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Berberine, a natural plant product, Inhibits Cell Growth in Human Cancer cells compared with healthy cells

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Abstract

Berberine, an isoquinoline derivative alkaloid, has recently been shown to have cytotoxicity and antitumor activity. The present study aimed to investigate the effect of Berberine at different concentrations (400, 200, 100, 50, 25µg/ml) on cell culture (PA-1:ovary derived from metastatic site, PC-3:Prostate cancer cell line, A375:skin cancer cell line, and WRL-68: human hepatic normal cell, by cytotoxicity and DNA damage, through MTT assay and alkaline comet assay. The results of cytotoxicity showed that berberine induced a decrease in the viability of PA-1: ovary-derived compared with the WRL-68: human hepatic normal cell at 400 and 200 µg/ml of berberine. And indicated that tail length, tail DNA%, and Tail mean moment were significantly different in different concentrations of berberine at 400, 200 µg/ml, when compared with the control non-treatment. These results suggest that berberine can reduce DNA damage and cytotoxicity.

Keywords: comet assay, MTT assay, berberin, ATCC.

Introduction

Berberine-containing plants are used medicinally in virtually all traditional medical systems, and have a long history of usage in Ayurvedic and Chinese systems of medicine, dating back to at least 5,000 years [1]. Berberine is an isoquinoline alkaloid isolated from several traditional Chinese herbal medicines (TCM) such as *Coptis chinensis*, *Berberis aristata*, and *Coptis japonica* [2] Figure (1). Berberine has been used extensively to treat diarrhea, clear heat. Multiple pharmacological observations, resulting from modern research of the effects of berberine, have demonstrated activities relating to the efficacy of antioxidants [3], hepatoprotective effect [4,5], lowering blood glucose [6–8], lipid-lowering [9], and antineoplastic [10, 11] and antiarrhythmic effect [12]. A recent report indicated that berberine could induce hepatoma cell apoptosis through a mitochondria/caspases pathway while eliciting no cytotoxic effects in healthy hepatocytes [13].

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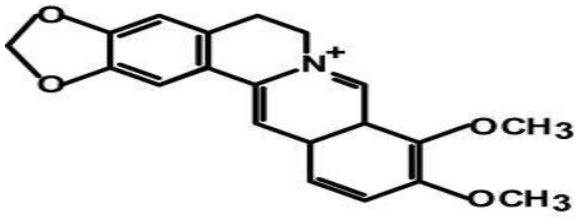

Figure (1): Structure of berberine (C20H18NO4+)

Several studies have reported that berberine has synergistic effects against cancer in combination with irradiation. Berberine has been shown to radiosensitize lung cancer cells by inducing autophagy [14] and esophageal cancer cells by the downregulation of the homologous recombination repair protein, RAD51[15]. DNA damage plays a pivotal role in most mechanisms underlying the action of anticancer drugs that interact with DNA and subsequently kill neoplastic cells [16].

The comet assay determines the amount of DNA damage (both single and double-strand breaks and conformational changes) in a cell exposed to DNA-damaging agents. The single-cell gel electrophoresis or comet assay was first designed by Östling and Johanson [17] to estimate the DNA damage in the single cells [18-20]. The present study was undertaken to obtain an insight into the DNA-damaging effects of berberine by alkaline comet assay and its correlation with cell survival in PA-1: ovary derived.

Material and method

Study effect Berberine was from Sigma (St. Louis, MO) at different concentration (400, 200, 100, 50, 25 µg/ml) on cell culture (PA-1: ovary derived from metastatic site, PC-3:Prostate cancer cell line, A375:skin cancer cell line, and WRL-68: human hepatic normal cell were obtained from American Type Culture Collection (ATCC), and maintained in RPMI (Gbico, Carlsbad, CA), supplemented with 10% FBS (Sigma), 1% 5,000 units/mL penicillin, 5,000 lg/mL streptomycin (Sigma). The cells were incubated in an atmosphere of 5% CO_2 at 37Ċ. incubated with berberine at various concentrations (400–80 mM) for 24 hours. There after the medium was aspirated, and the cells were fixed with 0.2 mL of 10%cold TCA/ per well at 4 ℃ for 30 min, washed with deionized water, dried at room temperature overnight, and incubated with MTT (0.5 mg/mL) for 4 hours. The viable cell number was directly proportional to the production of formazan solubilized with isopropanol, which could be measured spectrophotometrically at 563 nm [21]. The inhibition rate was calculated according to the following formula:

% inhibition rate = (control – test/ control) x 100

The Statistical Analysis System- SAS (2012) program [22] was used to evaluate different factors (concentration and cell line) in study parameters. Least significant difference –The LSD test was used to significantly compare between means in this study.

Comet assay

This test was done using the Oxiselect comet assay kit, Cat number STA-350 [23-24]. The best concentration of berberine effect on cell culture, which was used in this assay, cells were centrifuged at 1500 rpm for 2 min. The supernatant was discarded, and the pellet was washed once with ice-cold PBS and centrifuged at 1500rpm for 2 min; then the supernatant was discarded. Oxiselect Comet Agarose was heated to 90-95ºC in a water bath for 20 minutes until agarose liquefied, then transferred to a

37 ºC water bath for 20 min. The cell sample was combined with comet agarose at a 1:10 ratio (v/v), and the mixture (75µl/ well) was immediately added to the slide comet. The slides were held horizontally, then transferred to 4 ºC in a dark container for 15 min. The slide was transferred to a small basin containing lysis buffer, and the slide was immersed in the buffer for 30-60 min at 4 ºC in a dark container. The lysis buffer was aspirated from the container and replaced with an alkaline solution. The slide was immersed in the solution for 30 min at 4 ºC in a dark container. The alkaline solution was aspirated from the container and replaced with TBE electrophoresis solution, and immersed for 5 min (repeated once more). The slide was transferred to a horizontal electrophoresis chamber (1volt/cm voltage for 10-15 min). The slides were transferred from the electrophoresis chamber into a small basin containing dH2O for 2 min (repeated twice). The slides were transferred into a container containing 70% ethanol for 5 min, then air-dried. Diluted Vista Green DNA dye (100 µl) was added to each well and incubated at room temperature for 15 min. The slides were examined by fluorescence microscopy using a FITC filter green.

Result and discussion

The results of the trypan blue in MTT assay demonstrated that berberine induced decrease in the viability of **PA-1: ovary derived** compared with the **WRL-68: human hepatic normal cell** at 400 and 200 µg/ml from berberine (Table. 1), indicating that berberine has highest cytotoxic effects on of **PA-1: ovary derived** when using 400 µg/ml (48.03 ± 3.53) compared with other concentration, and decreased cytotoxic effect at 200 µg/ml to 25 µg/ml of berberine to reach from 71.14 ± 8.55 to 97.58 ± 2.61 respectively, through increase viability of cell. And Table (1) represented the result of the cytotoxic effect on A375: skin cancer cell and PC-3 prostate cancer cells that were incubated with different concentration of berberine at 400, 200,100, 50, 25µg/ml to show that, the lowest concentration at 200 to 25µg/ml respectively appeared to effect no significantly ($p \leq 0.006$) on viability of cell comparing to WRL-68: human hepatic normal cell at the same concentration. However, exposure to the highest concentration from berberine 400µg/ml showed a significant about 71.84 ± 6.76 and 74.42 ± 8.62 in A375:skin cancer cells and PC-3: Prostate cancer, respectively, compared with normal cells about (90.95 ± 3.95).

Table 2 compares three cell lines that were used in the present study (PA-1: ovary-derived, A375: skin cancer cell, PC-3 prostate cancer cell, and WRL-68: human hepatic normal cell) in viability under different concentrations of berberine. The result appeared that the best cell line was PA-1:ovary derived through the effect of berberine on cell viability compared with other cell lines.

Berberine sulfate has been reported to significantly inhibit the tumor yield and incidence of tumor-bearing animals in two stage skin carcinogenesis induced [25], may of study referred to used berberine to antitumor effects on many cancer cell lines, including leucocytes, liver, lung, stomach, colon, skin, oral, esophageal, brain, bone, breast and genital cancer cells [26- 28]. And Animal studies have demonstrated that berberine can suppress chemically-induced carcinogenesis [29], tumor formation [30], and tumor invasion [31].

Comet assay

Results in table (3) indicated that tail length was significantly different in different concentrations of berberine at 400, 200 µg/ml, to show mean values of 5.2 ± 0.384% and 6.4 ± 0.357% respectively, when compared with control non-treatment (8.6 ± 0.107%). There was a significant about ($P < 0.0001$) between the two groups, treatment and non-treatment of PA-1: ovary-derived cells, as shown in Table 3. Tail DNA% and Tail mean moment and figure (2) referred to DNA damage of PA-1:ovary derived cells, non-treatment, however, low DNA damage of cells after adding different concentrations of berberine.

Table 1. Effect of concentration on cell viability

Concentration (µg/ml)	Mean ± SD of viability (%)	Mean ± SD of viability (%)	Mean ± SD of viability (%)	Mean ± SD of viability (%)
	PA-1:ovary derived	**A375:skin cancer cell**	**PC-3:Prostate cancer**	**WRL-68: human hepatic normal cell**
25	97.58 ± 2.61 a	94.04 ± 3.84 a	94.87 ± 1.28 a	96.24 ± 2.27 a
50	97.87 ± 3.87 a	93.77 ± 2.18 a	93.06 ± 1.93 a	93.34 ± 3.96 a
100	94.21 ± 4.18 a	90.43 ± 5.03 a	88.81 ± 5.85 a	92.94 ± 1.84 a
200	71.14 ± 8.55 b	87.69 ± 6.02 a	87.42 ± 4.92 a	92.77 ± 3.99 a
400	48.03 ± 3.53 c	71.84 ± 6.76 b	74.42 ± 8.62 b	90.95 ± 3.95 a
LSD value	9.0968 **	9.165 **	9.572 **	6.0814 NS
P-value value	0.0001	0.0016	0.0060	0.4621
** (P<0.01).				

Mean having different small later in columns is significant.

Table 2. Compare between differences in lines % viability

Concentration (µg/ml)	Cell line				LSD value
	PA-1	A375	PC-3	WRL-68	
25	97.58 ± 2.61 A	94.04 ± 3.84 A	94.87 ± 1.28 A	96.24 ± 2.27 A	5.016 NS
50	97.87 ± 3.87 A	93.77 ± 2.18 A	93.06 ± 1.93 A	93.34 ± 3.96 A	5.899 NS
100	94.21 ± 4.18 A	90.43 ± 5.03 A	88.81 ± 5.85 A	92.94 ± 1.84 A	8.450 NS
200	71.14 ± 8.55 B	87.69 ± 6.02 A	87.42 ± 4.92 A	92.77 ± 3.99 A	11.517 **
400	48.03 ± 3.53 C	71.84 ± 6.76 B	74.42 ± 8.62 B	90.95 ± 3.95 A	0.0002 **
** (P<0.01), NS: Non-significant.					

Mean having different big later in row is significant.

Table (3): DNA damages in PA-1:ovary derived and control (Non-treatment) using the comet assay

No	Type	Tail length (mean ±St.error)	Tail DNA% (mean ±St.error)	Tail mean moment
1	No treatment	8.6 ± 0.107%	53.692± 0.387 %	**2.019±0.048 %**
2	400	5.2 ± 0.384%	34.122 ± 2.612%	**0.716±0.109%**
3	**200**	**6.4 ± 0.357%**	**41.952 ± 0.721%**	**1.315± 0.024%**

*Different letters: Significant difference ($P \leq 0.001$) between means (Duncan test).

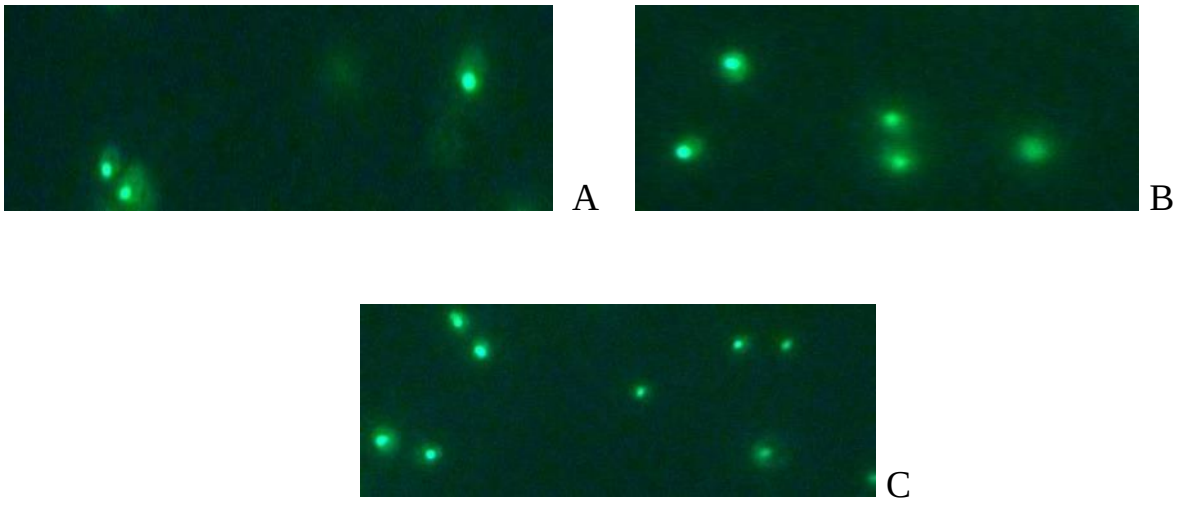

Figure 2: Photomicrograph of a fluorescent microscope green comet analysis. (A) PA-1: ovary derived (Non treatment), (B) and(C), PA-1:ovary derived was treated with 200 and 400 µg/ml of berberine, respectively.

Single cell gel (SCG) allows the detection of DNA alternative of diverse kinds, such as double-strand breaks, single-strand breaks, alkali–labile sites, incomplete repair sites, cross-links, and repair in individual cells [32,33]. Tail Moment and Tail DNA% are the two most common parameters to analyze Comet assay results. The Tail Moment has been suggested to be an appropriate index of induced DNA damage in considering both the migration of the genetic material as well as the relative amount of DNA in the tail [23], Tail DNA% = 100 x Tail DNA Intensity/Cell DNA Intensity and Tail Moment can be measured using one of the following methods: (a) Tail Moment = Tail DNA% x Tail Moment Length(measured from the center of the head to the center of the tail), (b) Tail Moment = Tail DNA% x Length of Tail.

Jagetia and Rao [34] referred to the cell survival and molecular DNA damage in HeLa cells treated with berberine as having an inverse correlation, indicating that with increased DNA damage, cell survival declined. Another study showed to indicate that berberine selectively induces cell death in HepG2 cells while has no cytotoxicity in normal Chang liver cells [35], and showed that cell viability was significantly decreased when berberine concentrations were higher than 0.05mg/mL. Berberine at a concentration above 0.1mg/mL altered the morphology of murine fibroblast (L929) cells, and the DNA damage indicator score increased in groups where the concentration of berberine was above 0.025mg/mL [36]. The conclusions: this study supports the fact that berberine at high concentration has cytotoxicity in the cell cycle and DNA damage.

Conflict of interest: NIL

Funding: NIL

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