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Role of Autophagy in the Antitumor Activity of Aloin in Breast Cancer Cells

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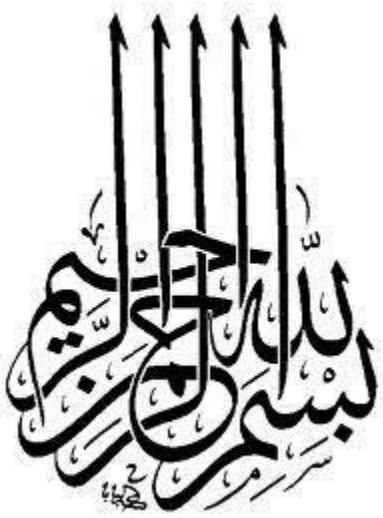

(قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنا إِلاَّ مَا
عَلَّمْتَنا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيم)

صدق الله العظيم

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This thesis has not been submitted for a degree at this or any other university

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ABSTRACT

Aloin is a natural bioactive anthraquinone extracted from *Aloe* sp. and has the potential of tumor regression by inhibition of cell proliferation and induction of cell apoptosis in different human cancer cell lines. The ability of cancer cells to evade apoptosis, which often limits the efficacy and accounts for the resistance to chemotherapy, strives the search of autophagy process as an alternative target to promote cell death. The present study was undertaken to verify the autophagy process as a probable mechanism for the antitumor activity of aloin in 2 types of breast cancer cell lines; estrogen receptor positive (T47D) and triple negative (MDA-MB231), compared to an anthraquinone analog, doxorubicin. Initially, the cytotoxicity of increasing concentrations of aloin and doxorubicin were assessed using MTT and clonogenic assays at 2 exposure periods (24 and 72h) to determine the half maximal inhibitory concentration (IC_{50}) of aloin and doxorubicin in both types of cell lines. The formation of autophagy process in the treated tumor cells was initially detected by using transmission electron microscope (TEM). Emphasis of autophagy process was then achieved by monitoring the formation of acidic vesicular organelles (AVOs) qualitatively by confocal fluorescence microscope, and quantitatively by fluorescence activated cell sorting (FACS). Finally, the protein expression levels of some

autophagy-related genes were determined by Western blotting. Results obtained from this study revealed that aloin inhibited the cell growth of both T47D and MDA-MB231 cells, with a more pronounced effect in the 72 exposure regimen. TEM of tumor cells treated with IC_{50} of aloin revealed the presence of autophagosomes, as early and autolysophagosomes, as late autophagic compartments. The autophagic activity of aloin was then emphasized by the accumulation of acidic vesicular organelles (AVOs) in the treated tumor cells and the up-regulation in the protein expression of some autophagy-related genes, such as microtubule-associated proteins 1A/1B light chain 3B (LC3B II), beclin1, phosphorylated AKT (p-PKB), and in contrast down-regulation of p62 and phosphorylated mammalian target of rapamycin kinase (p-mTOR). These findings concluded that autophagy is regarded as one of the modes of the cytotoxic action of aloin in T47D and MDA-MB231 breast cancer cells via modulating mTOR cell signaling pathway.

Keywords: Aloin, Doxorubicin, Breast cancer cells, Autophagy, Transmission electron microscopy, Confocal fluorescence microscopy, Flow cytometry and Western blotting.

List of Abbreviations

AVOs	Acid Vesicular Organelles
AMPK	Adenosine monophosphate-activated kinase
AL	Aloin
ATCC	American Type Culture Collection
AO	Acridne orange
ATG	Autophagy related genes
BRCA	Breast cancer gene
ECM	Extracellular matrix
CMA	Chaperone-mediated autophagy
JNK	C-jun N-terminal kinase
CLSM	Confocal laser scanning microscopy
DARK	Death-associated protein kinase 1
DMSO	Dimethyl sulfoxide
Dox	Doxorubicin
ER	Estrogen receptor
ELISA	Enzyme-linked immunosorbent assay
HER2	Human epidermal growth factor receptor 2
EREs	Estrogen receptor elements
4EBP1	Eukaryotic initiation factor 4E binding protein
ERK	Extracellular signal-regulated kinase
FACS	Fluorescence activated cell sorting
FBS	Fetal bovine serum
FOXO1	Forkhead box O protein O1
GFs	Growth factors

List of Abbreviations (Cont.)

NSCLC	Human non-small cell lung carcinoma
Hsc70	Heat shock protein
HUVECs	Human umbilical vascular endothelial cells
IC50	Inhibitory concentration that kill 50%
ICF	Inhibition of colony formation
IRS1	Insulin receptor substrate 1
IRS2	Insulin receptor substrate 2
LC3B	Microtubule-associated proteins 1A/1B light chain 3B
mTORC1	Mammalian target of rapamycin complex1
MTD	Maximum tolerated dose
MDR	Multidrug resistance
MAPK	mitogen-activated protein kinase
MTT	3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
NRF2	Nuclear factor erythroid-2–like 2
SQSTM1/p62	Sequestosome-1 (ubiquitin-binding protein p62)
PDK1	Pyruvate dehydrogenase kinase 1
P-gp	P-glycoprotein
PCD	Programed cell death
PTEN	Phosphatase and tensin homolog
PBS	Phosphate-buffered saline
PIP3	Phosphatidylinositol 3, 4, 5 trisphosphate
PI3K	Phosphoinositol-3-kinase
PARP	Poly ADP-ribose polymerase
PR	Progesterone receptor
PKB/AKT	Protein kinase B

List of Abbreviations (Cont.)

ROS	Reactive oxygen species
RTKs	Receptor tyrosine kinases
RPMI	Complete RPMI-1640 medium
siRNA	small interfering RNA
SPF	S-phase fraction
STAT	Signal transducers and activators of transcription
TNF-α	Tumor necrosis factor alpha
Topo II	Topoisomerase II
TEM	Transmission Electron Microscopy
TNBC	Triple-negative breast cancers
TSC	Tuberous sclerosis complex
ULK1	51-like serine threonine kinase complex
WHO	World health organization

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