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RESEARCH ARTICLE

Cytotoxic effects investigation of nanomicelle and free curcuminoids against cancer and normal cells

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ABSTRACT

Curcumin, which is derived from the turmeric rhizomes (curcuma longa) as a natural polyphenol, is a substantially lipophilic molecule. This commonly used substance is employed as a coloring agent and spice in food and contains potent antioxidant, as well as anti-inflammatory, and anti-proliferative tumor activities. The developed nanomicelle formulations of curcumin are used to promote the bio-availability and solubility of the above-mentioned lipophilic molecule. The present investigation aimed to examine the anti-proliferative activity of nanomicelle and free curcuminoids by using different cancer and normal cells using a tetrazolium dye-based assay. To this end, various cell lines were treated with nanomicelle or free curcuminoids at different concentration of 5, 10, 20, 30, and 40 µM for 48 hours at 37 °C. Our results demonstrated that the half maximal inhibitory concentrations of the micellar form of curcuminoids for different cancer cell lines were as high as its levels measured for its free form but in normal cells, the toxicity of nanomicelles is lower than free form of curcuminoids.

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INTRODUCTION

Curcuminoids are a class of natural polyphenolic compounds derived from turmeric (Curcuma longa), which belong to the Zingiberaceae (ginger family). Among them a yellowish compound, curcumin, is the most abundant composition and one of the most active constituents of turmeric (1, 2).

It is widely consumed by people around the world in the Traditional Chinese Medicine, cooking as well as the food industry. Curcuminoids have been introduced to have an array of biological effects, namely as "anti-inflammatory", "antioxidant", "antimicrobial, lipid modifying", "anticancer", and "anti-angiogenic" "(2-8).

Various studies in recent years showed that these class of compounds can act as a potent preventive substance in the initiation and promotion of tumors formed and induced by chemical carcinogen in animals (3-5). Curcuminoids have been reported by numerous researchers as a cancer chemo-preventive in various types of animal tumors, including colon (7, 9), duodenal (10), stomach (2), prostate (11), and breast (12) both in vitro and in vivo.

They are shown to have certain growth inhibition and also apoptosis induction effects on the human cancerous cells. Moreover, these substances target multiple cellular signaling pathways without toxicity induction in the normal cells (9, 13). For further information, there is an in-depth review of literature that introduces curcumin, the most active curcuminoids, as an excellent alternative

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among the diverse natural compounds used for cancer therapy (14).

According to numerous reports, curcumin is also capable of effectively modulating the expression of a large number of genes that are responsible for various phases of proliferation, angiogenesis, invasion, and metastasis of cancerous cells. Furthermore, it is capable of suppressing the progression and metastasis of tumors by blocking a range of signal transduction pathways including "p53, Ras, Wnt-β, MAPKs, ERK, PI3K, and Akt" in the cancerous cells (2).

This substance is reported to influence CSCs (Cancer Stem Cells) through diverse mechanisms, such as deactivation of "transcriptional factors and inflammatory cytokines", or by suppressing various protein kinases activities. Other mechanisms include modulation of the activity of enzymes that are in charge of inflammation and tumorigenesis, inhibition of the receptors signaling cytokine as well as growth factors. Moreover, the suppression of the "expression of adhesion molecules" and inhibition of "anti-apoptotic proteins and other targets" are also mentioned. According to a set of clinical trials exploring its safety or toxicity, the acceptable dose of curcumin for achieving the optimal therapeutic effects is reportedly between 4 and 8 grams per day that is the tolerable dose for human beings. On the other hand, it imposes "limited toxicity on the Neural Stem Cells (NSCs)", but exerts remarkable "cytotoxic effects on the CSCs" (2). Furthermore, curcumin is capable of lowering the concentration of the "circulating TNF-α" that is a multifunctional cytokine associated with different cell events such as immunity, inflammation, cell survival and apoptosis (15, 16). Curcumin also acts as "a MicroRNA regulator in cancer" (34-36).

The fact that curcumin, as the major component of curcuminoids, can be prescribed up to 8 g per day in human clinical trials without any limitations imposed by the dose-toxicity reveals how effective it might well be in the prevention and treatment of cancers (17, 18).

However, a major limitation of these compounds is their low solubility in water (i.e., 0.0004 mg/mL at pH 7.3 for curcumin) and their extreme sensitivity at physiological pH (14, 16-20). What is more, the low bioavailability of curcumin has been reported in many pre-clinical and clinical research conducted on mice, rats, and humans (16). In a study conducted on humans, the oral administration of 10 or 12 g/day curcumin led to the observation of curcumin level of 50 ng/mL in serum. Nevertheless, this lowered the availability of curcumin in blood circulation to the minimum (19). Another study revealed that the use of nanomicelles highly promoted both the in-vitro cellular uptake and the in-vivo corneal permeation and enhanced the anti-inflammatory efficacy, compared to the employment of a free curcuminoids solution (2).

With this background, the present study was performed with the aim of examining the efficiency of curcuminoid nanomicelles on cancer and normal cells.

To this end, the study investigated the "anti-proliferative effects» of free and curcuminoid nanomicelles through a tetrazolium dye-based (MTT) assay on ten different cell lines (Table 1).

MATERIALS AND METHOD

Nanomicelle containing curcuminoids is registered as SinaCurcumin® obtained from Exir Nano Sina Company, Tehran, Iran (IRC: 1228225765).The curcuminoids powder was purchased from Sami Lab Limited (Bengaluru, Karnataka, India).

In addition, U-87-MG (human glioblastoma cell line), NIH 3T3 (mouse embryonic fibroblast cells), A549 (human fetal lung fibroblast cell line), and Hela (human cervical carcinoma) were purchased from the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Furthermore, B16F0

Table 1. Cancer cell lines and normal cell line data in this study

Cell line	Tissue Type	Tissue	Phenotype	Primary
NIH	Mouse	Fibroblast	Adherent	No
4T1	Mouse	Breast/Mammary	Adherent	No
TUBO	Mouse	Breast/Mammary	Adherent	No
SKBR3	Human	Breast/Mammary	Adherent	No
MDA-MB -231	Human	Breast/Mammary	Adherent	No
B16F0	Mouse	Skin	Adherent	No
HELA	Human	Cervix	Adherent	No
J774	Mouse	Macrophage	Adherent	No
U87-mg	Human	Astrocyte	Adherent	No
A549	Human	Lung	Adherent	No

(melanoma cell line) was obtained from the Sigma-Aldrich (USA). Above-mentioned cell lines were maintained in Dulbecco's Modified Eagle medium (DMEM, Sigma), supplemented with 10% (v/v) fetal cow serum (FCS) (Gibco, BRL), 100 IU/mL penicillin and 100 mg/mL streptomycin, and 2 mM L glutamine and incubated at 37 °C in "a humidified atmosphere" containing "5% CO_2 and 95% air".

TUBO, a cloned cell line overexpressing the rHER2/ neuprotein, was kindly provided by Doctor Pier-Luigi Lollini from the Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy. This cell line was cultured in the DMEM supplemented with 20% FCS. Other cell lines included SK-BR-3 (human breast adenocarcinoma cells), MDA-MB-231 (human breast adenocarcinoma cells), and 4T1 (mouse mammary tumor cell line), purchased from the National Cell Bank of Iran and were cultured in "RPMI 1640 medium containing 25 mM HEPES", as well as "2 mM L-glutamine supplemented with 10% (v/v) heat-inactivated" FCS, "100 IU/mL penicillin, and 100 mg/mL streptomycin (all from Gibco)" and incubated in the humidified atmosphere of 5% CO_2 and 95% air, at 37 °C.

Cell Proliferation Assay

The cancer and normal cell proliferations in "the presence of various concentrations of nanomicelle and free curcuminoids" were determined through the MTT assay (Roche Applied Sciences, Germany). Briefly, "monolayer cultures were trypsinized in the exponential growth phase", after which by using trypan blue exclusion, the viable cell were counted. Subsequently, the seeding of the cells was conducted in "96 well flat-bottom microtitration plates (SPL Life Sciences, South Korea) at an appropriate density of cells/well (200 µL media/well)".

After 24 h incubation to reach 85% confluence, cells treated with various concentrations of nanomicelle and free curcuminoids. First a standard "stock solution of curcuminoids" (10 mg/ml) was prepared in extra pure dimethyl sulfoxide and diluted with free FCS medium. Nanomicelles were diluted directly in free-FCS medium. Afterwards, the nanomicelle and free curcuminoids were added to the cells at different concentrations (i.e. 5, 10, 20, 30, and 40 µM). After 48 h incubation, cells were washed twice with "fresh and free-FCS medium. Subsequently, a fresh FCS-containing medium was used to remove nanomicelle or free curcuminoids remained at the cell surface and then they were incubated again for 48 hours. This step was added to eliminate the reducing effect of these compounds on MTT and interference with the spectroscopic measurments in the final step. After 48 hours of incubation, the complete medium was replaced with 100 µL of free FCS medium that contained 10 µL MTT (5 mg/ml in PBS). After incubating the cells "for 4 hours at 37°C", the medium was carefully substituted with 200 µL dimethyl sulfoxide in order to solve formazan crystals. Finally, with the help of a spectrophotometric micro plate reader (Bio Tek Elx. 808), the optical density was assessed "at a wave length of 570 nm with background subtraction at 630 nm". The viability of the cells was calculated using the following formula:

$$\text{Cell viability (\%)} = \frac{OD_{toxin\ exposure}}{OD_{control}} \times 100$$

where OD represents optical density.

RESULTS AND DISCUSSION

In the present study, the time-dependent cytotoxicities of free and nanomicelle form of curcuminoids were measured on different cancer cells. The nanomicelle form of curcuminoids, SinaCurcumin®, is marketed by Exir Nano Sina Company in Tehran, Iran (IRC: 1228225765). The encapsulation efficiency of curcuminoids in nanomicelles is almost 100%. The mean diameter of nanomicelles is around 10 nm, according to dynamic light scattering. The curcuminoid content and size distribution of nanomicelles remains constant for at least 24 months. The oral absorption of SinaCurcumin is at least 59 times more than the conventional powder of curcumin in mice (21)

The different cell lines including 4T1, HELA, and SKBR3, TUBO, MDA-MB-231, J774, B16F0, U87-MG, A549 and also on a normal cell line, NIH3T3, were used (Table 1). The Half maximal inhibitory concentration (denoted as IC50s) of the curcuminoids during the 48-hour incubation period of time is presented in Table 1. Data represented as µM ± standard deviation (SD), (n=3). As depicted in Table 1, the cytotoxicity of the drugs varied among different cell lines with the highest in 4T1 and the lowest for normal cells, NIH3T3. Also, the results indicated that except for a significant difference between cytotoxicity of free and nanomicelle form of curcuminoids in NIH3T3 cells ($p<0.007$), there was no statistical difference, observed in other cell lines. A comparison of IC50s of two different forms of curcuminoids between studied cell lines is shown in Fig. 1.

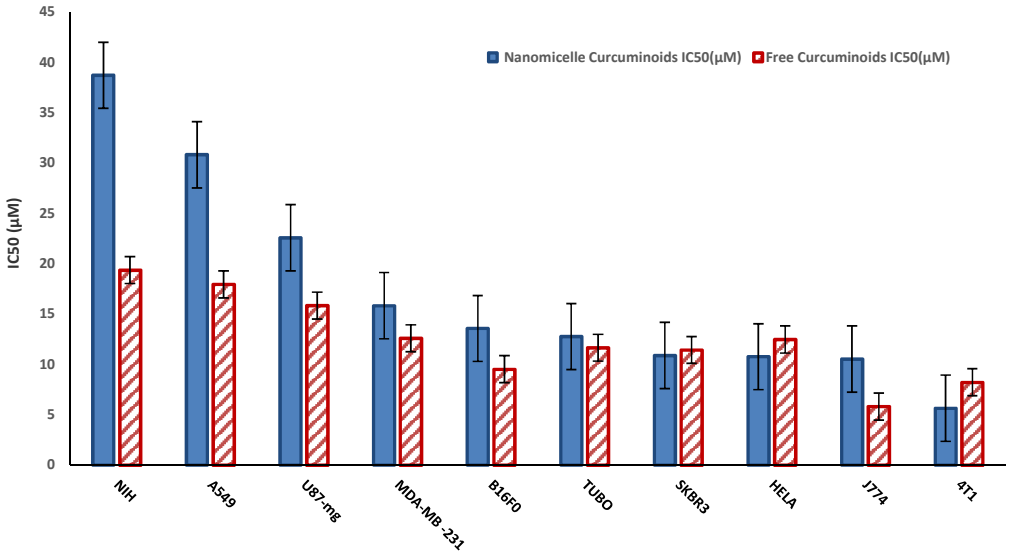

Fig. 1. Normal and cancer cells Half maximal inhibitory concentration (IC50; μM) of free and nanomicelle form of curcuminoids after the 48-hour incubation period (**: P-value <0.01).

Table 2. Normal and cancer cells Half maximal inhibitory concentration (IC50; μM) of free and nanomicelle form of curcuminoids after the 48-hour incubation period.

	IC_{50}±SD (μM)		
Cell line	*NC*	*FC*	*P-value*
NIH	38.71±2.88	19.37±6.05	0.007
4T1	5.64±0.40	8.23±2.21	0.117
TUBO	12.77±10.58	11.66±6.89	0.886
SKBR3	10.88±4.68	11.43±5.66	0.904
MDA-MB -231	15.83±7.88	12.59±2.83	0.54
B16F0	13.57±7.64	9.53±3.11	0.305
HELA	10.77±2.74	12.48±1.40	0.515
J774	10.52±3.14	5.82±1.47	0.31
U87-mg	22.57±5.45	15.85±1.54	0.235
A549	30.82±2.33	17.94±2.63	0.106

NC: curcuminoids nanomicelle, *FC*: free curcuminoids
Statistically *Significant: P-value <0.05*

Different in vitro experiments have shown the cytotoxic impacts of curcuminoids on cancer cells at high concentrations. After taken orally, however, these concentrations are never provided in the tumor microenvironment due to its low bioavailability and extensive metabolism. Therefore, this limits the potential impact of oral curcuminoids prescription on cancer treatment. So far, different strategies have been developed to overcome these limitations and many formulations have been further studied to increase solubility and enhance the bioavailability of curcuminoids, such as prescription of curcuminoids with piperine, synthetic analogues of curcumin, and nanotechnology-based drug delivery systems, which include curcumin phytosomes, liposomes, polymeric nanoparticles, etc. (22-28). Sina Curcumin is a recently developed formulation of curcuminoids introduced by Nano Exir Sina Company (Tehran. Iran), based on nano micellar form of curcuminoids.

Various in vitro experiments have revealed that by being exposed to high doses of curcuminoids, ranging from 5–50 μM, cancer cells would die. The results of the present study also confirmed different cytotoxic effects of free and nanomicelles form of curcuminoids, Sina Curcumin, on various cancer cell lines. According to these findings, the highest IC50 value was observed in normal cell line, NIH3T3,

meaning lower toxicity, which could be related to the less harvesting of curcuminoids by these cells because of their lower proliferation rate. In this cell line, a lower significant toxicity was observed for curcuminoids nanomicelles compared to its free form ($P<0.01$) as well, which could be related to slow releasing of curcuminoids from the nanomicelles resulting in slow harvesting of the drug.

As mentioned before, there is a wide range of reports confirming that curcumin and other curcuminoids may lead to cell death under certain conditions. Goodpasture and Arrighi have shown that in several mammalian cell lines, turmeric could induce chromosome aberrations in terms of dose and time dependence (29). According to the accumulated data, DNA damage and chromosomal alterations induced by curcumin occurs at doses similar to those reported to exert beneficial effects (30-35). These reports raise concerns about "curcumin safety", due to the importance of "DNA alterations" in carcinogenesis. There is an abundance of evidence that «reactive oxygen species» (ROS) may have a significant role in molecular mechanisms underlying its negative effects. Some researchers showed that although curcumin induce antioxidant effects at lower concentrations, it can increase the cellular levels of ROS at higher concentrations (36-39). "Two α,β-unsaturated" ketone groups in the curcumin chemical structure are known to be involved in a reaction, called Michael addition. In this reaction, these unsaturated ketone groups covalently react with " thiol groups of cysteine residues" of an array of proteins. It can explain the reason why curcumin generates ROS through irreversible modification of "the antioxidant enzyme thioredoxin reductase", and why it causes other protein in-activations such as topoisomerase II, tumor suppressor protein p53 and exert its cytotoxic effects at high concentrations. (27).

CONCLUSION

The results of present study confirmed the cytotoxic effects of free and nanomicellar form of curcumin in both normal and cancer cells. However, it should be noted that the effects on normal cells reduced for nanomicells compared to free curcuminoids and this finding requires further studies to clarify the probable mechanisms through which different dosage forms of curcumin induce such effects.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. Li L, Leung PS. Pancreatic Cancer, Pancreatitis, and Oxidative Stress. Gastrointestinal Tissue: Elsevier; 2017. p. 173-86.
2. Chen A, Xu J, Johnson AC. Curcumin inhibits human colon cancer cell growth by suppressing gene expression of epidermal growth factor receptor through reducing the activity of the transcription factor Egr-1. Oncogene. 2005;25(2):278-87.
3. Azuine MA, Bhide SV. Chemopreventive effect of turmeric against stomach and skin tumors induced by chemical carcinogens in Swiss mice. Nutrition and Cancer. 1992;17(1):77-83.
4. Huang M-T, Smart RC, Wong C-Q, Conney AH. Inhibitory effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-O-tetradecanoylphorbol-13-acetate. Cancer research. 1988;48(21):5941-6.
5. Rao CV, Desai D, Kaul B, Amin S, Reddy BS. Effect of caffeic acid esters on carcinogen-induced mutagenicity and human colon adenocarcinoma cell growth. Chemico-Biological Interactions. 1992;84(3):277-90.
6. Satoskar R, Shah S, Shenoy S. Evaluation of anti-inflammatory property of curcumin (diferuloyl methane) in patients with postoperative inflammation. International journal of clinical pharmacology, therapy, and toxicology. 1986;24(12):651-4.
7. Sharma OP. Antioxidant activity of curcumin and related compounds. Biochemical Pharmacology. 1976;25(15):1811-2.
8. Srimal RC, Dhawan BN. Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent*. Journal of Pharmacy and Pharmacology. 1973;25(6):447-52.
9. Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: A short review. Life Sciences. 2006;78(18):2081-7.
10. Perkins S, Verschoyle RD, Hill K, Parveen I, Threadgill MD, Sharma RA, et al. Chemopreventive efficacy and pharmacokinetics of curcumin in the min/+ mouse, a model of familial adenomatous polyposis. Cancer Epidemiology and Prevention Biomarkers. 2002;11(6):535-40.
11. Huang M-T, Lou Y-R, Ma W, Newmark HL, Reuhl KR, Conney AH. Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. Cancer research. 1994;54(22):5841-7.
12. Singh SV, Hu X, Srivastava SK, Singh M, Xia H, Orchard JL, et al. Mechanism of inhibition of benzo [a] pyrene-induced forestomach cancer in mice by dietary curcumin. Carcinogenesis. 1998;19(8):1357-60.
13. Cucuzza LS, Motta M, Miretti S, Accornero P, Baratta M. Curcuminoid-phospholipid complex induces apoptosis

in mammary epithelial cells by STAT-3 signaling. Experimental and Molecular Medicine. 2008;40(6):647.
14. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": From kitchen to clinic. Biochemical Pharmacology. 2008;75(4):787-809.
15. van Horssen R, ten Hagen TL, Eggermont AM. TNF-α in cancer treatment: molecular insights, antitumor effects, and clinical utility. The oncologist. 2006;11(4):397-408.
16. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer research. 2003;23(1/A):363-98.
17. Cheng A, Hsu C, Lin J, Hsu M, Ho Y, Shen T, et al. Phase 1 Clinical Trial of Curcumin, a Chemopreventive Agent. Patients with High-risk of Pre-malignant Lesions Anticancer Res.21:2895-001.
18. Choudhuri T, Pal S, Das T, Sa G. Curcumin Selectively Induces Apoptosis in Deregulated Cyclin D1-expressed Cells at G2Phase of Cell Cycle in a p53-dependent Manner. Journal of Biological Chemistry. 2005;280(20):20059-68.
19. Kostarelos K, Emfietzoglou D, Papakostas A, Yang W-H, Ballangrud ÅM, Sgouros G. Engineering Lipid Vesicles of Enhanced Intratumoral Transport Capabilities: Correlating Liposome Characteristics with Penetration into Human Prostate Tumor Spheroids. Journal of Liposome Research. 2005;15(1-2):15-27.
20. Li M, Xin M, Guo C, Lin G, Wu X. New nanomicelle curcumin formulation for ocular delivery: improved stability, solubility, and ocular anti-inflammatory treatment. Drug Development and Industrial Pharmacy. 2017;43(11):1846-57.
21. Hatamipour M, Sahebkar AH, Alavizadeh SH, Dorri M, Jaafari MR. Novel nanomicelle formulation to enhance bioavailability and stability of curcuminoids. Iranian Journal of Basic Medical Sciences. 2019;22(3):282-9.
22. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas P. Influence of Piperine on the Pharmacokinetics of Curcumin in Animals and Human Volunteers. Planta Medica. 1998;64(04):353-6.
23. Marczylo TH, Verschoyle RD, Cooke DN, Morazzoni P, Steward WP, Gescher AJ. Comparison of systemic availability of curcumin with that of curcumin formulated with phosphatidylcholine. Cancer Chemotherapy and Pharmacology. 2006;60(2):171-7.
24. Bisht S, Feldmann G, Soni S, Ravi R, Karikar C, Maitra A, et al. Polymeric nanoparticle-encapsulated curcumin ("nanocurcumin"): a novel strategy for human cancer therapy. Journal of Nanobiotechnology. 2007;5(1):3.
25. Sun A, Shoji M, Lu YJ, Liotta DC, Snyder JP. Synthesis of EF24–Tripeptide Chloromethyl Ketone: A Novel Curcumin-Related Anticancer Drug Delivery System. Journal of Medicinal Chemistry. 2006;49(11):3153-8.
26. Sandur SK, Pandey MK, Sung B, Ahn KS, Murakami A, Sethi G, et al. Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. Carcinogenesis. 2007;28(8):1765-73.
27. Burgos-Morón E, Calderón-Montaño JM, Salvador J, Robles A, López-Lázaro M. The dark side of curcumin. International Journal of Cancer. 2010:NA-NA.
28. Muqbil I, Masood A, Sarkar FH, Mohammad RM, Azmi AS. Progress in Nanotechnology Based Approaches to Enhance the Potential of Chemopreventive Agents. Cancers. 2011;3(1):428-45.
29. Goodpasture CE, Arrighi FE. Effects of food seasonings on the cell cycle and chromosome morphology of mammalian cells in vitro with special reference to turmeric. Food and Cosmetics Toxicology. 1976;14(1):9-14.
30. Giri A, Das SK, Talukder G, Sharma A. Sister chromatid exchange and chromosome aberrations induced by curcumin and tartrazine on mammalian cells in vivo. Cytobios. 1990;62(249):111-7.
31. Sakano K, Kawanishi S. Metal-mediated DNA damage induced by curcumin in the presence of human cytochrome P450 isozymes. Archives of Biochemistry and Biophysics. 2002;405(2):223-30.
32. Blasiak J, Trzeciak A, Kowalik J. Curcumin damages DNA in human gastric mucosa cells and lymphocytes. Journal of environmental pathology, toxicology and oncology: official organ of the International Society for Environmental Toxicology and Cancer. 1999;18(4):271-6.
33. Cao J, Jia L, Zhou H-M, Liu Y, Zhong L-F. Mitochondrial and Nuclear DNA Damage Induced by Curcumin in Human Hepatoma G2 Cells. Toxicological Sciences. 2006;91(2):476-83.
34. Urbina-Cano P, Bobadilla-Morales L, Ramírez-Herrera MA, Corona-Rivera JR, Mendoza-Magaña ML, Troyo-Sanromán R, et al. DNA damage in mouse lymphocytes exposed to curcumin and copper. Journal of Applied Genetics. 2006;47(4):377-82.
35. Verschoyle RD, Steward WP, Gescher AJ. Putative Cancer Chemopreventive Agents of Dietary Origin—How Safe Are They? Nutrition and Cancer. 2007;59(2):152-62.
36. López-Lázaro M. Anticancer and carcinogenic properties of curcumin: Considerations for its clinical development as a cancer chemopreventive and chemotherapeutic agent. Molecular Nutrition & Food Research. 2008.
37. Syng-ai C, Kumari AL, Khar A. Effect of curcumin on normal and tumor cells: role of glutathione and bcl-2. Molecular Cancer Therapeutics. 2004;3(9):1101-8.
38. McNally S, Harrison E, Ross J, Garden O, Wigmore S. Curcumin induces heme oxygenase 1 through generation of reactive oxygen species, p38 activation and phosphatase inhibition. International Journal of Molecular Medicine. 2007.
39. Sandur SK, Ichikawa H, Pandey MK, Kunnumakkara AB, Sung B, Sethi G, et al. Role of pro-oxidants and antioxidants in the anti-inflammatory and apoptotic effects of curcumin (diferuloylmethane). Free Radical Biology and Medicine. 2007;43(4):568-80.