

**Cairo University**

**Faculty of Veterinary Medicine**

**Department of Cytology and Histology**

# **Cell Apoptosis and Its Relation to Cancer Treatment**

**A thesis presented**

**By**

**Zainab Sabry Othman Ahmed**

**(B.V. Sc. 2010; Cairo University)**

**(M.V. Sc. 2013; Cairo University)**

**For the Degree of**

**Ph.D.**

**(Cytology & Histology)**

**Under supervision of**

**Prof. Gehad Abd El-Fattah  
Hassan Elbargeesy**

Professor of Cytology and  
Histology, Faculty of Veterinary  
Medicine, Cairo University

**Prof. Fawzy Abd Elhakeem  
Mohamed Elnady**

Professor of Anatomy and  
Embryology, Faculty of Veterinary  
Medicine, Cairo University

**Prof. El-Sayed Mosallam  
Mohammed Mosallam**

Professor of Cytology and Histology,  
Faculty of Veterinary Medicine,  
Cairo University

**Prof. Q. Ping Dou**

Professor of Oncology, Pharmacology and  
Pathology, Barbara Ann Karmanos Cancer  
Institute, School of Medicine, Wayne State  
University, Detroit, Michigan, USA.

**2018**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

# **APPROVAL SHEET**

## **Cell Apoptosis and Its Relation to Cancer Treatment**

**Ph.D. thesis**

**By**

**Zainab Sabry Othman Ahmed**

**(B.V.Sc.2010; Cairo University)**

**(M.V. Sc.2013; Cairo University)**

### **APPROVAL COMMITTEE**

**Prof. Mohammed Ibrahim Abd EL-Aziz**

Professor of Cytology and Histology, Faculty of Veterinary Medicine, Alexandria University.

**Prof. Moukhtar Hanafy Gad Moussa**

Professor of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

**Prof. Gehad Abd El-Fattah Hassan Elbargeesy**

Professor of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

**Prof. El-Sayed Mosallam Mohammed Mosallam**

Professor of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

**Prof. Fawzy Abd Elhakeem Mohamed Elnady**

Professor of Anatomy and Embryology, Faculty of Veterinary Medicine, Cairo University.

# **SUPERVISION SHEET**

## **Cell Apoptosis and Its Relation to Cancer Treatment**

**Ph.D. thesis**

**By**

**Zainab Sabry Othman Ahmed**

**(B.V.SC.2010; Cairo University)**

**(M.V. Sc.2013; Cairo University)**

### **SUPERVISION COMMITTEE**

**Prof. Gehad Abd El-Fattah Hassan Elbargeesy**

Professor of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

**Prof. El-Sayed Mosallam Mohammed Mosallam**

Professor of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

**Prof. Fawzy Abd Elhakeem Mohamed Elnady**

Professor of Anatomy and Embryology, Faculty of Veterinary Medicine, Cairo University.

**Prof. Q. Ping Dou**

Professor of Oncology, Pharmacology and Pathology, Barbara Ann Karmanos Cancer Institute, School of Medicine, Wayne State University, Detroit, Michigan, USA.

**Name of candidate:** Zainab Sabry Othman Ahmed

**Date of Birth:** 10-09-1988, Giza

**Nationality:** Egyptian

**Specialization:** Cytology and Histology

**Degree:** Doctorate of Philosophy

**Title of thesis:** Cell apoptosis and its relation to cancer treatment

**Supervisors:**

**Prof. Gehad Abd El-Fattah Hassan Elbargeesy**

Professor of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

**Prof. El-Sayed Mosallam Mohammed Mosallam**

Professor of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

**Prof. Fawzy Abd Elhakeem Mohamed Elnady**

Professor of Anatomy and Embryology, Faculty of Veterinary Medicine, Cairo University.

**Prof. Q. Ping Dou**

Professor of Oncology, Pharmacology and Pathology, Barbara Ann Karmanos Cancer Institute, School of Medicine, Wayne State University, Detroit, Michigan, USA.

## ABSTRACT

Apoptosis is one of programmed cell death pathways, it is a physiological process through which the animal could organize the number of cells in tissues. Failure of apoptosis results in different diseases including cancer. Prostate cancer remains the second leading cause of cancer related death in men. Therefore, induction of apoptosis has become one of the most effective strategies for cancer treatment. In our study, we induced apoptosis via targeting the ubiquitin proteasome system (UPS). Inhibition of UPS was achieved by using proteasome inhibitors mainly natural 19S inhibitors as isothiocyanates (ITCs) which found abundantly in cruciferous vegetables. We hypothesize that ITCs as electrophiles could interact with the catalytic triads (CYS, HIS and ASP) of the 19S associated USP14 and UCHL5, ultimately inhibiting their activities. Docking and biochemical results suggest that ITCs are potent inhibitors of UCHL5 than USP14. Indeed, Ub-VS assay confirmed the inhibitory activity of each ITC as the ubiquitin binding activity of UCHL5 and USP14. This inhibition of USP14 and UCHL5 caused increased levels of USP14 and UCHL5 proteins but not the third 19S DUB, RPN11 suggesting feedback loop activation and further supporting that ITCs are inhibitors of proteasomal cysteine DUBs. Also, DUBs inhibition was associated with significant accumulation of ubiquitinated proteins, induction of apoptosis, inhibition of cells proliferation, suppression of cell invasion and degradation of androgen receptor which is considered an important driver of castration resistance prostate cancer (CRPC). Curcumin treatment induced apoptosis and downregulation of androgen receptor in a dose and time dependent manner. Chemical inhibitors of proteasome exhibited antiproliferative effect and induced apoptosis in a dose and time dependent manner. Bortezomib showed a synergetic effect with b-AP15 in induction of apoptosis and downregulation of androgen receptor variant 7. Knocking down of USP14 or UCHL5 increased the sensitivity of the knocked down cells to the anticancer therapy. Our finding of ITCs as proteasomal cysteine DUB inhibitors should provide insightful information for designing, discovering and developing potent, specific 19S-DUB inhibitors for cancer therapies.

**Key words:**

Apoptosis, Ubiquitin proteasome system, Deubiquitinating enzymes, USP14, UCHL5, Isothiocyanate, BITC, PEITC, SFN, Curcumin, Androgen receptor, Castration resistant prostate cancer.

# DEDICATION

*I dedicate this work to whom my heart feels thanks; to the first teacher of mankind, prophet Muhammed (Peace and blessings be upon him), to all members of my great family, especially my kind father (Sabry Othman Ahmed), my mother (Amaal Hussein) , my lovely sisters; Huda and Eman, my brother Ahmed, Eman's family especially Mariam and Yahia and to my aunt Fatimah Hussein, to the souls of who passed away and left this world; my grandparents, my uncle Muhammed Hussein, my aunts especially my aunt Karimah Hussein ,Sheikh Saeed Al-Sharaawy and my professors especially my dear supportive Prof. Samir El-Shafeey who taught me a lot, may Allah forgive all of them, accept their good deeds and grant them with Al-Ferdous Al-A'ala, to all my scholars, shyoukh, my dear professors especially my supervisors; Prof. Gehad El-Bargeesy, Prof. El-Sayed M. M. Mosallam, Prof. Fawzy Elnady and Prof. Q. Ping Dou and also to my dear professor Moukhtar H.G. Moussa, to my colleagues at Cytology and Histology department, Faculty of Veterinary Medicine, Cairo University, to my colleagues at Dou Lab, to the beautiful and supportive people of Michigan especially the Muslim community and the volunteers of the University Islamic Center of Detroit (Cass Masjid), Muslim breakfast program and the Muslim center, to my lovely friends in Egypt and all over the world and to my dear students who I really feel proud of them, and I wish for all the best in this life and hereafter.*

# Acknowledgement

*In fact, the prayerful thanks, at first, to Allah, our merciful GOD, who gives me everything I have.*

*I would like to express my sincere thanks, deepest gratitude and appreciation to my supervisors; Prof. Gehad Abd Elfattah Hassan Elbargeesy, professor of Cytology and Histology, Faculty of Veterinary Medicine - Cairo university for his kind supervision and guidance, Professor EL-Sayed M. M. Mosallam, professor of Cytology and Histology, Faculty of Veterinary Medicine - Cairo university for his kind supervision and endless support, professor Fawzy Abd Elhakeem Mohamed Elnad, professor of Anatomy and Embryology, Faculty of Veterinary Medicine- Cairo university for his kind supervision, wisdom and valuable advice and I really appreciate his continuous support and encouragement and professor Q. Ping Dou for his kind supervision, continuous support, commitment and his endless help and guidance and I'm grateful to him for this opportunity to be one of his lab members for two years that taught me many things.*

*Deep gratitude and appreciation to professor Moukhtar H.G. Moussa, professor of Cytology and Histology, Faculty of Veterinary Medicine - Cairo university for his endless support and help and I really appreciate his kind supervision during my Master study which indeed helped me a lot during my PhD work,*

*Special thanks to my colleagues at Dou lab for their continuous help and support, thanks to my co-authors for their hard work in the published research article, thanks to Dr. Xin Li and Dr. Feng Li for UB-VS data and to Kush Patel and Hassan Ali Cheaito for their work in the computer docking. Thanks to all other lab members who spent time with me and taught me anything especially Dr. Reda Ahmed, Eunice and Claire Soave. Thanks to all members at Barbara Ann Karmanos institute and to its police service which used to give me a ride to my home at midnight because of my late work,*

*Grateful thanks to the beautiful community of Michigan who supported me a lot, special thanks to my dear friend Dr. Amal Hamza Hejab who spent her time and efforts to support me and to encourage me for studying and writing my thesis, and I prayed for her to succeed in her life. Thanks to my dear volunteers in the University Islamic Center of Detroit (Cass Masjid), Muslim breakfast program and the Muslim Center for their beautiful souls that inspired me and supported me a lot. Special thanks to my Indonesian and Malaysian friends who provide a peaceful and wonderful environment in Michigan. Endless thanks and appreciation to the Egyptian community in Michigan and our Egyptian friends who helped and supported me a lot during that time especially Nada Khodair.*

*Thanks are extended to my professors and to the head of Cytology and Histology department; Professor. Saad Elgharabawy for his support and help. Also, my appreciation to Prof. Mamdouh El-Shammaa and Prof. Hany Elhabbak for their role in my academic life and their continuous encouraging and support. Special and grateful thanks to the soul of my dear professor Samir El-Shafeey for his endless support, guidance and help, may Allah accept his good deeds.*

*Thanks to Prof. Hosny Elbanna, the professor of Pharmacology, Faculty of Veterinary Medicine, Cairo University for his continuous support and help. Thanks to my Shyokhi especially Sheikh Mohammed Abd El-Rahman Faried Al-Azhari, to my scholars, my colleagues, to all my lovely friends especially those who helped and encouraged me to apply for this mission; Ghazal Nabil, Hanaa Salah, Nehal Kama, Zeinab Ibrahim and Eman El-Maghraby. Special thanks to my dear students who always support and inspire me a lot.*

*Great thanks to my beloved family members for their patience, help and encouragement, especially to my kind parents who supported me too much during this work, may Allah bless them more, grant them with the best life and accept their good deeds.*

# CONTENTS

|                                                                                       | Page |
|---------------------------------------------------------------------------------------|------|
| INTRODUCTION.....                                                                     | 1    |
| REVIEW OF LITERATURE.....                                                             | 4    |
| ▪ Apoptosis.....                                                                      | 4    |
| ▪ The ubiquitin proteasome system.....                                                | 6    |
| ▪ Deubiquitinating enzymes, deubiquitinases (DUBs).....                               | 12   |
| ▪ DUBs inhibition and its relation to cancer treatment.....                           | 17   |
| ▪ Prostate cancer.....                                                                | 19   |
| ▪ Androgen receptor and its relation to prostate cancer.....                          | 20   |
| ▪ The relation between proteasome inhibition and androgen<br>receptor expression..... | 32   |
| ▪ Chemical inhibitors of the 20S proteasome.....                                      | 34   |
| ▪ Chemical inhibitors of the 19S-associated DUBs.....                                 | 36   |
| ▪ Chemoprotective compounds.....                                                      | 40   |
| • Isothiocyanates.....                                                                | 40   |
| • Curcumin.....                                                                       | 44   |
| ▪ Knockdown of 19S-associated DUBs.....                                               | 45   |
| MATERIALS AND METHODS.....                                                            | 47   |
| RESULTS.....                                                                          | 55   |
| A- Results of isothiocyanates.....                                                    | 58   |
| B- Results of curcumin.....                                                           | 100  |
| C- Results related to different chemical inhibitors of the 26S<br>proteasome.....     | 116  |
| DISCUSSION.....                                                                       | 140  |
| CONCLUSION.....                                                                       | 147  |
| SUMMARY.....                                                                          | 149  |
| REFERENCES.....                                                                       | 154  |
| LIST OF ABBREVIATIONS.....                                                            | 203  |
| ARABIC SUMMARY.....                                                                   |      |

## **List of figures**

| <b>No. of figure</b> | <b>Title</b>                                                                                                                                 | <b>Page number</b> |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| <b>1</b>             | <b>Ubiquitin proteasome pathway</b>                                                                                                          | <b>8</b>           |
| <b>2</b>             | <b>Protein degradation by 26S proteasome</b>                                                                                                 | <b>9</b>           |
| <b>3</b>             | <b>The molecular structure of 26S proteasome</b>                                                                                             | <b>10</b>          |
| <b>4</b>             | <b>Domain structures of full-length androgen receptor and androgen receptor splice variants</b>                                              | <b>29</b>          |
| <b>5</b>             | <b>The chemical structure of the selected drugs</b>                                                                                          | <b>48</b>          |
| <b>6</b>             | <b>Morphology and proteins expression level in the parental, non-targeted and Knocked down 22Rv1 prostate cancer cells without treatment</b> | <b>55</b>          |
| <b>7</b>             | <b>Determination of interactions of BITC, PEITC and SFN with 19S-associated DUBs USP14 and UCHL5 by computational docking.</b>               | <b>58</b>          |
| <b>8</b>             | <b>Inhibition of active site-directed labeling of proteasomal DUBs by BITC, PEITC and SFN</b>                                                | <b>59</b>          |
| <b>9</b>             | <b>The cytotoxic effect of isothiocyanates on parental 22Rv1 prostate cancer cells.</b>                                                      | <b>60</b>          |
| <b>10</b>            | <b>BITC shows morphological changes and cell death of 22Rv1 in a dose dependent manner at 50X magnification.</b>                             | <b>61</b>          |
| <b>11</b>            | <b>BITC shows morphological changes and cell death of 22Rv1 in a dose dependent manner at 100X magnification</b>                             | <b>62</b>          |
| <b>12</b>            | <b>SFN shows morphological changes and cell death of 22Rv1 in a dose dependent manner at 50X magnification</b>                               | <b>63</b>          |
| <b>13</b>            | <b>SFN shows morphological changes and cell death of 22Rv1 in a dose dependent manner at 100X magnification.</b>                             | <b>64</b>          |

| <b>Figure</b> | <b>Title</b>                                                                                                                                          | <b>Page number</b> |
|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| 14            | Crystal violet staining of prostate cancer 22Rv1 cells treated with BITC                                                                              | 65                 |
| 15            | Crystal violet staining of prostate cancer 22Rv1 cells treated with SFN                                                                               | 66                 |
| 16            | Inhibition of prostate cancer 22Rv1 cell invasion (wound healing assay) by BITC                                                                       | 67                 |
| 17            | Inhibition of prostate cancer 22Rv1 cell invasion (wound healing assay) by SFN                                                                        | 68                 |
| 18            | Inhibition of MDA-MB-231 breast cancer cells invasion (wound healing assay) by BITC                                                                   | 69                 |
| 19            | Inhibition of MDA-MB-231 breast cancer cells invasion (wound healing assay) by PEITC.                                                                 | 70                 |
| 20            | Inhibition of MDA-MB-231 breast cancer cells invasion (wound healing assay) by SFN                                                                    | 71                 |
| 21            | The Kinetic effect of BITC on 22Rv1 prostate cancer cell line                                                                                         | 72                 |
| 22            | The Kinetic effect of SFN on 22Rv1 prostate cancer cell line                                                                                          | 73                 |
| 23            | ITCs cause degradation of ARv7, accumulate ubiquitinated proteins and induce cell apoptosis in prostate cancer 22Rv1 cells in a time dependent manner | 74                 |
| 24            | Inhibition of calpain and caspase activity by using calpain inhibitor (CAPT) and caspase inhibitor (Z-VAD) in 22Rv1 prostate cancer cells.            | 75                 |
| 25            | Pretreatment of BITC treated cells with CAPT or Z-VAD for inhibition of calpeptin and caspase activity.                                               | 76                 |
| 26            | Comparison between the effect of galetrone and BITC treatment on AR expression level in 22Rv1 cells pretreated with CAPT                              | 77                 |
| 27            | The effect of BITC on the cell viability of KD 22Rv1 cell lines.                                                                                      | 78                 |
| 28            | Crystal violet staining of KD 22Rv1 cells treated with different concentrations of BITC                                                               | 79                 |

| <b>Figure</b> | <b>Title</b>                                                                                                                                                                                   | <b>Page number</b> |
|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| <b>29</b>     | <b>BITC induces morphological changes and apoptosis in addition to degradation of androgen receptor and accumulation of ubiquitinated proteins in KD 22Rv1 prostate cancer cells</b>           | <b>80</b>          |
| <b>30</b>     | <b>Degradation of androgen receptors in parental and KD 22Rv1 and accumulation of ubiquitinated protein after their treatment with BITC for short time.</b>                                    | <b>81</b>          |
| <b>31</b>     | <b>The cytotoxic effect of curcumin on prostate cancer 22Rv1 cell line.</b>                                                                                                                    | <b>100</b>         |
| <b>32</b>     | <b>Curcumin shows morphological changes and cell death of 22Rv1 in a dose dependent manner.</b>                                                                                                | <b>101</b>         |
| <b>33</b>     | <b>Crystal violet staining of prostate cancer 22Rv1 cells treated with curcumin</b>                                                                                                            | <b>102</b>         |
| <b>34</b>     | <b>Inhibition of prostate cancer 22Rv1 cell invasion (wound healing assay) by curcumin</b>                                                                                                     | <b>103</b>         |
| <b>35</b>     | <b>Inhibition of MDA-MB-231 breast cancer cells invasion (wound healing assay) by curcumin</b>                                                                                                 | <b>104</b>         |
| <b>36</b>     | <b>Curcumin induces cell apoptosis in prostate cancer 22Rv1 cells associated with degradation of ARV7 and accumulation of ubiquitinated proteins in a time dependent manner</b>                | <b>105</b>         |
| <b>37</b>     | <b>The effect of curcumin on the 19S associated DUBs, USP14 and UCHL5</b>                                                                                                                      | <b>106</b>         |
| <b>38</b>     | <b>The effect of different doses of curcumin on KD 22Rv1 cell lines</b>                                                                                                                        | <b>107</b>         |
| <b>39</b>     | <b>Crystal violet staining of KD 22Rv1 cells treated with different concentrations of curcumin</b>                                                                                             | <b>108</b>         |
| <b>40</b>     | <b>Curcumin induces morphological changes and cell apoptosis in addition to degradation of androgen receptor and accumulation of ubiquitinated proteins in KD 22Rv1 prostate cancer cells.</b> | <b>109</b>         |
| <b>41</b>     | <b>The cytotoxic effect of BTZ on prostate cancer 22Rv1 cells</b>                                                                                                                              | <b>116</b>         |

| <b>Figure</b> | <b>Title</b>                                                                                                      | <b>Page number</b> |
|---------------|-------------------------------------------------------------------------------------------------------------------|--------------------|
| 42            | BTZ induces morphological changes and cell death of 22Rv1 in a dose dependent manner                              | 117                |
| 43            | Crystal violet staining of prostate cancer 22Rv1 cells treated with different concentrations of BTZ               | 118                |
| 44            | The cytotoxic effect of different concentrations of BTZ on KD prostate cancer 22Rv1 cells                         | 119                |
| 45            | The effect of 20nM BTZ on KD prostate cancer 22Rv1 cells                                                          | 120                |
| 46            | The effect of 100nM of BTZ on different KD 22Rv1 prostate cancer cell lines                                       | 121                |
| 47            | The cytotoxic effect of combination of BTZ and b-AP15 on prostate cancer 22Rv1 cells                              | 122                |
| 48            | Combination of BTZ and b-AP15 induces more cell apoptosis and degradation of ARV7 in 22Rv1 prostate cancer cells. | 123                |
| 49            | The cytotoxic effect of b-AP15 on 22Rv1 prostate cancer cells                                                     | 124                |
| 50            | The effect of pretreatment of BTZ treated 22Rv1 cells with IU1                                                    | 125                |
| 51            | The cytotoxic effect of the combination of BTZ with BITC or PEITC on prostate cancer 22Rv1 cells.                 | 126                |
| 52            | The cytotoxic effect of RA-9 and VLX1570 on prostate cancer 22Rv1 cells                                           | 127                |
| 53            | Comparison between the effect of VLX1570 and RA-9 on 22Rv1 prostate cancer cell lines.                            | 128                |
| 54            | The cytotoxic effect of VLX1570 on KD 22Rv1 prostate cancer cells                                                 | 129                |
| 55            | The cytotoxic effect of RA-9 on KD 22Rv1 prostate cancer cells                                                    | 130                |
| 56            | The effect of 7 $\mu$ M RA-9 on the cell viability of KD 22Rv1 cell lines                                         | 131                |

## Introduction

Apoptosis is one of programmed cell death pathways (**Ouyang *et al.*, 2012**), it is a physiological process through which organisms could regulate the number of cells in tissues (**Ellis *et al.*, 1991**). Failure of apoptosis results in many diseases including cancer due to the uncontrolled proliferation of cells that leads to tumor growth (**Hoeppner *et al.*, 1996** and **Evan and Vousden, 2001**). Therefore, one of the most important strategies in cancer treatment is induction of apoptosis (**Hong and Sporn, 1997; Jaffrézou *et al.*, 1998; Debatin, 2000; Kelloff *et al.*, 2000** and **Woynarowska and Woynarowski, 2002**). Induction of apoptosis could be through targeting the ubiquitin proteasome system (UPS) which controls different cellular processes and is considered the major regulator of protein degradation in eukaryotes (**Hershko and Ciechanover, 1998; Goldberg, 2007; Finley, 2009; Schrader *et al.*, 2009** and **D'Arcy and Linder, 2012**).

The ubiquitin proteasome system (UPS) is composed of a small molecule of ubiquitin and large multiunit complex called 26S proteasome, ubiquitin molecule is responsible for tagging of the protein substrate to the 26S proteasome for its degradation. The 26S proteasome is composed of 20S barrel shaped structure which is responsible for the proteolytic activity of the proteasome capped on each end by one or two 19S regulatory particles (**Hershko *et al.*, 1983; Groll *et al.*, 1997; Amerik and Hochstrasser, 2004; Goldberg, 2007; Lee *et al.*, 2010; D'Arcy and Linder, 2012** and **Nguyen *et al.*, 2013**). Ubiquitination is a reversible process that includes ubiquitin

ligating (E3 ligase) and could be reversed by deubiquitination through deubiquitinating enzymes (DUBs) **(Liao *et al.*, 2017)**. Deubiquitinating enzymes (DUBs) are responsible for removal of ubiquitin or ubiquitin like chain from the target protein before its degradation in the 20S proteasome in eukaryotes **(Song and Rape, 2008 and Aressy *et al.*, 2010)**.

There are approximately 100 DUBs encoded by human genome **(Liao *et al.*, 2017)**, three of them are associated with the 19S proteasome in the mammalian cells **(Lee *et al.*, 2011 and D'Arcy *et al.*, 2015)**. Ubiquitin specific protease 14 (USP14) and Ubiquitin carboxyl-terminal hydrolase isozyme L5 (UCHL5) belong to cysteine proteases class while the third one is proteasome regulatory particle lid subunit RPN11 (RPN11) and it belongs to the metalloprotease class **(Lee *et al.*, 2011; D'Arcy *et al.*, 2015 and Wang and Linder, 2015)**. These proteasome's associated DUBs play an important role in the maintenance of ubiquitin homeostasis **(Lee *et al.*, 2011 and D'Arcy *et al.*, 2015)**.

Since tumor tissues show overexpression of different DUBs **(Daviet and Colland, 2008; Wang *et al.*, 2016 and Liao *et al.*, 2017)**, DUBs could be an important target for treatment of different cancers including prostate cancer which is considered the second leading cause of cancer related death in men **(Jemal *et al.*, 2007; Knudsen and Kelly, 2011 and Lu and Luo, 2013)**. Also, DUBs are associated with the stabilization, co-regulation and transcription of androgen receptor (AR) **(Liao *et al.*, 2017)** which is considered the fundamental driver

for the progression of castration resistant prostate cancer (CRPC) **(Tamura *et al.*, 2007; Chen *et al.*, 2008 and Attard *et al.*, 2009).** Therefore, inhibition of these proteasome associated DUBs, or their silence may be a promising strategy for downregulation of androgen receptor (AR) and induction of apoptosis in prostate cancer cells. The aim of this study is to obtain a potent natural or chemical compound that could inhibit the development of cancer, particularly prostate cancer and breast cancer which remain the second leading cause of cancer related death in men and women, respectively. In our study, we used different proteasome inhibitors including chemical inhibitors and natural compounds which present in cruciferous vegetables as broccoli, these natural compounds include isothiocyanates such as benzyl isothiocyanate (BITC), phenethyl isothiocyanate (PEITC) and sulforaphane (SFN) in addition to curcumin which was extracted from *curcuma longa* plant. We tested the inhibitory activity of these compounds on DUBs and observed the antiproliferative activity of these compounds on cell growth in addition to the relation between DUBs inhibition or silence and the expression of androgen receptor, particularly androgen receptor variant 7 (AR-V7) which has an important role in the progression of Castration resistant prostate cancer (CRPC). The present study should provide insightful information for designing, discovering and developing potent, specific 19S-DUB inhibitors for cancer therapies.