UNIVERSITI PUTRA MALAYSIA

MOLECULAR EFFECTS OF FREE AND DOXORUBICIN-LOADED ARAGONITE CALCIUM CARBONATE NANOPARTICLES ON MCF-7 CELL LINES

HAMIDU AHMED

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By

HAMIDU AHMED

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

This thesis is dedicated to my late Daddy, Alhaji. Ahmadu M. Boderel, for this librettos of encouragement and reinforcement in search of excellence, May your soul rest in perfect peace Ameen summa ameen and to my Mother Hajja Hauwa. Alh. A. M. Boderel moral supports, guidance and patient.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Professor Md Zuki bin Abu Bakar @ Zakaria, PhD
Institute : Biosciences

Cancer is one of the prime causes of death and breast cancer is the most erratic malignant disease which causes serious burden to women worldwide. Cockle shell-derived aragonite calcium carbonate nanoparticles (Ar-CC-NPs) encapsulated with doxorubicin (DOX) has transpire as an efficacious therapy against breast tumour, but still the use of targeted therapy in cancer treatment appears to be ineffective and often associated with some set back, hence necessitates the need for improved targeted therapy. This study determines the therapeutic potential of doxorubicin-loaded aragonite $CaCO_3$ nanoparticles using proteomic approach. For the proteomic study, Liquid Chromatography/Mass Spectrometry analysis (LCMS/MS) was employed to investigate the MCF-7 related protein in human breast cancer cells after treatment with the DOX and DOX-Ar-CC-NPs. Aragonite $CaCO_3$ nanoparticles are synthesized from cockle shells and characterized for physiochemical properties using Transmission electron microscope (TEM), Field emission scanning electron microscope (FESEM), Zeta potential, Fourier transmission infrared (FTIR) and X-ray diffraction (XRD) techniques. The aragonite nanoparticles are synthesized from the cheaply available natural sea water cockle shells, which are cleaned with banana peels, homogenized and then stirred vigorously in dodecyl dimethyl betane (BS-12) solution using a rotary pulvering blending machine in order to reduce the a stringent temperature and unsafe chemicals associated with nanoparticles production and are then characterized for particle geometry using electron microscopy. An IC_{50} which is inhibitory concentration of 50% of the tested cells that signifies drug concentration, for the indication of cell viability was also determined and then, the synthesized aragonite calcium carbonate nanoparticles (Ar-CC-NPs) are then loaded with doxorubicin (DOX), an antineoplastic agent, which formed Doxorubicin-aragonite-nanoparticles (DOX-Ar-CC-NPs). The cytotoxic effect of DOX-Ar-CC-NPs was determined using superoxide dismutase commercial ELISA kit for cell membrane integrity, and flow cytometry, fluorescent imaging and electron microscopy for

programmed cells death evaluation. Enzymes-linked immunosorbent assay was also used to assay oxidative stress biomarkers and apoptotic enzymes from MCF-7 cell treated using DOX-Ar-CC-NPs and the proteomic profile of cancer cell treated with DOX-Ar-CC-NPs are examined and evaluated the release profile, cytotoxicity and uptake, in pursuance of understand the molecular sound effects of free and DOX-Ar-CC-NPs on MCF-7 cell line and to further improve the anticancer effects of doxorubicin through early prediction and resolution of its drug resistance problems. The results of the study shows that Ar-CC-NPs with average diameter of 35.5 nm, 19.3% loading content and 97% encapsulation efficiency has a surface potential and intensity of -19 ± 3.9 mV and 100%, respectively. In addition, DOX-Ar-CC-NPs have an IC_{50} at 24, 48 and 72 hours of 1.829µg/mL, 0.902µg/mL and 1.0377µg/mL while that of DOX alone were 0.475µg/mL, 0.2483µg/mL and 0.0723µg/mL, respectively. However, even at higher concentration, no apparent toxicity was recorded with Ar-CC-NPs, which reveals its anticancer effect with MCF-7 cells with a viability of 92%. The DOX-Ar-CC-NPs had significant inhibitory effect on cell feasibility compared to DOX alone ($p<0.05$), similar trend was noticed in cellular apoptosis, oxidative stress markers and cellular uptake evaluation. However, treatment with DOX-Ar-CC-NPs significantly decreased the elevated level of superoxide dismutase 2 (SOD2) compared to untreated MCF-7 cells. Thus, the present findings revealed the capability of DOX-Ar-CC-NPs to induce apoptosis in MCF-7 cells, which indicates the high potency of Ar-CC-NPs in drug delivery. For the proteomic study, a total of 408 MCF-7 related proteins for DOX-Ar-CC-NPs and 128 proteins for DOX alone were identified from MCF-7 cells. The proteomic profiling analysis outcomes reveals new developments for the advancement of proteomics technologies which could yield a good result on discovery of potential significant breast cancer biomarkers for MCF-7 cells pertinent studies with archival samples. Shotgun LC-MS/MS studies could also serve to determine new biomarkers.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN MOLEKULAR DOXORUBICIN DAN PARTIKEL NANO CALCIUM KARBONAT ARAGONIT DIMUATKAN DENGAN DOXORUBICIN KE ATAS SEL MCF-7

Oleh

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Ogos 2019

Pengerusi : Profesor Md Zuki bin Abu Bakar @ Zakaria, PhD
Institut : Biosains

Kanser adalah salah satu penyebab utama kematian dan kanser payudara adalah penyakit malignan yang paling biasa yang menyebabkan beban serius kepada wanita di seluruh dunia. Partikel nano kalsium karbonat aragonit (Ar-CC-NPs) yang berasal dari cengkerang kerang dan dimuatkan dengan doxorubicin (DOX) telah muncul sebagai terapi yang berkesan terhadap kanser payudara, namun begitu penggunaan terapi yang disasarkan dalam rawatan kanser nampaknya masih tidak berkesan dan sering dikaitkan dengan beberapa kekurangan, oleh itu keperluan untuk meningkatkan terapi sasaran adalah sangat diperlukan. Kajian ini menentukan potensi terapeutik partikel nano $CaCO_3$ aragonit yang dimuatkan dengan doxorubicin menggunakan pendekatan proteomik. Untuk kajian proteomik, analisis Spektrometri Massa Kromatografi Cecair (LCMS/MS) digunakan untuk menyiasat protein MCF-7 yang berkaitan dengan sel-sel kanser payudara manusia selepas rawatan dengan DOX dan DOX-Ar-CC-NPs. Partikel nano $CaCO_3$ disintesis daripada cengkerang kerang dan dicirikan untuk sifat fisiokimia menggunakan mikroskop elektron penghantaran (TEM), mikroskop elektron pengimbasan pelepasan (FESEM), potensi Zeta, teknik inframerah transmisi Fourier (FTIR) dan X-ray difraksi (XRD). Partikel nano aragonit disintesis dari cengkerang kerang semulajadi yang murah, yang dibersihkan dengan menggunakan kulit pisang, dihomogenkan dan kemudian diaduk bersungguh-sungguh dalam larutan dodecyl dimethyl betane (BS-12) menggunakan mesin pengaduk berputar untuk mengurangkan suhu dan bahan kimia tidak selamat yang dikaitkan dengan penyediaan partikel nano, dan kemudian dicirikan untuk geometri zarah menggunakan mikroskop elektron. Partikel nano kalsium karbonat aragonit (Ar-CC-NPs) yang disintesis kemudian dimuatkan dengan doxorubicin (DOX), satu agen antineoplastik, yang membentuk Doxorubicin-partikel nano kalsium karbonat aragonit (DOX-Ar-CC-NPs). Kesan sitotoksik DOX-Ar-CC-NPs ditentukan dengan menggunakan kit ELISA komersial superoxide dismutase untuk integriti membran

sel, dan aliran sitometri, pencahayaan pendarfluor dan mikroskopi elektron untuk penilaian sel mati yang telah diprogramkan. Ujian imunosorben yang berkaitan dengan enzim juga digunakan untuk menguji biomarker tekanan oksidatif dan enzim apoptotik dari sel MCF-7 yang dirawat dengan DOX-Ar-CC-NPs dan profil protein sel kanser yang dirawat dengan DOX-Ar-CC-NPs diperiksa dan dinilai profil pelepasan, sitotoksisiti dan pengambilan, untuk memahami kesan molekul bagi Dox dan DOX-Ar-CC-NPs ke atas sel MCF-7 dan untuk meningkatkan lagi kesan antikanser doxorubicin melalui ramalan awal dan penyelesaian masalah ketahanan dadah (Dox). Hasil kajian menunjukkan bahawa Ar-CC-NPs dengan purata diameter 35.5 nm, 19.3% kandungan pemuatan dan 97% kecekapan enkapsulasi mempunyai potensi permukaan dan intensiti masing-masing -19 ± 3.9 mV dan 100%. Selain itu, DOX-Ar-CC-NPs mempunyai IC_{50} pada 24, 48 dan 72 jam masing-masing adalah 1.829µg / mL, 0.902µg / mL dan 1.0377 µg / mL manakala DOX sahaja masing-masing ialah 0.475µg / mL, 0.2483µg / mL dan 0.0723µg / mL. Walau bagaimanapun, pada kepekatan yang lebih tinggi, tiada ketoksikan yang jelas dicatatkan dengan Ar-CC-NPs, ini menunjukkan keserasiannya dengan sel-sel MCF-7 dengan daya maju sebanyak 92%. DOX-Ar-CC-NPs mempunyai kesan perencatan yang ketara ke atas daya tahan sel berbanding DOX sahaja ($p < 0.05$). Trend yang sama telah diperhatikan dalam apoptosis selular, penanda stres oksidatif dan penilaian pengambilan selular. Walau bagaimanapun, rawatan dengan DOX-Ar-CC-NPs mengurangkan paras kenaikan superoxide dismutase 2 (SOD2) dengan ketara berbanding dengan sel MCF-7 yang tidak dirawat. Penemuan ini mendedahkan keupayaan DOX-NP untuk mendorong apoptosis dalam sel-sel MCF-7, yang menunjukkan potensi tinggi Ar-CC-NPs dalam penghantaran dadah. Untuk kajian proteomik, sebanyak 408 protein berkaitan MCF-7 untuk DOX-Ar-CC-NPs dan 128 protein untuk DOX sahaja telah dikenal pasti dari sel-sel MCF-7. Hasil daripada analisis profil proteomik membuka lembaran baharu untuk penggunaan teknologi proteomik yang maju yang menjanjikan penemuan daripada biomarker kanser payudara berpotensi besar untuk kajian sel MCF-7 menggunakan sample ark. Kajian LC-MS /MS juga boleh digunakan untuk mencari biomarker bahru dan manikin memberi petunjuk kepada genom profil proteomik kawalan kanser payudara, rawatan dan pengubahsuaian pada asas molekul. Kajian lanjut diperlukan untuk pengesahan biomarker dimasa hadapan untuk profil proteomik MCF-7 dan diharapkan rawatan dan pencegahan yang lebih baik dapat dicapai pada masa akan datang.

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patiently the length of last 3 years, being assiduously waiting for me over there in Nigeria to finish my study, despite we live so far from each other. ALHAMDULILAH.YA RABB accepts from us.

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ATCC	American Type Culture Collection
ANOVA	Analysis of variance
ANC	Cockle-shell derived $CaCO_3$ nanoparticles aragonite polymorph
AO/PI	Acridine Orange and Propidium Iodine
Ar-CC-NPs	Aragonite Calcium Carbonate Nanoparticles
BCRP	Breast Cancer Resistance Protein
BCA	Bicinchonic acid
BSA	Bovine Serum Albumin
BS-12	Dodecyl Dimethyl Betaine ($RN+(CH_3)_2CH_2COO-$)
^0C	Degree Celsius
CGM	Complete growth Media
$CaCO_3$	Calcium Carbonate
$CaCl_2$	Calcium Chloride
$Ca(NO_3)_2$	Calcium nitrate
$Ca(OH)_2$	Calcium hydroxide
CC-NPs	Calcium Carbonate Nanoparticles
CO_2	Carbon dioxide
DDS	Developing Drug Delivery Systems
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
DOX	Doxorubicin
DOX-NPs	Doxorubicin-Nanoparticles
DOX-Ar-CC-NPs	Doxorubicin Aragonite Calcium Carbonate Nanoparticles

dUTP	Deoxyuridine triphosphate
DTT	Dithiothreitol
EE	Encapsulation efficacy
Erb	Estrogen receptor beta
FBS	Foetal Bovine Serum
FDA	Food and Drug Administration
Fe_3O_4	Iron (III) Oxide
FESEM	Field Emission Scanning Electron Microscope
FT-IR	Fourier Transform Infrared
GSSG/GSH	Glutathione
HPP	Human Proteome Project
hrs	Hours
IC_{50}	Half maximal Inhibitory Concentration
IEF	Isoelectric focusing
IUPAC	International Union of Pure and Applied Chemists
IR	Infrared
ISO	International Standards Organization
KDa	Kilo Dalton
PBS	Phosphate Buffered Saline
PDI	Polydispersity index
PI	Protein Isoelectric Point
pH	Potential of Hydrogen
QDS	Quantum Dots
MARK	Mutagen Activated Protein Kinase
MCF-7	Michigan Cancer Foundation-7
MC3T3	Osteoblastic Cell line

MDR1	Mutagen Drug Resistance Protein 1
min	Minute(s)
MTT	3-(4, 5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
mV	Millivolt
µm	Micrometre
MS	Mass Spectrometry
$NaCO_3$	Sodium Carbonate
NADH	Nicotinamide Adenine Dinucleotide
NCBI	National Centre for Biotechnology Information
$(NH)_2CO_3$	Ammonium Carbonate
NPs	Nanoparticles
nm	Nanometre
PCC	Precipitated Calcium Carbonate
µg/ml	Microgram per millilitre
µL/ml	Microliter per millilitre
RNA	Ribonucleic acid
ROD	Renal Osteodystrophy in Dialysis
ROS	Reactive Oxygen Species
RIPA	Radio Immuno precipitation Assay
rpm	Rounds per minute
RT	Room Temperature
SEM	Scanning Electron Microscopy
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SD	Standard Deviation

SiRNA	Small interfering RNA
SOD	Superoxide Dismutase Activity
TEM	Transmission Electron Microscopy
TGA	Thermogravimetric Analysis
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labelling
TN	Triple negative
UPM	Universiti Putra Malaysia
UV	Ultraviolet
Wt.	Total weight of drug fed
Wf	Weight of free drug
Wnp	Weight of nanoparticles
LC	Loading Capacity
WHO	World Health Organization
XRD	X-Ray Diffraction
1 D-gel	One Dimensional gel
2 D-gel	Two dimensional gel
%	Percentage
Zp	Zeta potential

CHAPTER 1

INTRODUCTION

1.1 Study Background

Cancer is one of the prominent causes of death and breast tumour is the most common malignant disease which causes serious burden on women worldwide (Siegel *et al*., 2017). In an attempt to relief and improved the condition and health of cancer patients, Scientist across the world discovered new drugs, screening new targets and focus on more reliable diagnostic method of identifying cancer in its early stages. However scientist are have developed an approach, of using nanotechnology based novel system for the delivery of various anticancer drugs and specifically, in situ molecular self-assembly and self-delivery carriers to target cancer cells selectively (Wang *et al.,* 2016).Cancer are known to occur when there is a continuous cells division, in an uncontrolled manner as a result of genetic damaged which control either one or many cells (Noor den *et al.,* 1998).

Exposure to certain agents have been attributed to cause damage to the genetic material of a cells, thereby causing uncontrolled division and countless generation of cells division have taken place in all the living being for the purpose of life sustenance. To avoid such exposure, healthy lifestyle such as regular exercises, taking rich nutrients food, and avoid any exposure to DNA damaging agents in the environment are good measures which helps in control and prevention of cancer (Stephens *et al.,* 2009).

Chemotherapeutics are been used in management of neoplastic cells, although they lack precision and accompanied high toxicity in normal or non-neoplastic cells of the tissues (Bazak *et al*., 2015). Developing means that can be applied for early cancer diagnosis and effective therapy remains as a major challenges in cancer management, considering that the conventional cancer therapy is associated with serious side effects.

However, nanoparticles platforms are currently gaining attention as potential solution to deliver the targeted mediators which can be used for cancer diagnosis and treatment (Bazak *et al.,* 2015; Sanna *et al.,* 2014). Conventional methods such as chemotherapy and radiation therapy limitations can be overcome by designing new nanoparticle delivery system to carry the drugs of interest and other cancer-targeting modules (Shafiu *et al.,* 2014). Developing alternative nanomaterial's for therapeutic delivery system remains as a hurdle in the field of nanomedicine. As a solution, inorganic materials were been utilize recently, as a delivery carriers in the field of Nano therapeutics. Proteomic also, has evolved as a powerful tool for providing unbiased interpretation, thereby distinguishing cancer from non-cancer patients as reported by Mata *et al*. (2009). In addition, proteomics are used to identify and

quantify the novel biomarkers of various molecular targets in order detect, diagnose and predict the outcome of a cancer therapy (Lu *et al.*, 2018; Posadas *et al.*, 2005).

Furthermore, SDS page particularly 2D gel, electrophoresis is known to be a common molecular biology method which differentiates between the two protein expression and more distinct samples. Besides that, greater numbers of lower abundance of expressed proteins in a defined cell population could be detected and identified by mass spectrometry. Characterization of lysates from few tumour cells could be analysed by surface-enhanced laser ionization/desorption time of flight matrix-assisted laser ionization and desorption time of flight which may act as a suitable biomarker for cancerous study (Esteva *et al.*, 2004).

1.2 Statement of Problems

Targeted therapies have been observed to be effective in treating cancer; they are often inevitably associated with drug resistance and often been off-target. Additionally, there are existence problems with the prediction of response to targeted anticancer therapeutics (Lam *et al.*, 2014). However, this response prediction is vital for the understanding of therapeutic efficacy. Though, there are arrays of molecular markers in cancer diagnosis and management, there exist problems with identification of molecular markers to evaluate the efficacy of anticancer (Lam *et al*., 2014). Understanding the mechanism of nanoanticancers is significant for improved targeted therapeutics, thereby achieving early prediction of drug efficacy as well as overcoming various side effects using the proteomic approach.

1.3 Justification

The studies identify the major mediators in anticancer efficacy as it relates to doxorubicin, and reduce their effects using the cockle shell derived $CaCO_3$ nanoparticles. Molecular biomarkers of doxorubicin efficacy were identified as well as measure the anticancer efficacy of DOX when loaded on cockle shell derived $CaCO_3$ nanoparticle as targeted drug carrier.

1.4 Hypothesis

i. Doxorubicin-loaded cockle shell-derived CaCO3 nanoparticles elicit oxidative stress in breast cancer cell lines (MCF-7 cell).
ii. Doxorubicin-loaded cockle shell-derived CaCO3 nanoparticles elicit apoptotic effects in breast cancer cell lines (MCF-7 cell).
iii. Doxorubicin-loaded cockle shell-derived CaCO3 nanoparticles elicit proteomic responses in breast cancer cells lines (MCF-7 cell).

1.5 General Objective

To determine the molecular effects of free Doxorubicin and Doxorubicin-loaded aragonite calcium carbonate nanoparticles on MCF-7 cell lines.

1.6 Specific Objectives

i. To synthesize and characterize Doxorubicin-loaded calcium carbonates nanoparticles with the help of banana peels agent and it's in vitro release profiles.
ii. To determine changes in oxidative stress markers following treatment with free Doxorubicin and Doxorubicin-loaded calcium carbonate nanocrystals on MCF-7 cancer cell line.
iii. To determine the in vitro apoptotic pathways in cancer cell line following treatment with free Doxorubicin and Doxorubicin-loaded calcium carbonate loaded nanoparticles in MCF-7 cells.
iv. To determine the proteomic profiles of MCF-7 cells treated with free Doxorubicin and Doxorubicin-loaded calcium carbonate nanoparticles.

REFERENCES

Abak, A., Abhari, A. & Rahimzadeh, S. (2018). Exosomes in cancer: small vesicular transporters for cancer progression and metastasis, biomarkers in cancer therapeutics. *PeerJ*, *6*: e4763.

Abdelaziz, H. M., Gaber, M., Abd-elwakil, M. M., Mabrouk, M. T., Elgohary, M. M., Kamel, N. M., … Elzoghby, A. O. (2018). Inhalable particulate drug delivery systems for lung cancer therapy : Nanoparticles , microparticles , nanocomposites and nanoaggregates. *Journal of Controlled Release*. 269 :374–392.

Acar, H., Srivastava, S., Ji, E., Schnorenberg, M. R., Barrett, J. C., Labelle, J. L., & Tirrell, M. (2017). Self-assembling peptide-based building blocks in medical applications. *Advanced Drug Delivery Reviews*. 110–111: 65–79.

Ahamed, M., Akhtar, M. J., Alhadlaq, H. A., Khan, M. A. M., & Alrokayan, S. A. (2015). Comparative cytotoxic response of nickel ferrite nanoparticles in human liver HepG2 and breast MFC-7 cancer cells. *Chemosphere*. 135: 278–288.

Ahamed, M., Akhtar, M. J., Khan, M. A. M., Alrokayan, S. A., & Alhadlaq, H. A. (2019).Oxidative stress mediated cytotoxicity and apoptosis response of bismuth oxide (Bi2O3) nanoparticles in human breast cancer (MCF-7) cells. *Chemosphere*. 216:823–831.

Ahamed, M., Javed, M., Alhadlaq, H. A., & Alshamsan, A. (2016). Colloids and Surfaces B : Biointerfaces Copper ferrite nanoparticle-induced cytotoxicity and oxidative stress in human breast cancer MCF-7 cells. *Colloids and Surfaces B: Biointerfaces*. 142: 46–54.

Ahamed, M., Javed, M., Alhadlaq, H. A., Alshamsan, A., Bhosle, S. M., Huilgol, N. G., … Oliver, J. (2005). Apoptotic index as predictive marker for radiosensitivity of cervical carcinoma : Evaluation of membrane fluidity , biochemical parameters and apoptosis after the first dose of fractionated radiotherapy to patients. *Colloids and Surfaces B: Biointerfaces*. 29(9), 369–375.

Al-Khanbashi, M., Caramuta, S., Alajmi, A. M., Al-Haddabi, I., Al-Riyami, M., Lui, W. O., & Al-Moundhri, M. S. (2016).Tissue and serum mirna profile in locally advanced breast cancer (labc) in response to neo-adjuvant chemotherapy (nac) treatment. *PLoS One*.11 (4): e0152032.

Al- Obaidi, J. R., Jamil, N. A. M., Rahmad, N., & Rosli, N. H. M.(2018).Proteomic and metabolomic study of wax apple (Syzygium samarangense) fruit during ripening process. *Electrophoresis.* 39(23): 2954-2964.

Aldana-Masangkay, G. I., & Sakamoto, K. M. (2011).The Role of HDAC6 in Cancer. *Journal of Biomedicine and Biotechnology*. 1–10.

Alfarouk, K. O., Stock, C. M., Taylor, S., Walsh, M., Muddathir, A. K., Verduzco, D.Rauch, C. (2015). Resistance to cancer chemotherapy : failure in drug response from ADME to P ‐ gp. *Cancer Cell International*. 1–13.

Ali, M. E., Hashim, U., Mustafa, S., Man, Y. C., Yusop, M. H. M., Bari, M. F.& Hasan, M. F. (2011). Nanoparticle sensor for label free detection of swine DNA in mixed biological samples. *Nanotechnology*. 22(19): 195503.

Alibolandi, M.,Abnous, K., Mohammadi, M., Hadizadeh, F., Sadeghi, F., Taghavi, S. Jaafari, MR., Ramezani, M (2017).Extensive preclinical investigation of polymersomal formulation of doxorubicin versus Doxil-mimic formulation. *Journal of Controlled Release.*264:228-236.

Alshammari, G. M., Ignacimuthu, S., & Alshatwi, A. A. (2017). ScienceDirect Epoxy clerodane diterpene inhibits MCF-7 human breast cancer cell growth by regulating the expression of the functional apoptotic genes Cdkn2A , Rb1 , mdm2 and p53. *Biomedicine et Pharmacotherapy*. 87: 388–396.

Amin, S., Rattner, J., Keramati, M. R., Farshidfar, F., McNamara, M. G., Knox, J. J.& Bathe, O. F. (2019).A strategy for early detection of response to chemotherapy drugs based on treatment-related changes in the metabolome. *PloS one*. 14(4): e0213942.

Anchordoquy, T. J., Barenholz, Y., Boraschi, D., Chorny, M., Decuzzi, P., Dobrovolskaia, M. A. Simberg, D.(2017).Mechanisms and Barriers in Cancer Nanomedicine: Addressing Challenges, Looking for Solutions. *ACS nano*. 11(1): 12–18.

Annadurai, G., Juang, R. S., & Lee, D. J. (2003).Adsorption of heavy metals from water using banana and orange peels.*Water science and technology*.47(1):185-190.

Anadon, A., Martínez, M. A., Castellano, V., & Martínez-Larrañaga, M. R(2014).The role of in vitro methods as alternatives to animals in toxicity testing. *Expert opinion on drug metabolism & toxicology*.10 (1): 67-79.

Anwar, A., Gould, E., Tinson, R., Iqbal, J., & Hamilton, C. (2018).Redox Modulation at Work: Natural Phytoprotective Polysulphides from Alliums Based on Redox-Active Sulfur.*Current pharmacology reports*. 4(5): 397-407.

Anhwange, B. A., Ugye, T. J., & Nyiaatagher, T. D.(2008). Chemical composition of Musa sapientum (banana) peels. *Journal of Food Technology*.6(6):263-266.

Arber, N., Shapira, I., Ratan, J., Stern, B., Hibshoosh, H., Moshkowitz, M & Halpern, Z. (2000).Activation of cK-ras mutations in human gastrointestinal tumors.*Gastroenterology*. 118(6): 1045-1050.

Akakuru, O. U., Louis, H., Oyebanji, O. O., Ita, B. I., & Amos, P. I. (2018).*Utility of Nanomedicine for Cancer Treatment. J Nanomed Nanotechnol* 9*: 481.*

Arms, L., Smith, D. W., Flynn, J., Palmer, W., Martin, A., Woldu, A., & Hua, S. (2018).Advantages and limitations of current techniques for analyzing the biodistribution of nanoparticles. *Frontiers in pharmacology*. 9.

Bazak, R., Houri, M., El Achy, S., Kamel, S., & Refaat, T. (2015). Cancer active targeting by nanoparticles: a comprehensive review of literature.*Journal of cancer research and clinical oncology*. 141(5): 769-784.

Boja, E., Rivers, R., Kinsinger, C., Mesri, M., Hiltke, T., Rahbar, A., & Rodriguez, H.(2010).Restructuring proteomics through verification. *Biomarkersin medicine*. 4(6):799-803.

Berardi, A., Bisharat, L., Cespi, M., Basheti, I. A., Bonacucina, G., Pavoni, L.& Alkhatib, H. S. (2017). Controlled release properties of zein powder fi lled into hard gelatin capsules. *Powder Technology*. 320: 703–713.

Biswas, S., Kumari, P., Lakhani, P. M., & Ghosh, B. (2016). European Journal of Pharmaceutical Sciences Recent advances in polymeric micelles for anti-cancer drug delivery. *PHASCI*. 83:184–202.

Blanco, E., Shen, H., & Ferrari, M.(2015).Principles of nanoparticle design for overcoming biological barriers to drug delivery.*Nature biotechnology*.33(9):941.

Bobo, D., Robinson, K. J., Islam, J., Thurecht, K. J., Corrie, S. R., & Corrie, S. R. (2016). Nanoparticle-Based Medicines : A Review of FDA-Approved Materials and Clinical Trials to Date. *Pharmaceutical Research*. 2373–2387.

Bodoor, K., Abu-Sheikha, A., Matalka, I., Alzou'bi, H., Batiha, O., Abu-Awad, A & Albatayneh, K.(2018).Immunohistochemical analysis of heat shock proteins in triple negative breast cancer: HSP60 expression is a marker of poor prognosis. *Eur. J. Gynaecol. Oncol*, 39(6)

Boenink, M. (2010).Molecular medicine and concepts of disease: the ethical value of a conceptual analysis of emerging biomedical technologies. *Medicine, health care and philosophy*.13 (1):11-23.

Boiziau, C., Nikolski, M., Mordelet, E., Aussudre, J., Vargas-Sanchez, K., & Petry, K. G. (2018). A Peptide Targeting Inflammatory CNS Lesions in the EAE Rat Model of Multiple Sclerosis. *Inflammation*. 41(3):932–947.

Boyd, L. S., Gozuacik, D., & Joubert, A. M. (2018). The in vitro effects of a novel estradiol analog on cell proliferation and morphology in human epithelial cervical carcinoma. *Cellular and Molecular Biology Letters*.23(1):1–23.

Brannon-peppas, L., & Blanchette, J. O. (2012). Nanoparticle and targeted systems for cancer therapy.*Advanced Drug Delivery Reviews*. 64: 206–212.

Braunstein, M., Liao, L., Lyttle, N., Lobo, N., Taylor, K. J., Krzyzanowski, P. M., Spears, M. (2016).Downregulation of histone H2A and H2B pathways is associated with anthracycline sensitivity in breast cancer. *Breast Cancer Research*. 18(1):1–16.

Brenneisen, P. & Reichert, A. (2018).Nanotherapy and reactive oxygen species (ROS) in cancer: A novel perspective.*Antioxidants*.7(2): 31.

Brigger, I., Dubernet, C., & Couvreur, P. (2012). Nanoparticles in cancer therapy and diagnosis. *Advanced Drug Delivery Reviews*. 64: 24–36.

Brown, A., Kai, M., DuRoss, A., Sahay, G., & Sun, C.(2018).Biodistribution and toxicity of micellar platinum nanoparticles in mice via intravenous administration. *Nanomaterials*. 8(6):410.

Bussard, K. M., Mutkus, L., Stumpf, K., Gomez-manzano, C., & Marini, F. C. (2016). Tumor-associated stromal cells as key contributors to the tumor microenvironment. *Breast Cancer Research*. 1–11.

Blue, C. R., Giuffre, A., Mergelsberg, S., Han, N., De Yoreo, J. J., & Dove, P. M.(2017).Chemical and physical controls on the transformation of amorphous calcium carbonate into crystalline CaCO3 polymorphs. *Geochimica et Cosmochimica Acta*.196:179-196.

Bharatham, H.M. D., & Perimal, E. K.(2014).Mineral and physiochemical evaluation of Cockle shell (Anadara granosa) and other selected Molluscan shell as potential biomaterials. *Sains Malaysiana*. 43(7):1023-1029.

Cabeza, L., Ortiz, R., Prados, J., Delgado, Á. V., Martín-Villena, M. J., Clares, B. & Arias, J. L. (2017). Improved antitumor activity and reduced toxicity of doxorubicin encapsulated in poly (ε-caprolactone) nanoparticles in lung and breast cancer treatment: an in vitro and in vivo study. *European Journal of Pharmaceutical Sciences*.102:24-34.

Camerini, S., & Mauri, P. (2015). The role of protein and peptide separation before mass spectrometry analysis in clinical proteomics. *Journal of Chromatography*. 1381: 1–12.

Campos, R., Maria, D., Britto, C., Fátima, W. De, Eustáquio, G., Brito-melo, A. Mattos, M. (2018). Original article Effect of short- and medium-term toxicity of doxorubicin on spermatogenesis in adult Wistar rats. *Reproductive Biology*. 18(2):169–176.

Cannell, I. G., Merrick, K. A., Morandell, S., Tsao, M., Hemann, M. T., Yaffe, M. B.,Grant, R. A. (2015). A Pleiotropic RNA-Binding Protein Controls Distinct Cell Cycle Checkpoints to Drive Resistance of p53 - Defective Tumors to Chemotherapy Article A Pleiotropic RNA-Binding Protein Controls Distinct Cell Cycle Checkpoints to Drive Resistance of p53 . *Cancer Cell*. 1–15.

Chang, A. G., Hu, J., Lake, E., Bouley, D. M., & Johns, J. L.(2015).Biochemical and hematologic reference intervals for aged Xenopus laevis in a research colony.*Journal of the American Association for Laboratory Animal Science*. 54(5): 465-470.

Chang, F. W., Fan, H. C., Liu, J. M., Fan, T. P., Jing, J., Yang, C. L., & Hsu, R. J. (2017). Estrogen Enhances the Expression of the Multidrug Transporter Gene ABCG2—Increasing Drug Resistance of Breast Cancer Cells through Estrogen Receptors.*International journal of molecular sciences*. 18(1):163.

Chen, S. T., Pan, T. L., Tsai, Y. C., & Huang, C. M. (2002).Proteomics reveals protein profile changes in doxorubicin–treated MCF-7 human breast cancer cells.*Cancer letters*. 181(1): 95-107.

Christiana, A., & Aebersold, R. (2018). Advancing translational research and precision medicine with targeted proteomics. *Journal of Proteomics*.189:1–10.

Colombo, S., Beck-broichsitter, M., Peter, J., Malmsten, M., Rantanen, J.& Bohr, A. (2018).Transforming nanomedicine manufacturing toward Quality by Design and micro fl uidics. *Advanced Drug Delivery Reviews*. 128:115–131.

Danmaigoro, A., Selvarajah, G. T., Noor, M. H. M., Mahmud, R., Zakaria, M., & Bakar, Z. A. (2017).Development of cockleshell (Anadara granosa) derived CaCO3 nanoparticle for doxorubicin delivery. *Journal of Computational and Theoretical Nanoscience*. 14(10):5074-5086.

Davies, K. J.(2000).Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. *IUBMB life*.50(4- 5): 279-289.

Deavall, D. G., Martin, E. A., Horner, J. M., & Roberts, R. (2012).Drug-induced oxidative stress and toxicity.*Journal of toxicology*.2012.

Development, P. (2018). Ac ce pt us, *7450*. https://doi.org/10.1080/10837450.2018.1545236

Dhand, C., Dwivedi, N., Loh, J., & Jie, N. (2015).Methods and strategies for the synthesis of diverse nanoparticles and their applications : *RSC Advances*. 5:105003–105037.

Dimitriou, N. M., Tsekenis, G., Balanikas, E. C., Pavlopoulou, A., Mitsiogianni, M., Mantso, T.Georgakilas, A. G. (2017).Pharmacology & Therapeutics Gold nanoparticles , radiations and the immune system : Current insights into the physical mechanisms and the biological interactions of this new alliance towards cancer therapy. *Pharmacology and Therapeutics*.178:1–17.

Dirks, P. B., Meyer, M., Reimand, J., Lan, X., Head, R., Zhu, X., … Bader, G. D. (2014). Single Cell Derived Clonal Analysis of Human Glioblastoma Links Functional and Genomic Heterogeneity. *Neuro-Oncology*.16(3): iii14-iii14.

Dou, M., Chouinard, C. D., Zhu, Y., Nagy, G., Liyu, A. V., Ibrahim, Y. M. & Kelly, R. T. (2018).Nanowell-mediated multidimensional separations combining nanoLC with SLIM IM-MS for rapid, high-peak-capacity proteomic analyses. *Analytical and bioanalytical chemistry*, 1-10.

Dudefoi, W., Villares, A., Peyron, S., Moreau, C., Ropers, M., Gontard, N., & Cathala, B. (2018). Nanoscience and nanotechnologies for biobased materials , packaging and food applications : New opportunities and concerns. *Innovative Food Science and Emerging Technologies.* 46: 107–121.

Durand, N., Monger, H. C., Canti, M. G., & Verrecchia, E. P. (2018). Calcium carbonate features. In *Interpretation of micromorphological features of soils and regoliths* (pp. 205-258). Elsevier.

Dixon J & Kuldell N (2011)BioBuilding using banana-scented bacteria to teach synthetic biology.Methods Enzymol. 497:255-71.

Elanthikkal, S., Gopalakrishnapanicker, U., Varghese, S., & Guthrie, J. T. (2010). Cellulose microfibres produced from banana plant wastes: Isolation and characterization.*Carbohydrate Polymers*. 80(3):852-859.

Essien, J. P., Akpan, E. J., & Essien, E. P.(2005).Studies on mould growth and biomass production using waste banana peel. *Bioresource Technology*. 96(13):1451-1456.

Elmore, S. (2007).Apoptosis: a review of programmed cell death. *Toxicologic pathology*.35 (4): 495-516.

Eccles, S. A. & Welch, D. R. (2007).Metastasis: recent discoveries and novel treatment strategies.*The Lancet*. 369(9574):1742-1757.

Elebro, K., Borgquist, S., Rosendahl, A. H., Markkula, A., Simonsson, M., Jirström, K., Jernström, H. (2017). High estrogen receptor β expression is prognostic among adjuvant chemotherapy-treated patients - Results from a population-based breast cancer cohort. *Clinical Cancer Research*. 23(3):766–777.

Esteva, F. J., & Hortobagyi, G. N. (2004). Prognostic molecular markers in early breast cancer. *Breast Cancer Research* .6(3):109–118.

Farhane, Z., Bonnier, F., Maher, M. A., Bryant, J., Casey, A., & Byrne, H. J.(2017). Differentiating responses of lung cancer cell lines to Doxorubicin exposure: in vitro Raman micro spectroscopy, oxidative stress and bcl- 2 protein expression. *Journal of biophotonics*. 10(1), 151-165.

Felder, S., Morley, L., Ng, E., Chan, K., Ballantyne, H., Di Tomasso, A. & Rink, A.(2018).Brachytherapy patient safety events in an academic radiation medicine program. *Brachytherapy*.17(1):16-23.

Filice, F. P., Henderson, J. D., Li, M. S. & Ding, Z.(2019).Correlating Live Cell Viability with Membrane Permeability Disruption Induced by Trivalent Chromium. *ACS omega*. 4(1):2142-2151.

Fukui, Y., & Fujimoto, K.(2012).Bio-inspired nanoreactor based on a miniemulsion system to create organic–inorganic hybrid nanoparticles and nanofilms. *Journal of Materials Chemistry*.22(8), 3493-3499.

Fu, W., Mohd Noor, M. H., Yusof, L. M., Ibrahim, T. A. T., Keong, Y. S., Jaji, A. Z., & Zakaria, M. Z. A. B. (2017). In vitro evaluation of a novel pH sensitive drug delivery system based cockle shell-derived aragonite nanoparticles against osteosarcoma.*Journal of Experimental Nanoscience*. 12(1): 166-187.

Gérard, C., & Goldbeter, A. (2014).The balance between cell cycle arrest and cell proliferation: control by the extracellular matrix and by contact inhibition. *Interface focus*.4(3):20130075.

Ghaji, M. S., Abu, Z., Zakaria, B., Shameha, A. R. I., Noor, M., Hezmee, M., & Hazilawati, H. (2017). Novelty to Synthesis Nanoparticles from Cockle Shall via Mechanical Method to Delivery and Controlled Release of Cytarabine, *14*(xx), 1–9.

Ghorbani, M., & Morgan, M. R.(2010).Protein characterisation and immunochemical measurements of residual macadamia nut proteins in foodstuffs.*Food and agricultural immunology*. 21(4):347-360.

González-Fernández, Y., Imbuluzqueta, E., Zalacain, M., Mollinedo, F., Patiño-García, A., & Blanco-Prieto, M. J.(2017).Doxorubicin and edelfosine lipid nanoparticles are effective acting synergistically against drug-resistant osteosarcoma cancer cells. *Cancer letters*. *388*: 262-268.

Ghafar, M. A., Liyana, S., Hussein, M. Z., & Abu Bakar Zakaria, Z. (2017). Synthesis and characterization of cockle shell-based calcium carbonate aragonite polymorph nanoparticles with surface functionalization.*Journal of Nanoparticles*. 2017.

González-Fernández, Y., Imbuluzqueta, E., Zalacain, M., Mollinedo, F., Patiño-García, A. & Blanco-Prieto, M. J. (2017).Doxorubicin and edelfosine lipid nanoparticles are effective acting synergistically against drug-resistant osteosarcoma cancer cells.*Cancer letters*. 388:262-268.

Gottesman, M. M., Fojo, T., & Bates, S. E.(2002).Multidrug resistance in cancer: role of ATP–dependent transporters.*Nature Reviews Cancer*. 2(1): 48.

Gottlieb, E., & Vousden, K. H.(2010).P53 regulation of metabolic pathways. *Cold Spring Harbor perspectives in biology*.2 (4): a001040.

Guan, X. (2015).Cancer metastases: challenges and opportunities. *Acta pharmaceutica sinica B*. 5(5): 402-418.

Gurunathan, S., Han, J. W., Eppakayala, V., Jeyaraj, M., & Kim, J. H.(2013).Cytotoxicity of biologically synthesized silver nanoparticles in MDA-MB-231 human breast cancer cells. *BioMed research international*, 2013.

Gillespie and, J. P., Kanost, M. R., & Trenczek, T. (1997). Biological mediators of insect immunity.*Annual review of entomology*. 42(1):611-643.

Hammadi, N. I., Abba, Y., Hezmee, M. N. M., Razak, I. S. A., Jaji, A. Z., Isa, T., & Zakaria, M. Z. A. B. (2017). Formulation of a sustained release docetaxel loaded cockle shell-derived calcium carbonate nanoparticles against breast cancer.*Pharmaceutical research*. 34(6):1193-1203

Hanash, S.(2003).Disease proteomics.*Nature*. 422(6928):226.

Hariharan, M., Varghese, N., Cherian, A. B.& Paul, J. (2014).Synthesis and characterisation of CaCO3 (Calcite) nano particles from cockle shells using chitosan as precursor.*International Journal of Scientific and Research Publications*. 4(10):1-5.

Hassan, S., Prakash, G., Ozturk, A. B., Saghazadeh, S., Sohail, M. F., Seo, J. & Khademhosseini, A.(2017).Evolution and clinical translation of drug delivery nanomaterials. *Nano Today*. 15:91-106.

Hilal, N., Al-Zoubi, H., Darwish, N. A., Mohamma, A. W., & Arabi, M. A.(2004).A comprehensive review of nanofiltration membranes: Treatment, pretreatment, modelling, and atomic force microscopy.*Desalination*. 170(3):281-308

Hong, S. M., Hwang, S. W., Wang, T., Park, C. W., Ryu, Y. M., Jung, J. H. & Yoon, G. (2019).Increased nicotinamide adenine dinucleotide pool promotes colon cancer progression by suppressing reactive oxygen species level.*Cancer science*. 110(2):629.

Hoque, M. E., Shehryar, M., & Islam, K. N. (2013).Processing and characterization of cockle shell calcium carbonate (CaCO3) bioceramic for potential application in bone tissue engineering. *J Mater Sci Eng*. 2(4):132.

Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., & Sarkar, S. (2014). Drug resistance in cancer: an overview. *Cancers*. 6(3):1769-1792.

Hu, X., & Zhang, Z. (2015). Understanding the Genetic Mechanisms of Cancer Drug Resistance Using Genomic Approaches. *Trends in Genetics*. *xx*, 1–11.

Huang, S. (2009). Non-genetic heterogeneity of cells in development: more than just noise. *Development*. 136(23):3853–3862.

Ilyin, S. E., Belkowski, S. M., & Plata-Salamán, C. R.(2004).Biomarker discovery and validation: technologies and integrative approaches.*Trends in biotechnology*. 22(8):411-416.

Jameel, R. A. O., Yusmin, M. Y., Tey, C. C., Normahnani, M. N., & Rofina, Y. O. (2016). Identification of a partial oil palm polygalacturonase-inhibiting protein (EgPGIP) gene and its expression during basal stem rot infection caused by Ganoderma boninense. *African Journal of Biotechnology*.9(46):7788–7797.

Jemal, A., Center, M. M., DeSantis, C., & Ward, E. M. (2010).Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiology and Prevention Biomarkers*.19 (8):1893-1907.

Jhaveri, A., Deshpande, P., & Torchilin, V. (2014).Stimuli-sensitive nanopreparations for combination cancer therapy. *Journal of controlled release*.190: 352-370.

Jaji, A. Z., Bakar, M. Z. B. A., Mahmud, R., Loqman, M. Y., Hezmee, M. N. M., Isa, T.& Hammadi, N. I.(2017).Synthesis, characterization, and cytocompatibility of potential cockle shell aragonite nanocrystals for osteoporosis therapy and hormonal delivery.*Nanotechnology, science and applications*.10: 23

Jones, P. A., Issa, J. P. J., & Baylin, S. (2016).Targeting the cancer epigenome for therapy. *Nature Reviews Genetics*.17(10):630–641.

Joseph, M. M., Aravind, S. R., George, S. K., Raveendran Pillai, K., Mini, S., & Sreelekha,T.T.(2015). Anticancer activity of galactoxyloglucan polysaccharide-conjugated doxorubicin nanoparticles: Mechanistic insights and interactome analysis. *European Journal of Pharmaceutics and Biopharmaceutics*. 93:183–195.

Kamba, S., Ismail, M., Hussein-Al-Ali, S., Ibrahim, T., & Zakaria, Z.(2013). In vitro delivery and controlled release of doxorubicin for targeting osteosarcoma bone cancer. *Molecules*. 18(9):10580-10598

Kamba, A. S., Ismail, M., Ibrahim, T. A. T., & Zakaria, Z. A. B. (2013). Synthesis and Characterisation of Calcium Carbonate Aragonite Nanocrystals from Cockle Shell Powder (Anadara granosa). *Journal of Nanomaterials.2013*.

Kamper, P., Ludvigsen, M., Bendix, K., Hamilton-Dutoit, S., Rabinovich, G. A., Møller, M. B & d'Amore, F. (2011).Proteomic analysis identifies galectin-1 as a predictive biomarker for relapsed/refractory disease in classical Hodgkin lymphoma. *Blood*. 117(24):6638-6649

Khan, I., Saeed, K. & Khan, I. (2017). Nanoparticles : Properties , applications and toxicities. *Arabian Journal of Chemistry*. 10:1016

Khan, M. S. (2017). Doctor of Philosophy.

Kiranda, H. K., Mahmud, R., Abubakar, D., & Zakaria, Z. A. (2018).Fabrication, characterization and cytotoxicity of spherical-shaped conjugated gold-cockle shell derived calcium carbonate nanoparticles for biomedical applications. *Nanoscale research letters*. 13(1):1-10.

Kooijmans, S.A.,Gitz-Francois, J. J.,Schiffelers, R. M.& Vader, P. (2018).Recombinant phosphatidylserine-binding nanobodies for targeting of extracellular vesicles to tumor cells: a plug-and-play approach. *Nanoscale*. 10(5):2413-2426

Kulasingam, V. & Diamandis, E. P. (2008).Strategies for discovering novel cancer biomarkers through utilization of emerging technologies. *Nature Reviews Clinical Oncology*. 5(10):588.

Kuleš, J., Mrljak, V., Rafaj, R. B., Selanec, J., Burchmore, R., & Eckersall, P. D. (2014). Identification of serum biomarkers in dogs naturally infected with Babesia canis canis using a proteomic approach. *BMC veterinary research*. 10(1):111.

Lam, S. W., Jimenez, C. R., & Boven, E. (2014). Breast cancer classification by proteomic technologies: Current state of knowledge. *Cancer Treatment Reviews*.40(1):129–138.

Lee, J. A., Kim, M. K., Kim, H. M., Lee, J. K., Jeong, J., Kim, Y. R. & Choi, S. J. (2015). The fate of calcium carbonate nanoparticles administered by oral route: absorption and their interaction with biological matrices.*International journal of nanomedicine*. 10:2273.

Li, L., Andrén, P. E., & Sweedler, J. V. (2018).Editorial and Review: 29th ASMS Sanibel Conference on Mass Spectrometry—Peptidomics: Bridging the Gap between Proteomics and Metabolomics by MS. *Journal of the American Society for Mass Spectrometry*. 29(5):801-806.

Lin, Y. R., Kuo, C. J., Lin, H. Y. H., Wu, C. J., & Liang, S. S. (2014).A proteomics analysis to evaluate cytotoxicity in NRK-52E cells caused by unmodified nano-Fe3O4.*The Scientific World Journal*. 2014

Liu, Z., Bi, Y., Sun, Y., Hao, F., Lu, J., Meng, Q. & Xie, J.(2017).Pharmacokinetics of a liposomal formulation of doxorubicin in rats.*Saudi Pharmaceutical Journal*. 25(4): 531-536.

Liu, M., Zheng, S., Zhang, X., & Guo, H.(2018).Cerenkov luminescence imaging on evaluation of early response to chemotherapy of drug-resistant gastric cancer. *Nanomedicine: Nanotechnology, Biology, and Medicine*. 14(1): 205–213.

Liu, Y., Wang, W., Yang, J., Zhou, C., & Sun, J. (2013). ScienceDirect pH-sensitive polymeric micelles triggered drug release for extracellular and intracellular drug targeting delivery. *Asian Journal of Pharmaceutical Sciences*. 8(3):159–167.

Lu, M., & Zhan, X. (2018)The crucial role of multiomic approach in cancer research and clinically relevant outcomes.*EPMA Journal*. 9(1):77-102.

Lucas, M., Stuart, L. M., Savill, J., & Lacy-Hulbert, A.(2003). Apoptotic cells and innate immune stimuli combine to regulate macrophage cytokine secretion. *The Journal of Immunology*. 171(5): 2610-2615.

Lu, L., Wang, W., Wang, M., Kang, T. S., Lu, J. J., Chen, X. P & Ma, D. L.(2016).A luminescent G-quadruplex-selective iridium (III) complex for the label-free detection of lysozyme. *Journal of Materials Chemistry B*. 4(14):2407-2411.

Luqmani, Y. A.(2005).Mechanisms of drug resistance in cancer chemotherapy.*Medical Principles and Practice*. 14(1):35-48.

Mahmood, S. K., Zakaria, M. Z., Razak, I. S. A., Yusof, L. M., Abubakar, A. A., Mahmood, Z. K., & Ab Latip, M. B. (2018). In Vivo evaluation of the novel nanocomposite porous 3D scaffold in a rabbit model: haematology and biochemistry analysis. *Iraqi Journal of Veterinary Sciences*. 32(2): 219-230.

Majno, G. & Joris, I.(1995).Apoptosis, oncosis, and necrosis. An overview of cell death. *The American journal of pathology*. 146(1).3.

Manatunga, D. C., de Silva, R. M., de Silva, K. N. & Neelika, G. (2018).Effective delivery of hydrophobic drugs to breast (MCF-7) and liver (HepG2) cancer cells: a detailed investigation using cytotoxicity assays, fluorescence imaging and flow cytometry. *Cytometry*. 2018.001.

Maliepaard, M., Scheffer, G. L., Faneyte, I. F., van Gastelen, M. A., Pijnenborg, A. C., Schinkel, A. H. & Schellens, J. H. (2001).Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Research*. 61(8): 3458-3464.

Maruyama, N., Nishihara, K., Nakasone, T., Saio, M., Maruyama, T., Tedokon, I. & Yoshimi, N. (2018).Triple primary malignancies of surface osteosarcoma of jaw, myelodysplastic syndrome and colorectal cancer as a second primary cancer detected by PET2-[18F]-fluoro-2-deoxy-D-glucose positron emission tomography: A case report. *Oncology letters*. 15(6): 9901-9907.

Mao, B., Chen, Z., Wang, Y., & Yan, S. (2018). Silver nanoparticles have lethal and sublethal adverse effects on development and longevity by inducing ROS-mediated stress responses. *Scientific Reports*. (500): 1–16. 20728.

Marcazzan, S., Varoni, E. M., Blanco, E., & Lodi, G. (2018). Nanomedicine , an emerging therapeutic strategy for oral cancer therapy. *Oral Oncology*.76:1–7.

Mihoub, A. B., Saidat, B., Bal, Y., Frochot, C., Vanderesse, R., & Acherar, S. (2018). Development of new ionic gelation strategy: Towards the preparation of new monodispersed and stable hyaluronic acid/β-cyclodextrin-grafted chitosan nanoparticles as drug delivery carriers for doxorubicin. *Frontiers of Materials Science*. 12(1): 83-94.

Mitchison,T.J. (2012).The proliferation rate paradox in antimitotic chemotherapy. *Molecular biology of the cell*. 23(1):1-6.

Mohd Abd Ghafar, S. L., Hussein, M. Z., Rukayadi, Y., & Zakaria, M. Z. A. B. (2017). Surface-functionalized cockle shell-based calcium carbonate aragonite polymorph as a drug nanocarrier. *Nanotechnology, Science and Applications*. 10: 79–94.

Nebbia, C., Girolami, F., Carletti, M., Gasco, L., Zoccarato, I., & Giuliano, A. (2017). In vitro interactions of malachite green and leucomalachite green with hepatic drug-metabolizing enzyme systems in the rainbow trout (Onchorhyncus mykiss). *Toxicology Letters*. 41–47.

Nel, M., Joubert, A. M., Dohle, W., Potter, B. V., & Theron, A. E. (2018).Modes of cell death induced by tetrahydroisoquinoline-based analogs in MDA-MB-231 breast and A549 lung cancer cell lines. *Drug design, development and therapy*.12:1881.

Nyåkern, M., Cappellini, A., Mantovani, I., & Martelli, A. M.(2006).Synergistic induction of apoptosis in human leukemia T cells by the Akt inhibitor perifosine and etoposide through activation of intrinsic and Fas-mediated extrinsic cell death pathways. *Molecular Cancer Therapeutics*. 5(6):1559-1570.

NNg, V. Y., Scharschmidt, T. J., Mayerson, J. L., & Fisher, J. L. (2013). Incidence and survival in sarcoma in the United States: A focus on musculoskeletal lesions. *Anticancer Research*. 33(6): 2597–2604.

Nicholls, C., Pinto, A. R., Li, H., Li, L., Wang, L., Simpson, R., & Liu, J.-P. (2012). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) induces cancer cell senescence by interacting with telomerase RNA component. *Proceedings of the National Academy of Sciences*.109(33):13308–13313.

Nurul, K., Ali, E., Bin, Z., Bakar, A., Loqman, M. Y., Islam, A., Ullah, M. (2013). A novel catalytic method for the synthesis of spherical aragonite nanoparticles from cockle shells. *Powder Technology*. 246: 434–440.

Nurul, K., Bin, Z., Bakar, A., Ali, E., Zobir, M., Hussein, B., Hashim, U. (2013). A novel method for the synthesis of calcium carbonate (aragonite) nanoparticles from cockle shells. *Powder Technology*. 235: 70–75.

Oatley-Radcliffe, D. L., Walters, M., Ainscough, T. J., Williams, P. M., Mohammad, A. W. & Hilal, N. (2017).Nano filtration membranes and processes: A review of research trends over the past decade. *Journal of water process engineering*. 19:164-171.

Okada, K. I., Minamino, T., Tsukamoto, Y., Liao, Y., Tsukamoto, O., Takashima, S. & Yutani, C. (2004). Prolonged endoplasmic reticulum stress in hypertrophic and failing heart after aortic constriction: possible contribution of endoplasmic reticulum stress to cardiac myocyte apoptosis. *Circulation*. 110(6):705-712.

Pawar, V. K., Singh, Y., Sharma, K., Shrivastav, A., Sharma, A., Singh, A. & Datta, D. (2017).Doxorubicin Hydrochloride Loaded Zymosan-Polyethylenimine Bio polymeric Nanoparticles for Dual 'Chemo immunotherapeutic' Intervention in Breast Cancer. *Pharmaceutical research*. 34(9): 1857-1871.

Paramasivam, G., Kayambu, N., Maximus, A., Sundramoorthy, A. K., & Sundaramurthy, A. (2017). Acta Biomaterialia Anisotropic noble metal nanoparticles : Synthesis , surface functionalization and applications in biosensing , bioimaging , drug delivery and theranostics. *Acta Biomaterialia*. 49: 45–65.

Pradas del Real, A. E., Vidal, V., Carrière, M., Castillo-Michel, H., Levard, C., Chaurand, P., & Sarret, G. (2017).Silver nanoparticles and wheat roots: a complex interplay. *Environmental science & technology*. 51(10):5774-5782.

Parmar, H. S., & Kar, A. (2008).Medicinal values of fruit peels from Citrus sinensis, Punica granatum, and Musa paradisiaca with respect to alterations in tissue lipid peroxidation and serum concentration of glucose, insulin, and thyroid hormones. *Journal of Medicinal Food*.11(2):376-381.

Park, S., Koh, E., Koo, J. S., Kim, S. Il, Park, B.-W., & Kim, K.-S. (2017). Lack of both androgen receptor and forkhead box A1 (FOXA1) expression is a poor prognostic factor in estrogen receptor-positive breast cancers. *Oncotarget*, 8(47), 82940–82955.

Parveen, S., Misra, R., & Sahoo, S. K. (2012).Nanoparticles : a boon to drug delivery therapeutics,diagnostics and imaging. *Nanomedicine: Nanotechnology, Biology, and Medicine*. 8(2): 147–166.

Patra, J. K., Das, G., Fraceto, L. F., Vangelie, E., Campos, R., Rodriguez, P., … Grillo, R. (2018). Nano based drug delivery systems : recent developments and future prospects. *Journal of Nanobiotechnology*. 1–33.

Peppas, N. A., Hilt, J. Z., Khademhosseini, A. &Langer, R.(2006).Hydrogels in biology and medicine: from molecular principles to bionanotechnology.*Advanced materials*. 18(11): 1345-1360.

Piktel, E., Niemirowicz, K., Wątek, M., Wollny, T., Deptuła, P., & Bucki, R. (2016). Recent insights in nanotechnology ‐ based drugs and formulations designed for effective anti-cancer therapy. *Journal of Nanobiotechnology*. 1–23.

Plemel, J. R., Caprariello, A. V., Keough, M. B., Henry, T. J., Tsutsui, S., Chu, T. H. & Stys, P. K. (2017). Unique spectral signatures of the nucleic acid dye Acridine orange can distinguish cell death by apoptosis and necroptosis. *J Cell Biol*. 216(4): 1163-1181.

Posadas, E. M., Simpkins, F., Liotta, L. A., MacDonald, C., & Kohn, E. C. (2005).Proteomic analysis for the early detection and rational treatment of cancer—realistic hope? *Annals of oncology*. 16(1):16-22.

Postow, M. A. (2015). Managing Immune Checkpoint-Blocking Antibody Side Effects. *American Society of Clinical Oncology Educational Book*. 35: 76–83.

Rajabnia, T., & Meshkini, A.(2018).Fabrication of adenosine 5′-triphosphate-capped silver nanoparticles: Enhanced cytotoxicity efficacy and targeting effect against tumor cells. *Process Biochemistry*. 65: 186-196.

Ramasamy, K. A. J. V. V. (2016). Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity. *Applied Nanoscience*. 6(3): 399–408.

Rathmell, J. C & Thompson, C. B. (2002).Pathways of apoptosis in lymphocyte development, homeostasis, and disease. *Cell*. 109(2): S97-S107.

Reboredo-rodríguez, P., González-barreiro, C., Cancho-grande, B., Simal-gándara, J., Giampieri, F., Forbes-hernández, T. Y., Battino, M. (2018). E ff ect of pistachio kernel extracts in MCF-7 breast cancer cells : Inhibition of cell proliferation , induction of ROS production , modulation of glycolysis and of mitochondrial respiration. *Journal of Functional Foods*. 45: 155–164.

Render, D., Samuel, T., King, H., Vig, M., Jeelani, S., Babu, R. J., & Rangari, V. (2016).Biomaterial-derived calcium carbonate nanoparticles for enteric drug delivery. *Journal of nanomaterial's*. 2016.

Saidykhan, L., Bakar, M. Z. B. A., Rukayadi, Y., Kura, A. U., & Latifah, S. Y. (2016). Development of nanoantibiotic delivery system using cockle shell-derived aragonite nanoparticles for treatment of osteomyelitis. *International Journal of Nanomedicine*. 11: 661–673.

Sakinah, S. S., Handayani, S. T., & Hawariah, L. A.(2007).Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio. *Cancer cell international*. 7(1), 4.

Susman, S., Berindan-Neagoe, I., Petrushev, B., Pirlog, R., Florian, I. S., Mihu, C. M. & Tomuleasa, C. (2018).The role of the pathology department in the preanalytical phase of molecular analyses. *Cancer management and research*. 10: 745

Saidykhan, L., Bakar, M. Z. B. A., Rukayadi, Y., Kura, A. U., & Latifah, S. Y. (2016). Development of Nano antibiotic delivery system using cockle shell-derived aragonite nanoparticles for treatment of osteomyelitis. *International journal of nanomedicine*. 11: 661.

Sali SS. (2016). MTech Thesis dissertation: Natural calcium carbonate for biomedical applications. Centre for Research in Nanotechnology and Science, Indian Institute of Technology, Bombay Powai Mumbai India.

Saleha, A. A., Khor, K. H., Zunita, Z., & Jalila, A. (2015). Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia, *27*, 7–11.

Sanna, V., Pala, N., & Sechi, M. (2014).Targeted therapy using nanotechnology: focus on cancer. *International journal of nanomedicine*. 9:467.

Seabra, A., & Duran, N.(2015).Nano toxicology of metal oxide nanoparticles. *Metals*. 5(2), 934-975.

Schmitz, I., Kirchhoff, S & Krammer, P. H. (2000).Regulation of death receptor-mediated apoptosis pathways. *The international journal of biochemistry & cell biology*. 32(11-12):1123-1136.

Semkina, A., Abakumov, M., Grinenko, N., Abakumov, A., Skorikov, A., Mironova, E. Zarghami, N. (2016). A Review of Breast Cancer Research in Malaysia. *Molecular Oncology*. 4(3): 1–11.

Sgarbi, G., Gorini, G., Liuzzi, F., Solaini, G., & Baracca, A. (2018).Hypoxia and IF1 expression promote ROS decrease in cancer cells. *Cells*. 7(7): 64

Shafei, A., El-bakly, W., Sobhy, A., Wagdy, O., Reda, A., Aboelenin, O., … Ellithy, M. (2017). Biomedicine & Pharmacotherapy A review on the e ffi cacy and toxicity of di ff erent doxorubicin nanoparticles for targeted therapy in metastatic breast cancer. *Biomedicine & Pharmacotherapy*. 95: 1209–1218.

Shafiu Kamba, A., Ismail, M., Tengku Ibrahim, T. A., Zakaria, Z. A. B., & Hassan Gusau, L. (2014). In Vitro Ultrastructural Changes of MCF-7 for Metastasise Bone Cancer and Induction of Apoptosis via Mitochondrial Cytochrome C Released by CaCO 3 /Dox Nanocrystals. *BioMed Research International*. 1–14.

Shaheed, S., Tait, C., Kyriacou, K., Linforth, R., Salhab, M., & Sutton, C. (2018). Evaluation of nipple aspirate fluid as a diagnostic tool for early detection of breast cancer. *Clinical Proteomics.* 1–15.

Shariatgorji, M., Nilsson, A., Goodwin, R. J., Svenningsson, P., Schintu, N., Banka, Z. & Andren, P. E. (2012).Deuterated matrix-assisted laser desorption ionization matrix uncovers masked mass spectrometry imaging signals of small molecules. *Analytical chemistry*. 84(16): 7152-7157.

Sharma, S., Parmar, A., Kori, S., & Sandhir, R. (2016). Trends in Analytical Chemistry PLGA-based nanoparticles : A new paradigm in biomedical applications. *Trends in Analytical Chemistry*.80: 30–40.

Shi, J., Kantoff, P. W., Wooster, R., & Farokhzad, O. C. (2017).Cancer nanomedicine: progress, challenges and opportunities. *Nature Reviews Cancer*. 17(1): 20.

Siegel, R. L., Miller, K. D., Fedewa, S. A., Ahnen, D. J., Meester, R. G., Barzi, A., & Jemal, A. (2017).Colorectal cancer statistics, 2017. *CA: a cancer journal for clinicians*. 67(3):177-193

Singh, M., Kumar, S. V., Waghmare, S. A., & Sabale, P. D. (2016). Aragonite–vaterite–calcite: Polymorphs of CaCO3 in 7th century CE lime plasters of Alampur group of temples, India. *Construction and Building Materials*.112: 386-397.

Singh, R., & Jr, J. W. L. (2009). Nanoparticle-based targeted drug delivery. *Experimental and Molecular Pathology*. 86(3): 215–223.

Singh, S. K., Jr, J. W. L., & Singh, R. (2018).Reversal of drug resistance by planetary ball milled (PBM) nanoparticle loaded with resveratrol and docetaxel in prostate cancer. *Cancer Letters*. 427: 49–62.

Sklirou, A., Papanagnou, E., Fokialakis, N., & Trougakos, I. P. (2018). Cancer chemoprevention via activation of proteostatic modules. *Cancer Letters*. 413: 110–121.

Sommer, A. K., Hermawan, A., Ljepoja, B., Fröhlich, T., Arnold, G. J., Wagner, E., & Roidl, A. (2018).A proteomic analysis of chemo resistance development via sequential treatment with doxorubicin reveals novel players in MCF-7 breast cancer cells. *International journal of molecular medicine*. 42(4): 1987-1997.

Stephens, F. O., Aigner, K., & Allen-Mersh, T. G.(2009).*Basics of oncology* (pp. 259-68). Dordrecht: Springer.

Strasser, A., & Vaux, D. L. (2018).Viewing BCL2 and cell death control from an evolutionary perspective. *Cell death and differentiation*. 25(1): 13-20.

Surinova, S., Schiess, R., Hüttenhain, R., Cerciello, F., Wollscheid, B., & Aebersold, R.(2010).On the development of plasma protein biomarkers. *Journal of proteome research*. 10(1): 5-16.

Szklarczyk, D., Morris, J. H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M. & Jensen, L. J.(2016).The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. *Nucleic acids research*. gkw937.

Schaebitz, M., Wirth, R., Janssen, C., & Dresen, G. (2015).First evidence of CaCO3-III and CaCO3-IIIb high-pressure polymorphs of calcite: Authigenically formed in near surface sediments. *American Mineralogist*. 100(5-6), 1230-1235.

Thapa, B., Bahadur KC, R., & Uludağ, H.(2018).Novel targets for sensitizing breast cancer cells to TRAIL- induced apoptosis with siRNA delivery. *International journal of cancer*.142 (3): 597-606.

Thomas, P., & Smart, T. G. (2005). HEK293 cell line : A vehicle for the expression of recombinant proteins. *Journal of Pharmacological and Toxicological Methods*. 51:187–200.

Timachi, M. H., Hutter, C. A., Hohl, M., Assafa, T., Böhm, S., Mittal, A., ... & Bordignon, E. (2017).Exploring conformational equilibria of a heterodimeric ABC transporter. *Elife*. 6: e20236.

Teixeira, D.M., Bondancia, T. J., Teodoro, K. B., Corrêa, A. C., Marconcini, J. M., & Mattoso, H.C.(2011).Sugarcane bagasse whiskers: extraction and characterizations.*Industrial Crops and Products*. 33(1): 63-66.

Vanichtantikul, A., Hodge, K. G., Somparn, P., Saethang, T., Triratanachat, S., Pisitkun, T., & Lertkhachonsuk, R. (2019). Proteomic identi fi cation of predictive biomarkers for malignant transformation in complete hydatidiform moles. *Placenta*. 77: 58–64.

Vu, H. T., Scarlett, C. J., & Vuong, Q. V. (2017).Optimization of ultrasound- assisted extraction conditions for recovery of phenolic compounds and antioxidant capacity from banana (Musa cavendish) peel. *Journal of food processing and preservation*. 41(5), e13148

Wagner, V., Hüsing, B., Gaisser, S., & Bock, A. K. (2008).Nanomedicine: Drivers for development and possible impacts. *JRC-IPTS, EUR*. 23494.

Wang, L., Chen, Q., Qi, H., Wang, C., Wang, C., Zhang, J., & Dong, L. (2016).Doxorubicin-induced systemic inflammation is driven by upregulation of toll-like receptor TLR4 and endotoxin leakage. *Cancer research*. 76(22):6631-6642.

Wang, H., Feng, Z., & Xu, B.(2017).D-amino acid-containing supramolecular nanofibers for potential cancer therapeutics. *Advanced drug delivery reviews*. 110:102-111.

Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *International Journal of Nanomedicine*. 12: 1227–1249.

Wang, Z., Deng, X., Ding, J., Zhou, W., Zheng, X., & Tang, G. (2018). Mechanisms of drug release in pH-sensitive micelles for tumour targeted drug delivery system : A review. *International Journal of Pharmaceutics*.535(1–2): 253–260.

Wunnicke, D., & Hänelt, I.(2017).The synergetic effects of combining structural biology and EPR spectroscopy on membrane proteins. *Crystals*. 7(4):117.

Wei, L., Surma, M., Gough, G., Shi, S., Lambert-Cheatham, N., Chang, J., & Shi, J. (2015). Dissecting the mechanisms of doxorubicin and oxidative stress-induced cytotoxicity: The involvement of actin cytoskeleton and ROCK1. *PLoS ONE*. *10*(7). e0131763.

Wei, W., Ma, G. H., Hu, G., Yu, D., Mcleish, T., Su, Z. G., & Shen, Z. Y.(2008).Preparation of hierarchical hollow CaCO3 particles and the application as anticancer drug carrier. *Journal of the American Chemical Society*. 130(47):15808-15810.

Wu, J., & Zeng, R. J. (2017).Biomimetic regulation of microbially induced calcium carbonate precipitation involving immobilization of sporasarcina pasteurii by sodium alginate. *Crystal Growth & Design*. 17(4):1854-1862.

Zhang, H., Zhu, Y., Qu, L., Wu, H., Kong, H., Yang, Z. & Hai, M. (2018).Gold Nano rods conjugated porous silicon nanoparticles encapsulated in calcium alginate nano hydrogels using micro emulsion templates. *Nano letters*. 18(2):1448-1453.

Wilk-Zasadna, I., Bernasconi, C., Pelkonen, O., & Coecke, S. (2015). Biotransformation in vitro: an essential consideration in the quantitative in vitro-to-in vivo extrapolation (QIVIVE) of toxicity data. *Toxicology*. 332:8-19.

Wunnicke, D., & Hänelt, I.(2017).The synergetic effects of combining structural biology and EPR spectroscopy on membrane proteins. *Crystals*. 7(4):117.

Xu, A. W., Ma, Y. & Cölfen, H(2007).Biomimetic mineralization. *Journal of Materials Chemistry*.17 (5):415-449.

Yamagishi, Y., Watari, A., Hayata, Y., Li, X., Kondoh, M., Yoshioka, Y. & Yagi, K.(2013).Acute and chronic nephrotoxicity of platinum nanoparticles in mice. *Nanoscale research letters*. 8(1):395.

Yang, J., Guo, W., Wang, L., Yu, L., Mei, H., Fang, S. Liu, G. (2017).Notch signalling is important for epithelial-mesenchymal transition induced by low concentrations of doxorubicin in osteosarcoma cell lines. *Oncology Letters*.13: 2260-2268.

Yu, Z.-g., & Lai, R. Y.(2013).Effect of Signalling Probe Conformation on Sensor Performance of a Displacement-Based Electrochemical DNA Sensor. *Analytical Chemistry*. 85(6): 3340-3346.

Young, J. M., Durcinoska, I., DeLoyde, K., & Solomon, M. J. (2018).Patterns of follow up and survivorship care for people with colorectal cancer in new South Wales, Australia: a population-based survey. *BMC cancer*. 18(1):339.

Yang, M. H., Yuan, S. S., Chen, Y. L., Chu, P. Y., Jong, S. B., Huang, Y. F.& Tyan, Y. C. (2014). Mass spectrometry in clinical diagnosis: a preliminary application in tumor cellular proteomics for biomarker discovery. *Journal of Analytical & Bio analytical Techniques*, (S2), 1.

Yuan, Y. G., Peng, Q. L., & Gurunathan, S. (2017). Combination of palladium nanoparticles and tubastatin-A potentiates apoptosis in human breast cancer cells: A novel therapeutic approach for cancer. *International journal of nanomedicine*. 12:6503.

Yea, K., Jones, T. M., Jung, D., Shin, S., Arlian, B. M., Han, K. H. & Lerner, R. A.(2019). An agonist antibody prefers relapsed AML for induction of cells that kill each other. *Scientific reports*. 9(1): 3494.

Zahreddine, H., & Borden, K.(2013).Mechanisms and insights into drug resistance in cancer. *Frontiers in pharmacology*. 4:28.

Zeng, L., Pan, Y., Tian, Y., Wang, X., Ren, W., Wang, S. & Wu, A. (2015). Doxorubicin-loaded NaYF4: Yb/Tm–TiO2 inorganic photosensitizers for NIR-triggered photodynamic therapy and enhanced chemotherapy in drug-resistant breast cancers. *Biomaterials*. 57:93-106.

Zhang, Y., Gu, A. Z., Xie, S., Li, X., Cen, T., Li, D., & Chen, J. (2018). Nano-metal oxides induce antimicrobial resistance via radical-mediated mutagenesis. *Environment International*,.121: 1162–1171.

Zhang, Y., Ma, P., Wang, Y., Du, J., Zhou, Q., Zhu, Z., … Yuan, J. (2012). Biocompatibility of Porous Spherical Calcium Carbonate Microparticles on Hela Cells. *World Journal of Nano Science and Engineering*. 2(1): 25–31.

Zhao, Y. & Brasier, A. R.(2013). Applications of selected reaction monitorin(SRM) mass spectrometry(MS)for quantitative measurement of signaling pathways. *Methods*. 61(3): 313–322.

Zhang, H., Zhu, Y., Qu, L., Wu, H., Kong, H., Yang, Z. & Hai, M. (2018).Gold nanorods conjugated porous silicon nanoparticles encapsulated in calcium alginate nano hydrogels using microemulsion templates. *Nano letters*. 18(2): 448-1453.

Zoppino, F. C., Guerrero-Gimenez, M. E., Castro, G. N., & Ciocca, D. R.(2018).Comprehensive transcriptomics analysis of heat shock proteins in the molecular subtypes of human breast cancer. *BMC cancer*.18(1):700.

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LIST OF PUBLICATIONS

Hamidu Ahmed, Mokrish Ajat, Rozaihan Mansor, Intan Shameha Abdul Razak, Abubakar Danmaigoro, Alhaji Zubair Jaji; and Md Zuki Abu Bakar@Zakaria (2019). Modified methods of nanoparticles synthesis in pH sensitive Nano-carriers production for doxorubicin delivery on MCF-7 breast cancer cell-line. *International journal of nanomedicine*.14:3615–3627.

Hamidu Ahmed, Mokrish, Ajat, Rozaihan Mansor, Intan Shameha Abdul Razak, Abubakar Danmaigoro, Alhaji Zubair Jaji, Krishnan Nair Balakrishnan and Md Zuki Abu Bakar@Zakaria (2019). Optimization of Doxorubicin Encapsulated $CaCO_3$-nanoparticles in terms of cytotoxic and apoptotic on MCF-7 (Breast Cancer) Cell-line.*European Journal of Cancer*. Manuscript ID: EJC-D-19-01266. Under Review.

Hamidu Ahmed, Mokrish Ajat, Rozaihan Mansor, Intan Shameha Abdul Razak, Abubakar Danmaigoro, Alhaji Zubair Jaji; and Md Zuki Abu Bakar@Zakari (2019).Cytotoxic and Apoptotic Effects of Doxorubicin Encapsulated $CaCO_3$-nanoparticles on MCF-7 (Breast Cancer) Cell-line.*Cancer Management and Research*. Manuscript ID: 218087. Under review

Conferences

Hamidu Ahmed, Mokrish Ajat, Rozaihan Mansor, Intan Shameha Abdul Razak, Abubakar Danmaigoro, Alhaji Zubair Jaji, and Md Zuki Abu Bakar@Zakaria(2017).Synthesis of Aragonite Calcium Carbonate Nanoparticles as Delivery Carrier for DOX-Based Anti-Cancer Therapy. Symposium on Advanced Materials and Nanotechnology. 18^{the} – 19th July 2017 (Oral presentation)

Hamidu Ahmed, Mokrish Ajat, Rozaihan Mansor, Intan Shameha Abdul Razak, Abubakar Danmaigoro, Alhaji Zubair Jaji, Jaafaru Sani Mohammed and Md Zuki Abu Bakar@Zakaria; Apoptotic evaluation and cytotoxic effects of Doxorubicin conjugated CaCO3-nanoparticles on breast cancer cell-line. Front. Pharmacol. Conference Abstract: International Conference on Drug Discovery and Translational Medicine 2018 (ICDDTM '18) "Seizing Opportunities and Addressing Challenges of Precision Medicine. doi:10.3389/conf.fphar.2018.63.00096 Received: 19 Oct 2018; Published Online: 17 Dec 2018.* Correspondence: Prof. Md Zuki Abu Bakar, Faculty of Veterinary Medicine, Putra Malaysia University, Serdang, 43400, Malaysia, zuki@upm.edu.my https://www.frontiersin.org/10.3389/conf.fphar.2018.63.00096/event_abstract (Oral presentation)

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