AN IN-VITRO STUDY ON THE EFFECT OF *Carica papaya* CRUDE AQUEOUS EXTRACT ON THE VOLTAGE-GATED SODIUM CHANNEL (VGSC) MEDIATED MOTILITY ON MDA-MB 231 BREAST CANCER CELL LINE

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LIST OF ABBREVIATION

Abs	Absorbance
bp	base pair
CO^2	carbon dioxide
C	Control
ddH_2O	Nuclease Free Water
DHA	Docosahexaenoic acid
DMSO	*Dimethyl Sulfoxide*
Etbr	Ethidium Bromide
EM	Epithelial-menchymal transition
ER	Estrogen receptor
g	gravity
HV	Hyperladder V
h	hour(s)
mL	mililiter
min	minutes
nm	nano-meter
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

nNav1.5	neonatal splice variant Nav1.5 gene
PUFA	Polyunsaturated fatty acid
RT	Reverse transcription
SEM	Standard error of mean
SD	Standard deviation
siRNA	small interfering RNA
TTX	Tetradotoxin
VGSC	Voltage Gated Sodium Channel
V	Voltage
μL	micro-Litre
μM	micro-Molar
°C	Degree celcius
1x	one reaction volume
12x	twelve reaction volume

ABSTRAK

Pelbagai kaedah penyelidikan telah dijalankan oleh saintis di seluruh dunia bagi membuktikan bahawa voltan saluran natrium berpagar (VGSC) adalah sesuai sebagai satu sasaran untuk terapi kanser. Terdapat banyak penyelidikan yang telah membuktikan penglibatan VGSC dalam mengawal pertumbuhan dan perkembangan sel kanser serta peranannya dalam metastasis. VGSC yang utama di dalam sel kanser payudara adalah neonatal Nav1.5 (nNav1.5). Objektif penyelidikan kami bertujuan untuk targetkan penanda VGSC nNav1.5 gen ke arah mengawal kadar metastasis dalam kanser payudara. *Carica papaya* adalah calon pilihan kami untuk mengawal atur gen nNav1.5 disebabkan oleh sifat anestetik yang dimiliki olehnya serta peranannya yang terkenal sebagai agen anti-kanser atau anti-proliferatif. MTT assay dan Trypan Blue assay telah dijalankan bagi menguji ketoksikan dan ketahanan sel kanser payudara MDA-MB -231 sebagai tindak balas terhadap rawatan *C. papaya* pada kepekatan 1 μg/mL, 1.0 μg/mL dan 10.0 μg/mL. Selain itu, kami juga menjalankan assay pergerakan (*motility assay*) dan PCR untuk menentukan kesan rawatan C. papaya pada kepekatan yang sama terhadap sel MDA-MB -231 dan expresi gen *nNav1.5*.

Keputusan dan analisis data yang telah dijalankan menunjukkan bahawa pada kepekatan yang telah diuji, ekstrak *C.papaya* adalah tidak toksik dan juga tidak menjejaskan pertumbuhan sel MDA-MB-231. Menariknya, walaupun ekstrak *C. papaya* tidak memberi kesan kepada pertumbuhan sel, namun pergerakan sel MDA-MB-231 telah dikurangkan. Ini menunjukkan bahawa kesan ekstrak untuk mengurangkan pergerakan sel MDA-MB-231 adalah tidak dapat dinafikan. Expresi gen nNav1.5 gen didapati menurun apabila kepekatan C.papaya menaik. Hal ini demikian, mencadangkan bahawa

mekanisma ekstrak untuk mengurangkan pergerakan sel mungkin melibatkan VGSC nNav1.5. Namun analisis data yang dijalankan tidak mendapati kedua-duanya tidak mempunyai hubungan untuk disignifikasikan. Oleh itu, dalam tesis ini, kami menyediakan cadangan untuk kajian masa hadapan demi mendapatkan hasil yang lebih baik dan membuktikan keupayaan *C. papaya* dalam mengawal metastasis kanser payudara.

ABSTRACT

Voltage-gated sodium channel (VGSC) in the line of suitable target markers for cancer therapy has been extensively studied by researchers over recent years. Variety of study provides suggestive evidence on the involvement of VGSC in regulating growth and expansion of cancer cell together with its role in metastasis. The predominant and highly expressed VGSC in breast cancer cells is the neonatal splice variant (nNav1.5). We aimed to target the specific VGSC markers, nNav1.5 gene expression towards controlling the rate of metastasis in breast cancer. *Carica papaya* is our choice candidate in down regulating the gene expression of nNav1.5 due to its local anesthetic properties as well as well-known role in exhibiting the anti-cancer or anti-proliferative properties. MTT assay and Trypan Blue assay procedure were conducted to test the toxicity and viability of MDA-MB-231 breast cancer cell in response to *C. papaya* aqueous crude extract treatment at 0.1 µg/mL, 1.0 µg/mL and 10.0 µg/mL. Moreover, we performed the lateral motility assay and PCR in order to determine the effects of *C. papaya* treatment at similar concentration on MDA-MB-231 cells motility and nNav1.5 gene.

Results and data analysis provide suggestive evidence that at the concentrations tested, the aqueous crude extract of *C. papaya* was non-toxic and also did not affect the growth of MDA-MB 231 cells. Interestingly, whilst the extract was none effective on the cell's growth, lateral motility of MDA-MB-231 cells was reduced, suggesting that the effect of the extract on reducing motility was indisputable. Consequently, the expression of nNav1.5 gene was down-regulated as the concentration of *C. papaya* treatment increased, suggesting the mode of action of the extract on reducing motility might

involve VGSC nNav1.5. However the data analysis appears to be insignificant for both. Therefore, in this thesis, we provide suggestion for future study to obtain a better result and prove the ability of *C. papaya* in controlling breast cancer metastasis.