adscendens* (Roxb.) extract on cell viability of HT-29 cell lines by MTT assay**

Figure 4: Effect of PLGA-PEG encapsulation of *Caralluma adscendens* (Roxb.) extract on cell viability of HT-29 cell lines by MTT assay

Lane L: Ladder (100 bp), **Lane A:** HT-29 cells (Untreated)
Lane B: HT-29 cells treated with Doxorubicin (5 µg/ml), **Lane C:** HT-29 cells treated with crude extract sample (1000µg/ml)
Lane D: HT-29 cells treated with crude extract sample (500µg/ml), **Lane E:** HT-29 cells treated with encapsulated test sample (1000µg/ml)
Lane F: HT-29 cells treated with encapsulated test sample (500µg/ml)

Figure 5: Effect of PLGA-PEG encapsulation of *Caralluma adscendens* (Roxb.) extract on DNA fragmentation in HT-29 colon cancer cell lines

RESULTS

SEM And TEM Analysis

The nanoparticles prepared by the single emulsion solvent evaporation method were observed by Scanning Electron Microscope and Transmission Electron Microscope and the micrographs were given in Figure 1 and 2.

From the Figure 1 and 2, it was clear that the shape of *Caralluma adscendens* (Roxb.) loaded PLGA-PEG nanoparticles were spherical and smooth surfaced. The well dispersed individual particles with spherical core-shell structure were visible with some aggregations. The nanoparticles have narrow dimensional distribution with an average size of about 40-50 nm.

In Vitro Cytotoxicity Assay

Effect of PLGA-PEG encapsulation of *Caralluma adscendens* (Roxb.) extract on cell viability of HT-29 cell lines was evaluated by MTT assay and the results were presented in Table 1, Figure 3 and Figure 4.

From the Table 1 and Figure 3 and 4, it was clear that HT-29 cells treated with crude and encapsulated extract at various concentrations of 62.5 to 1000µg/ml for 24 h showed dose-dependent decrease in cell viability of HT-29 cells with a CTC₅₀ value above 1000 µg/ml.

Free crude extract and extract loaded nanoparticles induced similar cytotoxicity, demonstrating that the triggering mechanism for the release of the drug from the endosome/lysosome into the cytosol is highly efficient. PLGA nanoparticles can be internalized by phagocytic processes followed by endosomal escape and delivery of encapsulated agents to the cytosol¹⁹.

DNA Fragmentation Assay

The ability of crude and encapsulated extract to induce apoptosis in HT-29 cells was confirmed using DNA fragmentation assay and the results were shown in Figure 5. The lane L represents the well which was loaded with Marker DNA fragments having different molecular weight. The lane A represents the untreated control HT-29 cell lines and did not show any ladder formation. Lane B represents the HT-29 cell lines treated with Doxyrubicin

(5µg/ml) where ladder formation was observed and the lanes C,D,E and F represent the cell lines which was treated with 1000 and 500µg/ml of *Caralluma adscendens* (Roxb.) extract and PLGA-PEG encapsulated sample 1000 and 500µg/ml which showed similar pattern in ladder formation.

DISCUSSION

PLGA-based nanoparticles present many advantages for drug delivery. They can protect drugs from degradation and enhance their stability. Moreover, due to their nano size it can penetrate specific tissues via (i) endothelium of cancer and inflamed tissue or (ii) via receptors over expressed by target cells or in the blood brain barrier, This allows a specific delivery of drugs, proteins, peptides or nucleic acids to their target tissue. PLGA- based nanoparticles can increase the efficacy of treatments because of the sustained release of the therapeutic agents from stable nanoparticles.

PLGA- PEG nanoparticles loaded with *Caralluma adscendens* (Roxb.) were successfully prepared by the emulsion solvent-evaporation method. The PLGA-PEG nanoparticles loaded with *Caralluma adscendens* (Roxb.) extract were characterized by SEM and TEM analysis and the result showed that the nanoparticles were in the size range of 40-50nm and spherical in shape.

The cytotoxic activity of both crude *Caralluma adscendens* (Roxb.) extract and PLGA-PEG loaded *Caralluma adscendens* (Roxb.) was determined by MTT assay and the results revealed that cytotoxic action increases with increasing *Caralluma adscendens* (Roxb.) concentration. The CTC value was found to be above 1000µg/ml which indicated its efficacy as antiproliferative agent. Free crude extract and extract loaded nanoparticles induced similar antiproliferative and cytotoxic action, demonstrating that the triggering mechanism for the release of the drug from the endosomes/lysosomes into the cytosol is highly efficient.

DNA fragmentation is regarded as the hall mark of apoptosis and a late event during apoptosis and the nuclear DNA of apoptotic cells showed characteristic laddering pattern^{20,21}. The ability to induce tumor cell apoptosis is an important property of a candidate anticancer drug, which discriminates between anticancer drugs and toxic compounds. Much effort has been directed towards searching for compounds that influence apoptosis and understanding their mechanism of action²².

It has been proposed that the transformation of normal colorectal epithelium to carcinomas involves progressive apoptotic inhibition. Apoptosis entails the execution of specialized machinery, central components of which are the family of Bcl-2-related proteins along with other mitochondrial proteins. Defects in the cascade of apoptosis-related events during neoplastic development could well affect the execution of apoptotic death and disrupt homeostasis regulation of the colonic tissue. Strategy for colon cancer chemoprevention is the search for nutritional components directed at inducing apoptosis of cancer cells²³.

In DNA fragmentation assay, *Caralluma adscendens* (Roxb.) plant extract and PLGA –PEG nanoparticles loaded with *Caralluma adscendens* (Roxb) showed similar pattern in programmed cell death related DNA fragmentation.

CONCLUSION

The present research ascertains that PLGA-PEG loaded *Caralluma adscendens* (Roxb.) is a good choice for further experiments in drug delivery systems because of less cytotoxicity with regard to the herbaceous nature of the anticancer agent. Since PLGA-PEG loaded nanoparticles are highly biocompatible and do not possess any Significant toxicity *in vitro*, the prepared nanoparticles can very well used as a carrier for drug delivery.

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REFERENCES

1. Koning GA, Krijger GC. Targeted multifunctional lipid-based nanocarriers for image-guided drug delivery. *Anticancer Agents Med Chem* 2007; 425–40.
2. Rangasamy M, Parthiban KG. Recent advances in novel drug delivery systems. *IRJAP* 2010; 1 suppl 2: 316-326.
3. Riehemann K, Schneider SW, Luger TA, Godin B, Ferrari M, Fuchs H. Nanomedicine. challenge and perspectives. *Angew Chem Int Ed Engl* 2009; 48:872–97.
4. Slatery M L, Edwards S L, Boucher K M, Anderson K, Caan BJ. Life style and colon cancer: an assessment of factor associated with risk. *Am J Epidemiol* 1999; 150: 866-877.
5. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. *Adv Drug Deliv Rev* 2002; 54:631–51.
6. Molinski TF. Developments in marine natural products. Receptor-specific bioactive compounds. *J Nat Prod* 1993; 1, 56: 1–8.
7. Grabley S, Thiericke R. Bioactive agents from natural sources: trends in discovery and application. *Adv Biochem Eng Biotechnol* 1999; 64:101–54.
8. Amin AR, Kucuk O, Khuri FR, Shin DM. Perspectives for cancer prevention with natural compounds. *J Clin.Oncol* 2009; 27: 2712–25.
9. Huang Q, Yu H, Ru Q. Bioavailability and delivery of nutraceuticals using nanotechnology. *J Food Sci* 2000; 75:R50–57.
10. Van Vlerken LE, Amiji MM. Multi-functional polymeric nanoparticles for tumour-targeted drug delivery. *Expert opin drug deliv* 2006; 3: 205–16.
11. Al-Yaha MA, Abdel-Sattar E. Pregnane glycosides from *caralluma russeliana*. *J. Nat. Prod* 2000; 63: 1451-1453.
12. Odey MO, Iwara IA, Udiba UU, Johnson JT, Inekwe UV, Asenye ME, Victor O. Preparation of Plant Extracts from Indigenous Medicinal Plants. *IJST* 2012; 1 suppl12:688-692.
13. Bhavani and Leelavathi. Investigation on *in vitro* cytotoxic activity of a selected wild cucurbitaceae plant *Corallocarpus epigeaus* against cancer. *IJPSR* 2015; 6(8): 3554-3557.
14. Ibrahim MM, Sammour OA, Hammad M, Megrab NA, Li X, Jast B. Integrity and Bioactivity of Insulin Loaded PLGA Nanoparticles Prepared by a Novel Aqueous Method and its Comparison to Emulsion Solvent Evaporation Method. *Curr Trends Biotechnol Pharm* 2011; 5 (2):1084-1097.
15. Elumalai EK, Kayalvizhi K, and Silvan S. Coconut water assisted green synthesis of silver nanoparticles. *J Pharm Bioallied Sci* 2014; 6(4): 241–245.

16. Hussain JI, Kumar S, Hashmi AA, Khan Z. Silver nanoparticles: preparation, Characterization, and kinetics. *Advanced material letters* 2011; 2(3):188-194.
17. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application of proliferation and cytotoxicity assay. *J. Immunol. Methods* 1983; 65: 55–63.
18. Basnakian AG, James SJ. A rapid and sensitive assay for the detection of DNA fragmentation during early phases of apoptosis. *Nucleic Acids Res* 1994; 22(13):2714-5.
19. Shen H, Ackerman AL, Cody V, Giodini A, Hinson ER, Cresswell P, et al. Enhanced and prolonged cross-presentation following endosomal escape of exogenous antigens encapsulated in biodegradable nanoparticles. *Immunology* 2006; 117:78-88.
20. Yu Z, Li W. Induction of apoptosis by puerarin in colon cancer HT-29 cells. *Cancer Letters* 2006; 238:53–60.
21. Taraphdar AK, Roy M, Bhattacharya RK. Natural products as inducers of apoptosis: Implication for cancer therapy and prevention. *Current Science* 2001.80(11):1387-1396
22. Sivagami G, Vinothkumar R, Preethy CP, Riyasdeen A, Akbarsha, MA, Menon VP, Nalini N. Role of hesperetin (a natural flavonoid) and its analogue on apoptosis in HT-29 human colon adenocarcinoma cell line – A comparative study. *Food and Chemical Toxicology* 2012; 50: 660–671.
23. Lavi I, Friesem D, Geresh S, Hadar Y, Schwartz B. An aqueous polysaccharide extract from the edible mushroom *Pleurotus ostreatus* induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells. *Cancer Letters* 2006; 244: 61–70.

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