## Original paper

# The In-Vitro Effect of Curcumin and Arsenic Trioxide on The Level Of NF-kB and Induction of Apoptosis in B16 Cell Lines

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## Abstract

**Background:** Malignant Melanoma (MM) is one of aggressive skin tumors that had increase in incidence during the past two decades. Curcumin is a natural product that had been utilized for a variety of maladies like rheumatic, cardiac, neurological and numerous other disorders while arsenic trioxide 'ArT' (not approved for treatment of MM) is well known cytotoxic agent. NF-kB is a specific complicated pertinacious element that participates in controlling of DNA transcription, regulating cytokine expression and, hence, cell survival. It was found that high level of NF-kB is highly associated with occurrence of MM. Induction of programmed cell-death (apoptosis) is another target, through which; destruction of tumor cells can be triggered.
**Aim of the study:** to demonstrate the effectiveness of curcumin on expression of NF-kB and induction of apoptosis as compared to the use of arsenic trioxide on B16 cell line.
**Material and Methods:** in this study we utilized B16 cell lines that divided into four categories (curcumin treated, ArT treated, curcumin combined with ArT, and control categories). The first 3 categories where treated with serial concentrations of each drug (at concentrations of 1, 2, 4, 8, 16, and 32 µg/ml), then the supernatant of 96 well of cell culture plate was used for ELISA detection of NF-kB level while cell pellet was used for detection of percentage of apoptotic cells.
**Results:** there was significant reduction of NF-kB level when curcumin was used from 390±2.646 pg/ml to 223.67±11.02 (at P<0.001) while ArT reduced NF-kB from 356+27.683 to 231.67+10.07 pg/ml (at P<0.001). The percentages of apoptotic cells elevated with using of curcumin from 37.4±0.8 to 99.93+0.06% (at P<0.001), while ArT increase in percentages of apoptotic cells from 11.1±0.2% to 99.17+0.17% (at P<0.001).
**Conclusion**: Curcumin had very potent concentration dependent anti-cancer effects against B16 MM cell line. ArT had similar concentration dependent anti-cancer activity but to less extent effect than curcumin against B16 melanoma cell line. Combination of curcumin with ArT had no significant potentiation effect for activity of curcumin on reduction of NF-kB level or increase in the percentage of apoptotic cells.
**Keywords**: curcumin, arsenic trioxide, NF-kB, flow cytometry, apoptotic cell, B16 cell line.

## Introduction

Melanocytes are particularized cells that derived from neural-crest that is paramount for the skin homeostasis; they are accountable for protection of keratinocytes from exposure to detrimental UV radiation [(1)]. MM is one of malignant tumors that carry a high mortality rate (about 75% of skin cancer-linked deaths) [(2)] with high resistance rate for treatment [(3)]. MM became the 5th and 7th most commonly seen malignancy in males and females respectively. Males are somewhat have higher risk for MM (2.9%) than females (about 1.9%) [(4)]. NF-kB "abbreviation of nuclear factor kappa-light-chain-enhancer of activated B cells"

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is a specific complicated pertinacious element that participate in controlling of DNA transcription, regulating cytokine expression and, hence, cell survival [5]. Abnormal function or uncontrolled generation of NF-kB can be highly associated with MM, [6].

Curcumin is a fat-soluble yellow pigmented substance derived from the rhizomes of "Curcuma longa L" oftentimes utilized as a yellow coloring-agent and flavoring-agent in foods [7]. Curcumin can affect a lot of cellular and extracellular target-area s such as regulatory cytokines, wide range of kinases for numerous protein, multiple transcriptional-inducing factors, variable adhesion directing molecules, diverse inflammatory specialization mediators and free O2 species-enzymes [8]. As a new proved fact, curcumin appears to be completely clog TNF-provoked NF-kB over-expression by way of the retarding phospho-activation of NF-kB, leading to subsequent suppressing of cell surviving, proliferative in addition to inflammatory-provoked gene assembly [9]. In addition, curcumin has been shown in vitro studies to inhibit both malignant melanoma cell migration and invasion, and induce apoptosis by down-regulation of "JAK-2/STAT3" signaling pathway [10]. A remarkable cross-talk relating Notch-1 and NF-kB passageway was found to be there, for which, down-regulation by both curcumin and the curcumin-provoked reduction in Notch-1 levels, subsequently, resulting in down-expression of tumor-induced gene-generation [11].

Arsenic trioxide (ArT) is one of oldest cytotoxic medicinal substance regarded as an inorganic compound with a chemical formula of "As2O3". ArT is an old traditional remedy utilized by Chinese people since ages and , nowadays, it is therapeutically utilized in treating acute pro-myeloid leukemia [12]. It is obviously that ArT has two main mechanisms of action recognized (for both in vivo and in vitro studies) include promoting cellular differentiation at low potion of ArT and enhancement of spontaneous apoptotic machinery at high potion of ArT [13]. It was found that degradation of what is called the fusion-dependent protein is most distinctly possible mechanism by which ArT provokes cellular differentiation to normal morphology in malignant white blood cells allows malignant pro-myelocytes to transcend their maturations' prohibition [14]. Extracellular signal-linked protein kinases [ERKs] are stimulated after exposure to various concentrations of ArT which eventually prevents malignant transformation, while 'JNKs' are activated by higher levels of ArT which plays a vital role in pro-apoptotic pathways while the virtual effect of ArT on suppression of NF-kB directly was not well demonstrated [15]. ArT can efficiently activate nicotinamide adenine di nucleotide phosphate (NADPH) oxidase which is highly impactful for the production of superoxides which ultimately generating H2O2 enhancing directed pro-apoptotic action imposed on the mitochondria [16]

## Materials and methods

The study was done in specialized research laboratory for cell culture at University of Babylon/College of Medicine/Department of Pharmacology under almost full aseptic conditions and optimum environment. The design of this study was planned to expose B16 MM cell line to serial concentrations (1, 2, 4, 8, 16, and 32µg/ml) of the curcumin, ArT, and their combinations. Regarding ArT, it was already water soluble, while curcumin is lipid soluble, so that curcumin was converted to water soluble stock solution by using 10% solution of (DMSO) which made curcumin highly water soluble. Preparation of proper target for these drugs involved using of optimal in vitro cells that had high resemblance to the cancer cell in vivo. This required the utilization of B16 cell

line as an in vitro model. The cultured 96 well plate, then, was treated with curcumin, ArT, and their combinations. Each concentration of each drug was added to 3 wells (each well containing at least 1x104 cells) of the tissue culture plate. The experiment was repeated in 6 different plates to increase the sample size (no. = 6). The content of each well was centrifuged after 48 hours of incubation and then the supernatant was used to estimate the level of NF-kB by ELISA technique while cell pellet refrigerated until used for detection of percentage of apoptotic cells by flow cytometry.

## Results

There was significant dose dependent reduction in the level of NF-kB mostly noted for curcumin when was used as compared to ArT. Combination of both drugs did not potentially affect the efficacy of curcumin as shown in table (1).

There was significant dose dependent elevation in percentage of apoptotic cells mostly noted for curcumin when was used as compared to ArT. Combination of both drugs did not potentially affect the efficacy of curcumin as shown in table (2).

## Discussion

**Effect of treatment with serial concentrations of curcumin, arsenic trioxide, and their combinations on NF-kB level in B16 cell line:**

NF-kB, nowadays, became the target-area of interest for many researchers for treatment of numerous types of cancers include MM [(17)]. The degree of NF-kB level reduction was smooth, dose dependent, notably for curcumin alone or in combination with ArT as shown in in table (1). Certain study showed that curcumin prohibits the activation of NF-kB, and subsequently decrease cell growth, by induction of oxidative stress in the mitochondria [(18)].

Arsenic trioxide in a study done to demonstrate its effect on breast cancer showed significant reduction of NF-kB in low doses (less than 10µg/ml) [(19)].

**Table 1.** Effect of treatment with serial concentrations of curcumin, arsenic trioxide, and their combinations on NF-kB level compared to control category.

| Treatment categories | Drug(s) concentration | NF-kB pg/ml (mean± SD) |
|---|---|---|
| **Cur** | 1 µg/ml | 390±2.646 |
| | 2 µg/ml | 335.00±21.66 |
| | 4 µg/ml | 247.33±20.60* |
| | 8 µg/ml | 263.33±19.86** |
| | 16 µg/ml | 297.33±4.04** |
| | 32 µg/ml | 223.67±11.02** |
| **ArT** | 1 µg/ml | 356±27.683 |
| | 2 µg/ml | 328.33±2.52 |
| | 4 µg/ml | 304.33±6.51 |
| | 8 µg/ml | 264.33±8.51** |
| | 16 µg/ml | 232.67±16.62** |
| | 32 µg/ml | 231.67±10.07** |
| **Cur + ArT** | 1 µg/ml | 364±33.181 |
| | 2 µg/ml | 326.00±26.06 |
| | 4 µg/ml | 290.67±4.51* |
| | 8 µg/ml | 255.67±8.62** |
| | 16 µg/ml | 248.67±33.56** |
| | 32 µg/ml | 184.67±23.97** |
| **Control group** | 362 ± 35.2 | |

Data were presented as (mean±SD)
** Significant (p<0.05) as compared with control category.*
*** Highly significant (p<0.01) as compared with control category.*

No significant difference was observed in reduction of NF-kB level on B16 cell line when curcumin utilized solely or in combination with ArT in comparability to using of ArT solely, with few exceptions as shown in table (1).

In another study, curcumin and ArT possessed significant additive effect on specific concentration was due to mainly by activation of oxidative stress of both drugs at low concentration [20] and by prohibition of ERK, that eventually causes reduction in level of NF-kB at somewhat higher doses [20].

**Effect of treatment with serial concentrations of curcumin, arsenic trioxide, and their combinations on percentage of apoptotic cells in B16 cell line:**

The lower doses of curcumin showed superior significant difference in induction of apoptosis when compared to ArT, whereas the difference became not statistically impactful at higher doses as shown in tables (2).

These effects were mainly explained by another study suggested that curcumin exhibits high inducing effect on pro-apoptotic caspase pathway at lower doses while ArT induced reactive oxygen species activity in higher doses [21] i.e. curcumin potentiate apoptotic pathway by stimulation of caspases, JNK activation, and Foxo3a nuclear translocation while ArT causes disruption of mitochondrial function by free oxygen radical formation [21, 22].

**Table 2.** Percentage of apoptotic cells in B16 cell line treated with curcumin, arsenic trioxide and their combinations compared to control category.

| Treatment categories | Drug(s) concentration | Percentage of apoptotic cells |
|---|---|---|
| **Cur** | 1 µg/ml | 37.4±0.8* |
| | 2 µg/ml | 77.74±2.17** |
| | 4 µg/ml | 95.34±2.56** |
| | 8 µg/ml | 97.20±1.68** |
| | 16 µg/ml | 99.52±0.32** |
| | 32 µg/ml | 99.93±0.06** |
| **ArT** | 1 µg/ml | 11.1±0.2 |
| | 2 µg/ml | 18.64±1.81 |
| | 4 µg/ml | 30.72±1.88* |
| | 8 µg/ml | 51.39±0.83** |
| | 16 µg/ml | 98.22±0.78** |
| | 32 µg/ml | 99.17±0.17** |
| **Cur +ArT** | 1 µg/ml | 46.6±0.7** |
| | 2 µg/ml | 97.14±0.76** |
| | 4 µg/ml | 98.37±2.55** |
| | 8 µg/ml | 99.51±0.30** |
| | 16 µg/ml | 99.80±0.29** |
| | 32 µg/ml | 99.87±0.21** |
| **Control group** | 6.693±0.544 | |

Data were presented as (mean±SD)
** Significant (p<0.05) as compared with control category.*
*** Highly significant (p<0.01) as compared with control category.*

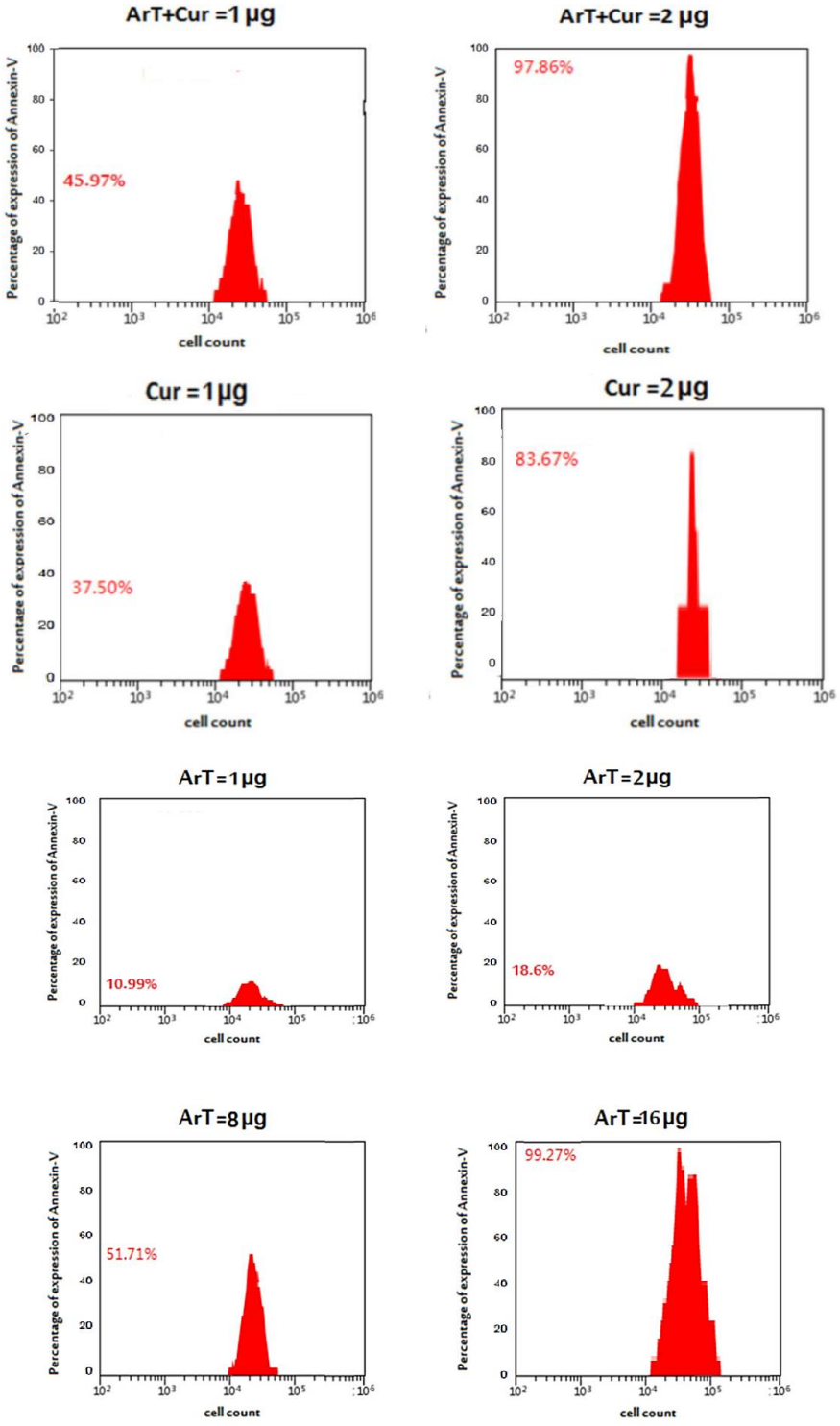


**Figure 1.** Flow cytometric histograms showed the percentage of apoptotic cells for some treatment categories at variable concentrations.

## References

1. Vandamme N, Berx G. From neural crest cells to melanocytes: cellular plasticity during development and beyond. Cellular and Molecular Life Sciences. 2019;76:1919-34.
2. Law MH, Bishop DT, Lee JE, Brossard M, Martin NG, Moses EK, et al. Genome-wide meta-analysis identifies five new susceptibility loci for cutaneous malignant melanoma. Nature genetics. 2015;47:987.
3. Okano Y, Satoh T, Horiguchi K, Toyoda M, Osaki A, Matsumoto S, et al. Nivolumab-induced hypophysitis in a patient with

advanced malignant melanoma. Endocrine journal. 2016:EJ16-0161.
4. Liu G, Mattick J, Taft RJ. A meta-analysis of the genomic and transcriptomic composition of complex life. Cell cycle. 2013;12:2061-72.
5. Ruiz MP, Tarifa CM, Qureshi R, Yoon H, Shin M, Kurani H, et al. Mammary adipocytes mediate cytokine production and malignant progression of ER-positive breast cancer through NF-kB activation. AACR; 2018.
6. Poźniak J, Nsengimana J, Laye JP, O'Shea SJ, Diaz JMS, Droop AP, et al. Genetic and Environmental Determinants of Immune Response to Cutaneous Melanoma. Cancer research. 2019;79:2684-96.
7. Panahi Y, Saadat A, Beiraghdar F, Sahebkar A. Adjuvant therapy with bioavailability-boosted curcuminoids suppresses systemic inflammation and improves quality of life in patients with solid tumors: a randomized double-blind placebo-controlled trial. Phytotherapy Research. 2014;28:1461-7.
8. Gupta SC, Prasad S, Kim JH, Patchva S, Webb LJ, Priyadarsini IK, et al. Multitargeting by curcumin as revealed by molecular interaction studies. Natural product reports. 2011;28:1937-55.
9. Mouzaoui S, Rahim I, Djerdjouri B. Aminoguanidine and curcumin attenuated tumor necrosis factor (TNF)-α-induced oxidative stress, colitis and hepatotoxicity in mice. International immunopharmacology. 2012;12:302-11.
10. Esatbeyoglu T, Huebbe P, Ernst IM, Chin D, Wagner AE, Rimbach G. Curcumin—from molecule to biological function. Angewandte Chemie International Edition. 2012;51: 5308-32.
11. Sha J, Li J, Wang W, Pan L, Cheng J, Li L, et al. Curcumin induces G0/G1 arrest and apoptosis in hormone independent prostate cancer DU-145 cells by down regulating Notch signaling. Biomedicine & Pharmacotherapy. 2016;84:177-84.
12. Bael TE, Peterson BL, Gollob JA. Phase II trial of arsenic trioxide and ascorbic acid with temozolomide in patients with metastatic melanoma with or without central nervous system metastases. Melanoma research. 2008;18:147-51.
13. Zhang X-W, Yan X-J, Zhou Z-R, Yang F-F, Wu Z-Y, Sun H-B, et al. Arsenic trioxide controls the fate of the PML-RARα oncoprotein by directly binding PML. science. 2010;328:240-3.
14. Emadi A, Gore SD. Arsenic trioxide—an old drug rediscovered. Blood reviews. 2010;24:191-9.
15. Binet F, Chiasson S, Girard D. Arsenic trioxide induces endoplasmic reticulum stress-related events in neutrophils. International immunopharmacology. 2010;10:508-12.
16. Lengfelder E, Hofmann W, Nowak D. Impact of arsenic trioxide in the treatment of acute promyelocytic leukemia. Leukemia. 2012;26:433.
17. Zehavi L, Schayek H, Jacob-Hirsch J, Sidi Y, Leibowitz-Amit R, Avni D. MiR-377 targets E2F3 and alters the NF-kB signaling pathway through MAP3K7 in malignant melanoma. Molecular cancer. 2015;14:68.
18. Liao W, Xiang W, Wang F-F, Wang R, Ding Y. Curcumin inhibited growth of human melanoma A375 cells via inciting oxidative stress. Biomedicine & Pharmacotherapy. 2017;95:1177-86.
19. Shi Y, Cao T, Huang H, Lian C, Yang Y, Wang Z, et al. Arsenic trioxide inhibits cell growth and motility via up-regulation of let-7a in breast cancer cells. Cell Cycle. 2017;16:2396-403.
20. Li Y, Kong D, Ahmad A, Bao B, Sarkar FH. Augmenting the efficacy of chemo-and radio-therapy by nutraceuticals: Evidence from pre-clinical and clinical trials. Nutraceuticals and Cancer: Springer; 2012. p. 355-76.
21. Weibin Z, Min T, Gang X, Shaowei H. Influence of curcuma aramatica oil on apoptosis and Bcl-2 expression of HepA liver cancer cells in mice [J]. Modern Journal of Integrated Traditional Chinese and Western Medicine. 2009;4.
22. Yu T, Ji J, Guo Y-l. MST1 activation by curcumin mediates JNK activation, Foxo3a nuclear translocation and apoptosis in melanoma cells. Biochemical and biophysical research communications. 2013;441:53-8.