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# Selective Cytotoxic and Chemoprotective effect of nanocurcumin against human HCT-116 cell line

**Youssef M. Shehata [1], Nermin Rafaat[2], Haytham A. Gad[3,4], Sara F. Gaafar[5]**

[1]. *Professor of Biochemistry, Faculty of Veterinary Medicine, Zagazig University.*

[2]. *Assistant Professor of Medical Biochemistry, Faculty of Medicine, Zagazig University.*

[3]. *Associate Professor of Biochemistry, Faculty of science, University of Jeddah, KSA.*

[4]. *Assistant Professor of Biochemistry, Faculty of Veterinary Medicine, Zagazig University.*

[5]. *B.V. SC., Diploma in Biochemistry, Faculty of Veterinary Medicine, Zagazig University.*

**ARTICLE INFO**

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**ABSTRACT**

Background: limitation of curcumin use as antioxidant and anticancer may related to its low bioavailability so its use as nanocurcumin may improve its effect.

Aim: this study was designed to investigate the possible chemoprotective and cytotoxic effect of nanocurcumin against Human colorectal carcinoma (HCT-116) cell line

Material and methods: MTT assay was performed to detect the cytotoxic effect of nanocurcumin against normal and HCT-116 cells, and determination of gene expression of P53, BAX, Caspase-9 and BCL-2 was performed to detect the apoptotic effect of nanocurcumin

Results: nanocurcumin has a selective cytotoxic effect on human HCT-116 cells with no cytotoxic effect on healthy cells. At the same time, nanocurcumin increases the expression of P53, BAX and Caspase-9 with reduction in expression of BCL-2 gene in medium and high doses with no effect in lower dose

Conclusion: nanocurcumin has selective cytotoxic and chemoprotective effect against human HCT-116 cells according to its dose used.



Corresponding author: Mobile:+20120551740,

E-mail address: sarafekry111088@gmail.com

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## INTRODUCTION

Colorectal cancer representing a major public health problem as it considered the third most common cancer worldwide, following tumors of the lung and breast and the fourth most common cause of oncological death [(1)]. Hereditary factors, lynch syndrome, red meat and high fat diets with low fruits and vegetables were considered the risk factors affecting the development of colon [(2,3)]. Chemoprevention by use of natural compounds that have the potential to delay, prevent or reverse the development of colorectal cancer is a good option [(4)]. The search for new chemo preventive and antitumor agents that are more effective and less toxic has great interest in phytochemicals [(5)].

Curcumin (diferuloylmethane) (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is an active ingredient of turmeric, which is derived from the dried roots of the plant Curcuma Longa [(6)]. Curcumin has many beneficial functions, however, the limitation of curcumin practically returns to its low bioavailability that resulted in poor metabolism, poor absorption, rapid elimination and Poor aqueous solubility [(7,8)]. Therefore an efficient drug delivery system is made to be a breakthrough technology for the successful medical application of curcumin.

Nanocurcumin is a compound, which preserves the properties of curcumin and ensures that it reaches the affected tissue to show its therapeutic effects, efficiently [(9)]. Nanocurcumin is better absorbed, more target to affected tissue and required low dosages as compared to curcumin [(10-12)]. Curcumin has been shown to be an anti-oxidant,anti-proliferating, anti-inflammatory [(13)],anti-atherogenic & anti-cancer properties [(14,15)]. Several reports suggested the chemoprotective effect of curcumin against many types of cell line such as on HT-29 cells [(16,17)], Colo 205 cells [(18)], HCT-116 cells [(17,19,20)] and many others. The chemoprotective effect of curcumin depends mainly on suppression of several cellular signal transduction pathways which have role in growth, differentiation, and malignant transformation [(21)], or activation of several apoptotic enzymes including P53, Caspase-8 that induced cleavage of BID, leading to activation of BAX that lead to mitochondrial Cytochrome c release and induced Caspase- 3 activation with inhibition of BCL-2 gene expression [(5)]. In order to prevent rapid metabolism and increase bioavailability, curcumin has been used polymeric nanoparticles with a hydrophobic core and a hydrophilic shell have also been used for encapsulation of curcumin [(22,23)].

According to the above mentioned, this study was designed aiming to investigate the chemopreventive effect of nanocurcumin against HCT-116 cell line.

## MATERIAL AND METHODS

### *Chemicals*

Nanocurcumin was purchased from NanoTech Egypt for photo-electronics, Al Giza, Egypt, dissolved in sterile 0.1% (v/v) Dimethyl sulfoxide (DMSO).

The HCT-116 cell line was purchased from Vacsera, AL Giza, Egypt.

All other chemicals used in this experiment were purchased from sigma Aldrich. Egypt.

### *Cell-line preparation*

The cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin in a humidified incubator containg 95% air and 5% $CO_2$ at 37ºC. HCT-116 cells ($5x10^5$ cells / ml) were seeded on six well culture plate containing

2 ml DMEM after 24 hr incubation the medium was removed and fresh medium containing various concentrations of nanocurcumin 10-15-25 µmol/ml were added & one well seeded without drug as untreated control and incubated for 24 hr at 37 $^{o}$C in 5% $CO_2$ incubator until further analysis [24].

***Cytotoxic effect on human cell lines using MTT assay***

This cytotoxic activity test was conducted and determined by the Bioassay Cell Culture Laboratory, National Research Centre, Dokki, Egypt. The MTT assay is a colorimetric assay depend on reduction of yellow MTT (3- (4,5- dimethylthiazol-2-yl) -2,5- diphenyl tetrazolium bromide) to purple formazan [25]. Briefly, $l0^4$ cells/well were treated with various concentrations of nanocurcumin. After 48 hr incubation 2.5 µg /ml of MTT was added to each well and incubated at 37°C for 4hr. The formazan crystals that formed were dissolved by adding 200 µl/well of 10% Sodium dodecyl sulphate. A positive control was used that gives 100% lethality under the same conditions [26,27] and the absorbance was read at 595 nm. The percentage of change in viability was calculated according to the formula: (Reading of extract / Reading of negative control) -1) x 100.

***Molecular determinations***

Total RNA was extracted from human HCT-116 cell using PureLink® RNA Mini Kit purchased from Ambion by life technologies by Thermo Scientific, Catalog numbers: 12183018A and using the manufacture instructions. The purity of RNA samples were checked using NanoDrop® ND-1000 Spectrophotometer, NanoDrop Technologies Wilmington, Delaware, USA. The Synthesis of cDNA was occurred by using High Capacity cDNA Reverse Transcription Kit purchased from Thermo Scientific, code 4374966. Real time PCR amplification was performed using Maxima SYBR Green qPCR Master Mix (2X) kit purchased from Thermo scientific, cataloug #K0251, to detect the gene expression of P53, BAX, BCL-2, Caspase-9. The amount of target gene expression levels were quantified using the formula of $2^{-\Delta\Delta ct}$ [28]. The primer sequences of the desired genes, was designed according to **Table 1**.

**Statistical analysis**

Data were statistically analyzed using the software SPSS/PC+ 2001 for obtaining mean data and standard error. Data were analyzed using one-way ANOVA to determine the statistical significance of differences among experimental groups.

**RESULTS**

***Cytotoxic effect of Nanocurcumin on HCT-116 cell line:***

The MTT assay was performed to assess the rate of proliferation of HCT-116 cells and normal retina cell line (RPE1) after treatment with varying concentrations of nanocurcumin. We examined the in vitro effects of different concentrations of nanocurcumin ranged between 0.78 to 100 µmol /ml on the viability of HCT-116 cells after 48 hr using the MTT assay **(figure 1)**. The result showed that nanocurcumin had a concentration inhibitory effect on HCT-116 cells and no effect on normal cells. Nanocurcumin inhibited the growth of HCT-116 cells in a concentration-dependent manner. At the concentration of 69.7 µmol, 50% viability was detected during the 48 hr treatment, whereas maximum cytotoxicity $LC_{90}$ was observed at a concentration of 123.2 µmol, we also examined the in vitro cytotoxic effect of nanocurcumin on normal cells RPE1 after 48 hr treatment with different concentrations of Nanocurcumin

ranged between 0.78 to 100 µmol /ml. The result shows that had no inhibitory effect on the viability of RPE1 cells **(Table 2)**.

### *Effect of Nanocurcumin on the level of gene expression of p53, Bax, Bcl-2, Caspase-9 by using real time PCR :*

The results of gene expression showed that, there were an increase in the level of gene expression P53, BAX, and Caspase-9 in HCT-116 by increasing the concentrations of nanocurcumin with the decrease in the expression level of BCL-2 gene in HCT-116 cells by increasing the concentration of nanocurcumin **(figure 2)**. The expression of P53, BAX and Caspase-9 genes were significantly elevated while BCL-2 gene expression were significantly reduced with 15 µmol and 25 µmol nanocurcumin treatment while showing non-significant reduction with 10 µmol of nanocurcumin in HCT-116 cell line.

## DISCUSSION

The present study revealed the cytotoxic effect of nanocurcumin on HCT-116 cell line and normal cells RPE1 to confirm the efficacy of nanocurcumin as a chemo-protcetive agent by using MTT assay. Our results represented that nanocurcumin has a concentration inhibitory effect on HCT-116 cells and no effect on normal cells. These results were supported by the experiment of Shakibaei *et al.,* who approved the anti-proliferative effects of curcumin in CRC cells [(29)]. Another reports showed the powerful cytotoxic effect of curcumin on HCT-116 cell line by MTT assay [(30,31)]. Khosropanah *et al*., concluded that, these nanocurcumin are effective at low dose rates, when examined the in vitro cytotoxic activity of curcumin and nanocurcumin on human breast adenocarcinoma cell line (MDA-MB231) by MTT assay and could be applied as an anticancer strategy [(32)]. Anitha *et al*., confirmed our MTT results that nanocurcumin had no cytotoxic effect on healthy cells L-929, but showed specific toxicity towards cancer cells PC-3 (prostate) and MCF-7 (breast) cell line [(33)]. Nanocurcumin seemed to induce more cell death than free curcumin at relatively lower equivalent concentrations. Reasons for the discrepancy of the cytotoxicity of curcumin and nanocurcumin may resulted from the different uptake efficiency [(34)]. The mechanism of this selective cytotoxic and chemoprotective effect of nanocurcumin was studied through detection of the expression levels of some apoptotic genes such as P53, BAX, Caspase-9 and BCL-2. The results showed that the mRNA expression levels of these genes were significantly increased according to nanocurcumin concentrations except BCL-2 gene which is decreased which indicates the apoptotic effect of nanocurcumin on HCT-116 cell line. Guo *et al*., investigated that, curcumin inhibited the growth of LoVo cells by inducing apoptosis. Western blotting analysis indicated that curcumin induced the release of Cytochrome c, a significant increase of BAX and P53 and a marked reduction of BCL-2 and measurement of Caspase-3 and Caspase-9 activities were upregulated by a 24 hr curcumin treatment in a dose-dependent manner [(35)]. Another study proved that curcumin upregulated Caspase-9, Caspase-3, Caspase-8 in human hepatocellular carcinoma cell lines treated with curcumin by flow cytometry and confirmed the result by western blotting technique [(36)]. Zhu *et al*., suggested that the cell apoptosis in SW872 cells human adipocytes induced by treatment with curcumin is dependent on alterations in the expression of BCL-2 family proteins and Cytochrome c, and is associated with the mitochondrial pathway [(37)]. In another report curcumin could inhibit

PC-3 growth, decrease tumor volume, reduce tumor weight, and induce cell apoptosis under the skin of nude mice by up-regulating BAX and down-regulating BCL-2, by injected PC-3 cells subcutaneously to the nude mice to establish the tumor model [(38)]. Dilnawaz *et al*., utilized curcumin loaded magnetic nanoparticle formulations to K562 cells and induced a rapid decrease in mitochondrial membrane potential with release of Cytochrome c into cytosol, followed by cleavage of Caspase-9 and Caspase-3 [(39)]. In neo HL-60 cells, curcumin induced apoptosis through mitochondrial pathway involving Caspase-8, BID cleavage, Cytochrome c release, and Caspase-3 activation and also suggested that BCL-2 and BCL-XL are critical negative regulators of curcumin-induced apoptosis [(5)]. Down-regulation of BCL-2 could contribute to curcumin-induced apoptosis, this approved by assess the effect of curcumin on mRNA level of BCL-2 in both DNR-insensitive AML cell lines (KG1a and Kasumi-1) and in DNR-sensitive U937 cells by reverse transcription PCR [(40)]. Taken together, our results these findings suggest a mechanism of curcumin action on HCT-116 cells and should further establish its use as a valid chemo preventive and chemotherapeutic agent in colorectal cancer.

From all of the above we can conclude that, Nanocurcumin revealed a possible prophylactic and protective role against colorectal cancer due to its anti-carcinogenic effect and improve the poor solubility problem than free curcumin. The prophylactic role of nanocurcumin against colorectal cancer might be due to its apoptotic activities through its ability to up regulation the gene expression of P53, BAX, Caspase-9 and down regulation of BCL-2.

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**Table 1 primer sequence of P53, BAX, BCL-2, Caspase-9.**

| Gene | Sequence |
|---|---|
| **P53** | F 5- ACTTGTCGCTCTTGAAGCTAC-3<br>R 5-GATGCGGAGAATCTTTGGAACA-3 |
| **BAX** | F 5-CCTGTGCACCAAGGTGCCGGAACT-3<br>R 5-CCACCCTGGTCTTGGATCCAGCCC-3 |
| **BCL-2** | F 5-TTGTGGCCTTCTTTGAGTTCGGTG-3<br>R 5-GGTGCCGGTTCAGGTACTCAGTCA-3 |
| **Caspase-9** | F 5 -CACTTCCCCTGAAGACGAGTC-3<br>R 5-GTGGGCAAACTAGATATGGCG-3 |

**Table 2. $LC_{50}$ &$LC_{90}$ of Nanocurcumin on HCT-116 & normal cells RPE1 by using MTT assay.**

| Sample Code | $LC_{50}$ (µmol/ml) | $LC_{90}$ (µmol/ml) | Remarks |
|---|---|---|---|
| Nanocurcumin treated HCT116 cells | 69.7 | 123.2 | 64.2% at 100 µmol |
| Nanocurcumin treated normal cells RPE1 | 0 | 0 | 4.5 % at 100 µmol |

$LC_{50}$: Lethal concentration of the sample which causes the death of 50% of cells in 48 hr.

$LC_{90}$: Lethal concentration of the sample which causes the death of 90% of cells in 48 hr.

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**Figure 1.`** Show the effect of different concentration of nanocurcumin on HCT-116 on cell viability by using MTT assay after 48 hr incubation.

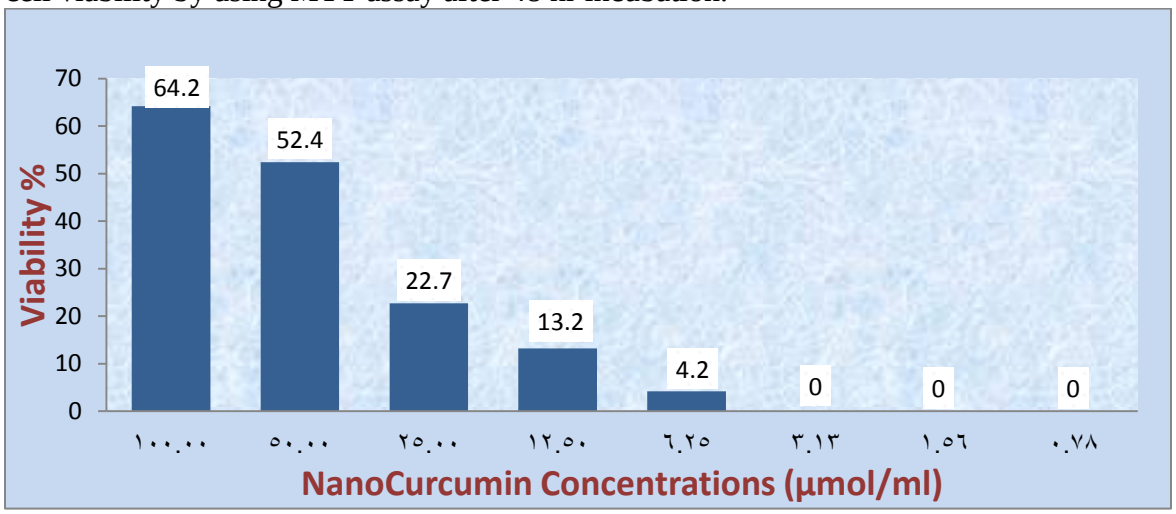
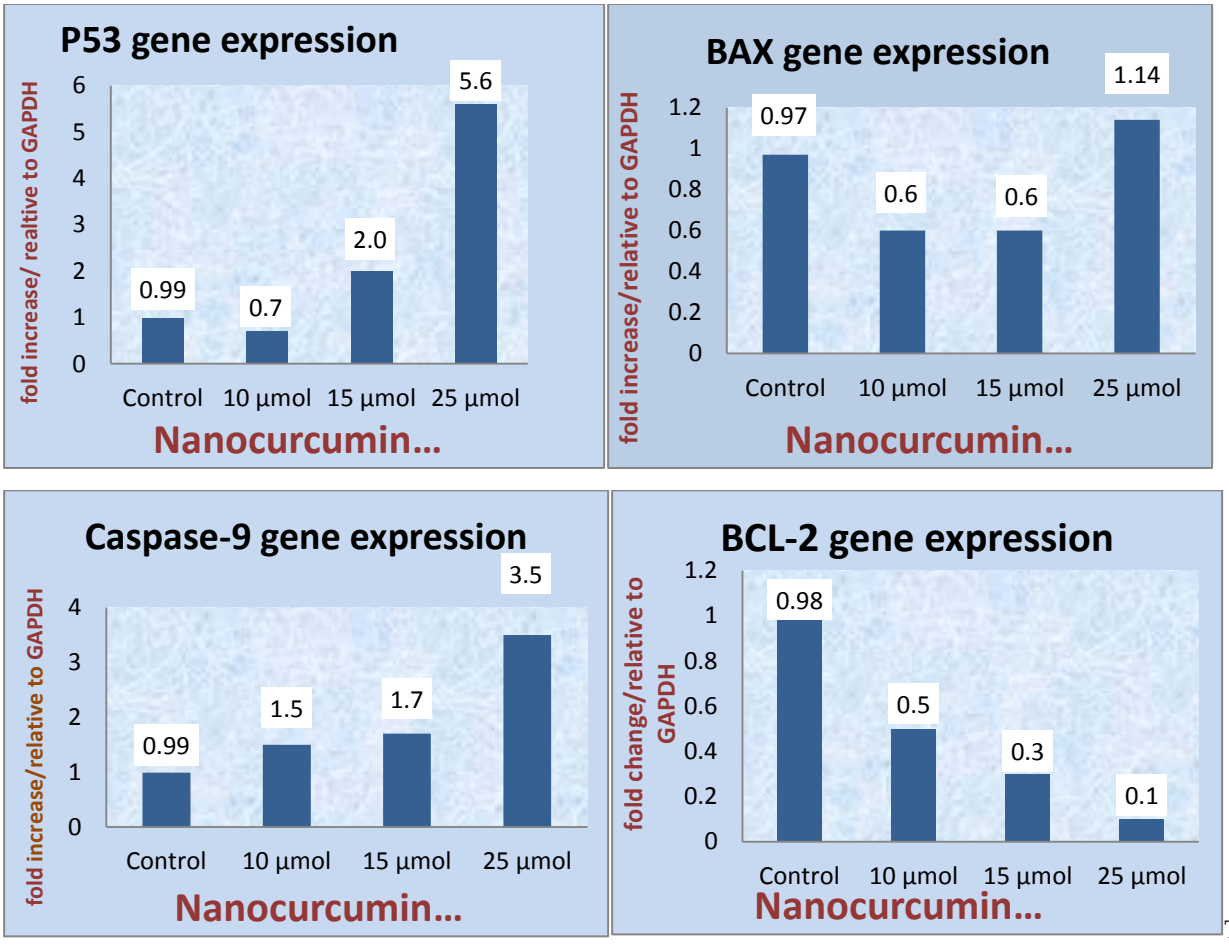



**Figure 2.** Analysis of PCR product of P53, BAX, Caspase-9 and BCL-2 genes in HCT-116 cell line by using real time PCR.




The expression of P53, BAX and Caspase-9 genes were significantly elevated while BCL-2 gene expression were significantly reduced with 15 µmol and 25 µmol nanocurcumin treatment while showing non-significant reduction with 10 µmol of nanocurcumin in HCT-116 cell line. Data representing as Mean ± SE %. P-values <0.01.