

**INDUCTION OF MITOCHONDRIAL-MEDIATED
APOPTOSIS IN HT-29 HUMAN COLORECTAL
ADENOCARCINOMA CELLS BY AQUEOUS FRACTION
OF *NEPHELIUM RAMBOUTAN-AKE* RIND.**

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ABSTRACT

The objective of this study was to evaluate the cytotoxic effect and elucidate the possible underlying apoptotic mechanisms of *Nephelium ramboutan-ake* (pulasan) rind in selected human cancer cell lines. The crude ethanol extract and fractions (ethyl acetate and aqueous) of *N. ramboutan-ake* reduced the cell viability of HT-29, HCT-116, MDA-MB-231, Ca Ski cells by MTT assay. The *N. ramboutan-ake* aqueous fraction (NRAF) was found to exert the most potent cytotoxic effect against HT-29 cells in a dose-dependent manner. Further investigation for its plausible mechanisms was conducted by using flow cytometry and fluorescence microscopy. This study confirmed the induction of apoptosis by a series of archetypal apoptotic features such as chromatin condensation, DNA fragmentation, cell shrinkage and apoptotic body formation by Hoechst 33342/PI dual staining. The apoptotic mechanisms were further substantiated with the detection of DNA fragmentation by TUNEL assay and phosphatidylserine (PS) externalization on the outer leaflet of plasma membrane which detected by annexin V-FITC/PI binding confirming the early stage of apoptosis. In addition, mitochondrial permeability transition is a vital step in the induction of cellular apoptosis, and data clearly revealed that NRAF resulted in disruption of mitochondrial transmembrane potential ($\Delta\psi_m$) in HT-29 cells. Dissipation of $\Delta\psi_m$ was indicated through a series of evidences such as increased generation of ROS and depletion of GSH, upregulation of Bax protein expression, as well as activation of caspase-3/7 and caspase-9. Collectively, these results suggested that NRAF induced mitochondrial-mediated apoptosis.

ABSTRAK

Objektif kajian ini adalah untuk menilai kesan sitotoksik dan menyiasat mekanisme apoptosis oleh kulit *Nephelium ramboutan-ake* (pulasan) terhadap beberapa jenis sel kanser yang terpilih. Dengan menggunakan asai MTT (3,4 [dimetiltiazol-2-yl]-2, 5-difeniltetrazolium bromida), ekstrak etanol dan pecahan-pecahan lain (etil asetat dan akueus) dari kulit *N. ramboutan-ake* didapati menurunkan kebolehidupan sel HT-29, HCT-116, MDA-MB-231 dan Ca Ski. Pecahan akueus kulit *N. ramboutan-ake* (NRAF) mempamerkan kesan sitotoksik yang paling kuat terhadap sel HT-29 secara bersandarkan dos. Kajian mendalam bagi menjelaskan mekanisme apoptosis telah dilakukan dengan menggunakan sitometer alir dan mikroskopi pendarfluor. Kajian menggunakan Hoechst 33342/PI membuktikan apoptosis dengan mendedahkan ciri-ciri seperti kondensasi kromatin, serpihan DNA, pengecutan sel dan pembentukan jasad apoptotik. Mekanisme apoptotik telah dijelaskan melalui pengesanan serpihan DNA menggunakan asai TUNEL dan pendedahan fosfatidilserina sisi luar membrane plasma yang diperhatikan melalui asai Annexin-V/PI. Di samping itu, kebolehtelapan peralihan mitokondria merupakan langkah yang penting bagi apoptosis. Oleh itu, data menunjukkan NRAF menyebabkan gangguan dalam potensi diantara membran mitokondria ($\Delta\psi_m$) pada sel HT-29. Gangguan $\Delta\psi_m$ telah dijelaskan serta berkorelasi dengan bukti-bukti seperti peningkatan penjanaan spesies oksigen reaktif intrasel dan pengurangan GSH intrasel, tahap ekspresi protein Bax yang tinggi serta pengaktifan kaspase-3/7 dan kaspase-9. Keseluruhannya, keputusan mengesahkan bahawa NRAF dapat menginduksikan apoptosis dengan mengantarakan mitokondria.

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TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vi
LIST OF FIGURES	x
LIST OF TABLES	xii
LIST OF ABBREVIATIONS	xii
LIST OF SYMBOL	xvi
LIST OF PUBLICATIONS/ PRESENTATIONS	xvii
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEW	
2.1 Cancer	4
2.2 Colorectal cancer and carcinogenesis	5
2.3 Treatments for colorectal cancer	7
2.4 Mode of cell death	9
2.5 Apoptosis and cancer	9
2.6 Apoptotic pathways	12
2.6.1 Extrinsic pathway	12
2.6.2 Intrinsic pathway	15
2.7 Redox homeostasis and cancer	18
2.7.1 Oxidative stress	18
2.7.2 Reactive Oxygen Species	19
2.7.3 Antioxidants	24

2.7.4	Glutathione	25
2.8	Caspases	26
2.9	Bcl-2 family	31
2.10	<i>Nephelium ramboutan-ake</i>	34

CHAPTER 3 MATERIALS AND METHODS

3.1.	Materials	
3.1.1	Solvents	37
3.1.2	Cell lines	37
3.1.3	Growth medium	37
3.1.4	Antibodies, chemical, drugs and reagents	37
3.1.5	Assay kits	38
3.1.6	Miscellaneous	38
3.1.7	Instrument/ Equipment	39
3.2.	Methods	
3.2.1.	Plant Material	39
3.2.2.	Preparation of Crude <i>N. ramboutan-ake</i> Extract and Fractions	39
3.2.3.	Cell culture	
3.2.3.1	Maintenance of cells	40
3.2.3.2	Subculturing cells	40
3.2.3.3	Cryopreservation of cells	41
3.2.3.4	Reviving cells	41
3.2.3.5	Cell counting	41
3.2.3.6	Treatment of cells	42
3.2.4.	Evaluation of cytotoxic effects of extract and fractions of <i>N. ramboutan-ake</i>	

3.2.4.1	In vitro MTT cell viability assay	42
3.2.4.2	Total cell count	43
3.2.5.	Assessment of apoptotic effect of the bioactive fraction of <i>N.ramboutan-ake</i>	
3.2.5.1	Nuclear morphology detection using Hoechst 33342/PI	44
3.2.5.2	Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) Assay	45
3.2.5.3	Annexin V/PI staining for the assessment of phosphatidylserine externalization	45
3.2.5.4	Measurement of mitochondrial membrane potential ($\Delta\psi_m$)	46
3.2.5.5	Determination of intracellular total glutathione (GSH) content	47
3.2.5.6	Measurement of intracellular reactive oxygen species (ROS)	48
3.2.5.7	Determination of Bax and Bcl-2 protein expression level	49
3.2.5.8	Measurement of Caspase-3/7 and Caspase-9 activities	50
3.2.6	Preliminary phytochemical screening	
3.2.6.1	Test for alkaloids	51
3.2.6.2	Sakowski test for sterols	51
3.2.6.3	Frothing Test for saponins	51
3.2.6.4	Test for tannins	51
3.2.6.5	Test for flavonoids	51

3.2.7	Statistical analysis	52
CHAPTER 4 RESULTS		
4.1	Reduction of HT-29 cell viability by NRAF	53
4.2	The effect of NRAF on nuclear morphological changes of HT-29 cells by Hoescht 33342 and PI staining	57
4.3	Induction of DNA fragmentation detected by TUNEL assay	60
4.4	Externalization of phosphatidylserine by using Annexin V/PI staining	62
4.5	Alteration of Mitochondrial membrane potential ($\Delta\Psi_m$)	66
4.6	Effect of NRAF induced formation of ROS	69
4.7	Depletion of Glutathione Content by NRAF	75
4.8	Modulation of apoptotic proteins by NRAF	77
4.9	NRAF induced caspase-3/7 and caspase-9 activation	83
4.10	Phytochemical content analysis	88
CHAPTER 5 DISCUSSION		89
CHAPTER 6 CONCLUSION		100
REFERENCES		102
APPENDIX		118

LIST OF FIGURES

		Page
Figure 2.1	The development of colorectal cancer	6
Figure 2.2	Diagram illustrated key molecular events which is death receptor-mediated procaspases-activation pathway.	14
Figure 2.3	Extrinsic and intrinsic pathways which in turn initiate caspases activation are illustrated.	17
Figure 2.4	A diverse of mechanisms are involved in the generation of ROS.	21
Figure 2.5	Schematic illustrated the caspases with the domains such as CARD (green), DED (yellow) which involved in recruitment and activation, N-peptide (red) which will be eliminated during the occurrence of apoptosis, large subunit (cyan) and small unit (blue).	27
Figure 2.6	Caspase-dependent death receptor-mediated and mitochondrial-mediated apoptosis mechanism.	30
Figure 2.7	Bcl-2 proteins family members classified into proapoptotic and anti-apoptotic.	33
Figure 2.8	The outlook and inner look of the <i>N. ramboutan-ake</i> fruits.	35
Figure 3.1	Reduction of tetrazolium 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) adapted from Ebada <i>et al.</i> , 2008.	43
Figure 3.2	Quadrants showed under the view of inverted light microscope.	44
Figure 3.3	The chemical structure of 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) dye.	47
Figure 3.4	Mechanism of 2', 7'- dichlorodihydrofluorescein diacetate (DCFH-DA) convert to DCF, DCFH as intermediate through de-esterification and oxidation, adapted from (Gomes <i>et al.</i> , 2005).	49
Figure 4.1	The cytotoxicity effect of NRAF extract and fractions against selected cancer cell lines at 72 h.	55
Figure 4.2	Graph represented the cytotoxicity effect of <i>N. ramboutan-ake</i> rind ethanol extract (NREE), ethyl	56

acetate fraction (NREAF) and aqueous fraction (NRAF)
against HT-29 cell line.

Figure 4.3	Nuclear morphological changes of HT-29 cells by NRAF.	59
Figure 4.4	Effect of NRAF on DNA fragmentation of HT-29 cells. HT-29 cells were treated with different concentrations of NRAF (50 µg/ml, 100 µg/ml and 200 µg/ml) at 24 h incubation period.	61
Figure 4.5	Dose-dependent induction of early and late apoptosis by NRAF (25-200 µg/ml) at 24 h.	63
Figure 4.6	Dose-dependent attenuation of mitochondrial membrane potential in HT-29 cells elicited by NRAF.	67
Figure 4.7	Effect of NRAF on intracellular ROS level.	70
Figure 4.8	Effect of NRAF on intracellular total glutathione content of HT-29 cells at 24 h. There was a significant reduction in intracellular GSH content (> 50%) after treatment with varying concentrations of NRAF (50 – 200 µg/ml).	76
Figure 4.9	Effect of NRAF on protein expression level of Bax and Bcl-2 in HT-29 cells. Cells were treated with 50 µg/ml of NRAF for different times.	78
Figure 4.10	Effect of 50 µg/mL NRAF on caspase activities.	85
Figure 6.1	Schematic illustration of a hypothetical pathway of NRAF-induced apoptosis in human colorectal adenocarcinoma HT-29 cells.	101

LIST OF TABLES

		Page
Table 2.1	Table illustrated subfamily members of caspases.	28
Table 4.1	IC ₅₀ values of extract and fractions of <i>Nephelium ramboutan-ake</i> rind against different cancer cell lines and normal cell line.	54
Table 4.2	Preliminary phytochemical analysis of <i>N. ramboutan-ake</i> aqueous fraction (NRAF)	89

LIST OF ABBREVIATIONS

3'-OH	3' hydroxyl
AJCC	American Joint Committee on Cancer
APC	adenomatous polyposis coli
Apaf-1	apoptotic protease-activating factor 1
ATP	Adenosine triphosphate
BH	Bcl-2 homology
BrdU	Bromodeoxyuridine
CIN	chromosomal instability
CO ₂	Carbon dioxide
CRC	Colorectal cancer

CTC	computed tomographic colonography
dATP	deoxyadenosine 5'-triphosphate
DCF	dichlorofluorescein
DCFH-DA	2'-7'-dichlorofluorescein diacetate
DED	death effector domain
DISC	death-induced signaling complex
DMSO	dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DR	death receptor
DSBs	DNA strand breaks
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
FADD	Fas-associated death domain
FasL	Fas ligand
FBS	Fetal bovine serum
FITC	Fluorescein isothiocyanate
FL-1/FL-2	Fluorescence channel1/2
FOBT	fecal occult blood test
5-FU	5-fluorouracil

GR	Glutathione reductase
GCL	γ -glutamate-cysteine ligase
GSH	reduced glutathione
GS	Glutathione synthetase
GSSG	Glutathione disulfide
h	Hour
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IAP	inhibitor of apoptosis protein
IC ₅₀	50% inhibitory concentration
ICAD	inhibitor of caspase-activated deoxyribonuclease
Ig G	Immunoglobulin G
JC-1	5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide
MDA	malondialdehyde
miRNAs	microRNAs
min	Minutes
MMR	mismatch repair
MOMP	mitochondrial outer membrane permeabilization
MPTPs	mitochondrion permeability transition pores

MSI	microsatellite instability
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na ₂ CO ₃	Sodium bicarbonate
NADPH	Nicotinamide adenine dinucleotide phosphate
NRAF	N. ramboutan-ake rind aqueous fraction
NREAF	N. ramboutan-ake rind ethyl acetate fraction
NREE	N. ramboutan-ake rind ethanol extract
PBS	Phosphate buffer
PI	Propidium iodide
PS	phosphatidylserine
PT	permeability transition
RNase	Ribonuclease
ROS	reactive oxygen species
rpm	Rounds per minute
sDNA	Stool DNA
SSA	5-sulfosalicylic acid
TBHP	tert-butyl hydroperoxide
tBid	truncating Bid

TdT	polymerase terminal deoxynucleotidyl transferase
TNF	tumor necrosis factor
TNFR1	tumor necrosis factor receptor1
TRAIL	tumor necrosis factor related apoptosis inducing ligand
TRAIL-R1	tumor necrosis factor related apoptosis inducing ligand receptor I
TUNEL	Terminal Deoxynucleotidyl Transferase UTP Nick End Labeling
VDAC	voltage-dependent anion channel

List of symbols

G	gram
μg	microgram
mg	miligram
mL	mililiter
μl	microliter
$^{\circ}\text{C}$	Degree celcius
$\Delta\psi_m$	Mitochondrial membrane potential
%	Percentage

List of publication/ proceeding

1. **Chan, C.K.** and Kadir, H.A. (2011) Antiproliferative and apoptosis effects of *Nephelium mutabile* in HT-29 Human Colorectal adenocarcinoma cells. Proceedings of the 16th Biological Sciences of Graduate Congress held at National University of Singapore, Singapore on December 12-14, 2011. Abstract PP-2-03, pp. 70.
2. **Chan C.K.**, Goh B.H., Kamarudin M.N.A., Kadir H.A. (2012) Aqueous Fraction of *Nephelium ramboutan-ake* Rind Induces Mitochondrial-Mediated Apoptosis in HT-29 Human Colorectal Adenocarcinoma Cells. *Molecules*. 17(6), 6633-6657.