

# Dendrosomal nano-curcumin; The novel formulation to improve the anticancer properties of curcumin

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## ABSTRACT

Curcumin is a hydrophobic polyphenol extracted from the plant *Curcuma longa* with established anticancer properties. However, curcumin benefits have been impaired by its very low water solubility, low absorption, rapid metabolism and clearance from the body. Recently, nanotechnology promises to be helpful in development of drugs delivery systems by recent advances in macromolecular design of nanocarriers. In this review, we present the novel generation of nano-vehicles termed dendrosomes which are readily synthesized from esterification of oleic acid and polyethylene glycol residues. Dendrosomes efficiently encapsulate curcumin in a spherical micellar or polymersome structures which leads to increase aqueous solubility of this hydrophobic agent and higher bioavailability of curcumin. Anti-cancer potency of this nanoformulation was confirmed in different mouse and human cancer cells including fibrosarcoma, colon, glioblastoma, bladder, gastric, breast and hepatocellular carcinoma *in vitro* and *vivo*. It has also demonstrated that this nano preparation has no cytotoxicity effects on normal cells. Finally, these results introduce dendrosomal curcumin as potent anti-tumor agent although further clinical examinations are needed.

**Keywords:** cancer, curcumin, dendrosomal curcumin, dendrosome, nanotechnology.

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## Abbreviations

CMC: Critical Micelle Concentration  
DNC: Dendrosomal Nano Curcumin  
DDS: Drug Delivery System  
DLS: Dynamic Light Scattering  
PEG: Poly Ethylene Glycol  
RES: Reticuloendothelial system  
TEM: Transmission Electron Microscopy

## Amphiphilic block copolymers nanocarriers; Micelle and Polymersome, similarities and differences

Amphiphilic block copolymers in water, like natural phospholipids (which form liposome can make micelle or polymersome (vesicle based on hydrophilic domain part to total mass ratio ( $f$ )).

Polymeric micelles have been studied by many research groups in the field of drug delivery for the past two decades and extensively reviewed by Lavasanifar and her team (1-3. Wherever the concentrations of the block copolymers are above the critical micelle concentration (CMC, it is more likely that amphiphilic block copolymers self-assemble in aqueous solution to form micellar structures which are typically in a range of 10–100 nm. The hydrophilic surface layers of micelles usually consist of polyethylene glycol chains which hinder the interaction between the hydrophobic compartments and biological membranes, prevent the adsorption of plasma proteins onto nanoparticle surfaces, and therefore reduce their recognition and elimination by the immune system specially by the reticuloendothelial system (RES (4. A diverse kind of high molecular weight amphiphilic block copolymers have been utilized to form polymersomes. Among the pioneers in this field, Zhang and Eisenberg (1995 synthesized polystyrene/poly (acrylic

acid) (PS-PAA) block copolymers as self-assembled polymeric vesicles. Recently, much more advanced structures such as stimuli-responsive polymersomes for drug delivery have been developed and described by Mura *et al.* (5-7). Polymersomes (block copolymer vesicles) generally have thicker and more versatile membrane due to tunable physical and chemical properties by variation of block lengths and chemical structures. Manipulating elasticity and permeability of polymersome membranes leads to a higher stability and storability in comparison to the liposomes and simple lipid-based vesicles formed by low molecular weight surfactants and lipids (2, 8, 9). Unlike micelles which only can encapsulate hydrophobic small molecules, aqueous core compartment and thick hydrophobic/hydrophilic shell enable polymersomes to encapsulate both hydrophilic and hydrophobic biomaterials such as drugs, proteins and nucleic acids (8, 10). Additionally, it is believed that in addition to the micelles, amphiphilic block copolymers can also form polymersomes (vesicles) when the hydrophilic domain part to total mass ratio ( $f$ ) is about  $\approx 35\% \pm 10\%$  (11). In this study, we will review synthesis and application of a novel nanocarrier family comprised from a di-block copolymer (different types of pegylated oleic acid), which simultaneously form both micelle and polymersome.

## Dendrosomes: The novel micelle/polymersome vesicular nanocarrier

Dendrosomes are polymeric micelle/polymersome structures firstly introduced by Sarbolouki *et al.* (12, 13). These structures are readily and inexpensively synthesized from esterification of oleoyl chloride and polyethylene glycol in the presence of triethyl

amine and chloroform as the solvent (Figs.1 and 2) (14). Our data from dynamic light scattering showed that dendrosomes size are around 100 nm and put it in scale of nano structures (Fig. 3). Oleic fatty acid tails and PEG residues constitute the hydrophobic and hydrophilic parts, respectively. Similar to all of the amphipathic structures, in aqueous media, hydrophobic tails repel the water

while hydrophilic attract to water resulting to form micelle structures. Different derivatives of condense spacing including DenO400, DenO6, DenO1, DenS26 and Den123 (DenOA2000) were developed in our research group and the differences are related to polyethylene glycol residues or bulk groups produced different molecular weight or density.

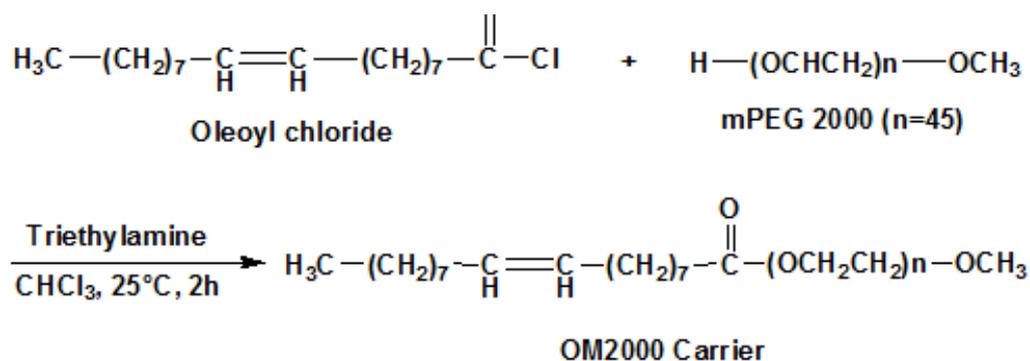


Figure 1. Schematic overview on dendrosome. The polymeric carriers were synthesized by esterification of oleoyl chloride and methoxy polyethylene glycol 2000 in the presence of triethyl amine and chloroform as solvent at 25°C for 2 h. Triethyl amine hydrochloride salt filtered from organic phase. Then, chloroform was evaporated and dendrosomes were dried in vacuum oven 40°C for 4 h.

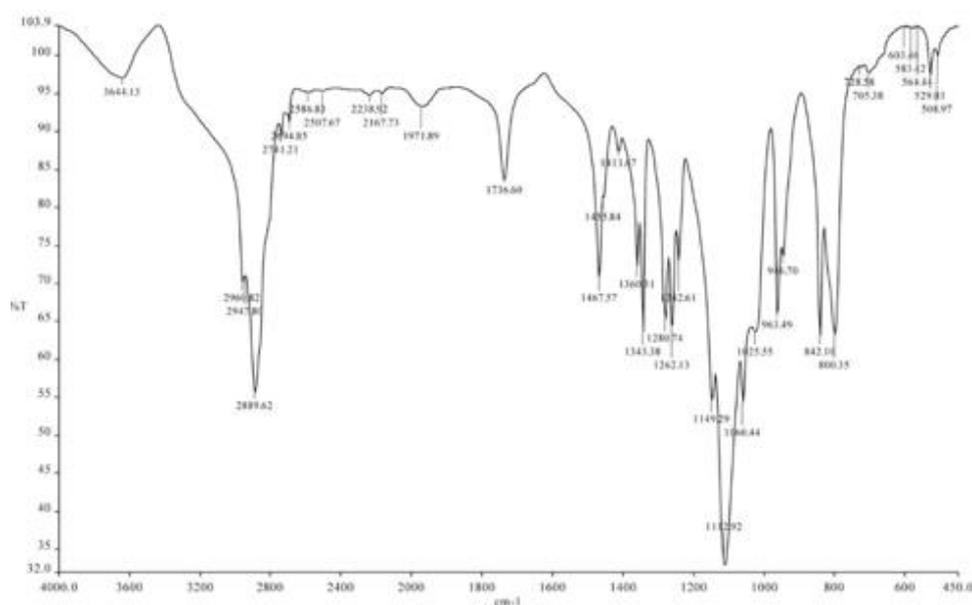


Figure 2. FT-IR spectrum of polymeric carrier OM2000. The FT-IR spectrum of A) OA 400 (14) and B) OM2000 shows stretching band of C-H aliphatic at 2889, 2947 and 2960  $\text{cm}^{-1}$ . The C=O stretching vibration of ester bands can be seen at 1736  $\text{cm}^{-1}$ . The C-H bending vibration of  $\text{CH}_2$  and C-H bending vibration of  $\text{CH}_3$  can be seen in 1467 and 1343  $\text{cm}^{-1}$ , respectively. The C-O stretching vibration was at 1112  $\text{cm}^{-1}$  as broad band.

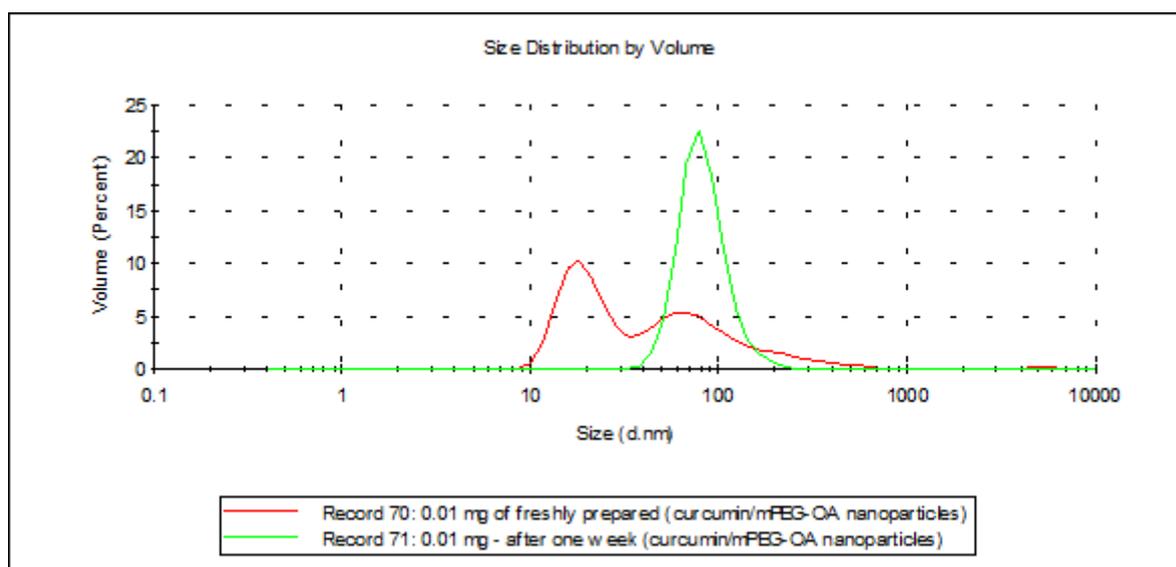


Figure 3. DLS diagram of effect of one week at room temperature without shaking on mPEG<sub>2000</sub>-OA nanoparticle size and PDI. Red line shows freshly prepared mPEG-OA nanoparticles and green line shows more uniform nanoparticles after one week at room temperature.

Table 1. *In vitro* characterization of dendrosome sub-types

Dendrosome	MW(Dalton)	CMC (mg/ml)
PEG <sub>400</sub> -OA	683	0.03
mPEG <sub>2000</sub> -OA	2283	0.03

Dendrosome mPEG<sub>2000</sub>-OA (formulated with 2000 Dalton mPEG; monomethoxy poly (ethylene glycol)-oleate) known as OM2000 and PEG<sub>400</sub>-OA (formulated with 400 Dalton PEG; poly (ethylene glycol)-oleate) known as OA400 dendrosome are two well-characterized derivatives of dendrosome (Table 1) in our laboratory and cover the main focus of this review. mPEG-OA had a bimodal size distribution, micelles (approximately 17-50 nm diameter) and polymersomes (>50 nm diameter) both were spherical in shape. Despite there are two classes, mPEG<sub>2000</sub>-OA nanocarrier was generally monodisperse (PDI<0.47) (15). mPEG<sub>2000</sub>-OA micelles has very low CMC (0.03 g/l) which is appropriate for drug release in diluted concentrations in natural

body situation (15). Similar results were observed for OA400 dendrosome (PEG<sub>400</sub>-OA) (unpublished data). There are two classes of micelles and polymersomes in PEG<sub>400</sub>-OA. Additionally, observation showed that there is no significant difference between measured CMC of PEG<sub>400</sub>-OA and mPEG<sub>2000</sub>-OA (unpublished data).

### Dendrosomes as an efficient gene porter

Dendrosomes were firstly considered as gene porters by Sarbolouki *et al.* It was hypothesized that micellar/polymersomal nature of dendrosomes may increase the uptake of gene through the cells (12, 13). Different types of cells are well transfected

with dendrosomes with any cytotoxicity connected to these carriers. Similar to lipofectamin, dendrosomes are nicely capable to deliver the desired genes into the cells especially smaller nucleic acids. Additionally, dendrosomes have lower cytotoxicity than conventional lipofectamin protocols promising it as an alternative approach in cell transfection with a desired gene (16). Recently, Karimi *et al.* showed that dendrosome can efficiently transfer *hsp27*-siRNA into the Huh-7 cell lines and promote the downregulation of *hsp27* (17). All of these observations suggest that dendrosome are potent as a vehicle for small gene delivery and maybe of DNA vaccine in near future.

### **Dendrosomes from gene porter to drug's carrier**

The high potential of dendrosomes as gene porter led to the hypothesis that it can be exploited as a vehicle for hydrophobic drugs in aqueous systems. Micelles/ polymersomes efficiently have ability to encapsulate hydrophobic chemicals in their structures. In the next section, we will discuss that dendrosomes were successfully applied for encapsulation of plant-based anticancer extract known as curcumin.

### **Curcumin**

Curcumin is a polyphenol extracted from the rhizome of plant *Curcuma longa*. Traditional medicine is so familiar with this extract where the Curcumin has been advised as a curing agent for wide range of medical conditions including asthma and inflammation diseases (18). Most of the clinical benefit of Curcumin has been approved in modern medicine. Among which, the anti-cancer property of Curcumin is well-established and curcumin is introduced as a

pleiotropic molecule. It means several molecular targets can be recognized by this small biomolecule. Being a pleiotropic molecule makes curcumin as a preferable candidate relative to other mono targeted drugs and agents in cancer (19, 20). Like many of medications, Curcumin is not free from disadvantage and has some limitations that shed light on its way as therapeutically agent. Water insolubility, low adsorption, rapid metabolism and inactivity of related metabolites are the main obstacles in curcumin therapy (21). Recently, nanotechnology has presented different kind of strategies to cope with this problem. Lipid-formulations including micelles and liposome preparation as well as natural/ synthetic analogues or adjuvants are the methods undertaken by scientists in curcumin adventures (22). In this session, we present that how Dendrosome nano-carriers solve the curcumins' problems.

### **Dendrosomal curcumin nanoformulation**

The first report for curcumin-dendrosome formulation published at 2012 by Babaei *et al.* in which optimum encapsulation of 1:25 weight: weight ratio (curcumin to dendrosome) was determined using spectrophotometry technique (23). The obtained data showed that dendrosome increased the water solubility of curcumin, the fact that can be observed by naked eyes (Fig. 4). It is necessary to mention that after 24h, curcumin particles start to precipitate showing that the loading of curcumin to dendrosomes is not 100%. Based on the chemical structure of dendrosome, it is postulated that these PEGylated Oleic Acid monomers have been self-assembled in aqueous media meanwhile trapped curcumin particles in its hydrophobic core or membrane

part (Fig. 5) (23, 24). More investigations were performed at 2014 by Tahmasebi Birgani *et al.* on *in vitro* characterization of

dendrosomal curcumin (OA400 dendrosome). It was observed that dendrosomal curcumin is a polydispersed colloidal suspension.



Figure 4. Dendrosome increased the water solubility of curcumin. A) PEG<sub>400</sub>-OA dendrosome. Empty dendrosome at the left. Curcumin in PBS at the center and PEG<sub>400</sub>-OA dendrosomal curcumin nanoformulation at the right. B) Curcumin encapsulation in mPEG<sub>2000</sub>-OA dendrosome and its transparency over time. Cur/mPEG<sub>2000</sub>-OA remained transparent after 300 days of storage at 4°C, although loading efficiencies higher than 6% resulted in some curcumin precipitation (photo obtained from references (15)).

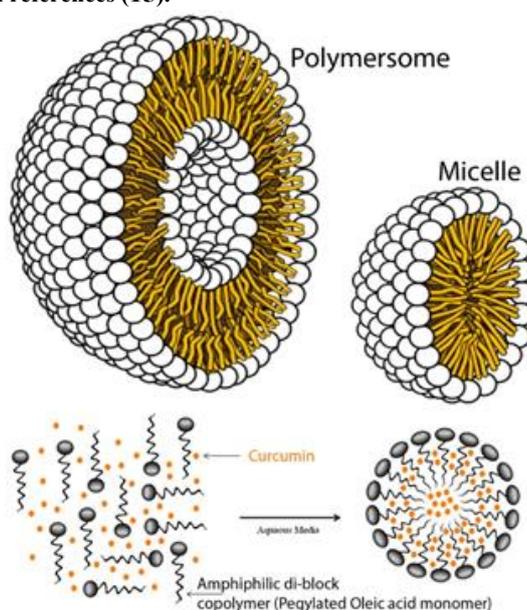
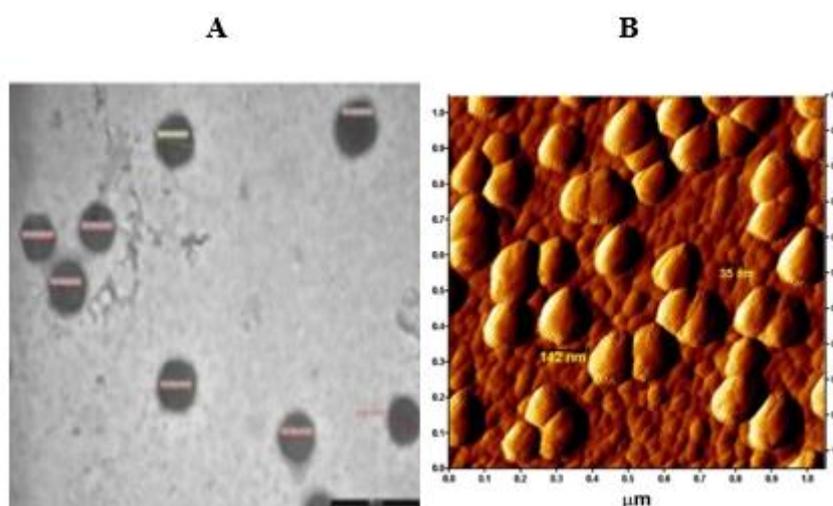


Figure 5. Schematic presentation of dendrosomes self-assembly and curcumin entrapment in aqueous media

Dynamic light scattering (DLS) beside transmission electron microscopy (TEM) confirmed that dendrosomal curcumin have sizes about 142 nm spherical particles in which curcumin efficiently encapsulated in dendrosomes (around 87%). This small size makes dendrosomal curcumin suitable for systemic administration (Fig. 6). Additionally, acceptable physical and chemical stability was recorded for this nanoformulation during DLS and HPLC (High Performance Liquid Chromatography) analysis and was compatible with obtained  $\zeta$ -potential (14).

Similarly, recently published data by Erfani-Moghadam *et al.* (15, 25) showed that mPEG<sub>2000</sub>-OA dendrosomes efficiently encapsulated curcumin. The encapsulation efficiency and drug loading (DL) were determined to be 87.1%±7.7% and 5.22%±0.46%, respectively, for the ratio of 0.06% (curcumin: mPEG<sub>2000</sub>-OA w/w). Curcumin-loaded mPEG<sub>2000</sub>-OA polymersomes (second class) have very stable structures over several weeks storage at room temperature and even in very low concentration about 1000

fold concentration lower than the CMC ( $3 \times 10^{-5}$  g/l) of micelles (15). Although the population of both forms; micelles and polymersomes increase in higher concentrations but micelles increase more. In the concentrations lower than CMC, the population of polymersomes is more stable than micelles, so that this form is stable even in the absence of micelles in the concentrations too much lower than CMC. Micelles tend to join together and form polymersomes which are thermodynamically more stable as described by Pearson and coworkers (26). The negative zeta potential induced by PEG in this nanoformulation increases the nanoparticles stability. mPEG-OA Zeta-potential  $-32.6 \pm 11.1$  mV, obtained from DLS, is at optimum range for developing stable particles with a more uniform size distribution. After shaking the nanoparticles, the size decreased and polymersomes transform to micelles or smaller polymersomes. Storing the samples at room temperature for one week generally resulted in an increased monodispersity of mPEG<sub>2000</sub>-OA nanoparticle (Fig. 3) (27).



**Figure 6. Morphology and particle size distribution of curcumin/nanoparticles. A) TEM image of Cur/ PEG 400 (14), B) AFM image of Cur/mPEG<sub>2000</sub>-OA nanoparticles redissolved after freeze-drying (0.05 mg/ml) (15)**

**Table 2. In vitro characterization of PEG<sub>400</sub>-OA and mPEG<sub>2000</sub>-OA formulation of curcumin using Dynamic Light Scattering and TEM/AFM microscopy**

Dendrosome + Curcumin	Morphology	PDI	%E.E	Size(nm)	ζ-potential	Ref.
PEG <sub>400</sub> /OA + Curcumin	Spherical	0.4± 0.03	87.65±1.62	142.97±4.27	-7.8±1.82	(14)
Technique of evaluation	TEM	DLS	Dialysis-HPLC	DLS and TEM	DLS	
mPEG <sub>2000</sub> /OA+ Curcumin	Spherical	0.47±0.071	87.1±7.7	18.33±5.32- 99.4±65 *	-32.6± 11.1	(40)
Technique of evaluation	AFM	DLS	Spectroscopy	DLS and AFM	DLS	

\*Size was different based on the micelle (18nm) or polyerosome(99.4nm) formation

### Tracking of curcumin uptake into the cells by dendrosome based on native fluorescent property of curcumin

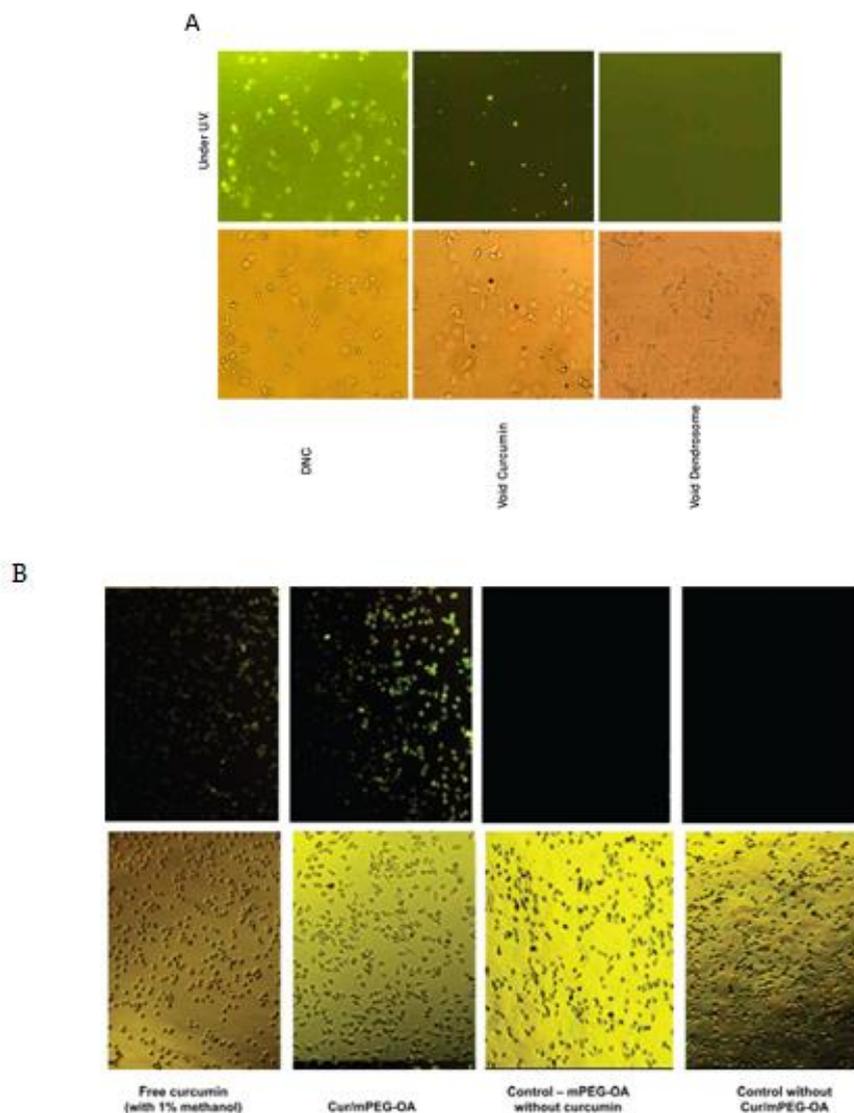
Native fluorescence property of curcumin provides simple way to analyze curcumin entrance using fluorescent microscopy. In Figure 7a green florescence shows curcumin uptake facilitated by dendrosome; PEG<sub>400</sub>-OA/cur (curcumin encapsulated in PEG<sub>400</sub>-OA) into the WEHI-164 cells. Additionally, mPEG<sub>2000</sub>-OA improves uptake of curcumin in U87MG cells (15, 25). Our results show that dendrosomes increased the curcumin water solubility and facilitate the uptake of curcumin into the U87MG glioblastoma cancer cells Figure 7b.

### Evaluation of anti-cancer properties of dendrosomal nanocurcumin

Anticancer efficacy of dendrosomal nanocurcumin was considered on different human and mouse cancer cells in *vitro* and *vivo*. All of these experiments showed that

dendrosomal curcumin efficiently suppressed cancer cells in comparison with naked curcumin. Besides, no cytotoxicity was connected to dendrosome alone. This suggests that dendrosome only increases the water solubility of curcumin. In a dose-dependent experiment, acute and chronic cytotoxicity of nanocurcumin has evaluated by Alizadeh *et al.* in mice model of BALB/c. All aspects including blood chemicals, hematological parameters, inflammatory responses, liver and kidney function were considered during one week of consecutive injection. It is found that nanocurcumin is a safe formulation even at dose of 31mg/kg, and in higher concentration can be observed (28).

Additionally, we found that dendrosomal curcumin follows the dose- and time-dependent manner in this way. Table 3 reviewed all of the examined cells in which dendrosomal curcumin was effective. The first report on anticancer property of dendrosomal curcumin was published by Babaei *et al.* where the anticancer potential of dendrosomal curcumin was investigated on mouse model of Fibrosarcoma in *vitro* and *vivo* (23).



**Figure 7.** Tracking the uptake of curcumin into the A) WEHI-164 and B) U87MG(40) cancer cells based on native fluorescent character of curcumin; 8 hours after treatment with DNC, void curcumin and void dendrosome. Above, cells under U.V. spectrum and below side shows the same cells from the same view under visible spectrum.

Data obtained from in vitro experiments demonstrated that dendrosomal curcumin inhibited A431 and WEHI cells at concentration of 5-20  $\mu\text{M}$  in a time- and dose-dependent manner. It seems that induction of apoptosis in a caspase dependent way as well as PARP cleavage is the reason of this inhibitory property. Interestingly, administration of 12.5mg/kg dendrosomal

curcumin significantly decreased the tumor size in BALB/C mice model of fibrosarcoma following increasing of survival (Fig. 8). Consequently, it is found that dendrosomal curcumin stimulate the  $\text{INF-}\gamma$  production. Therefore, it is postulated that anti-tumor immunity caused by dendrosomal nano-curcumin may involve in tumor removal (23).

**Table 3. Effective concentration (LD50) of dendrosomal curcumin preparation on different mouse and human cell lines using MTT assay after 24h drug exposure**

Cell line	Origin	Type	Type	24h- LD50 (μM)	48h- LD50 (μM)	Ref.
Wehi	Mouse	Cancerous	Fibrosarcoma	16.8	7.5	(23)
A431	Mouse	Cancerous	Epidermal carcinoma	19.2	14.3	(23)
4T1	Mouse	Cancerous	Breast	35	25	(37)
U87MG	Human	Cancerous	Glioblastoma	20	20	(14)
5637	Human	Cancerous	Bladder	20	15	(24)
HepG2	Human	Cancerous	Hepatocellular carcinoma	30	23	(32, 33)
Huh-7	Human	Cancerous	Hepatocellular carcinoma	30	21	(32, 33)
AGS	Human	Cancerous	Gastric adenocarcinoma	13	7.5	(41)
HFSF-PI3	Human	Normal	Fibroblast	25	25	(14)
MEF	Mouse	Normal	Fibroblast	>45	>45	(42)
hMSC	Human	Normal	Bone-marrow derived Mesenchymal stem cells	28	30	(14)



**Figure 8. Dendrosomal curcumin decrease the tumor size in mice model of fibrosarcoma. A) Mouse with fibrosarcoma tumors, B) Mouse treated with dendrosomal curcumin at day 36**

The inhibitory role of dendrosomal curcumin was evaluated in azoxymethane-induced colon cancer mice model. Similar to fibrosarcoma models, dendrosomal curcumin decreased the tumor size in mice in comparison with non-administrated control group. Additionally, dendrosomal curcumin resulted in reduction of nuclear/cytoplasmic ratio, epithelial stratification and nuclear dipolarity in histological staining of tissue taken from sacrificed mice. It has also confirmed that Bax/Bcl2 ratio was decreased

following DNC treatment showing the activation of apoptotic pathway (23). In Glioblastoma cells, master genes of proliferation pathways including *Oct4*, *Sox-2* and *Nanog* were significantly suppressed by dendrosomal curcumin leading to induction of apoptosis in these cells. In parallel, *miR-145*- the negative regulator of *Oct4*, *Sox-2* and *Nanog*- was upregulated after DNC treatment. Therefore, DNC indirectly downregulated proliferation genes via *miR-145* induction (14, 29).

**Table 4. Genes and Molecular pathways targeted by dendrosomal curcumin preparation**

Genes	Origin	Cancer cells	Up/down	Molecular methods	Ref.		
<i>OCT4 A</i>	Human	Brain and bladder	Down	Realtime PCR	(14, 24)		
<i>OCT4B1</i>			Down	Realtime PCR	(14, 24)		
<i>SOX2</i>			Down	Realtime PCR	(14, 24)		
<i>Nanog</i>			Down	Realtime PCR	(14, 24)		
<i>miR-145</i>			Up	Realtime PCR	(14)		
<i>DNMT1</i>			Down	RT-PCR	(32)		
<i>DNMT3A</i>			Down	RT-PCR	(32)		
<i>DNMT3B</i>			Down	RT-PCR	(32)		
<i>hTERT</i>			Down	RT-PCR	(33)		
<i>TGFβ1</i>			Human	Hepatocellular carcinoma	Up	RT-PCR	(33)
<i>Smad3</i>	Up	RT-PCR			(33)		
<i>Smad7</i>	Up	RT-PCR			(33)		
<i>MEG3</i>	Up	RT-PCR			(33)		
<i>miR-29a</i>	Up	Realtime PCR			(32)		
<i>miR-185</i>	Up	Realtime PCR			(32)		
<i>CAT</i>	Up	Realtime PCR			(39)		
<i>HO-1</i>	Up	Realtime PCR			(39)		
<i>VEGF</i>	Mouse	Breast			down	Realtime PCR	(37)
<i>NFκB</i>					down	Realtime PCR	(37)
<i>MMP-9</i>			down	Realtime PCR	(37)		
<i>BAX</i>			up	Realtime PCR	(42)		
<i>BCL2</i>			down	Realtime PCR	(42)		
<i>VEGF</i>			down	Realtime PCR	(42)		
<i>MMP9</i>			down	Realtime PCR	(42)		
<i>COX-2</i>			down	Realtime PCR	(42)		
			Mouse	Fibrosarcoma			

As the targeting of proliferation pathways is one of the suggested ideas in blocking the cancer cell (30), this potential of dendrosomal curcumin is valuable although further *in vivo* confirmation is urgent. Similarly, dendrosomal curcumin induced apoptosis by suppression of pluripotency genes in 5637 bladder cancer cells although this downregulation was *miR-145*-independent (24, 29). Dendrosomal curcumin induced DNA hypomethylation resulting in re-expression of a silenced tumor suppressor *MEG3*. Due to promoter hypermethylation, the expression of this long non-coding RNA is downregulated in hepatocellular carcinoma. Interestingly, it is found that dendrosomal curcumin downregulates DNA methyl transferases including *DNMT3A*, *DNMT3B* and *DNMT1* by upregulation of *miR-29a* and *miR-185*, respectively, and reverse the

epigenetic signature of cancer cells (31, 32). In Huh-7 hepatocellular carcinoma cells, dendrosomal curcumin also inhibited the promoter of telomerase gene (*hTERT*) through the induction of TGFβ signaling pathway. The genes *TGFβ*, *Smad3* and *Smad7* were upregulated after 72h post-treatment with 15μM dendrosomal curcumin. Similarly, hTERT was downregulated in AGS gastric adenocarcinoma cells following DNC treatment (33). As previously reported, telomerase is upregulated in most of the cancer cells leading to uncontrolled proliferation (34). Additionally, anti-apoptotic gene *survivin* was downregulated with DNC in AGS lines. The upregulation of apoptotic gene Bax also confirmed in these cells following treatment (35, 36). It has also demonstrated that nanocurcumin decreased the tumor size of mouse model of breast

cancer through downregulation of angiogenic and metastatic genes. The expression profile of *VEGF*, *MMP-9* and *NFκB* were downregulated after treatment with dendrosomal curcumin showing the anti-angiogenic and metastatic potential of this nano preparation (37). The inhibitory role of nanocurcumin on extracellular matrix of SW40 cell line of colorectal has been also demonstrated (38). Adversely, we found that dendrosomal curcumin can also act as a protective agent at low concentration. In such doses, dendrosomal curcumin decreases the ROS production and lipid peroxidation protecting the cells from oxidant damages. Furthermore, upregulation of catalase (*CAT*) and hemoxygenase-1 (*HO-1*) gene expression following DNC treatment is consistent with anti-oxidant role of this nano formulation (39). Therefore, it seems that concentration of dendrosomal curcumin determine that which pathway; anticancer or anti-oxidant can activate in target cells. All of these data confirmed the high potency of dendrosome nanocarriers to improve the anticancer potential of curcumin by increasing the solubility and cellular bioavailability of hydrophobic curcumin in aqueous media.

#### **Evaluation of toxic effects of dendrosomal curcumin on normal cells**

Regarding to cytotoxic side effects of most of anticancer drugs on normal cells, the cytotoxic properties of dendrosomal curcumin was also investigated in different normal cells to answer if DNC has inhibitory effects on these cells. Interestingly, it is found that DNC had no inhibitory properties on normal cells or inhibit normal cells in a concentration higher than the dose effective on cancerous one (14, 23). These observation confirmed that dendrosomal curcumin preferentially target cancer cells than normal

counterparts and can be considered as a promising agent in cancer therapy.

#### **Conclusion**

Here, we aimed to review a novel generation of nanocarrier termed dendrosome which is produced in our research group. We demonstrated that these biocompatible polymeric micelles/ polymersomes not only have no cytotoxicity on normal cells but also efficiently suppress tumor cells in *vivo* and *vitro*. Inhibitory effects of dendrosomal curcumin were only mediated by curcumin and dendrosome only play a role as a delivery vehicle. Physical characteristics of dendrosomes showed that they are spherical and have relatively high encapsulation efficiency and drug loading capacity and additionally high stability over time. CMCs of dendrosomes are relatively in a good and acceptable range for a micelle family, although designing novel heavier di- or tri-block nanocarriers are recommended with bigger hydrophobic and hydrophilic parts which will create nanocarriers with even lower CMC and more stability specially when they administrate and expose to extremely diluted condition of real blood circulation. Considering the results, dendrosome family has appropriate properties which make them good candidates for drug and small nucleic acid delivery. Although, there is a long way to finalize this formulation as a drug in pharmacy, our results until now confirmed nanocurcumin can be considered as a relatively safe formulation for cancer therapy.

#### **Perspective**

Recent year's combination therapy has received a lot of attention for treating chronic diseases such as cancers. This review study recommends the use of dendrosome as a nanocarrier for drug and gene (small

molecule nucleic acids) therapy together. Simultaneous delivery of curcumin and siRNA can be an effective approach for facing common problem of drug resistance in cancer, and application of dendrosome for such researches are highly recommended.

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## REFERENCES

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1. Ma, Z., Haddadi, A., Molavi, O., Lavasanifar, A., Lai, R. and Samuel, J. (2008) Micelles of poly(ethylene oxide)-b-poly(epsilon-caprolactone) as vehicles for the solubilization, stabilization, and controlled delivery of curcumin. *J Biomed Mater Res A*, 86, 300-310.
2. Xiong, X.B., Binkhathlan, Z., Molavi, O. and Lavasanifar, A. (2012) Amphiphilic block copolymers: preparation and application in nanodrug and gene delivery. *Acta Biomater*, 8, 2017-2033.
3. Adams, M.L., Lavasanifar, A. and Kwon, G.S. (2003) Amphiphilic block copolymers for drug delivery. *J Pharm Sci*, 92, 1343-1355.
4. Tong, R. and Cheng, J. (2007) Anticancer Polymeric Nanomedicines. *Polymer Reviews*, 47, 345-381.
5. Kim, H., Kang, Y.J., Kang, S. and Kim, K.T. (2012) Monosaccharide-responsive release of insulin from polymersomes of polyboroxole block copolymers at neutral pH. *J Am Chem Soc*, 134, 4030-4033.
6. Lee, J.S., Groothuis, T., Cusan, C., Mink, D. and Feijen, J. (2011) Lysosomally cleavable peptide-containing polymersomes modified with anti-EGFR antibody for systemic cancer chemotherapy. *Biomaterials*, 32, 9144-9153.
7. Mura, S., Nicolas, J. and Couvreur, P. (2013) Stimuli-responsive nanocarriers for drug delivery. *Nat Mater*, 12, 991-1003.
8. Hamidi, M., Shahbazi, M.A. and Rostamizadeh, K. (2012) Copolymers: efficient carriers for intelligent nanoparticulate drug targeting and gene therapy. *Macromol Biosci*, 12, 144-164.
9. Antonietti, M. and Förster, S. (2003) Vesicles and Liposomes: A Self-Assembly Principle Beyond Lipids. *Advanced Materials*, 15, 1323-1333.
10. Chen, W., Meng, F., Cheng, R. and Zhong, Z. (2010) pH-Sensitive degradable polymersomes for triggered release of anticancer drugs: a comparative study with micelles. *J Control Release*, 142, 40-46.
11. Discher, D.E. and Ahmed, F. (2006) Polymersomes. *Annu Rev Biomed Eng*, 8, 323-341.
12. Sarbolouki, M.N., Sadeghizadeh, M., Yaghoobi, M.M., Karami, A. and Lohrasbi, T. (2000) Dendrosomes: a novel family of vehicles for transfection and therapy. *J of Chem Technol and Biot*, 75, 919-922.
13. Sadeghizadeh, M., Ranjbar, B., Damaghi, M., Khaki, L., Sarbolouki, M.N., Najafi, F., Parsaee, S., Ziaee, A.A., Massumi, M. and Lubitz, W. (2008) Dendrosomes as novel gene porters-III. *J of Chem Technol and Biot*, 83, 912-920.
14. Tahmasebi Mirgani, M., Isacchi, B., Sadeghizadeh, M., Marra, F., Bilia, A.R., Mowla, S.J., Najafi, F. and Babaei, E. (2014) Dendrosomal curcumin nanoformulation downregulates pluripotency genes via miR-145 activation in U87MG glioblastoma cells. *Int J Nanomedicine*, 9, 403-417.
15. Erfani-Moghadam V, Nomani A, Zamani M, Yazdani Y, Najafi F and M, S. (2014) A novel

- diblock copolymer of (monomethoxy poly [ethylene glycol]-oleate) with a small hydrophobic fraction to make stable micelles/polymersomes for curcumin delivery to cancer cells. *Int J Nanomed*, 2014;9(1) 5541—5554
16. Pourasgari, F., Ahmadian, S., Salmanian, A.H., Sarbolouki, M.N. and Massumi, M. (2009) Low cytotoxicity effect of dendrosome as an efficient carrier for rotavirus VP2 gene transferring into a human lung cell line. *Mol Bio Rep*, 36, 105-109.
  17. Karimi, M. (2013), In 1st Tabriz International Life Science Conference and 12th Iran Biophysical Chemistry Conference. Tabriz University of Medical Sciences.
  18. Anand, P., Sundaram, C., Jhurani, S., Kunnumakkara, A.B. and Aggarwal, B.B. (2008) Curcumin and cancer: an “old-age” disease with an “age-old” solution. *Cancer Let*, 267, 133-164.
  19. Goel, A., Kunnumakkara, A.B. and Aggarwal, B.B. (2008) Curcumin as “*Curecumin*”: From kitchen to clinic. *Biochem Pharmacol*, 75, 787-809.
  20. Ravindran, J., Prasad, S. and Aggarwal, B.B. (2009) Curcumin and cancer cells: how many ways can curry kill tumor cells selectively? *The AAPS journal*, 11, 495-510.
  21. Anand, P., Kunnumakkara, A.B., Newman, R.A. and Aggarwal, B.B. (2007) Bioavailability of curcumin: problems and promises. *Mol Pharmaceut*, 4, 807-818.
  22. Yallapu, M.M., Jaggi, M. and Chauhan, S.C. (2013) Curcumin nanomedicine: a road to cancer therapeutics. *Curr Pharm Design*, 19, 1994.
  23. Babaei, E., Sadeghizadeh, M., Hassan, Z.M., Feizi, M.A.H., Najafi, F. and Hashemi, S.M. (2012) Dendrosomal curcumin significantly suppresses cancer cell proliferation in vitro and in vivo. *Int Immunopharmacol*, 12, 226-234.
  24. Tahmasebi Mirgani, M., Sadeghizadeh, M., Najafi, F. and Mowla, S.J. (2013) Dendrosomal curcumin induced apoptosis by suppression of pluripotency genes in 5637 bladder cancer cells. *Modares Journal of Medical Sciences: Pathobiology*, 16, 23-39.
  25. Esmatabadi, M.J.D., Farhangi, B., Safari, Z., Kazerooni, H., Shirzad, H., Zolghadr, F. and Sadeghizadeh, M. (2015) Dendrosomal Curcumin Inhibits Metastatic Potential of Human SW480 Colon Cancer Cells through Down-regulation of Claudin1, Zeb1 and Hef1-1 Gene Expression. *Asian Pac J of Cancer Prev*, 16, 2473-2481.
  26. Erfani-Moghadam V, Nomani A, Yazdani Y, Najafi F and Sadeghizadeh M (2014) Design and Synthesis of a Novel Dendrosome and a PEGylated PAMAM Dendrimer Nanocarrier to Improve the Anticancer effect of Turmeric (*Curcuma longa*) Curcumin. *Modares Journal of Medical Science: Pathobiology*, 17, 63-77.
  27. Pearson, R.T., Avila-Olias, M., Joseph, A.S., Nyberg, S. and Battaglia, G. (2013) Smart Polymersomes: Formation, Characterisation and Applications, chapter 7 at "Smart Materials for Drug Delivery". Royal Society of Chemistry, Cambridge, United Kingdom.
  28. Erfani-Moghadam, V., (2014) Design and application of some dendritic nanocarriers on anticancer properties of curcumin from Turmeric (*Curcuma longa*). PhD thesis, Tarbiat Modares University, Tehran, Iran.
  29. Alizadeh, A.M., Sadeghizadeh, M., Najafi, F., Ardestani, S.K., Erfani-Moghadam, V., Khaniki, M., Rezaei, A., Zamani, M., Khodayari, S. and Khodayari, H. (2015) Encapsulation of Curcumin

- in Diblock Copolymer Micelles for Cancer Therapy. *Biomed Res Int*, DOI 10.1155/2015/824746.
30. Alireza Panahi, R.N.S.a.M.S. (2012) Evaluation of Apoptosis Induction on Gastric Cancer AGS Cells Made by Polymer Nano Curcumin. *2, 1*, 200-207.
  31. Birgani, M.T. (2012). Study of the Effects of Dendrosomal-Curcumin on Cell Cycle and Pluripotency of Tumor Cell Lines and Adult Stem Cells by Measuring the Expression Profile of OCT4, SOX-2, Nanog, miR-302 and miR-145. PhD thesis, Tarbiat Modares University, Tehran, Iran.
  32. Jordan, C.T. (2004) Cancer stem cell biology: from leukemia to solid tumors. *Curr opin Cell Bio*, *16*, 708-712.
  33. Zamani, M., Sadeghizadeh, M. and Behmanesh, M. (2014) Dendrosomal Curcumin Upregulates Expression of the Long Non-coding RNA gene MEG3 in U87MG Glioblastoma Cells. *Modares Journal of Medical Sciences: Pathobiology*, *17*, 39-54.
  34. Zamani, M., Sadeghizadeh, M., Behmanesh, M. and Najafi, F. (2015) Dendrosomal curcumin increases expression of the long non-coding RNA gene MEG3 via up-regulation of epi-miRs in hepatocellular cancer. *Phytomedicine*, *22*, 961-967.
  35. Shariati, M. (2013). Investigation of polymeric nanocurcumin inhibitory effect on the Human Telomerase Reverse Transcriptase gene (hTERT) promoter through induction of TGF $\beta$ 1 signaling pathway in hepatocarcinoma cell line (Huh7). MSc thesis. Tarbiat Modares University, Tehran, Iran.
  36. Komata, T., Kanzawa, T., Kondo, Y. and Kondo, S. (2002) Telomerase as a therapeutic target for malignant gliomas. *Oncogene*, *21*, 656-663.
  37. Najmeh Ranji, A.P.a.M.S. (2010) Study the effects of Dendrosomal curcumin in induction apoptosis on cancer and stem cells. *Modares Journal of Medical Sciences: Pathobiology*, *4*, 37-49.
  38. Najmeh Ranji, A.P.a.M.S. (2014) Investigation of Survivin and hTERT gene expression in human gastric adenocarcinoma cell line (AGS) treated by nano Curcumin. *Journal of Cellular and Molecular Research* *27*, 233-241.
  39. Farhangi, B., Alizadeh, A.M., Khodayari, H., Khodayari, S., Dehghan, M.J., Khorrami, V., Heidarzadeh, A., Khaniki, M., Sadeghizadeh, M. and Najafi, F. (2015) Protective effects of dendrosomal curcumin on an animal metastatic breast tumor. *Eur J of Pharmacol*, *758*, 188-196.
  40. Nazem, S. (2012). Study the effects of nano-Curcumin as a stress inhibitor on Human Mesenchymal Stem cells exposed to hydroquinone, MSc thesis. Tarbiat Modares University, Tehran, Iran.
  41. Motahari, P., Sadeghizadeh, M., Behmanesh, M., Sabri, S. and Zolghadr, F. (2015) Generation of stable ARE-driven reporter system for monitoring oxidative stress. *DARU Journal of Pharmaceutical Sciences*, *23*, 1-7.
  42. Babaei, E. (2011), study of the anti-cancerous effect of dendrosomal nano-curcumin in vitro and in vivo. Tarbiat Modares University, Tehran, Iran.