UNIVERSITI PUTRA MALAYSIA

PHYSICOCHEMICAL CHARACTERIZATION AND IN VITRO ANTI-CANCER EFFECTS OF IRON–MANGANESE AND IRON-DOPED SULFATED ZIRCONIA NANOPARTICLES IN CANCER CELL LINES

MOHAMED QASIM KHLAIF

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By

MOHAMED QASIM KHLAIF

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the degree of Master of Science

April 2016

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DEDICATION

I wish to dedicate this thesis to my mother (Samerah Obaid) and father (Qasim K. Al-Fahdawi) for their love and giving me the genes for research. They have always believed in me and have always encouraged me not only during this master period but throughout life.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

PHYSICOCHEMICAL CHARACTERIZATION AND *IN VITRO* ANTI-CANCER EFFECTS OF IRON–MANGANESE AND IRON-DOPED SULFATED ZIRCONIA NANOPARTICLES IN CANCER CELL LINES

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April 2016

Chairman : Professor Rasedee Abdullah, PhD
Faculty : Institute of Bioscience

Crystal nanoparticle is a new system with potential as a therapeutic agent in the treatment of diseases. The objectives of this study are to synthesize, characterize and determine the anticancer cell effects of iron-manganese and iron-doped sulfated zirconia nanoparticles. In this study the iron-manganese- and iron-doped sulfated zirconia nanoparticles were prepared by hydrothermal impregnation method followed by calcination. The characterization of both nanoparticles were carried out using X-ray diffraction (XRD), thermal gravimetric analysis (TGA), fourier transform infrared spectroscopy (FT-IR), Brunner-Emmett-Teller (BET) surface area measurements, X-ray fluorescence (XRF), X-ray photoelectron spectroscopy, zeta potential (ZSP) measurement, and transmission electron microscopy (TEM). The cytotoxicity of the nanoparticles was determined via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay on human breast adenocarcinoma (MDA-MB-231), colorectal adenocarcinoma (HT29), and hepatocellular carcinoma (HepG2) cell lines, and two normal human cell lines, the Chang liver and human umbilical vein endothelial cells (HUVEC) cell lines. The results showed that the iron-manganese- and iron-doped sulfated zirconia nanoparticles were of average size 12.7 and 32.0 nm, respectively and zeta potential of 15.0 and 0.206 mV, respectively. These nanoparticles tend to aggregate in solution. The iron-manganese- and iron-doped sulfated zirconia nanoparticles are highly toxic to the MDA-MB-231 and HepG2 cells, respectively, showing dramatic morphological changes suggesting loss of cell viability. The nanoparticles are comparatively less toxic to the HT29 cells compared to the other cancer cell lines. The study suggests that the anticancer effects of iron-manganese- and iron-doped sulfated zirconia nanoparticles implicate caspase-3, 8 and -9 in their anticancer cells activities. The findings from the study highlight the potential of iron-manganese- and iron-doped sulfated zirconia nanoparticles as therapeutic agents in the treatment of cancers, while showing lesser effect on normal cells.

Keywords: iron-manganese-doped sulfated zirconia nanoparticles, iron-doped sulfated zirconia nanoparticles, physiochemical characterization, cytotoxicity, HepG2, MDA-MB-231, HT29, HUVEC, Chang cell.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENCIRIAN FISIKOKIMIA DAN KESITOKSIKAN NANOZARAH ZIRKONIA BERSULFAT TERDOP FERUM-MANGAN DAN FERUM

Oleh

MOHAMED QASIM. KHLAIF

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Nanozarah hablur merupakan suatu system baharu yang berpotensi sebagai agen terapi dalam rawatan penyakit. Objektif kajian ini ialah untuk mensintesis, menciri, dan menentukan kesan antisel kanser nanozarah zirkonia bersulfat terdop ferum-mangan dan ferum. Dalam kajian ini nanozarah zirkonia bersulfat terdop ferum-mangan dan ferum disediakan melalui kaedah pengisian hidroterma diikuti dengan pengkalsinan. Pencirian kedua-dua nanozarah dijalankan mengguna pembelauan X-sinar (XRD), analisis gravimetric terma, spektroskopi inframerah tranformasi Fourier (FT-IR), pengukuran kawasan permukaan Brunner-Emmett-Teller (BET), pendarfluoran X-sinar (XRF), spektroskopi fotoelektron X-sinar, pengukuran keupayaan zeta (ZSP), mikroskopi electron pancaran (TEM). Kesitoksikan nanozarah ditentukan melalui assai (4,5-dimetiltiazol-2-yl)-2,5-difeniltetrazolium bromide (MTT) terhadap titisan sel adenokarsinoma payudara (MDA-MB-231), adenokarsinoma kolorektum (HT29), karsinoma hepatosel (HepG2) manusia, dan dua titisan sel normal manusia iaitu sel hati (Chang) dan sel endothelium vena umbilikus (HUVEC). Hasil kajian menunjukkan nanozarah zirkonia bersulfat terdop ferum-mangan dan ferum masing-masing berpurata saiz 12.7 dan 32.0 nm, dan keupayaan zeta masing-masing 15.0 dan 0.206 mV. Nanozarah ini cenderung untuk mengagregat dalam larutan. Nanozarah zirkonia bersulfat terdop ferum-mangan dan ferum adalah paling toksik masing-masing kepada sel MDA-MB-231 dan HepG2, menunjukkan perubahan morfologi ketara yang menyaran hilangnaya kebolehhidupan sel. Nanozarah ini kurang toksik terhadap sel HT29 berbanding titisan sel kanser lain. Kajian ini menyarankan yang kesan antikanser nanozarah zirkonia bersulfat terdop ferum-mangan dan ferum melibatkan kaspase-3, -8, dan -9 dalam aktiviti antisel kansernya. Hasil kajian ini mengutarakan potensi nanozarah zirkonia bersufat terdop ferum-mangan dan ferum sebagai agen terapi dalam rawatan kanser, sambil tidak menunjukkan banyak kesan terhadap sel normal.

Katakunci: nanozarah zirkonia bersulfat terdop ferum-mangan, nanozarah zirkonia bersulfat terdop ferum, pencirian fisiokimia, kesitoksikan, HepG2, MDA-MB-231, HT29, sel Chang.

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Thank you.

I certify that a Thesis Examination Committee has met on 27 April 2016 to conduct the final examination of Mohammed Qasim Khlaif on his thesis entitled "Physicochemical Characterization and *In Vitro* Anti-Cancer Effects of Iron-Manganese and Iron-Doped Sulfated Zirconia Nanoparticles in Cancer Cell Lines" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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ATCC	The American Type Culture Collection
CO_2	Carbon dioxide
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulphoxide
DTT	Dithiothreitol
dUTP	2'-deoxyuridine 5'-triphosphate
EDTA	Ethylendiaminetetraacetic acid
ELISA	Enzyme-linked Immunosorbent Assay
EtOH	Ethanol
FBS	Fetal bovine serum
XRD	X-ray diffraction
XRF	X-ray fluorescence
XPS	X-ray photoelectron spectroscopy
TGA	Thermogravimetric analysis
FTIR	Fourier transform infrared
TEM	Transmission electron microscopy
HCl	Hydrochloric acid
IC_{50}	Inhibition concentration at 50 percent
KCl	Potassium Chloride
KH_2PO_4	Potassium dihydrogen phosphate
LDH	Lactate Dehydrogenase
BET	Brunauer, Emmett and Teller analysis
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaCl	Sodium Chloride
NADH	Nicotinamide adenine dinucleotide
NaHPO4	Disodium hydrogen phosphate anhydrous
NaOH	Sodium hydroxide
PBS	Phosphate buffer saline

HepG2	Human hepatocellular carcinoma cell line
HT29	Human colorectal adenocarcinoma cell line
MDA-MB231	Human breast adenocarcinoma cell line
HUVEC	Human umbilical vein endothelial cell line
Chang	Human normal hepatocyte cell line

CHAPTER 1

INTRODUCTION

1.1 Introduction

Cancer is one of the common reason of death in the developed countries and has exceeded the cardiovascular diseases as the main death cause 4for people under 80 years old (Siegel et al., 2014). Cancers are induced by changes at molecular level leading to uncontrolled growth, and probably metastasized to other parts of the body (Leemans et al., 2011). There is continuous developing and growth in strategies used for the development of anticancer drugs (Seddon & Workman 2014). Using nanotechnology to treat cancer is one of these strategies. In fact, nanotechnology is one of the most current research area in cancer treatments (Jain et al., 2014). The mode of cancer treatments are gradually changing from surgical removal, chemotherapies, radiotherapy, immunotherapy (Lewis et al., 2014; Feldman et al., 2015) to the use of innovative means such as nanoparticle drug delivery systems (Liao et al., 2014) that facilitate controlled release of drugs (Jain et al., 2014) at nano-scale range (Brannon-Peppas & Blanchette 2012; Alimohammadi & Joo 2014). Thus, parallel to the explosion of knowledge on the biomedical and biochemical understanding of cancer development, nanotechnology is now becoming a weapon in the war against cancer (Jain 2014; Tan et al., 2014). It is well-known that the metallic elements have unique biological properties (Wang et al., 2012; Liao et al., 2014). This is attributed to their ability to easily lose electrons and produce soluble cations in biological fluids, while functioning as charged vehicles in the body and cellular mechanisms, such as sodium-potassium pump. When in combination with biological molecules e.g. nucleic acids and proteins, metallic elements function to sustain life through the supply essential molecules such as oxygen (Turner et al., 2008; Greeley et al., 2009; van Rijt & Sadler 2009; Nordberg et al., 2014).

Positively charged metal ions can facilitate catalysis (Xu et al., 2014) through the control of flow of electrons in enzyme or its substrate (Wang et al., 2011; Fu et al., 2014; Pernicova & Korbonits 2014). Many of these oxidation-reduction reactions are provided by multivalent metal ions (Moodley 2014; Morant-Miñana 2014; Nkuna 2014). Since ancient times, transition metals have been used in medicine (Orvig & Abrams 1999; Nordqvist & Herva 2013; Owunari et al., 2015). Ancient Egyptian chemists used copper to disinfect the water (Grass et al., 2011; Murari et al., 2015) while the gold was incorporated into many traditional Chinese medicine (Owunari et al., 2015; Smardzewski 2015; Xin 2015). In spite of that, the study of anticancer potency of transition metals, such as cisplatin, began to pick up about 40 years ago (Dasari & Tchounwou 2014; Schefter et al., 2014). It is generally believed that the cisplatin targets both nucleic acids, DNA (Olaussen et al., 2006) and RNA (Xiang et al., 2014) along with mitochondria (Yang et al., 2006) and other functional proteins rich in sulfur groups (Dasari & Tchounwou 2014). Cisplatin can penetrate tumor cells, and this process is either via passive (Ciarimboli 2014) or active (Burger et al., 2011). Transition metals-containing nanoparticles have fewer side-effects and cheaper to produce than the available chemotherapeutic compounds (Muhammad & Guo 2014). In this study, two zirconium-based nanoparticles, ferric-doped sulfated zirconia and iron–

manganese doped-sulfated zirconia nanoparticles were developed as potential anticancer compounds. The anticancer properties of these nanoparticles were tested on three human cancer cell lines, the colorectal adenocarcinoma (HT29), hepatocellular carcinoma (HepG2), and breast adenocarcinoma (MDA-MB-231) cells. Application of these nanoparticles as chemotherapeutic agents are governed by the following concerns: suitability of hydrothermal impregnation method in the synthesis of zirconia-containing nanoparticles, cytotoxicity towards cancer cells, selectivity of the nanoparticles for cancer cell lines, and the death pathway by which the nanoparticles exert their actions. In this study two zirconium-based nanoparticles, ferric doped sulfated zirconia and iron–manganese doped sulfated zirconia nanoparticles were prepared, characterized and their *in vitro* cytotoxicity on cancer cell lines evaluated.

1.2 Objectives

1.2.1 General objective:

The present study was undertaken to prepare and characterize the physicochemical properties and anticancer cell effects of two nanoparticles containing zirconium.

1.2.2 Specific objectives:

The specific objectives of the study are to:

1. Synthesize and characterize the physical properties of iron–manganese- and iron-doped sulfated zirconia nanoparticles.
2. Assess the cytotoxicity of iron–manganese- and iron-doped sulfated zirconia nanoparticles on MDA-MB-231, HT29, and HepG2 cell lines.
3. Determine the activity of caspases -3, -8 and -9 in cancer cells treated with iron–manganese- and iron-doped sulfated zirconia nanoparticles.

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