

Original Research Paper

Anti-Proliferative Activity of Zerumbone Against Tumour Cell Lines

^{1,2}Adel Sharaf Al-Zubairi

¹Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Albaha University, Al-Baha, Kingdom of Saudi Arabia

²Department of Biochemistry and Molecular Biology, Faculty of Medicine and Health Sciences, Sana'a University, Sana'a Yemen

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Tell: +967734825031

+966540994415

Email: adelalzubairi@hotmail.com

Abstract: Zerumbone (ZER) is a cytotoxic phytochemical component extracted from the tropical herbal plant, *Zingiber Zerumbet* smith. It has been identified as an active anticancer compound. This study was aimed to evaluate in vitro the anti-proliferative activity of this bioactive compound, zerumbone, on seven cancer cell lines namely: CEMss (T4-lymphoplastoid), Chinese Hamster Ovary (CHO), 3T3 (mouse fibroblast), WEHI-3 (murine monomyelocytic leukemia), HeLa (Human cervical cancer), MCF-7 (human breast cancer) and MDA-MB-231 (human mammary cancer-estrogen negative). The antiproliferative activity of ZER was evaluated using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, together with the commercially available anticancer drug Cisplatin as a control drug. The most sensitive cells to ZER were found to be WEHI-3B, 3T3 and HeLa cell lines with an IC_{50} of 5, 6 and $6.4 \mu\text{g mL}^{-1}$, respectively, followed by CEMss cell lines with an IC_{50} of $12 \mu\text{g mL}^{-1}$. This indicates the possible potential use of ZER for treatment of hematological cancers in addition to the cervical cancer. Meanwhile, ZER was found to be less toxic to the normal cell lines CHO.

Keywords: Zerumbone, Cell Lines, Antiproliferative Activity, MTT

Introduction

Asian traditional medicine has introduced the medicinal herbs for treatment of various ailments including malignant cancers. The most frequently used medicinal herbal preparations in Asian traditional medicine were formulated from the Zingiberaceae family. It has been used for treatment of sores, swelling and loss of appetite. The boiled rhizome's juice has been used as medicines for treatment of worm infestations in children. The rhizomes have been known in Asian traditional medicine for treatment of many diseases and in particular for treatment of many gastrointestinal tract conditions (Jang *et al.*, 2004; Prakash *et al.*, 2011). Rhizomes extracts have been found to exhibit antispasmodic and anticonvulsant (Yob *et al.*, 2011), anti-inflammatory (Sulaiman *et al.*, 2010; Zakaria *et al.*, 2010), anticancer (Rashid and Pihie, 2005; Bustamam *et al.*, 2008; Abdul *et al.*, 2008a), antibacterial (Abdul *et al.*,

2008b; Kader *et al.*, 2011), antipyretic (Somchit *et al.*, 2005), antidiabetic (Tzeng *et al.*, 2013), antioxidant (Habsah *et al.*, 2000), antitumour (Wahab *et al.*, 2008) and antiulcer (Al-Amin *et al.*, 2012) effects, while the young shoots have been used as condiments. The main active constituents of the volatile oils of the rhizomes were found to contain zerumbone, humuline and camphene (Jang *et al.*, 2005).

The sesquiterpene compound, Zerumbone is isolated from the wild ginger, *Zingiber zerumbet* Smith plant, which is locally known in Malaysia as 'lempoyang' and belongs to the Zingiberaceae family. Zerumbone has been known for its activity in vitro and in vivo against many diseases. It has been shown to be actively suppress free radical generation and skin tumor initiation and promotion and inhibit inducible nitric oxide synthase, cyclooxygenase 2 expression and TNF- α release in activated leukocytes (Chen *et al.*, 2011), while suppress the formation of colonic tumour marker in rats and

ameliorate the proliferation of human colonic adenocarcinoma cell lines and induces apoptosis in human colorectal cancer cell lines (Murakami *et al.*, 2002; 2004). In addition, Zerumbone has been reported to modulate osteoclastogenesis induced by RANKL and breast cancer (Sung *et al.*, 2009) as well as carcinogenesis process suppression in mouse colon and lung (Kim *et al.*, 2009). Our previous *in vivo* studies have shown zerumbone to have an activity against Cervical Intraepithelial Neoplasia (CIN) in mice (Bustamam *et al.*, 2008).

Medicinal plants represent a substantial source for the development of new anticancer drugs, new drug precursors and new chemical entities despite the advantages of the synthetic ones (Newman *et al.*, 2000; Dikshit *et al.*, 2004). Medicinal plants have a significant contribution to the field of drug discovery as many modern drugs gain their origin from traditional medicine of different cultures. The aim of this work was to evaluate the *in vitro* antiproliferative activity of ZER against different haematological and solid tumours cell lines namely; CEMss, CHO, 3T3, WEHI-3B, HeLa, MCF-7 and MDA-MB-231.

Materials and Methods

Zerumbone (ZER)

ZER was extracted in the laboratory of cancer research MAKNA-UPM, University Putra Malaysia, from the rhizomes parts of the *Zingiber zerumbet* plant (Abdul *et al.*, 2008a). The rhizomes of the plant have been obtained locally from the wet market in Kuala Lumpur, Malaysia. Methanol extraction and Column Chromatography (CC) method has been used to isolate, extract and purify ZER. To determine its molecular weight and purity, the isolated and purified crystals were subjected to High Performance Liquid Chromatography (HPLC) and Liquid Chromatography Mass Spectrometry (LCMS) followed by ¹³C NMR and ¹H NMR analysis to ascertain its molecular structure. ZER stock solution has been prepared in absolute ethanol immediately before use.

Cell Cultures and Maintenance

Solid tumours cell lines used in this study included; HeLa (Human cervical cancer), MCF-7 (human breast cancer), MDA-MB-231 (human mammary cancer - estrogen negative) and 3T3 (mouse fibroblast) were obtained from ATTC, while hematological tumours cell lines included; WEHI-3B (murine monomyelocytic leukemia cell lines) were obtained from ATTC and CEMss (T4-lymphoplastoid) cell lines were obtained from NIH (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: USA). In addition to the normal cells, CHO (Chinese hamster ovary cell line) was obtained from ECACC. All the cell

lines were cultivated in RPMI 1640 supplemented with 10% fetal calf serum, 1% penicillin-streptomycin and 1% amphotericin B. The culture flasks were incubated in a humidified incubator supplied with 5% CO₂ and set at 37°C temperature. They were frequently checked under inverted microscope (Micros, Austria). On 80% confluency, the media was removed and the cells were washed 3 times with 7 mL of PBS (Phosphate Buffer Saline). Trypsin has been used for the detachment of adherent after 5 min incubation. The flasks then were gently tapped to detach the cells and 10 milliliters of RPMI 1640 with 10% FCS was added and the content of the flasks were resuspended to allow the cells to disperse. Then 6 mL of cell suspension was transferred into a 75 cm³ flask. Finally, ten milliliters of RPMI 1640 with 10% FCS was added and incubated in CO₂ incubator at 37°C. The cells were frequently checked under an inverted microscope for confluency and viability.

Cytotoxicity Assay (MTT)

DMSO (Sigma Aldrich, Germany) has been used to solubilize ZER crystals and diluted in RPMI 1640 media to give final concentrations of 100 and 120 µg mL⁻¹, while a stock solution of 60 and 100 µg mL⁻¹ cisplatin were prepared from 1 mg mL⁻¹ stock solution as a control for the test system. Confluent cultures were washed 3 times with PBS, detached trypsin and were incubated for 5 min in the CO₂ incubator. To the detached cells, 10 mL of RPMI with 5% FCS was added into the flask and counted using a hemocytometer. One hundred microliters of cell suspension were plated in each well of 96 well plates at concentration of 1×10⁵ cells mL⁻¹ and incubated for 24 h after which they were treated with different concentrations of ZER and cisplatin. The cells were incubated in CO₂ incubator at 37°C for 3 days (72 h). The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Amresco) assay method used according to that described by Mossman (1983). Five milligrams/mL MTT (Micro culture Tetrazolium) has been prepared, from which 20 µL was added into each well. The aluminum foil covered plates were then incubated in dark at 37°C (5% CO₂) for 4 hours to allow the active live cells to convert water soluble yellow MTT solution into water insoluble purple formazan. Four hours later, the media containing MTT solution was removed and the remaining purple formazan crystals were dissolved in 100 µL DMSO and measured using ELISA plate reader at 450 nm (TECAN, SunriseTM, Männedorf, Switzerland). The median lethal concentration (IC₅₀) value (Concentration at which 50% of the cells are viable and another 50% cells killed) was calculated from the dose-response curve (% cell viability versus concentration of ZER or cisplatin).

Results and Discussion

Seven cell lines have been exposed to increasing concentrations of ZER, four cell lines belong to solid tumours namely HeLa, MCF-7, MDA-MB-231 and 3T3 and two cell lines were of hematological origin WEHI-3B and CEMss. The seventh cell line was non-tumour derived, normal cell lines, CHO cell lines. Cell viability was determined by the MTT assay. In vitro cytotoxic activity (IC_{50} $\mu\text{g/mL}$) of ZER and Cisplatin tested against different tumour cell lines incubated for 72 h is shown in Table 1.

Table 1: *In vitro* cytotoxic activity (IC_{50} $\mu\text{g/mL}$) of ZER and Cisplatin tested against different tumour cell lines incubated for 72 h

Cell lines	ZER IC_{50} ($\mu\text{g/mL}$)	Cisplatin IC_{50} ($\mu\text{g/mL}$)
3T3	6.0	4.6
Hela	6.4	2.8
MCF-7	23.0	6.1
MDA-MB 231	24.3	13.0
CEMss	12.0	8.5
WEHI-3B	5.0	3.5
CHO	25.4	5.0

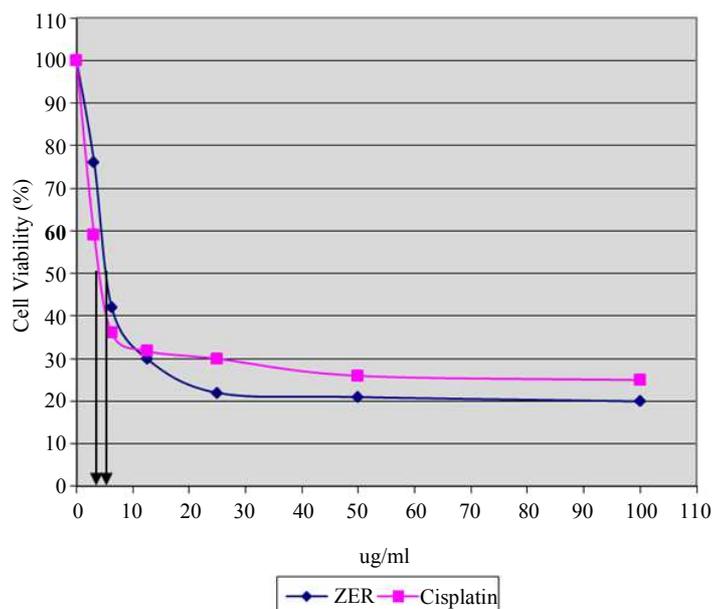


Fig. 1: Antiproliferative activity of ZER and Cisplatin on 3T3 cell lines

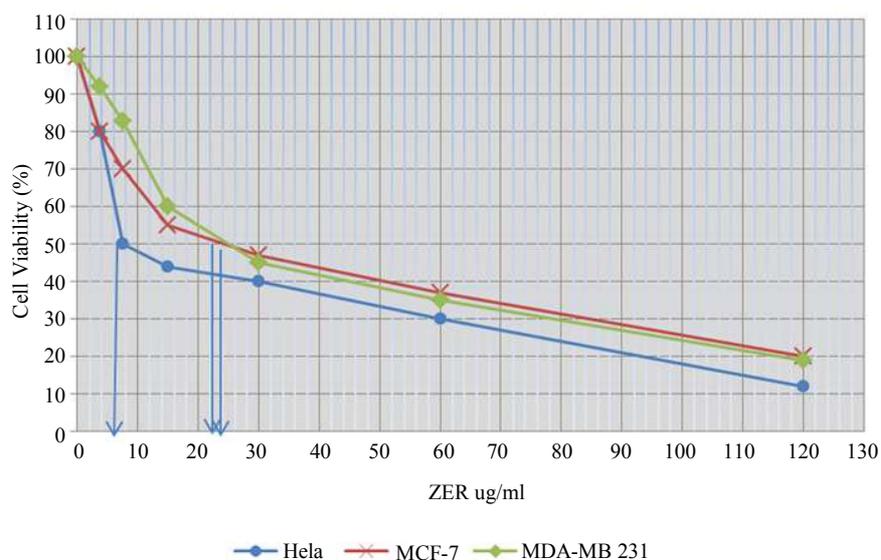


Fig. 2: Antiproliferative activity of ZER against HeLa, MCF-7, MDA-MB 231 cell lines

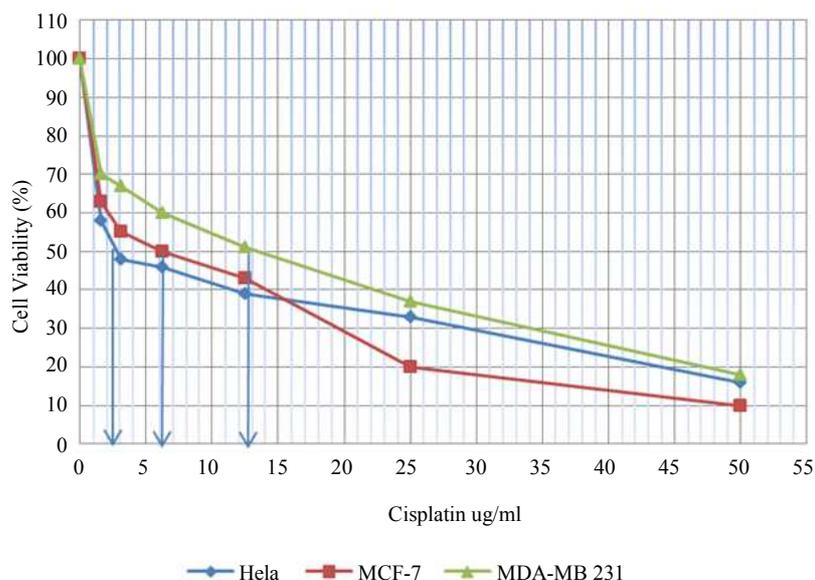


Fig. 3: Antiproliferative activity of Cisplatin against HeLa, MCF-7, MDA-MB 231 cell lines

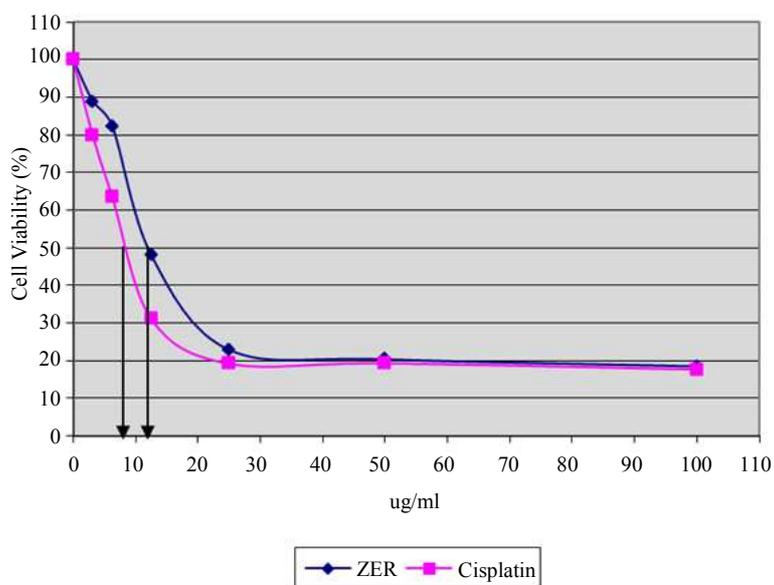


Fig. 4: Antiproliferative activity of ZER and Cisplatin on CEMss cell lines

This study indicated that the higher concentration of ZER, the lesser percentage of cell viability in all tested cell lines (Fig. 1 to 6). This suggests an increase in the toxic effects of ZER with the increase in the concentration. The smaller the IC_{50} of a compound the more toxic compound it was. ZER showed different antiproliferative activities towards the seven cell lines under investigation. Results present the IC_{50} values of ZER (the required concentration to reduce cell viability by 50% after 72 h of incubation compared to control wells) (Table 1). IC_{50} values were determined by plotting

dose response curves for the ZER in the range (1-120 $\mu\text{g mL}^{-1}$) and Cisplatin in the range of (2.5-100 $\mu\text{g mL}^{-1}$). The IC_{50} values of the reference drug Cisplatin were ranged from 2.8-8.5 $\mu\text{g mL}^{-1}$ against all tested cell lines. ZER exhibited effective antiproliferative activity towards WEHI-3B, 3T3 and HeLa cell lines with IC_{50} values of 5, 6 and 6.4 $\mu\text{g mL}^{-1}$ respectively. These three cell lines were shown to be the most sensitive to Cisplatin as well, with IC_{50} values of (3.5, 4.6 and 2.8 $\mu\text{g mL}^{-1}$, respectively). The antiproliferative activity of ZER displayed in this study against human cervical cancer cells (HeLa) was in

agreement with the finding of Alwi *et al.* (2007) while IC₅₀ value of ZER against human breast cancer cells MCF-7 was found to be higher. In accordance with the American Cancer Institute (NCI) protocol, which recommends considering the IC₅₀ values $\leq 30 \mu\text{g mL}^{-1}$ to be potent cytotoxic crude extracts of plant origin, while for pure substances the IC₅₀ values $\leq 4 \mu\text{g mL}^{-1}$ considered to be effective anticancer agent (Geran *et al.*,

1972), ZER with IC₅₀ values approaching the $4 \mu\text{g mL}^{-1}$ could be considered a potent cytotoxic pure substance. The antiproliferative effects of ZER against MCF-7 and MDA-MB-231 tumour cell lines were found to be less effective with IC₅₀ values of 23 and $24.3 \mu\text{g mL}^{-1}$, respectively. Meanwhile ZER cytotoxic activity against CHO non-tumour cell lines was found to be the lowest cytotoxic with IC₅₀ value of $25.4 \mu\text{g mL}^{-1}$.

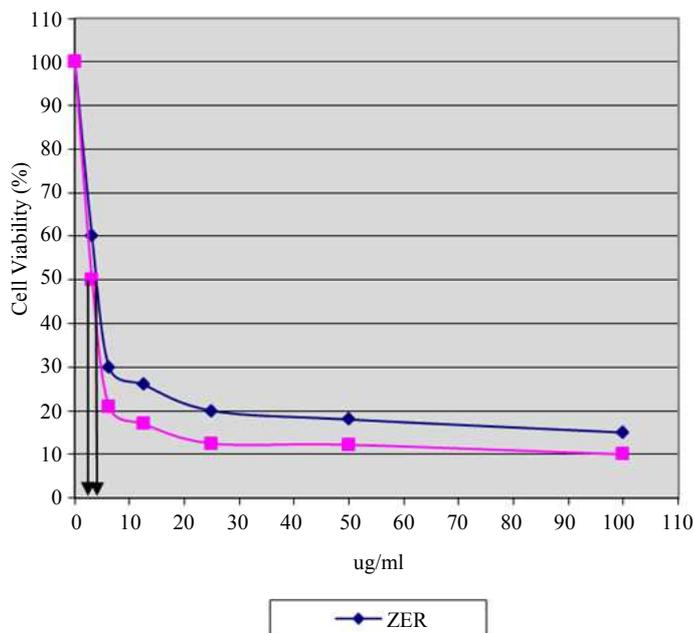


Fig. 5: Antiproliferative activity of ZER and Csiplatin on WEHI-3B cell lines

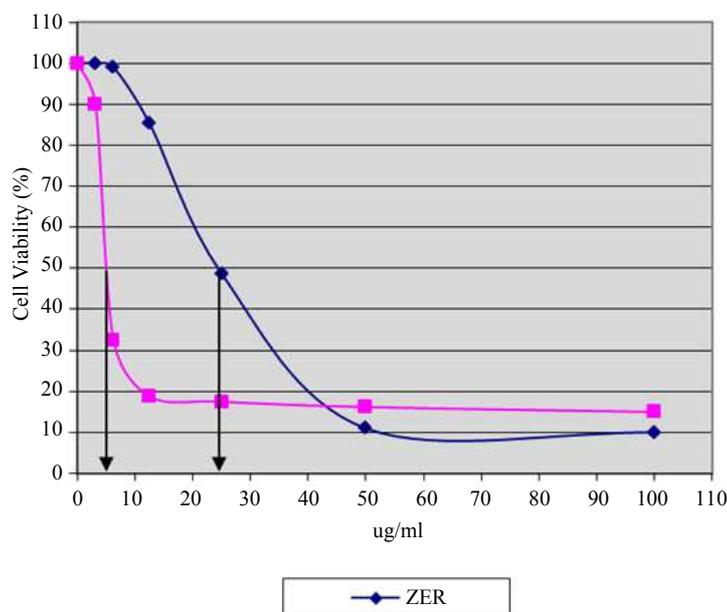


Fig. 6: Antiproliferative activity of ZER and Csiplatin on CHO cell lines

ZER is naturally occurring substance with potent anticancer cytotoxic effects and being extracted from edible plant gains added advantage due to low toxicity over the synthetic anticancer compounds. It has been suggested previously that ZER could act as effective as anticancer agent with a substantial chemotherapeutic potential against cervical cancer cells (Abdul *et al.*, 2008a) and against several tumour cell lines (Sadhu *et al.*, 2007). In the meantime the effects on non-malignant cell was found to be less compared to the malignant cells and this is in agreement with the earlier findings of Hoffmann *et al.* (2002) who observed that appropriate dose of ZER inhibited the proliferation of cancer cells but have no effect on the normal cells (Alwi *et al.*, 2007; Sadhu *et al.*, 2007).

Conclusion

ZER was most cytotoxic against WEHI-3B, 3T3 and Hela with promising concentrations to be used as an anticancer drug. In addition, CEMss cell line was found to be sensitive to ZER while other cell lines were shown to be sensitive to ZER with lesser degree, MCF-7 and MDA-MB 231. The non-tumour cell lines CHO cell lines were shown to be less sensitive to ZER. We can conclude that the effects of zerumbone seem to be cytoselective towards tumour cells.

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Conflicts of Interest

The authors declare no conflict of interest.

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