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Anti-Cancer and Anti-Angiogenesis Activities of Zerumbone Isolated from *Zingiber Zerumbet* – A systematic Review

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Abstract

Significant number of literatures has demonstrated the antiproliferative effect of Zerumbone and its role as anti-angiogenesis. The aims of this systematic review were to assess the anti-cancer effects of Zerumbone and the role of its antiangiogenic properties in treating cancer. Relevant articles were selected based on specific inclusion criteria. Articles chosen for this systematic review were between January 2008 and December 2018. Relevant articles were identified through an extensive search in Science Direct, PubMed, Google Scholar and Scopus. The literature searches of the electronic databases combined the following key words: anti-angiogenic, anticancer, Zerumbone and *Zingiber zerumbet*. Studies chosen for this review includes the following designs *in vitro*, *in vivo* and *ex vivo*.The initial literature search obtained a total of 352 related records and the final number of studies that met the inclusion criteria in the current review was 43 studies. *In vitro* studies were the commonest study design. Evidently, Zerumbone demonstrate a potential antiproliferative and antiangiogenic. The antiproliferative activities of Zerumbone was shown to induce by different signalling pathway. Zerumbone through its antiangiogenic effect play a great role in reducing invasion and metastasis. Some selected studies on Zerumbone were found to plague with limitation such as lack of toxic threshold value which may be needed for the clinical trials on this compound.

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Keywords

Anti-Angiogenesis;
Anti-Cancer;
Zerumbone;
Zingiber zerumbet.

Introduction

In the US and worldwide, the second most common cause of death is cancer. Dysregulation of the molecular pathways which alters protein expression resulting in uncontrolled cellular proliferation is the most identified underlying mechanism of cancer.[1] There has been a significant improvement in anticancer drug discovery using herbal medicine.

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Poor effectiveness, unwanted side effects, and tumour resistance were associated with drugs that target particular signalling pathways, while drugs that interfere with multiple molecules responsible for tumour growth have more efficacy and targeting different cancer cell line. Multi-targets drugs are considered to be more effective and less susceptible to drug-resistance.[2]

Angiogenesis, a process where new vasculature initiated from pre-existing normal vessels. Initiation of angiogenesis starts with localized release of pro and anti-angiogenesis growth factors by endothelial cells (ECs).[3]

New blood vessel inhibition properties of angiogenic proteins in cancer cells will induce endothelial cell apoptosis or inhibiting endothelial receptors. Plant derived anti-angiogenesis agents are unlike conventional chemotherapeutic agents. It can be used in lower doses with less cytotoxicity, and over a period of months to years to prevent recurrence from latent micro metastases.[2]

Around 100 new natural products have been established for cancer therapy and this type of research is still on going and needed in cancer therapeutic research area. To date only small part of rainforest plant have been studied in cancer research even the potential of this work have been more relevant and pressing from times to times. *Zingiber zerumbet* from Zingiberaceae family is one of natural product contains a range of different phytochemical groups. This plant is highly consumed in the traditional Asian diet. Zerumbone is one of these phytochemicals.[4] It inhibits cell proliferation in various cancer cell lines with negligible side effects on normal cells and it also exhibits antiangiogenic activities in cancer.[5]

Despite numerous researches focused on the effects of Zerumbone as anti-cancer and anti-angiogenesis compound, limited evidence-based systematic review regarding these topics available.

Zerumbone extracted from *Zingiber zerumbet* is proven to have anti-cancer and anti-angiogenesis properties. The effect of Zerumbone on cancer cells has been established through *in vitro*. *in vivo* and *ex vivo* researches. Receptor and mitochondrial signaling pathway are the first target in the cancer therapy. Studies has shown that the most cytotoxic drugs trigger cell death by targeting cytochrome-c/ Apaf-1/caspase-9–dependent multiple proapoptotic and anti apoptotics ignaling pathways. Various signaling pathways can modulate apoptosis and angiogenesis by converging on, thereby altering the activity of, common central control points within the apoptotic and angiogenic signaling pathways, which involve related proteins or mediators such as caspases, Bcl-2 proteins,VEGF and chemokines. Zerumbone displays promising anticancer effects through several mechanisms of actions such as cell cycle arrest, apoptosis, modulate multiple molecular targets and inhibition of tumour angiogenesis.[6,7]

This systematic review aimed to systematically summarize studies done on Zerumbone, including its mechanisms of actions, therapeutic and prophylactic potential against different cancers and its role as angiogenesis inhibitor.

Methodology

Search Strategy

For identifying articles used in this systematic review, two separated searches were conducted, both searches were performed through four databases which were Science Direct, PubMed, Google Scholar and Scopus. The last search was performed on 11/01/2019. The combinations of the following keywords were used in the first survey: "anticancer, Zerumbone, *Zingiber zerumbet*". The keywords for second search included "anti-angiogenesis, anti-angiogenic, anticancer, Zerumbone and *Zingiber zerumbet*".

Inclusion and Exclusion Criteria

The inclusion criteria for selecting relevant studies were as follows: article published between 1/01/2008 to 31/12/2018, articles on anticancer properties of Zerumbone extracted from *Zingiber zerumbet*, articles on antiangiogenic activity of Zerumbone as one of the roles for their anticancer effect, study designs using *in vitro*, *in vivo* and *ex vivo*. Articles which were written in language other than English, and articles that reported the activity of Zerumbone from unknown source were excluded.

Data Extraction

All selected articles that reported the effect of Zerumbone on cancer cells, and role of their angiogenic properties in anticancer effect were

further analysed. These following information were gathered: authors, publication year, design of a study, country, sample size, dose and duration, cytotoxic effect on cancer and normal cells and mechanism of its cytotoxic effect.

Article Selection

The articles retrieved from the databases were screened for duplication. After removing the duplicated articles, a reviewer independently screened the retrieved relevant articles by reading the full copy or abstract; inclusion and exclusion criteria were considered at this stage. In addition to that, manual search was conducted from the bibliography section of the selected articles. Finally, supervisor and co-supervisor reviewed the selected articles.

Results

Search literature

The literature searched conceded a total of 352 records (from January 2008 to December 2018). The numbers of articles obtained from the corresponding databases are as follows; Google scholar (n=142), Science Direct (n=134), PubMed (n=48) and Scopus (n=28). After the online search, duplicate articles were removed. The remaining 146 articles were screened by referring to the title and abstract. 84 articles were excluded due to nonfulfillment of inclusion criteria. From which 62 articles were further evaluated based on the full text, and a total of 19 articles were removed; two were using Zerumbone derivatives, two articles were review articles, six articles did not mention source of Zerumbone from *Zingiber zerumbet* clearly, one not related to anticancer nor antiangiogenic effect of Zerumbone, three were computational study and five no full text available. Finally, only 43 articles met the inclusion criteria and were further analysed in this review. The summary of search strategy was depictedin Figure 1.

The number of studies conducted on Zerumbone is strongly correlated to the geographical distribution of *Zingiber zerumbet*. The highest number of studies conducted on Zerumbone was recorded from Malaysia (15 studies), followed by India (7 studies), China (7 studies), USA (5 studies), Korea (4 studies), Japan (1 study) and Taiwan and Saudi (each of them 2 studies)

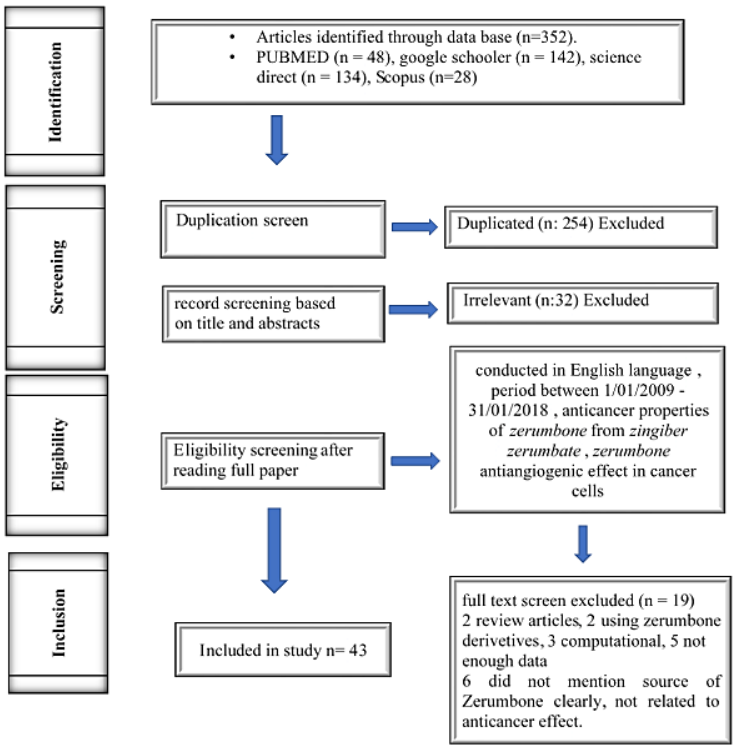

Fig.1: Summary of search methodology

Classification of Included Articles Describing Anticancer and Anti-Angiogenic Activities of Zerumbone

The total number of articles included in this systematic review is 43. The articles were classified according to the models of study applied; animal *in vivo*, *in vitro* and *ex vivo*. Two *in vivo* studies using animal models, 34 *in vitro* studies, and 7 studies combining *in vitro* and *in vivo* models. Two of the articles had combined *ex vivo*, *in vivo* and *in vitro* studies reported on the anti-angiogenic effect of Zerumbone.

***In vitro* Studies with Anti-Cancer and Angiogenetic Effect**

Thirty-four *in vitro* studies have been selected for this review. Majority of the papers are from tropical countries that are rich in ginger plant. Malaysia was the highest country that published articles on this compound.

Table 1: Summary of *in vitro* anticancer and antiangiogenic effects of Zerumbone

Cell line	Targeted proteins and pathways	Cytotoxic, invasion and migration effects	IC50 value	References
HeLA, Coav-3 and MCF-7	↑ caspase-3 in ZER treated HeLA ZER stimulate apoptosis of HeLA cells	ZER has stronger cytotoxic effects toward cancer cells compared to normal cells.	The IC_{50} value HeLa 20.30, Coav-3 24.30 & MCF-7 27.7 µM.	Abdel Wahab *et al.*, 2009[4]
Non-small cell lung cancer (A549) cells	↑ E-cadherin, ↓ TGF-β1 induced cell scattering and epithelial-mesen -chymal transition.	Inhibit invasion & migration of A549 cells.	IC_{50} values of 21 µM at 72 h.	Hseu *et al.*, 2015[23]
CEMss, CHO, 3T3, WEHI-3, HeLA, MCF-7 and MDA-MB-231		ZER was most cytotoxic against WEHI-3B, 3T3 and Hela. non-tumor cell lines CHO cell lines were shown to be less sensitive to ZER.	ZER IC_{50} value in µg/mL after 72hs, 3T3 6.0, Hel A 6.4, MCF-7 23. MDA-MB231 24.3, CEMss 12.0, WEHI-3B 5.0, CHO 25.4µM/ml.	Al-Zubairi, 2018[8]
MCF7/HER2, KBM-5, U266, H1299, SCC4, PANC-1, PANC -28, MIA PaCa-, AsPC-1, and A293	down-regulation of CXCR4at the transcrip -tional level → down-regulation of HER2 in breast cancer cells. ZER ↓ of NF-KB in MCF7/HER2 cells.	ZER Inhibit metastasis & invasion of cancer cells. ZER ↓ expression of CXCR4 protein in HER2-overexpre-ssing breast cancer cells.	Not mentioned	Sung *et al.*, 2008[39]
DU145 and PC3 cells prostate cancer cell line	ZER blocked IL-6-induced JAK2 and STAT3 phosphorylation in DU145 cells within 12 h, ZER induce cell cycle arrest at G0/G1 phase by 12 h.	ZER exerts anticancer effects against hormone refractory DU145 prostate cancer, ZER synergistically improves PTX sensitivity in DU145 cells.	IC_{50} value of ZER 24 µmol/l in DU145, IC_{50} of ZER in PC3 42 µmol/l IC_{50} of ZER in non-neoplastic human cells 57µmol/l.	Jorvig and Chakraborty, 2015[24]

	ZER ↑ PARP cleavage → ↓ caspase-3 cleavage during apoptosis, ZER ↓ Cyclin D1, STAT3 -regulated antiapoptotic proteins like survivin, c-Myc, and Bcl-2 were downregulated after 48h.			
DU145 and PC3 cells human hormone refractory prostate cancer cell line	MPM-2 expression was significantly ↑ in both PC-3 and DU-145 cells the induction of mitotic arrest inhibits tubulin assembly	ZER induce apoptosis and autophagy in HRPCs.	in PC-3 cells the IC_{50} values after 24 (16.9) and after 48-h 9.1 μM in DU-145 cells after 48h 6.9 and after 72-h 6.1μM .	Chan *et al.,* 2015[21]
Human CRC cell lines (HCT116 cells, HT29 and SW620 cells)	Pretreatment with ZER ↓ the radiation-induced expression of DNAPKcs at all the time points, and completely abolished the DNA-PKcs expression at 6 h post-IR. ZER significantly enh-anced radiation-induced G2/M arrest at 2 Gy radiation in cells treated with 25 μmole/L ZER.	ZER inhibited the proliferation of all CRC cell lines tested (HCT116, HT29 and SW620). ZER treatment significantly enhanced the radiation-induced apoptosis.	HCT116 cells (IC_{50} 30 ± 1.5 μmole/L), SW620 (IC_{50} 38.8 ± 1.2 μmole/L), HT29 HT29 (IC_{50}> 46 μmol/L).	Deorukhkar *et al.,* 2015[22]
Acute lympho-blastic leukemia cells CEM-ss cells	Caspase-3 enzyme is ↑ in ZER treated CEM -ss cells as compared to non-treated cells.	ZER inhibits the proliferation of T-acute lymphoblastic leukemia cells (CEM-ss) via the induction by apoptosis.	IC_{50} 8.4±1.9 μM/ml.	Abdel Wahab *et al.,* 2011[36]
Ovarian (Caov-3) and cervical (HeLa) cancer cells	ZER ↓ IL-6 in Caov-3 and HeLa, both cell lines were still expressing IL-6R after ZER treatment. ZER induce cell cycle arrest at G2/M phase.	ZER inhibits interleukin-6 and induces apoptosis and cell cycle arrest in ovarian and cervical cancer cells.	IC_{50}: Hela 20.0± 0.9μM,Caov-3 24.0± 0.9 μM	Abdel Wahab *et al.,* 2012[34]
Laryngeal carcinoma cells (Hep-2)	ZER arrested Hep-2 proliferation at S and G2/M phases of cell cycle.	ZER induce apoptosis in Hep-2 cell line, ZER at a concentration of 15 and 30 μM for 48 h	IC_{50} value of 15 μM.	Jegannathan *et al.*, 2016[19]
Human pancreatic carcinoma cell lines	ZER ↑ the activity of caspase-3 in PANC-1 cells 56%, to 197%,	ZER was able to induce apoptosis of pancreatic carcinoma cell lines,	IC_{50} ZER in PANC-1 at 24h, 48h, and 72h was 57.8± 6.2,	Zhang *et al.*, 2012[35]

PANC-1 and SW1990	ZER ↑ generation of ROS, pretreatment with ZER 30 µM significantly ↑ the expression of p53 protein in PANC-1 cells.	exposure of PANC-1 cells to ZER resulted in a significant reduction of cellular viability.	47.4 ± 7.5, and 33.6 ± 7.9 µM.	
Human hepato-cellular carcinoma (HepG2) cell line WRL68 cells normal cell line	ZER stimulate caspase -3 and-9 activities in the HepG2 cells, ZER induce cell cycle arrest by ↑ in the G2M phase of the cell cycle.	ZER inhibited the proliferation of HepG2 cells in a dose-dependent manner, distinct apoptotic ultra-morphological changes were observed in ZER-treated HepG2 cells.	In HepG2 IC_{50} (6.20 µg/ml)	Samad *et al.,* 2015[5]
Human peripheral blood lymphocyte, CHO cell line	Mitotic index in highest ZER dose (80 µm) revealed significant inhibitory effect, Treatment of CHO with ZER cause significant increase MN (micro-nucleus) induction at 50µg/ml.	ZER showed the ability to induce genotoxicity & cytotoxicity in CHO cells but less effects were observed on human PBL.	Not mentioned	Al-Zubairi, 2016[18]
WEHI 7.2 wild type murine thymoma cells, Chang liver cells	ZER induce apoptosis in treated WEHI 7.2 wild type cells	ZER is potent cytotoxic effect on WEHI 7.2 wild type murine thymoma cells, also ZER can induce genotoxicity in the cells.	IC_{50} values in WEHI 7.2 at 24, 48 and 72 hours were 13.832 µM, 12.503 µM and 12.137 µM respectively.	Hamid *et al.*, 2017[12]
Human cervical carcinoma lines SiHa	ZER induced block¬age of G1 cell cycle in SiHa cells, ZER markedly ↑ the production of ROS, MMP was ↓ by ZER, ZER induced the cleavage of the pro-apoptotic Bax protein and degrada¬tion of Bcl-2.	ZER significantly reduce cellular viability, ZER significantly increased the apoptotic rate of SiHa cells.	Not mentioned	Zhou *et al*., 2017[17]
SW480 cell lines human colon cancer	ZER induced apoptosis through ↑ cellular ROS and ↓ antioxidant level, ↑ cell in G2/M phase, ZER ↑ number of DNA fragmented cells, ZER alter the mitochon-drial membrane potential and induce extrinsic and intrinsic apoptotic pathways.	ZER lead to damage cell membrane integrity and DNA Fragmentation, ZER at high concentration demonstrated complete disappearance of actin filaments and total damage of the actin network in the cells.	IC_{50} value of 160 µM for 24 h and 102 µM for 48 h	Sithara *et al.,* 2018[10]

Human umbilical cord blood endothelial progenitor cells, HCT116 Ovcar8, HeLa, SW480 and T47D MCF-7 and SiHa	ZER induced cytochrome c release, ZER induced apoptosis through ++ Bax , Bcl 2 and Bcl-XL,	ZER induced cell death in most of the cell lines in time dependent manner, calcium dependent calpains and ROS contribute to Bax activation leading to cytochrome c release from mitochondria	Not mentioned	Sobhan *et al.,* 2013[32]
Hela	ZER ↑ Caspase-3	ZER able to induce cancer cell death.	IC_{50} value of 11.3 µM for 24 h	Abdel Wahab *et al.,* 2009[4]
HepG2, human umbilical Vein endothelial cell (HUVEC), and WRL68 cells	ZER downregulate phosphorylation of VEGFR 2 in HUVECs, ZER inhibited expression of VEGF, MMP-9, angiogenin, and angiopoietin ZER ↑ expressionof endostatin and thrombospondin. ZER downregulate VEGF, Matrix metalloproteinase 9 (MMP 9), and NF ϰB expressions.	ZER is an inhibitorangiogenesis, tumor growth, and spread, ZER inhibited neovascularization by 70%, ZER ↓ on HepG2 cell lines cell migration.	HepG2 cell IC_{50} concentration (6.2 µg/mL) of ZER for 72 h	Samad *et al.,* 2015[5]
Human melanoma cell line CHL 1	ZER significantly ↑ ROS production in CHL 1 cells. ZER ↓ MMP of CHL 1 cells,	ZER has chemotherapeutic effects on human melanoma cells by altering mitochondrial function. ZER significantly decreased cell proliferation & inhibited CHL 1 cell migration.	Not mentioned	Yan *et al.,* 2017[16]
Breast cancer cell line MDA-MB-231, MDA-MB-468, MDA-MB-361, T-47D MCF-7 and MCF-10A cells	ZER down-regulation of CD1d.	ZER treatment significantly decreased the expression of CD1d, LDL-r and prosaposin.	Not mentioned	Shyanti *et al.,* 2017[14]
Esophagus cancer EC-109 cells	ZER ↑ expression of P53 and ↓ expression of Bcl-2, Bcl-2 was significantly decreased at level of mRNA expression.	ZER can inhibit the proliferation and induce apoptosis of esophageal cancer EC-109 cells,	Not mentioned	Ma *et al.,* 2018[9]
Human RCC cell line 786-O and Caki-1	ZER down regulates the Expression of Cyclin D1, Bcl-2, Bcl-xL, Mcl-1,	ZER induce apoptosis in 786-O cells.	Not mentioned	Shanmugam *et al.*, 2015[29]

	Survivin, MMP-9, and VEGF in RCC Cells, ZER Inhibited STAT3 Phosphorylation in 786-O Cells. Tyrosine Phospha -tases are involved in ZER induced inhibition of STAT3 Activation, ZER suppressed phospho-rylation of JAK1 ZER increased accumu-lation of cell population in sub-G1 phase,			
Colon cancer cell line HCT15	ZER arrest HCT15 cell cycle in G2/M phase	ZER exhibited the cytotoxic effect on colorectal cancer cell line HCT15	IC_{50} of ZER 18µg/ml at 24h, 9 µg/ml at 48h	Thiyam and Narasu,2017[15]
Human renal clear cell carcinoma 786-O and 769-P cell lines	ZER activated caspase -3 and caspase-9 , ZER significantly suppressed the expression of Gli-1 and Bcl-2.	ZER-induced apoptosis by activation of the caspase cascade and deregulation of the Gli-1/Bcl-2 pathway.	Not mentioned	Sun *et al.*, 2013[33]
CML-K562 cells	ZER induces DNA damage, caspase-3, -9 activation, and PARP cleavage in K562 cells. ZER ↑ calcium, ROS, and loss of mitochondrial membrane potential in K562 cells.	Apoptosis induction by ZER was found to be mitochondria mediated, involving increased free intracellular Ca2+, ROS, and upregulation of soluble histone H2AX.	IC_{50} value: 3.5 µg/mL	Rajan *et al.,* 2015[28]
Oral squamous cell carcinoma (OSCC) ORL-48 and 115	ZER (30 µM) ↑ caspase3 and cleaved PARP levels, ↑ apoptotic cells in OSCC, ↑ cycle cell arrest in G2/M phase, ↓ of CXCR4 protein levels, ↓ active RhoA in ORL-48 & ORL-115 cells with no changes in total RhoA expression. inhibit Akt phosphorylation,	ZER inhibited OSCC proliferation and induced cell cycle arrest and apoptosis ZER caused reduction of cell proliferation by ~60% for both lines.	IC_{50} value: 0.8 –4.9 µM)	Zainal *et al.*, 2018[11]
Breast cancer cell line MDA-MB-231, MCF-7, and MCF-10A cell line	ZER ↓ of cyclin B1, cyclin-dependent kinase 1, Cdc25C, and Cdc25B, ZER ↑p53 and induction of PUMA,	ZER-induced cell cycle arrest and apoptosis.	IC_{50} for ZER in MDA-MB-231 cells after 24 and 48 h was 9.9 µM and 4.2	Sehrawat *et al.*, 2012[44]

	induce G2/M phase cell cycle arrest, ZER activate of Bax and Bak in both MDA-MB-231 and MCF-7 cells,		μM respectively, IC_{50} for ZER in MCF-7 cells after 24 and 48 h was 10.1 and 2.9 μM respectively.	
WM1552C human melanoma cell line	ZER ↑ level of PARP and cleaved PARP proteins. ZER ↓ the phospho-MAPK ZER inhibited TNF-α induced NF-κB activation in WM1552C cells.	ZER-induced apoptosis in WM1552C.	IC_{50} = ZER 25μm	Ni, 2013[43]
Human non–small lung cancer cell lines, NCI-H1299 and NCI-H460	ZER ↓ HSP27 protein expression, Inhibition of the monomeric form of HSP27 by ZER was correlated with production of cross-linking of HSP27, ZER before radiation inhibited the binding affinity between HSP27 and apoptotic molecules, such as cytochrome c and PKCd.	ZER exhibited sensitization to the radiation. ZER was involved in altered cross-linking of HSP27, which correlated to its sensitization to radiation.	Not mentioned	Choi *et al.,* 2011[45]
Human peripheral blood lymphocytes, Chinese Hamster Ovary (CHO) cells	ZER has a genotoxic activity after treatment with high concentrations (40 - 80 μM) inducing ↑ in the frequency of binucleated cells with MN (BNMN).	ZER can exert cytotoxic and genotoxic effect in human and CHO cells.	IC_{50} value = 20.0 (± 5.1) μM.	Al-Zubairi *et al.,* 2010[2]
Human umbilical vein endothelia cells (HUVECs)	ZER interacted with the VEGF/VEGFR2 signaling pathway. ZER at dose>100 μM inhibited the proliferation of HUVECs.	ZER had no effect on cytotoxicity in HUVECs.	Not mentioned	Park *et al.,* 2015[26]
Hs578T and MDA_MB231 Brest cancer cells	ZER ↓ basal level of IL-8 and MMP-3 mRNA expression in Hs578T breast cancer cells ZER inhibits IL-1ß induced IL-8 and MMP-3	ZER inhibits IL-1ß induced cell migration and invasion in Hs578T and MDA_MB231 breast cancer cells.	Not mentioned	Han *et al.,* 2014[30]

	expression in Hs578T cells.			
SGC-7901 cells gastric cancer	ZER downregulated cyp A expression ZER ↓expression of BcL-2	ZER suppress the viability and induced apoptosis of gastric cancer SGC-7901 cells	IC_{50} value obtained was around 12.27 µM.	Wang *et al.,* 2016[27]
A549 cells. Non-small cell lung cancer cell.	ZER ↓ osteopontin -induced ROCK/LIMK /cofilin signaling pathway. ZER ↓ osteopontin-induced lamellipodia formation in A549 cells ZER impairs osteopontin -induced the FAK/AKT pathway in A549 cells.	ZER suppresses osteopontin-induced invasion and of A549 cells.	Non cytotoxic concentration 10 µM	Kang *et al.,* 2015[25]
A375 cells melanoma cells.	ZER ↓ Bcl-2 and ↑ Bax expression. ZER ↑ cytochrome c and the activities of caspase-3,	ZER exerts anti prolife-rative effect on A375 cells through mitochondrial -mediated pathway.	A375 IC_{50} = 20 µM	Wang *et al.,* 2016[20]
Rat liver cells	↑ Bax and ↓ Bcl-2 protein	ZER reduce oxidative stress, inhibits proliferation, induces mitochondria-regulated apoptosis in liver cancer cell.	Not mentioned	Taha *et al.,* 2010[6]
HCT116, H1299, A293, PC3, DU145, MDA-MB-231, MCF-7 and AsPC-1.	ZER sensitize HCT 116 to TRAIL-ZER induces expression of TRAIL receptor DR4 and DR5in cancer cells which dependent on reactive oxygen species and reversed by MAPK. ZER ↓ the expression of cFLIP, and ↑ p53	ZER enhance TRAIL induced apoptosis through regulation of receptor of DRs in most cancer cells.	Not mentioned	Yodkeeree *et al.,* 2009[37]
MDA-MB-231 cells	ZER ↑ caspase-3 and caspase-9 activity ZER ↓ of Bcl-2 and Bcl-xl while Bax and cytochrome ↑.	ZER induced apoptosis via intrinsic pathway via activation of caspase-9 and caspase-3	IC_{50} = 5.96 µM/ml	Hosseinpour *et al.*, 2014[31]

↑ elevate or stimulate, ↓ inhibit or reduce, ZER Zerumbone

As summarized in Table I, all studies have demonstrated significant anticancer effect of Zerumbone on wide variety of cell lines. MCF-7 (human breast cancer) is the commonest cell line used (6 articles) followed by Hela (human cervical cancer) (5 articles), HCT116 (human colon cancer cell line) and PC3 (human prostate cancer cell line) which accounted for 4 studies each.

Discussion

Cell Viability

Cytotoxic evaluation of Zerumbone was carried out in different cell lines. The IC_{50} (the concentration needed to decrease cell viability by 50% after incubation for 72 h) value was reported in 25 articles. Interestingly, the included studies showed that Zerumbone can affect all tested cell lines and reduced the cell viability of cancer cells in a dose dependent fashion.[8] It is worth to mention that the highest concentration of Zerumbone associated with the lowest cell proliferation in all tested cell lines. The IC_{50} values was ranged between 3.5 to 46 µM. Zerumbone shown to be less toxic on normal cell line.[8] Range of IC_{50} value in the selected articles was illustrated in Figure 2.

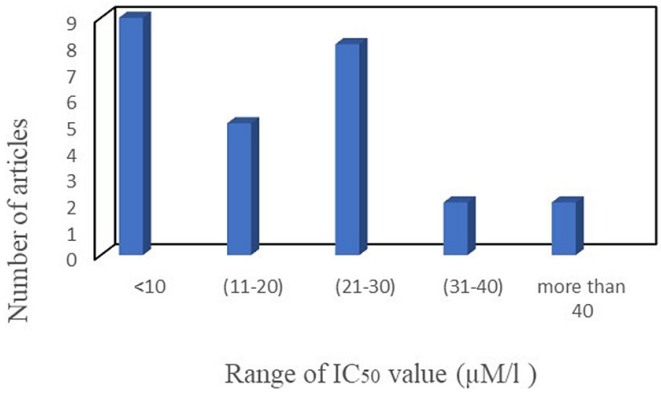

Fig.2: Range of IC_{50} values reported in the selected articles

The antiproliferative activities of Zerumbone showed variable results on a different types of cell lines. The lowest IC_{50} value was reported by Rajan *et al*.[28] on CML-K562 cells (chronic myelogenous leukemia cells) which was 3.5 µM, thus suggesting that Zerumbone might be significantly toxic on chronic myeloid leukemia.

Furthermore, Al-Zubairi[8] found that Zerumbone was effective on WEHI-3B (murine monomyelocytic leukemia), 3T3 (mouse fibroblast) and Hela (Human cervical cancer) cell lines with 5, 6 and 6.4 µg/mL as respective IC_{50} values, followed by CEMss cell line (T4-Lymphoplastoid) with an IC_{50} value of 12 µg/mL. This proposes the possibility of using Zerumbone in the treatment of hematological cancers and the cervical cancer.

Induction of Apoptosis and the Morphological Changes Induced by Zerumbone

Apoptosis is a biological phenomenon which is defined as programmed cell death, the findings from this indicated the role of Zerumbone in triggering apoptosis.Twenty-seven studies involved reported on apoptosis as an anti-cancer effect of Zerumbone. Abdel Wahab *et al*.[c] showed morphological changes of cell apoptosis after Zerumbone treatment by using scanning electron microscopy (SEM). In addition, Zerumbone caused significant elevation inthe cellular level of caspase-3 in treated cancer cells, as well as an increase in the number of TUNEL-positive stain, which are also characteristics of apoptosis. Similarly, Samad *et al*.[5] and Hosseinpour *et al*. [31] described Zerumbone-treated cell morphological changes in cancer cell lines which further suggested that Zerumbone induced apoptosis. Those morphological changes included membrane blebbing and hole formation, cell shrinkage, increase in surface irregularities and apoptotic body formation.

On the other hand, Rajan *et al*.[28] confirmed apoptosis in Zerumbone treated-CML (chronic myeloid leukemia) cells by demonstrating elevation of signaling molecules which have been responsible

on the induction of apoptosis (pro-caspase-3, -9 activation) and poly ADP-ribose polymerase (PARP) cleavage in western blot analysis, the latter is a hallmark of DNA damage.

Apoptosis Signaling Pathways of Zerumbone

Despite of a greatnumber of studies intended to understand signaling pathways of Zerumbone on cancer cell, the exact mechanism is still unclear. So far, there were multiple pathways targeted by Zerumbone recorded in the articles. This can explain the capability of Zerumbone in treating different type of human cell cancers.

Several findings reported various signal pathways targeted by Zerumbone. Nine studies demonstrate caspase 3 and caspase 9 activation as the key in Zerumbone induced apoptosis. Furthermore, six studies stated that Zerumbone induced cell cycle arrest in the G2/M phase. Zerumbone also showed potential in upregulating production of ROS (reactive oxygen species), downregulating protein BCl-2 (anti-apoptotic protein) and upregulating of Bax protein (pro-apoptotic). These were explained in 4 studies. Several studies also substantiated that the loss of mitochondrial membrane potential and caspase activation dependent on cytochrome c was frequently observed incell death induced by Zerumbone. The possible apoptosis signalling pathways of Zerumbone is illustrated in Figure 3.

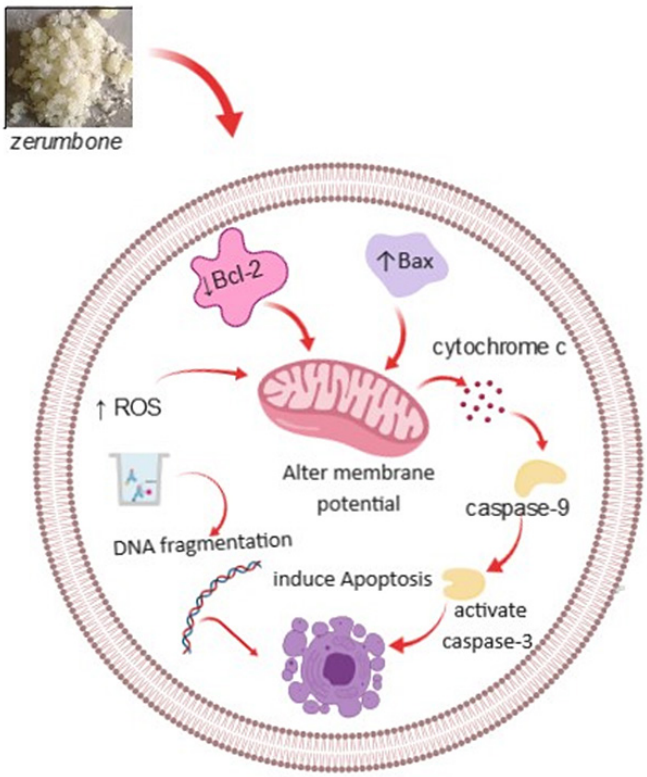

Fig. 3: Apoptosis signaling pathways of Zerumbone

Numerous biochemical processes have been distinguished as of great consequence in apoptosis and the key mediator for this process is caspases with caspase-3 as a main promoter of apoptosis. Abdel Wahab *et al*.[4] stated that Zerumbone induced significant elevation of caspase-3 that play crucial role in the initiation of apoptosis. This finding was supported by Samad *et al*.[5] results, which stated that the stimulation of caspase-3 and-9 showed by Zerumbone treatment in HepG2 cells. This indicated that Zerumbone induced apoptosis caused by the mitochondrial death pathway.

Moreover, sensitization of mitochondria can be driven under certain condition such as oxidative stress and, upshot of reactive oxygen species known activator of MPTP (mitochondrial permeability transition pore). Opening of these pores will lead to mitochondrial depolarization. Mitochondria will not be able to produce ATP anymore, and thus, the cell will die. Sithara *et al*. 10 reported that Zerumbone exposure will induce generation of reactive oxygen species, decrease the cellular antioxidant status and loss of mitochondrial membrane potential, the mitochondria membrane potential loss was preceded to initiate the intrinsic pathway of apoptosis through stimulate pro caspase 9 and activated caspase 3.

Correspondingly the data from Chan *et al*. [21] suggested that Zerumbone induces a crosstalk between endoplasmic reticulum (ER) stress which supported by upregulation of GRP-78 ER chaperone protein that induce assembly of newly-synthesized proteins), upregulation of CHOP expression (a transcription factor that regulates apoptosis in response to cellular stress), and rapid raise of intracellular Ca2+ levels together with the active calpain I (a protein belonging to the family of calcium-dependent proteolytic enzyme) formation. Through calpain and caspase-mediated cleavage of autophagy-regulated proteins Zerumbone can induce autophagy and apoptosis at the same time, this may have been explained by autophagic proteins implied to in regulate mitochondria-involved intrinsic apoptosis. Furthermore, Zerumbone suppressed the assembly of tubulin and elevated the expression of MPM-2 (anti-mitotic proteins).

Similarly, Sobhan *et al*.[32] reported that the activation of reactive oxygen species as well as calpains is the essential early step incell death induced by Zerumbone. Mitochondrial pathway was the main pathway in Zerumbone induced apoptosis.

Zerumbone induce apoptosis mainly through intrinsic pathway. The fundamental step in this way is of mitochondrial outer membrane permeabilization. This essential event was controlled by Bcl-2 family. Zerumbone implicated in decreasing the expression of anti-apoptotic proteins such as Bcl-2, Bcl-xL proteins, and increasing the expression of proapoptotic Bax protein. Additionally, over expression of Bcl 2 family proteins such as Bcl 2 and Bcl-XL generally inhibit Bax dependent cell death by Zerumbone. On the other hand, incells deficit of Bax expression (Bax knockout cells), Zerumbone failed to induce apoptosis or mitochondrial permeabilization.[9,31,32,44]

In the other hand, Zainal *et al*.[11] and Zhou *et al*.[17] demonstrated that Zerumbone had significant cytotoxic effect. Production of the reactive oxygen species (ROS) induced by Zerumbone, stimulated releases of cytochrome c (cyt-c) into the cytoplasm which has ability to downregulate mitochondrial membrane potential (MMP), Zerumbone increased Bax/Bcl-2 ratio, and concomitant with caspase-3 activation.

Yodkeeree *et al*. [37] reported the role of TRAIL (tumour necrosis factor–related apoptosis-inducing ligand) in apoptosis induced by Zerumbone. It mediated the activation of extracellular signal-regulated kinase 1/2 and p38 mitogen activated protein kinase which results in DR4 production and DR5 induction. This process then enhances the anticancer effects of TRAIL. TRAIL is one of the tumour necrosis factor family, itis postulated to regulate cancer cells death through different receptors, such as DR4 and DR5, which are transmembrane receptors mediating apoptosis. Stimulation of DR4 and DR5 will result in caspase-8 activation, which will lead tocaspase-9 and caspase-3 activation.

Zerumbone also induced p53 protein expression. P53 is tumour suppressor gene with a critical role in regulating apoptosis in cancer cells. Despite that, p53 expression has been linked to the expression of DR4 and DR5. DR5 expression induced by Zerumbone was mediated through a p53-independent mechanism.[9,37]

Zerumbone also shows anticancer activity in PANC-1 cells (pancreatic cancer cells) via promoting the upregulation of p53 protein expression and increasing the p21 and miR-34 level, ROS production was shown to be elevated by nearly 149% in PANC-1 cells. It was previously established that p53 activation, induced the increase expression of miR-34 and p21. Zerumbone increased miR-34 and p21 in Zerumbone-treated cancer cells, overexpression of miR-34 preceded growth arrest in cancer cells.[35]

Zerumbone has the potential to be used as an adjuvant to potentiate immunotherapy of advanced

stages of breast cancer, through inhibition of CD1d expression. CD1d is a lipid antigen presenting molecule and is an indicator for recognition by NKT cells. Advanced stage of breast cancer is associated with increased CD1d expression by the cell. Zerumbone reduced CD1d expression by modifying the antigen presentation pathway and thereby inhibiting cell proliferation.[14] Obviously, these findings confirmed that Zerumbone targeting multiple pathways which contribute to its anti-cancer properties.

Cell Cycle Analysis on Zerumbone

The review showed that Zerumbone induced growth inhibition, and showed that the effect of Zerumbone is refereed to changes in the recognized phase of cell cycle. Eight studies mentioned that Zerumbone arrests cancer cells in the G2/M phase of the cell cycle. The cell cycle confines a chain of events leading to division and replication. G2/M phase is a DNA damage checkpoint . It involves an arrest of the cell in G2 just before mitotic entry in response to stress.[46] Cancer cells divide at an uncontrollable way, and usually lose checkpoint controls that regulate replication of these cells. The effect of Zerumbonein arresting the cell cycle at the G2M phase is a proof to its inhibitory effect on cancer cells proliferation.

Contrary result was reported by Jorvig and Chakraborty 24 and Zhou *et al*.17 They demonstrated that Zerumbone significantly induced G1 cell cycle (first growth phase or post mitotic gap phase) arrested followed by apoptosis in SiHa cells (human cervical cancer cells) and DU145 cells (human prostate cancer cell line).

Zerumbone Suppresses Cancer Metastasis and Invasion

Four articles including Kang *et al*.,[25] Han *et al*.,[30] Sung *et al*.,[39] and Wang *et al*.,[47] reported that Zerumbone reduced ability of cancer cells to invade and metastasize.[4] different mechanism was described including upregulation of E-cadherin (cell adhesion proteins), downregulation of CXCR4 (chemokine receptor type 4) expression and a decrease in theosteopontin-induced ROCK/LIMK/cofilin signalling pathway. Zerumbonealso suppressed the expression of IL-1ß induced the expression of IL-8 and MMP-3 (matrix metalloproteinase-3) in the breast cancer cell line Hs578T.

Cadherins belong to a family of transmembrane glycoproteins among which E-cadherin withstand. This family is responsible for calcium-dependent intercellular adhesion. Low expression of E-cadherin might promote cancer metastasis. Zerumbone significantly increased expression of E-cadherin and by that reduce cancer metastasis.[47]

Rac1 (Ras-related C3 botulinum toxin substrate1) protein is member of the Rac subfamily under the Rho family. Rac1 participates in regulation of several cellular processes including tumour cell invasion and migration as well as cell to cell adhesion. It is highly expressed in many tumours. Wang *et al*.[47] demonstrated that Rac1 protein is down-regulatedwhen cell migration in inhibitedby Zerumbone. Rac1 knockdown could strengthen inhibition of cell migration by Zerumbone. Besides that, Rac1 promotes EMT (Epithelial-mesenchymal transition) and its expression level corresponds with tumour metastases and progression. Zerumbone inhibits cancer cell metastasis by inhibiting EMT process.

CXCR4 and Akt play a versatile part in cancer growth.Both, CXCR4 and Akt, have been involved in cell proliferation, migration and invasion. Zainal *et al*.[11] stated that Zerumbone repressed OSCC cells (oral squamous cell carcinoma)migration and invasion through suppressing CXCR4 protein expression. Suppression of the CXCR4-RhoA pathway was associated with inhibition of the PI3K-mTOR pathway via Akt and S6 protein inactivation. There was a number of evidencesthat supported CXCR4's role in acting as a marker of cancer aggressiveness. Zerumbone inhibited metastases in OSCCs through the suppression of CXCR4 signalling axis via IL-6 and IL-8 inhibition was previously reported. The finding from Zainal *et al*.[11] was consistent with Sung *et al*.[39] where Sung mentioned that Zerumbone downregulated CXCR4 protein expression whichled to CXCL12 suppression. CXCL12 is strongly chemotactic. It regulates hematopoietic cell migration, and that Zerumbone inhibited breast and pancreatic cell invasion.

Osteopontin (OPN) is a protein responsible for early T-lymphocyte activation. OPN induces invasion of human non-small cell lung cancer cells.[25] OPN

inactivates cofilin through theRho-associated kinase and activated LIM kinases (ROCK/LIMK) pathway. Rho-associated kinase and activate LIM kinases is moderated by the FAK/AKT pathway. OPN and OPN-regulated signalling pathways could play a crucial role for successful treatment of cancer. Kang *et al*.[25] suggested that invasion of A549 cells was induced by osteopontin and that Zerumbone repressed OPN-induced protein expression of ROCK1, the phosphorylation of LIM kinase 1 and 2 (LIMK1/2), and also cofilin. Through that, Zerumbone was able to strongly suppressthe invasion of A549 cells (human non-small cell lung cancer cell). Invasive breast cancers display high amounts of MMP-3, whereas non-invasive human breast cancer cells have undetectable amounts of MMP-3. MMP (Matrix metalloproteinases) are proteolytic enzymes which degrades components of protein in the extracellular matrix and basement membranes. MMPs play a crucial role in cancer invasion and metastasis. IL-1β plays a role as a major proinflammatory cytokine in most types of cancer. IL-1β and TNF-α augments the secretion of MMPs, which gives rise to EMT in human breast cancer growth. Zerumbone in this case was showed to be a potential inhibitor in the expression of IL-8 and MMP-3 leading to the suppression of cell migration and invasion in TNBC cells (breast cancer cell line) induced by IL-1β.[30]

The Enhancement of Sensitivity to Radiotherapy by Zerumbone

Deorukhkar *et al*.[22] described that Zerumbone increase radiation-induced apoptosis by inducingG2/M phase cell cycle arrest. Deorukhkar *et al* also mentioned that Zerumbone has little apoptotic effect by itself,but it significantly stimulates DNA damage induced by radiation, which was apparent by delayed resolution of post-irradiation nuclear cH2AX foci, whereas Zerumbone alone did not induce cH2AX foci formation. Zerumbone-mediated radiosensitization cellular glutathione (GSH) depletion.

Choi *et al*.[45] also agreed that pre-treatment with Zerumbone before radiation will induce sensitization to radiation, the authors found that Zerumbone induced sensitization by inhibiting HSP27 and apoptotic molecules (cytochrome c and Protein kinase C delta type) affinity to bind.

Genotoxicity Studies of Zerumbone

Zerumbone had no notable effect on normal human cells, the comet assay results by Al-Zubairi 18 showed that high concentration of Zerumbone could produce DNA damage. Furthermore, results from Al-Zubairi *et al*.[2] found that there was a direct relationship between the Zerumbone dose and the frequencies of micronuclei (MN assay is a widely used to assess *in vitro* chromosomal damage).This suggests that high concentrations of Zerumbone could be genotoxic, besides to its cytotoxic effect.

***In Vivo* Cell Proliferation and Angiogenesis Studies on Zerumbone**

The mice model was the most common model used to study the antiangiogenesis and anticancer effect of Zerumbone. Five studies used mice model and another 3 studies used rat model as shown in table II. According to Ni,[43] Zerumbone potentially reduced the tumor size and lung metastasis in C57 BL/6 mice. Additionally, Zerumbone was also found to cause apoptosis and autophagy in melanoma cell lines.

Prenatally exposed Female Balb/c mice to diethylstilbestrol were used in one of the selected studies in order to investigate the anticancer properties of Zerumbone.[40] Results from the experiment showed that Zerumbone effectively induces apoptosis in cervical neoplasia in diethylstilboestrol treated female Balb/c mice.

Park *et al*.[26] demonstrated marked reduction in hemoglobin content and in vascularization matrigel plug assay in Zerumbone-treated mice. These results indicated that Zerumbone inhibited cancer cell proliferation through different mechanisms which might include its registered antiangiogenics effects. Furthermore, Kim *et al*.[41] investigated the effectiveness of dietary administration of Zerumbone, where their result showed that mouse colon and lung carcinogenesis was inhibited by Zerumbone via various modulatory mechanisms of apoptosis, anti-inflammation and suppression of expression of NFxB and HO-I that were implicated in colon and lung carcinogenesis. Moreover, Al-Zubairi18 who observed polychromatic erythrocytes as well as the ratio of polychromatic to normochromic erythrocytes of male rats using the micronucleus

test (MN) of rat bone marrow reported that the acute exposure to Zerumbone resulted in a heighten number of micronuclei in polychromatic erythrocytes (MNPCEs). This suggested that a high dose of Zerumbone may have a genotoxic and cytotoxic effect.

In vivo data from Choi *et al*.[45] using nude mice after grafting of NCI-H460 (lower resistant to radiotherapy) and NCI-H1299 (higher radio resistant) cells indicated that Zerumbone builds up radio sensitization for both cell lines. Other than that, the ratio of radio sensitization was higher in NCI-H1299 cell-grafted mice.

Zerumbone suppressed DEN/AAF-induced carcinogenesis in rat liver. Taha *et al*.[6] reported that Zerumbone antiproliferative effect was mediated through reducing oxidative stress and inducing apoptosis via the mitochondrial pathway. Taha *et al*. reported that serum AFP (alpha-fetoprotein) concentration was significantly lesser in Zerumbone-treated rats. MDA (malondialdehyde) level which is a biomarker of hepatic lipid peroxidation in cancer cells, was considerably reduced in the cancerous rats treated with Zerumbone. Despite treatment with Zerumbone for 11 weeks, no abnormality was detected in the livers of normal rats.

Shanmugam *et al*. (2015) mentioned that when Zerumbone (I.P.) was administered to athymic nu/nu mice, activation of STAT3 in tumor tissues was supressed and growth of human RCC (renal cell carcinoma) xenograft tumors in athymic nu/nu mice was inhibited without any significant repercussions.

Additionally, Sehrawat *et al*.[44] stated that in conjunction with the apoptosis induction and suppression of cell proliferation, Zerumbone administration also significantly delayed growth of orthotropic MDA-MB-231 xenografts (an estrogen-independent cell line with mutant p53).

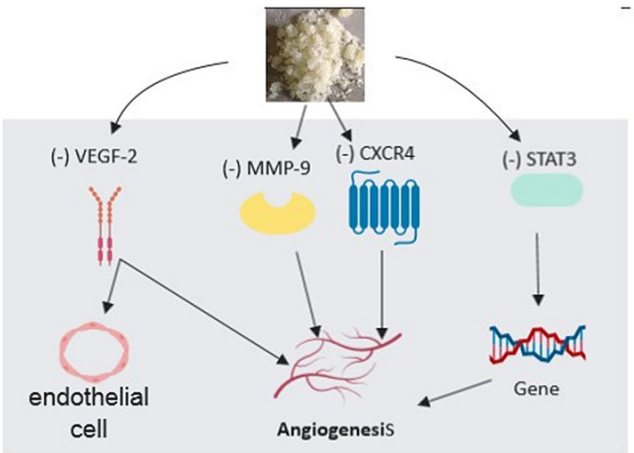

Fig. 4: The possible anti- angiogenesis signalling pathways by Zerumbone. (-) indicates inhibition of selected proteins

Antiangiogenic Effects of Zerumbone on Cancer Cell Lines

Four selected articles described the antiangiogenic effect of Zerumbone.[5,26,29,39] These results suggested that antiangiogenic effect of Zerumbone had multipath way also. The anti-angiogenic properties of Zerumbone were potentially controlled by CXCR4 expression, VEGF and STAT3. The possible anti-angiogenesis signalling pathways by Zerumbone was illustrated in Figure 4.

Results from Park *et al*.[26] found that Zerumbone has the potential in decreasing the phosphorylation of vascular endothelial growth factor receptor-2 and fibroblast growth factor receptor-1. These factors help regulate endothelial cell function and angiogenesis. *In vivo* Matrigel plug assay in Zerumbone treated mice displayeda significant reduction in vascularization counts and haemoglobin content in the plugs. Overall, these results evoke that Zerumbone inhibits different pathways in the

angiogenesis process, which might explain its antitumor effects.

According to Sung *et al*.,[39] Zerumbone can reduce CXCR4 expression.This chemokine receptor has been strongly associatedto cellproliferation, invasion, angiogenesis, and metastasis in cancer cells. This study suggests that downregulation of CXCR4 expression attributed to down-regulation of NF-ϰB. This leads to CXCL12 inhibition which then inhibits invasion in breast and pancreatic tumour cells as well. Sung also mentioned that Zerumbone inhibits the VEGF inflammatory cytokine which induces CXCR4 expression.

Samad *et al*.[5] demonstrated that Zerumbone inhibited cell growth and migration of HepG2 cell in adose-dependent fashion. This result also showed that Zerumbone decreased expression of angiogenesis molecular effectors such as, the matrix metalloproteinase-9, vascular endothelial growth factor (VEGF), and VEGF receptor proteins. Additionally, Zerumbone also reduced tube formation by HUVECs through inhibiting new blood vessel and tissue matrix formation.

From other aspect Shanmugam *et al*.[29] obtained results which established that Zerumbone inhibits STAT3 (signal transducer and activator of transcription) protein which has a crucialrole in both angiogenesis and cancer genesis. Shanmugam also suggested that STAT3 activation inhibition by Zerumbone resulted in the expression reduction of the genes involved in proliferation, survival, and angiogenesis which are mediated through protein inducer kinases (c-Src, JAK1, and JAK2) inhibition and t phosphatase (SHP-1) induction.

Thus, besides the antiproliferative effects, Zerumbone evidently hasanti-angiogenesis effects via inhibition of CXCR4 and MMP, sharing the same affected pathway of antimetastatic effect of Zerumbone. This suggests that Zerumbone inhibits the metastasis of tumours through its antiangiogenic activities.

Table 2: Summary on the *in vivo* effects of Zerumbone

Type of in vivo model study used	Mechanisms	Zerumbone dose / time	Results	References
Female offspring of female Balb/c mice exposed prenatally to diethylstilbestrol	ZER induced over -expression of pro -apoptotic protein Bax	8 and 16 mg/kg of ZER were given. Mice treated from 52 days of age to 60 days of age (4 dosages on alternate days).	Quantitative evaluation of PCNA using immunohisto-chemically-staining (percentage of apoptosis) : normal saline treated group (0%), with ZER treated (15.7%) and cisplatin treated (21.7%).	Abdel Wahab *et al.,* 2010 [40]
AOM/DSS-induced Colon cancer and NNK-induced lung cancer in (85 male ICR mice and 50 female A/J mice)	Feeding with ZER resulted in inhibition of proliferation, induction of apopto-sis, and suppression of NFϰB and heme oxygenase (HO)-1 expression in tumours developed in both tissues	fed the diet mixed with 100, 250 or 500 ppm ZER for 21 weeks	ZER feeding significantly decreased the PCNA labelling index at 3 doses and significantly increased TUNEL-positive apoptotic nuclei. at 250 and 500 significantly decreased the PCNA-labelling indexof adenoma cells and significantly increased TUNEL positive apoptotic nuclei at 3 doses.	Kim *et al.,* 2009[41]

C57BL/6 mice rat thoracic aorta ring assay	ZER interacted with the VEGF/VEGFR2 signalling pathway.	subcutaneously injection with 500 µl of matirgel containing (VEGF, bFGF and heparin) in the absence or presence of Zerum -bone 100, 200 µM than after 7 days the plugs were removed and assess plugs.	Zerumbone *in vivo* (200 µM) showed strong inhibition of vascular development.	Park *et al.,* 2015[26]
Rat bone marrow polychromatic erythrocytes (PCEs)		Three dose levels of Zerumbone (250, 500, 1000 mg/kg) were given intraperitoneal for 24h.	These results reveal that, ZER has a genotoxic activity only with high drug concentrations (40 and 80 µM). The acute exposure to ZER resulted in significant increase in the number of micronuclei in polychromatic erythrocytes	Al-Zubairi *et al.,* 2010[2]
Rats induced liver cancer with a single ip injection (DEN, 200 mg/kg) and dietary (AAF) (0.02%).	ZER increased Bax and decreased Bcl-2 protein expression in the livers of DEN/ AAF rats treated, DEN/AAF rats treated with ZER also showed less proliferating cell nuclear antigen (PCNA),	ZER injections intra peritoneal at 15, 30 or 60 mg/kg body wt. twice a week for 11 weeks after inj DEN by 4 days	Serum AFP concentrations were significantly lower in ZER-treated MDA level was significantly lower in the cancerous rats treated. treatment of cancerous rats with ZER at 30 and 60 mg/kg body wt. produced lower PCNA expression Zerumbone at 30 and 60mg /kg body wt. significantly increased Bax and decreased Bcl-2 expressions in the cancerous hepatocytes	Taha *et al.,* 2010[6]
6-week-old BALB/c athymic nude mice after grafting of NCI-H460 (lower expression of HSP27) and NCI-H1299 (higher expression of HSP27) cells	ZER inhibits mono -meric HSP27 protein expression, altered cross-linking of HSP27 by ZER affect HSP27-mediated radio-resistance	When the tumor reached a minimal volume of 200 mm 3 then treatment 5 times with DMSO, ZER after first radiation therapy (8 Gy) was delivered with a local regional application.	ZER exhibited sensitization to the radiation and inhibited the interaction between HSP27 and apoptotic molecule ZER has a greater radio sensitization effect in HSP27-overexpressed c Pre-radiation ZER treatment by 3 hr before radiation decreased clonogenic survival by radiation cells, such as NCI-H1299.	Choi *et al.,* 2011[45]

C57 BL/6 mice		For group 1 mous injected intraperi-toneal with ZER solution 50µl (20 µg/gin in DMSO), group 2&4 each mouse injected with ZER solution 50µl (40 µg/g in DMSO), group 3&5 (control group) no ZER injection ZER treatment 7 days before tumour implantation until tumour size of mice in the control group had exceeded 4 cm2, and after 19 days for group 4&5	ZER significantly reduced the tumor mass and lung metastasis in C57 BL/6 mice ZER significantly reduced the number of metastatic nodules growing in the lung	Ni, 2013[43]
Female SCID mice with MDA-MB-231 tumour	expression of prolife-ration marker Ki-67 was significantly lower in the tumours of ZER-treated mice compared with those of control mice	the mice intraperi-toneal administra-tion of either PBS (100 lL) or ZER (0.18 or 0.35 mg ZER/mouse; equates to about 7.5 and 15.7 mg ZER/kg body weight,respectively, in 100 lL PBS) five times/week	ZER administration resulted in a statistically significant increase in number of apoptotic bodies in the tumour as visualized by TUNEL assay	Sehrawat *et al.,* 2012[44]

Conclusion

Zerumbone (ZER), present in the subtropical ginger, *Zingiber zerumbet* has been shown to possess antiproliferative and anti-inflammatory properties. Studies reviewed in this literature proved that Zerumbone can be considered as a potent cancer therapeutic agent. Zerumbone promotes antiproliferative effects through apoptosis induction and cell cycle arrest. Furthermore, this compound exerted ant angiogenesis activities by inhibiting tumor growth and metastasis.

However, this systematic review has number of limitations. The limited data on the toxic dose of Zerumbone is lacking and could be improved by more analyses on the future studies of this compound. Most of the studies on Zerumbone employed *in vitro* model. *In vitro* models are generally more susceptible to artefacts due to various variables. The key requirement for*in vitro* application is validation and quality control. More animal modelsare required to determine the therapeutic and toxic threshold value of Zerumbone and is essential for translation of drug findings from bench to bedside.

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Conflict of interest

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