Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon

College of Medicine

Department of Pharmacology

Effect of Zoledronic Acid Alone and in Combination with Doxorubicin on Osteosarcom and Prostate CancerCell Line

A thesis

Submitted to the Council of the College of Medicine, University of Babylon, as a Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacology and Toxicology

By

Manar Omran Ali

B.Pharmacology (2007-2008)

Supervised by

Assist. Prof.
Majid K. Abbas

MSc. Pharmacology

Prof.Dr
Kaiser N. Madlum

Ph.D Biology.

1444 A.H

2022 A.

بسم الله الرحمن الرحيم

إن الله وملائكته يصلون على النبي يا أيها

الذين امنوا صلوا عليه وسلموا تسليما

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صدق الله العظيم

الاحزاب اية {٥٦}

Certification

We certify that this thesis entitled **"*The potential synergistic effect of combination of zoledronic acid with cytotoxic drug in tumor cell line* "** was prepared by (**Manar Omran Ali**) under supervision at the department of Pharmacology. College of Medicine, University of Babylon (Iraq) in partial fulfillment of the requirements for the master degree of sciences in pharmacology and toxicology.

Signature Signature

Assist Prof. Prof. Dr.

Majid K. Abbas ***Kaiser N. Madlum***

Supervisor Supervisor

Date: / /2022 Date: / /2022

The Recommendation of the Head of Department

In the view of the available recommendation, I forward this thesis for debate by the examining committee

Signature

Assist. Prof. Dr .

Selman M. Selman

The Head of Department of Pharmacology

College of Medicine

University of Babylon, Iraq

Date: / /2022

Dedication

I dedicated this work to my great father and mother

To My beautiful children: Baqir and Hasan

To my wonderful: sisters and brothers

To the soul of my dear husband

Manar Omran

Acknowledgments

Praise be to God, Lord of the worlds who helped me and gave me the strength, patience, and endurance to complete this thesis.

Great thanks to my supervisor Dr. ***Majid K. Abbas*** for his efforts, valuable advice and guidance during the study.

I would like to express my deepest thanks to my supervisor ***Dr. Kaiser N. Madlum*** for the support ,encouragement, and tolerability the course of this work.

My special thanks also go to **Dr. Hammed N. Obaid** for his help and support.

Special thanks and gratitude to the department of pharmacology for the infinite support and cooperation.

Finally; thanks to all the people who have supported me to complete the research work directly or indirectly.

Manar Omran

Summary :

Cancer is one of a major causes of death throughout the world. The malignant bone tumors are among the cancers that are most frequently linked to a high death rate. Its can either be primary bone tumors such osteosarcoma or secondary from solid cancer like breast or prostate cancer, which mostly associated with metastasized to bone. Nitrogen containing bisphosphonate such zoledronic acid is potent antiresorptive agent,and mainly used in osteoporosis as primary option , due to higher efficacy in suppression activity of osteoclast cells of bone , in addition to having direct potential anticancer activity.

In this study, MG-63 osteosarcoma cells line and LNCaP prostate cancer cells line were used as models, exposed to serial concentrations of zoledronic acid (500,250,125,62.5,31.25,15.5µg/ml) and serial concentrations of doxorubicin as cytotoxic drug (100,50,25,12.5,6.25,3µg/ml), each alone or in combination together , the incubation period was 24 hours .

This study involved three main parts:

In the first part, cytotoxicity assays were performed to evaluate the anti-proliferative effect of zoledronic acid and doxorubicin, each alone and in combination. The viablility of the treated cells was measured by MTT assay.

In the second part of the study, the anti-inflammatory effect of zoledronic acid was evaluated via the measurement of interlukine-6, tumor necrosis factor-alfa concentration using ELISA technique.

The last part was the evaluation of antioxidant effect of zoledronic acid at different concentrations by measuring the total antioxidant capacity assay.

The cytotoxicity results reaveled a significant decrease in cells number of MG-63 cell line at all serial concentrations used, while for LNCaP, a significant decrease was observed only at higher concentration of zoledronic acid. Regarding zoledronic acid-doxorubicin combination, a significant cytotoxic synergism was found at all concentrations used in both cell lines.

Zoledronic acid at higher concentrations causes a significant decrease in interlukin-6 and tumor necrosis factor- alfa concentrations in both cells lines. In MG-63 osteosarcoma cells line, zoledronic acid decreased the total antioxidant concentration, at higher concentration, while no significant difference demonstrated in LNCaP cells line .

In conclusion, the anti-proliferative effect of zoledronic acid is dose and cell type dependant. Zoledronic acid synergically increase doxorubicin activity against both cell lines. Finally, zoledronic acid have anti-inflamatory effect at higher concentrations by decreasing the proinflamatory cytokines Interlukine-6, Tumor Necrosis Factor-alfa levesl.

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Abbreviation	**Meaning**
ATP	Adenosine triphosphate
ANT	adenine nucleotide translocase
ApppI	triphosphoric acid 1-adenosine-5′-yl ester 3-[3-methylbut-3-enyl] ester
BP	Bisphosphonate
CrCl	creatinine clearance
CD4	Glycoprotein located on the surface of immune cells
C°	Centigrade
Conc	Concentration
c-Fms	macrophage-colony stimulating factor gene
DNA	Deoxyribonucleic Acid
DMSO	Dimethyl sulfoxide
DDW	deionized distilled water
Dox	Doxorubicin
EDTA	Trypsin-Ethylenediamine- tetraacetic acid
EPC	endothelial progenitor cells
FPP	farnesyl diphosphate
FDA	Food and Drug Administratio
FGF-2	fibroblast growth factor-2
GGPP	geranylgeranyl diphosphate

GTP	guanosine triphosphate
GPP	geranyl pyrophosphate
Gm	Gram
HHM	humoral hypercalcemia of malignancy
IPP	isopentenyl pyrophosphate
IGF-1	Insulin-like growth factor 1
IL	Interleukin
JNK	c-Jun NH2-terminal kinase
MAP	Mitogen activated protein
mTNF-α	transmembrane Tumor Necrosis factor- α
M-CSF	macrophage-colony stimulating factor
mRNA	Messenger ribonucleic acid
MTT	3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide)
NADH	Nicotinamide adenine dinucleotide phosphate oxidase
N-BP	Nitrogen Bisphosphonate
NF	nuclear factor
NF-kB	Nuclear factor kappa light chain enhancer of activated B cells
OD	optical density
ONJ	Osteonecrosis jaw
OS	Osteosarcoma
OPG	Osteoprotegerin
PTHrP	Parathyroid hormone-related protein
PDGF-BB	platelet-derived growth factor- BB
PSA	prostate-specific antigen
PCR	Polymerase chain reaction
PBMCs	peripheral blood mononuclear cells
PBS	Phosphate Buffer Saline
RANK	Receptor activator of nuclear factor K B
RANKL	Receptor activator of nuclear factor K B Ligand
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute
STAT	signal transducer and activators of transcription
sTNF-α	soluble TNF-α
SEM	scanning electron microscope
SRE	Skeletal related event
TMEV	Theiler's murine encephalomyelitis virus

TACE	TNF- α converting enzyme
TNF- α	Tumor necrosis factor-alfa
TGF-B	Transforming growth factor-beta
TNFR1	TNF receptor 1
TNFR2	TNF receptor 2
Th	T helper cells
VEGF	vascular endothelial growth factor
ZOL	Zoledronic acid

Chapter One

Introduction and Literature Review

1.1 Introduction

Cancer is uncontrolled growth of cells, consider a one of essential causes of death at the world. For cancer treatment utilizing surgery, chemotherapy, and radiotherapy either alone or in combination have given a better respond. The treatment of cancer related metastasis, was acting a great challenge In the past (Shewach and Kuchta, 2009). Solid tumor mostly metastasized to bone cause primarily osteolysis, increasing in osteoclasts activity, excepted those from prostate cancer, which it show as sclerotic secondary lesions(Ural *et al.*, 2006), increasing activity of osteoclasts may be leading to several diseases as (osteoporosis, periprosthetic osteolysis, bone tumors, and Paget's disease). Bone resorption mainly, belong to defecting in osteoclasts function(Bi.H, *et al.*, 2017).

Bisphosphonates, especially nitrogen-containing bisphosphonates, they are effective inhibitors of osteoclast-mediated bone resorption ,as a result they are used to inhibition bone destruction in cancer patients with bone metastasis(Clézardin, *et al* , 2011). The anti-tumor actions of zoledronic acid (zol) have been demonstrated in several studies in vitro, which including ability of zol in reducing tumor cell invasion, migration, and adhesion achieved by inhibition of farnesyl diphosphate (FPP) synthase and reduced prenylation of small GTPases (enzymes that hydrolyze guanosine triphosphate)(Wilson et al. 2015) . Osteoclast bone resorption are inhibition by zol, leading to decrease of bone-derived growth factors production , such as Transforming growth factor-beta (TGF-b) and Insulin-like growth factor (IGF-1), which are stimulate the growth of bone metastases. In other side the production parathyroid hormone-related protein and receptor activator of

nuclear factor K ligand(PTHrP and RANKL) of tumor-derived growth factors is decreased, which are promote osteoclast bone resorption(Young and Coleman, 2013)

Zoledronic acid was associated with potent clinical benefits appearant in the individual which suffering cancer with metastases to bone , involving (prostate cancer, lung cancer and renal cell carcinoma).through suppression bone tumor (Jiang, *et al* , 2014)

Cytokines, have potent role in bone resoption, mediated by, IL-1 and IL-6, and TNF-α which they stimulate osteoclastogenesis, detected osteoclast activity, while, IL-4 and gamma interferon, block osteoclast formation(Kenkre and Bassett, 2018) .

By cytotoxicity testing ,which used for measuring of cell viability in vitro, performance has attracted more attention recently, which utilized in oncological research to determine both the toxicity of an agent and the suppression of tumor cell growth through drug development. These assays depend on a wide range of cell functions, including cell membrane permeability, enzyme activity, cell adherence, ATP production, and coenzyme production. They are quick, available, and do not require the use of animals. They are also useful for testing a large number of samples(Aslantürk, 2018).

1.2 Aim of study:

1.Study the antiproliferative activity of zoledronic acid on osteosarcoma and prostate cancer cell lines

2. Study the possible synergistic effect of zoledronic acid with known anticancer drug on these two type of cancer cells.

3.Study the anti-inflammatory effect of zoledronic acid by measuring of IL-6, TNF-α in both cells lines.

4.Study the antioxidant effect of zoledronic acid in both cell lines.

1.3 Bonecancer:

Its result mainly, from increases in osteoclast bone resorption, and imbalance in RANKL and osteoprotegrein levels in local bone tissues, whereas RANKL/ RANK/OPG system; affects tumor vitality by controlling in osteoclast activity. In prior study have observsd expression levels of OPG and RANKL mRNA in massive cell tumors of the bone comparing with normal bone tissues are much rising(Bi *et al.*, 2017).

Cancer of bones are either been as a result of primary bone cancer or progressed of cancers that lead to metastasized to the bones especially with solid tumor ; osteosarcoma and Ewing's sarcoma are primary bone cancers, considered aggressive malignancies(González Díaz *et al.*, 2019), while metastasis cancer, including breast, prostate, lung and other solid tumors, that can severely impact on the quality of life of the patient(Kars *et al.*, 2007). Both solid tumors and hematopoietic malignancies, have deep effects upon the skeleton, leading to rising in osteoclast consistence and effectiveness(Rodan and Martin, 2000).

1.4 Osteosarcoma:

Osteosarcoma is an osteoid-producing malignancy of mesenchymal origins ,consider of most common primary malignancy of bone, acting less than 0.2% of all cancers (Lindsey, *et al.* , 2017).

Osteosarcoma histology: can be divided depending on location within bone into central (classic) and surface tumors, The classic central represent the high-grade primary osteosarcoma of bone, it's about 90% of all osteosarcoma cases acting. Classic osteosarcoma represents about 15% of all biopsy-analyzed primary bone , favorite affecting the metaphysis of long bones, distal femur, tibia, and proximal humerus (Friebele *et al.*, 2015),while surface tumor (Periosteal/Cortical,) represent 10-15% of all osteosarcoma cases represent low grade moreover extraskeletal third division osteosarcoma tumor this type is rare 5% af all cases(Klein and Siegal, 2006) , in figure (1.1) demonstrated biologic growth of osteosarcom.

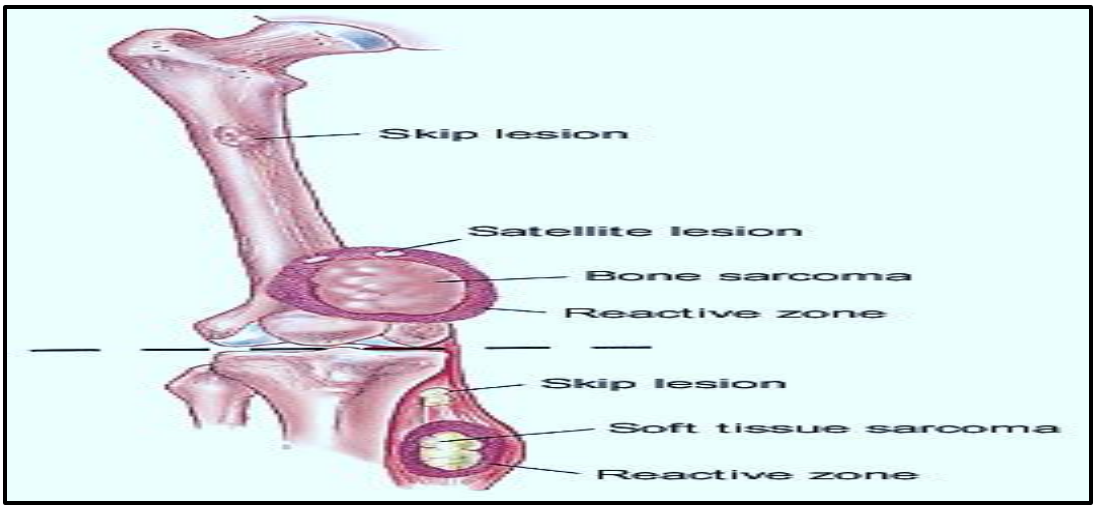

Figure1.1 :Biologic growth pattern of osteosarcomas (Wittig *et al.*, 2002a)

1.4.1 Epidemiology :

Osteosarcoma is a uncommon sarcoma, has histological outcome of osteoid secreation in combination with malignant mesenchymal cells. In adolescence, it's a third more widespread cancer, with only lymphomas and brain tumors being more widespread, and with an yearly occurring of 5.6 states per million children less than15 of age, mostly occurring at a second decade of life , prior the age of five rarely. It arises occasionally, with few states related by recognized inherited disorder in cell cycle controlling , but nearly 70% of tumor samples appear a chromosomal anomaly, mostly including mutations in tumor-suppressor genes or in DNA helicases (Misaghi *et al.*, 2018)

1.4.2 Risk factors of osteosarcoma:

- **Age** : Osteosarcoma have two group age ranking , the first related with young children and adolescents that represent higher incidence , while the second group certified in geriatric patients. The premature age was happen rate of osteosarcoma detection is comparatively proper nearly the world (3 to 4.5 cases/million population/year),while the high difference (1.5 to 4.5 cases/million population/year) were certified above 60 year (Leissan R Sadykova *et al.*, 2020).
- **Gender**: the malignant bone tumors was happen in males higher than females at adolescent and young adult population. Males are higher incidence for both osteosarcoma and Ewing sarcoma, moreover was improved a male predominance for remaining types of

malignant bone tumors, totally (Eary, 2015), while at another studies has been shown females less than 15 age, have a little rising cancer rates comparing with males at the same age group(Leissan R. Sadykova *et al.*, 2020).

- **Genetic**: osteosarcoma cells is highly associated with chromosomal aneuploidy, which hypothesis instability for somatic or germline chromosomal could being susceptible an individual to osteosarcoma, several researches about of the somatic changes existed in osteosarcoma cells, related with many cancer susceptible syndromes that a result of germline mutations that have highly penetrant(Savage and Mirabello, 2011)
- **Socio-economic status:** patients with lower socioeconomic groups appears, osteosarcoma and mortality have higher incidence rates. education, income, and occupation are examples for socioeconomic status, potent foreteller of morbidity and mortality**,** while education is a vital factor, can cause late in investigation or rejection of medical notice; in prefer, stand by methods as local bonesetters(Leissan R. Sadykova *et al.*, 2020)**.**

1.4.3:Dignosis of Osteosarcoma:

- Blood tests:
- Magnetic Resonance Imaging(MRI)
- CT Scanning

- Bone Scintigraphy
- Thalium Scintigraphy
- Biopsy(Wittig *et al.*, 2002)

1.5 Bone Metastases:

Potent effects of solid tumors on the skeleton include an increase in osteoclast activity and generation, either systemically as in humoral hypercalcemia of malignancy (HHM) or locally as in bone metastases. Solid tumors include breast, prostate, lung, kidney, and other cancers(Rodan and Martin, 2000).

The ability of malignant cells in the bone marrow cavity to secrete a wide range of paracrine factors that can boost bone cell function with activation of osteoclast function being of primary interest and leading to osteolysis is the basis cause for bone metastasis. Osteolysis is associated with disturbance of normal coupling between osteoblast and osteoclast function(Coleman, 2001).

Osteoclasts are causing bone degradation through dissolve bone mineral, leading to degrade the bone matrix, it a unique property for osteoclasts, makes it a dominant factor in bone metastasis formation(Le Pape, *et al*, 2016).

1.6 Biology of the Osteoclast:

Osteoclasts are multinucleated cells that resorb the bone matrix to maintain bone cycle as a result for maintaining the health of the skeleton. They develop from monocyte-macrophage progeny under the stimulation of mainly two cytokines: macrophage-colony motivating factor (M-CSF) and (RANKL), while osteoblasts, which are found primarily in the bone, play a critical role in controlling osteoclast formation (Maurizi and Rucci, 2018).

The receptor activator of nuclear factor-B ligand (RANKL) attachment by RANK originate signals essential osteoclast differentiation and the resorptive mission; and the survival of matured osteoclasts. M-CSF and macrophage-colony stimulating factor gene (c-Fms) provide the signals required for osteoclast precursor cell proliferation and survival(Kim and Kim, 2016), energizing of osteoclasts by RANKL, that produced by cancer cells, having a vital role in pathway of bone metastases and bone destruction(Bi.H, *et al.*, 2017).

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1.7 : Prostate Cancer:

Prostate cancer is a solid tumors, consider a second leading cause of cancer related death in men has high affinity to bone metastasize, estimated that >80% of men who die from prostate cancer develop bone metastases leading osteolysis and abnormal bone formation, (Ibrahim *et al.*, 2010).

The primary clinical complication of prostate cancer is bone metastasis, which occurs when osteoclast cells express nuclear factor kappa-B (RANK) receptor motivator and mature when they interact with RANKL, which is expressed on osteoblast cells. Cell proportion is controlled by the trio linkage of RANK, RANKL, and osteoprotegerin (OPG), a share receptor of RANK that links to RANKL, suppration of RANK- RANKL intraction leading to bone resoption, Inaddition to another factors contributed to metastasis of prostate cancer(Ziaee *et al.*, 2015).

1.7.1: Prostate Cancer Epidemiology:

Prostate cancer is the fifth main cause for cancer death in men about the world, especially in countries with prevalent black ethnic populatin have very mortality average as the Caribbean (29 per 100,000 males) and Africa (19.9). Mortality average from prostate cancer have mostly reduced about the world in the final decade, with the exclusion of a few Northern European and Asian(Merriel, *et al* , 2018).

1.7.2:Risk factor of prostate cancer:

Age: prostate cancer incidence linked robustly to age, with age-specific incidence rates rising clearly from the age of 50 years and being highest in men aged 90 and above.

Family history: a positive family history significantly affects a man's risk of prostate cancer. Collected risk assessment suggest that a man with one first- degree relative (father or brother) with prostate cancer has a relative risk .

Race: men from African and Caribbean ethnic backgrounds have the elevated prostate cancer incidence and mortality rates in contrast to other ethnic groups.(Merriel, *et al* , 2018)

1.7.3:Diagnosis of prostate cancer:

Prostate-specific antigen (PSA) testing has become more widely used recently, which has contributed to the decrease in incidence; nonetheless, prostate cancer fatality rates have remained largely stable over that time(Merriel, *et al* , 2018)

1.8 :Treatment of cancer:

The general common kind of cancer treatments such as chemotherapy, surgery and radiotherapy that are available nowadays(Arruebo *et al.*, 2011)

1.8.1 Surgery:

Still the primary treatment for solid cancer is tumor removal surgically. Many of investigators have improved independently the long-term survival associated with immediate postoperative chemotherapy.(Coffey *et al.*, 2006), mostly ; to monitory solid cancers, a surgical removal is the basic , However surgical excision of primary or yet metastatic tumors can keep or long-term of survival, but sometime may be exposure for challenge the surgical degradation itself may motivate or accelerate tumor recurrence(Tohme, *et al* , 2017).

1.8.2 Radiation Therapy:

Indication for high- energy X-rays or gamma rays that target a tumor or postsurgery tumor site as radiation therapy, it's have a potent influence in killing cancer cells that may still after surgery or recur, when tumor was removed(Sharma *et al.*, 2010), therefore using either before surgery (neoadjuvant therapy) to shrink the tumor, or after surgery (adjuvant therapy), to destroy microscopic tumor cell, may be remained beyond (Baskar *et al.*, 2012).

Dose of radiation should be potent enough to enclose the removal of cancer cells. period of treatments typically have given over a(five -seven) weeks, each treatment takes about 15 minutes(Sharma *et al.*, 2010)

1.8.3 Chemotherapy:

1.8.3.1 Doxorubicin:

Doxorubicin is an anthracyline compound taken away from Streptomyces peucetius var firstly, caesius in the 1970 ,usually utilized as therapeutic in different of cancers (breast, lung, gastric, ovarian, thyroid, non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma and sarcoma), have potent action (Micallef and Baron, 2020)

Mechanism of doxorubicin as anticancer effect through irreversible disrupt of tumor cell DNA. Antitumor effects mechanisms involving intercalation into DNA, causing suppress of micromolecule synthesis, generation reactive oxygen species (ROS) (Thorn *et al.*, 2011),inhibits topoisomerase type II(Ottewell *et al.*, 2008), and is accompanied by G2/M phase cell cycle stopping(Tsakalozou, *et al*, 2012)**.** As shown in (figure 1.2)

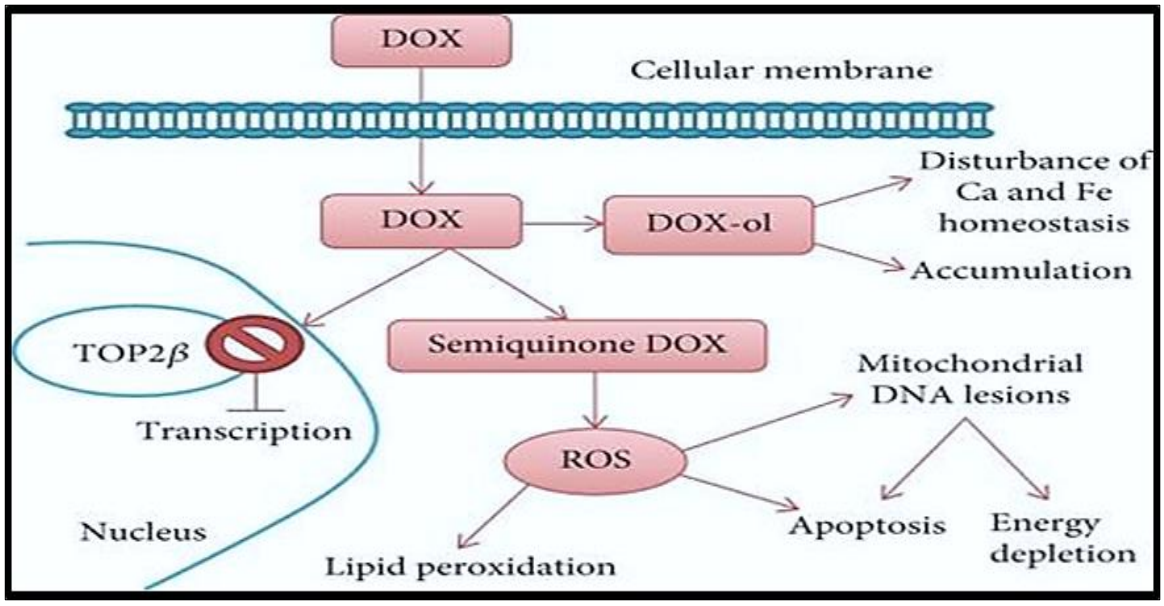

Figure1.2 :Mechanism of doxorubicin as antitumor (Lazăr et al., 2021)

Doxorubicin mainly is utilizing alone or combination with methotrexate and cisplatin in high-dose for osteosarcoma treatment. In spite of cardiotoxicity, associated with dox at cumulative dosing leading to congestive heart failure later in life(Kazantseva, *et al* , 2022).

1.9 Bisphosphonates:

Bisphosphonates(PBs) are category of molecules composed of phosphorus-carbon-phosphorus basis. The moieties that department from the germinal carbon impact the mineral linking and biologic charecterestic of the abundant BPs obtainable, related bone hydroxyapatite matrix and negatively impact osteoclast effectivty (Farrell *et al.*, 2018) Pyrophosphate is the simplest form of the polyphosphates, that suppress crystallization of calcium salts. Inorganic pyrophosphate, may render as an endogenous water softener to suppress calcification of soft tissue and regulate bone mineralization(Lewiecki, 2010).

BPS are classified into two classes, first non-nitrogen-containing compounds, such as etidronate and clodronate, while the second involve nitrogen-containing compounds bisphosphonate such as alendronate, pamidronate, and ibandronate are ten to a thousand times extra potent (more well absorbed by bone) than of the first generation. Risedronate and zoledronate are nitrogen containing bisphosphonates, but there are 100- 850 times further potent than pamidronate(Wiziack Zago *et al.*, 2018).

BPS was applied as therapeutic option for bone metastases disease, because the hypothesis show that every metastatic cancer in bone begins with osteolysis and sclerotic metastases may actually have increased activities of both osteoclasts and osteoblasts(Ullén *et al.*, 2005).

1.10 Zoledronic acid:

Zoledronic acid(zol) is a third-generation BP, heterocyclic imidazole with high potent suppression for activity of osteoclastic a bone resoption. (Kubista, B., 2006) , zol have a potent effect in decreasing and lateing of (SRE). Used mainly in tumor-induced hypercalcemia ,multiple myeloma and solid tumors with bone spreading (prostate cancer, lung cancer, and breast cancer) because potent inhibitor of osteoclastic bone resorption (Green and Lipton, 2010).

1.10.1.Chemical structure of Zoledronic acid:

Zoledronic acid is chemically designated as (1-hydroxy-2-imidazole-1-yl-phosphonoethylphosphonic acid mono- hydrate), is a white crystalline powder, which is commercially available as a sterile liquid concentrate solution(Burmaoglu and Aslan, 2019).

Zol were related to nitrogen- containing bisphosphonate (N-BP), is potent anti-resorptive drug, and for near 50 years has been used clinically, which is constant pyrophosphate analogues, where a carbon atom exchanged the central oxygen atom, formation the P-C-P backbone non-hydrolysable,

which is permit for BP linking to hydroxyapatite in bone tissue during chelation of Ca2+(Wang *et al.*, 2020). figure (1.3) shown structure of Zol.

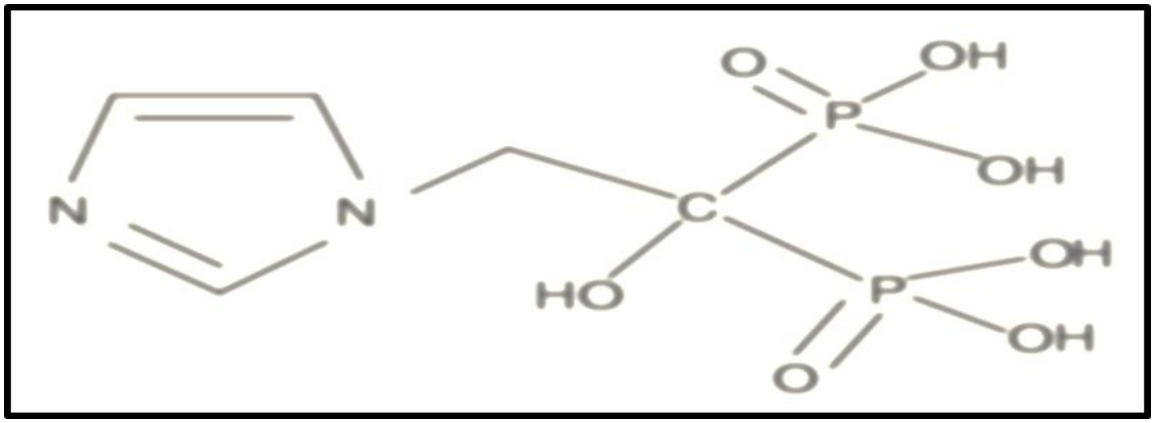

Figure 1. 3:chemical structure of zoledronic acid ((Young and Coleman, 2013)

1.10.2 Pharmacokinetic of zoledronic acid:

Zoledronic acid share with other available BP some pharmacological properties: are poorly absorbed by intestine , captured by the skeleton mainly where they bind robustly to hydroxyapatite crystals. Its suppress osteoclast-mediated bone resorption.(Sinigaglia, *et al*, 2007).

zoledronic acid approximately 56% plasma protein bound, while its concentration in blood is minimum. Plasma zoledronic acid concentration is separating of the range of protein binding, doesn't inhibit by human cytochrome P450 enzymes in vitro not metabolized in vivo.(Perry and Figgitt, 2004). Approximately 39% of an intravenous dose of 2–16mg was recovered in the urine within 24 hours, and remainder dosage is bound to bone then slowly released back into the systemic circulation, while renal

clearance during a first 24 hours after administration was 3.7 L/h. and has 146hr half-life. (Wellington *et al.*, 2003)

1.10.3 Mechanism action of Zoledronic acid:

1.10.3.1 Zoledronic acid as anticancer effect and enhance apoptosis:

Studies have been shown zol has antitumour activity mediated by inhibition of tumour cells proliferation, by suppression the key enzyme, farnesyl diphosphonate (FPP) synthase in the biosyntetic mevalonate pathway, was involved several intermediate such farnesyl pyrophosphate and geranylgeranyl pyrophosphate inhibition (Zekri, *et al*, 2014), suppression FPPS leading to two toxic effects, firstly suppresser of the prenylation of signalling GTPases, such as Ras, Rho and Rac, that are controlling of cell proliferation , cell duration and cytoskeletal regulation. while the second effective is production to ApppI via aminoacyltRNA-synthetases, as a result to converted an accumulation of isopentenyl pyrophosphate (IPP) to ApppI, which inhibits the mitochondrial adenine nucleotide translocase (ANT) emerge apoptosis(Bosch-Barrera *et al.*, 2011).

So according to preclinical and clinical studies were finding zol , in addition to have direct antitumor effect also having indirect effects, through FPP synthase inhibition lead to production of triphosphoric acid 1-adenosine-5′-yl ester 3-[3-methylbut-3-enyl] ester (ApppI), leading to osteoclast apoptosis induction(Liu *et al.*, 2019). Many studies were showed FPPS, is mainly for the division of osteoclast during stimulating the

biosynthesis of geranyl pyrophosphate GPP and farnesyl pyrophosphate FPP (Wang et al., 2020).

The direct antitumor effect of zoledronic acid may act out, of the suppression of tumor cell invasion adhesion, and proliferation ,moreover acting to enhance apoptosis in multiple human tumor cell lines (Bosch-Barrera et al., 2011). Demonstrated in figure 1.4 mechanism of zol as anticancer.

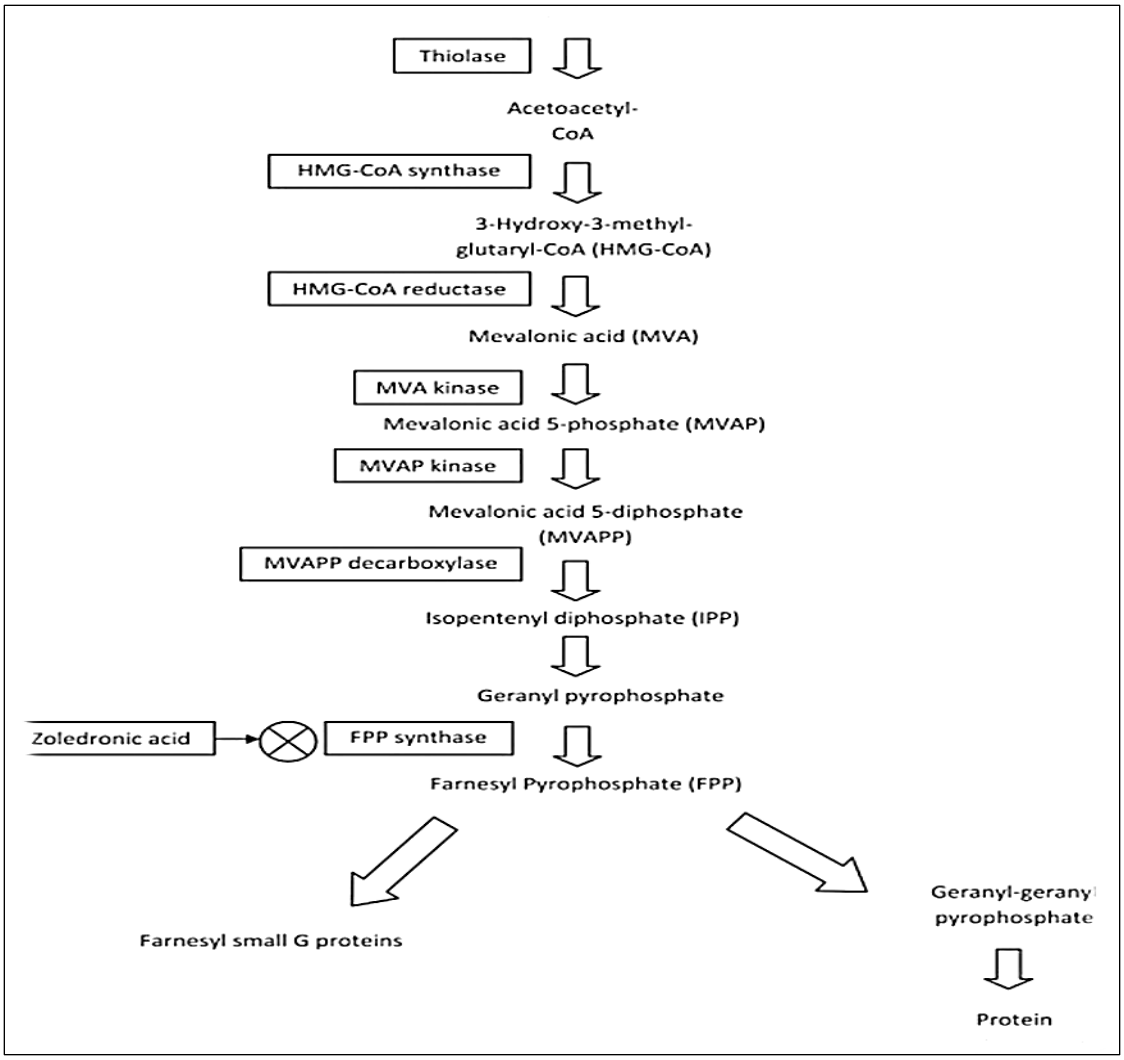

Figure1.4 :mechanism of action for zoledronic acid(Zekria, *et al*, 2014)

1.10.3.2:Zoledronic acid as antiangiogenesis:

Angiogenesis is term meaning a growth from pre-existing vessels to some extent of mature endothelial cells(Ziebart *et al.*, 2011)**.** Vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) are proangiogenic substances released by mesenchymal stromal cells that are linked to angiogenesis, immunological response, and cancer cell migration. The drug zoledronic acid was able to suppress them. Additionally, platelet-derived growth factor-BB, or PDGF-BB, is inhibited by zoledronic acid. which could encourage the development of endothelial progenitor cells into mature endothelial cells by osteoclast parent cellslls(Wang *et al.*, 2020)

1.10.3.3 :Zoledronic acid as synergistic with other cytotoxic drugs:

Zoledronic acid is one of potent drugs having direct and indirect antitumor, by enhancing synergism effect, when utilized with other anticancer agents, molecular target agents, and radiotherapy(Elsayed et al., 2016). Several studies of both in vitro and in vivo have demonstrate that zol was synergized with chemotherapeutic agents in a sequence-dependent; a higher potent effects on proliferation, apoptosis and angiogenesis will be obtained(Young and Coleman, 2013).

In preclinical research demonstrated (MCF7 and MDA-MB-436) human breast cancer cells treated with either paclitaxel (2 nm) or doxorubicin (50

nm) firstly then by zoledronic acid (1–25 μm) is followed, apoptosis grade were much higher comparing when was used alone , at same time treatment, or the inverse sequence(Green and Lipton, 2010)

1.10.3.4:Zoledronic acid inhibition of osteoclast cells:

Zoledronic acid have different pathways for inhibits the proliferation of osteoclasts in vitro, in addition to inhibition of mevalunate pathway of osteoclast, has been suppressing of receptor motivator of nuclear factor κB ligand RANKL /receptor energizing of nuclear factor- κB RANK pathway, non-canonical Wnt/ Ca2+/calmodulin based on protein kinase II CaMKII pathway, and prohibition of macrophage proliferation into osteoclasts(Wang *et al.*, 2020)

1.9.3.5:Activation of γδ T cells by zoledronic acid:

The immune system can be modulated by zoledronic acid within interaction with γδ T cells, have important role in innate immunity in cancer, as a result of internalization zol of (PBMCs) or cancer cells, FPPS suppression and following aggregation of IPP (Green and Lipton, 2010), leading to activation of γδTcells enhancing cytotoxicity against tumors(Rouce *et al.*, 2018), γδT cells was play role in tumor immunity , they have antitumor effect(Li *et al.*, 2021), moreover isopentenyl pyrophosphate was accumulated in monocyte will activated of γδT cell leading to proliferate and releasing proinflamatory cytokines (Karabulut *et al.*, 2010), moreover increase in γδT cells can exposed an antitumor afficacy by many pathway, involving the modulation of innate and adaptive immunity(Green and Lipton, 2010).

1.10.3.6:Zoledronic acid induce apoptosis mediated by release reactive oxygen species:

Reactive oxygen species (ROS) were at first renowned as toxic by-products of aerobic metabolism. In new years, it has become evident, that ROS plays an important signaling role in plants, regulating processes such as growth, development and especially response to biotic and abiotic environmental motivate. The main members of the ROS family involve free radicals such O•−2 ,OH• and non-radicals like H2O2 and O2.(Das and Roychoudhury, 2014).

Several studies, have demonstrated, which zol has enhancing the apoptosis of osteoclasts by different pathway, including inhibition of the mevalonate pathway through farnesyl pyrophosphate synthase (FPPS) suppression and enhancing of reactive oxygen species (ROS) that leading to inducing apoptosis, inhibition a prenylation of small GTP-binding proteins such as Rab, Rho and Rac induction of apoptosis of osteoclasts (Wang *et al.*, 2020). Another researchs have been found zoledronic acid induce apoptosis acts in a manner similar to chemotherapy agents, so it triggers ROS generation followed by ROS-mediated cell apoptosis in osteoclast precursors and mature osteoclast-like cells(Tai *et al.*, 2017)

zol can enhance apoptotic cell tumor alone and suppression of habitation formation associated by an increase of reactive oxygen species. These observation propose that ROS are including in apoptosis and suppression of habitation formation at zol treated SACC-83 cells(Ge *et al.*, 2014).

Using zol, needing to be supported by other antioxidant supplement for the clinical usefulness and investigation, If he was found zoledronic acid enhance rabbit liver oxidative stress and decreases with antioxidant levels in liver tissue. (Karabulut *et al.*, 2010). In cells death are involve the reactive oxygen species (ROS)release (Wu *et al.*, 2016) Zoledronic acid induce for apoptosis for osteoclast by inhibition of mevalonat pathway(Fukai, *et al* , 2014)

Elevating ROS levels when utilized zol for treatment of prostate carcinoma and salivary adenoid cystic carcinoma cell models, as result to action of zol in induction of apoptosis for cells(Wang *et al.*, 2020). In (figure 1.5) demonstrated activity of zol in apoptosis induction and suppression cell proliferation and division through inhibition FPPsynthase enzyme.

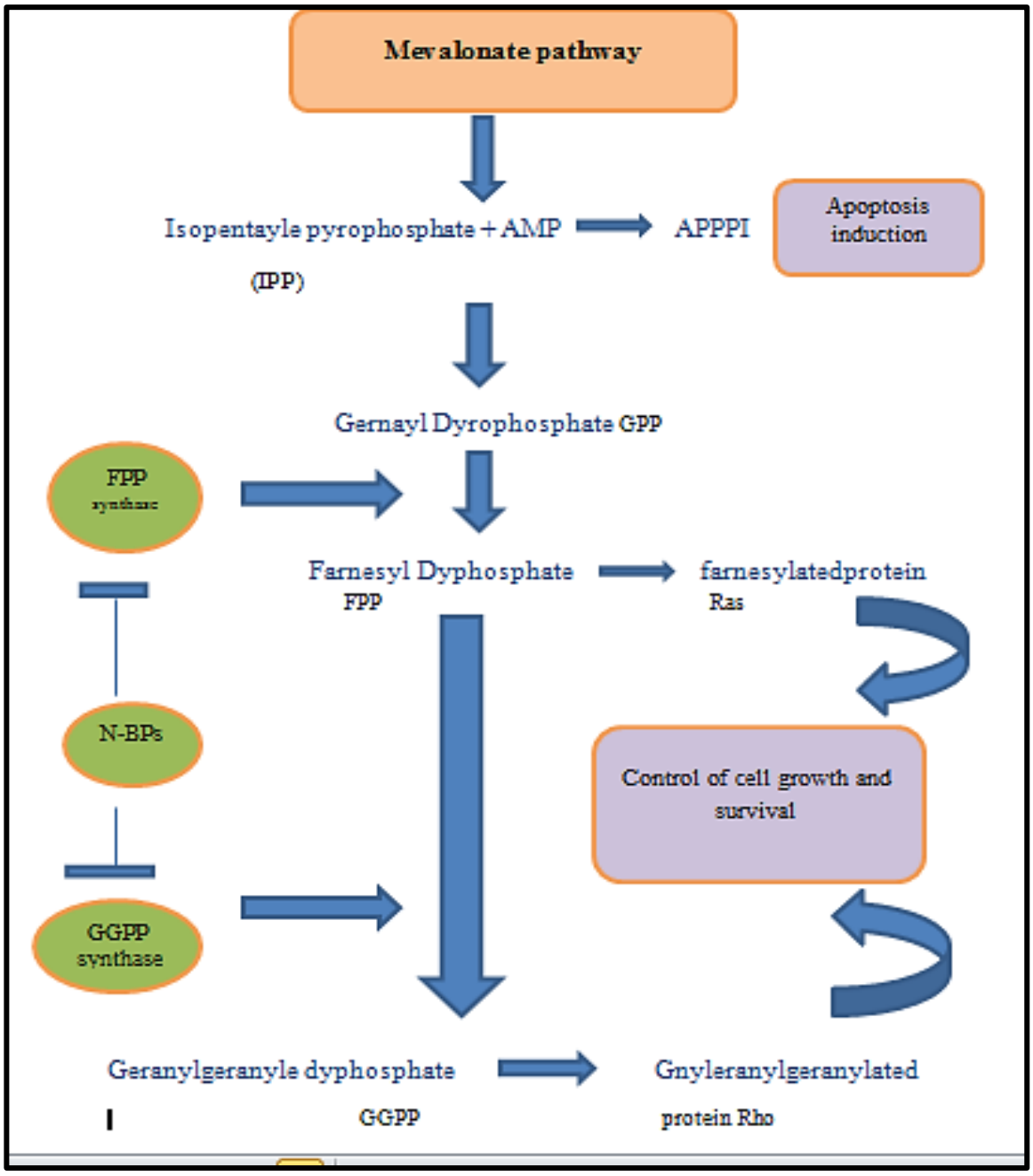

Figure 1.5 Schematic diagram of the molecular mechanism of action of N-BPs on the mevalonat pathway. FPPsynthase:farensyl pyrophosphate synthase , IPP: isopentyl pyrophosphate ,GGPP synthase:geranylgeranyle diphosphate synthase ,APPPI:triphosphoric acid 1-adenosin -5-yl ester 3(3-methylbut-3-enyl).(Wang *et al.*, 2020)

1.10.4Therapeutic efficacy for zoledronic acid :

- **Osteoporosis:** a skeletal condition marked by brittle bones and an increased risk of fractures. This medical disorder, which affects both men and women in different percentage, and is significantly connected with aging, is a leading source of morbidity and mortality in the elderly(Lin *et al.*, 2014). Zol has a powerful effect on bone, inhibiting osteoclast-mediated bone resorption. Similar to other bisphosphonates, zol binds to the calcium phosphate bone mineral hydroxyapatite with a strong affinity for calcified bone, particularly localizing at locations of significant bone turnover. (Räkel, *et al*, 2011)
- **Breast Cancer:** Approximately 70% of all metastatic breast cancer patients develop bone metastasis, which in turn increases the risk of fracture, chemotherapy that was induced bone loss is a critical problem, that including hypoestrogenism secondary to gonadotropin-releasing hormone antagonists in premenopausal women or aromatase inhibitors in postmenopausal women, as a result the treatment of both bone loss and bone metastasis is of great importance in order to improve quality of life and extend survival for breast cancer patients(Yan *et al.*, 2012)**,** zoledronic acid is potent for treatment bone loss that caused by cancer therapy-induced. Its consider soothing therapeutic of decided metastatic bone disease(Young and Coleman, 2013) several studies has been shown addition of zol to endocrine could be increases the disease-free survival of estrogen receptor (ER-positive) breast cancer patients by decreasing both locoregional and

distant metastases, suggesting that this compound might act directly on micrometastasis of breast cancer cells(Gallo *et al.*, 2012).

- **Hypercalcaemia of Malignancy:** Abnormalities in the normal bone formation and degradation cycle result a Hypercalcemia. In normal bone turnover, regulation osteoclast activity is by the binding of RANK surface receptor on the osteoclast to the receptor activator RANKL on the osteoblast, then leading to osteoclastogenesis regulation. Osteoblasts is secreted osteoprotegerin that consider potent inhibitor for bone resorption by binding to RANKL, then leading to inhibition of the interaction between RANK/RANKL, as a result the osteoclasts do not mature. While the interaction between RANK/RANKL is increased, leading to more osteoclastic expression and more bone resorption (Goldner, 2016). Extracellular and intracellular mechanisms of bisphosphonates decrease bone resorption, through binding, bisphosphonates to calcium phosphate and stabilize the bone matrix, as extracellulary, while intracellulary including blocker osteoclast activity through inhibition of the mevalonate pathway(Rosner and Dalkin, 2012). Pamidronate and zoledronate are approved for the treatment of hypercalcemia of malignancy, but the studies have been shown that zoledronic into both doses(4 or 8 mg) have superior, compromised by pamidronate(90 mg) in initial effect and duration of respond(Reagan, *et al*, 2014).
- **prostate cancer:** Zoledronic acid, is very potent of current bisphosphonates in prostate cancer with bone metastasis. due to its high bioavailability in bone, especially in vitro and in vivo models(Wang *et al.*, 2020), Zoledronic acid has demonstrate

antitumor activity emerging apoptosis, tumor cell growth, invasion, adhesion, and angiogenesis moving beyond its anti-osteoclastic activity(Finianos and Aragon-Ching, 2019). In hormone-refractory prostate cancer, zoledronic acid is standard of care for treating bone metastases, stronger in reduce the happens of skeletal related events when used in conjunction with chemotherapy or hormonal(Polascik and Mouraviev, 2008)

- **Osteosarcoma:** (OS) is a malignancy produced osteoid that was originated from mesenchymal, it's a primary malignancy of bone and fatal in both children and adults predominately (Lindsey, *et al*, 2017), secreation of osteoid or immature bone is characterized for osteosarcoma that consiste of centric calcium precipitate of hydroxyapatite crystals. preferentially zol may collected in primary osteosarcomas as well as their lung and bone metastases and continue for years. Farnesyl diphosphate synthase is suppressed by zol, which consider a vital enzyme in the mevalonate pathway, and thus decrease protein prenylation important for normal cell function and duration (Conry, *et al*, 2016). Suppression of osteolytic status is interesting step in blocking growth of osteosarcoma, belong to osteoclasts and osteosarcoma cells interaction to being a "vicious cycle", related to osteosarcoma growth and osteolysis. When utilized a combination of zoledronic acid with cisplatin for treatment of orthotopic osteosarcoma in tibia in mouse model, the results have been showed a significantly decrease bone tissue destruction than when was used alone(Liu L. *et al.*, 2021).

1.10.5:Side effects Zoledronic acid:

Initially studies have demonstrated the response for zoledronic acid at doses of 4mg and 8mg for treatment of metastatic bone disease, but risk of renal impairment is appear with 8mg dose , so the dose was reduced to 4 mg subsequently. At cute phase response demonstrated flu-like symptoms with pyrexia, bone pain and arthralgia, as adverse effects that occurs after first zoledronic acid infusion in up to 50% of patients, gradually will reduced with subsequent infusions. (Young and Coleman, 2013), Acute-phase reaction is believed as a result for release of inflammatory cytokines like TNF-α and IL-6(Kotian, *et al*, 2016).

Impairment in renal function is one of complication associated with this drug, occurrence of renal failure secondary to toxic acute tubular necrosis after treatment with zol will be showed(G.S. *et al.*, 2003), if creatinine clearance less than 30 ml/ min for patient zoledronic acid is not recommend using, while if CrCl values between 30–60 cc/min ,the dose should be adjusted for indications.(Perazella and Markowitz, 2008),

Osteonecrosis jaw(ONJ) is rare occurrence with bisphosphonate, long-term treatment by zoledronic acid is developed of ONJ in 1.2–3.8% when used in metastatic breast cancer patients(Kourie *et al.*, 2015). ONJ is more spread between patients having high cumulative doses of bisphosphonates or denosumab comparison in patients who have lower doses(Nicolatou-Galitis *et al.*, 2019).

1.11: Cytokines:

Cytokines are proteins that are secreted by cells that have a special impact on the communications and interactions between cells. The generic name for this substance is cytokine, whereas lymphokine other names refer to cytokines made by lymphocytes, cytokines made by monocytes called monokine, while chemokine refer to cytokines with chemotactic activities, and cytokines made by one leukocyte and acting on other leukocytes refer to interleukin. Although numerous cell types produce cytokines, helper T cells (Th) and macrophages are the most common ones(Zhang and An, 2007). Cytokines participating in acute and chronic inflammation, because they are consider akey modulators of inflammation.(Turner *et al.*, 2014)

1.11.1. Classification of Cytokines:

Cytokines are classified depended on their cellular source, into two typpe ,the first produced by bunch of division (CD4)+ T-helper 1 (Th1) cells, involving (IL-2,IL-12,IFN-y, and TNF-B); and second kind of cytokines, secreation by CD4+ Th2 cells, involving(IL-4, IL-5, IL-6, IL-10, and IL-13), moreover could be classified based on their role of cytokines as pro-inflammatory cytokines involving (IL-1B, IL-6, IL-8, IL-12, TNF-α, and interferon), that emerge inflammatory reactions and resort to motivate immunocompetent cells. And anti-inflammatory cytokines such as (IL-4, IL-6, IL-10, IL-11, IL-13, IL-1 receptor antagonist (IL-1RA), and TGF-B,) block inflammation and inhibit immune cells(Liu C. *et al.*, 2021)

1.11.2 Interlukin-6 (IL-6):

IL-6 is a soluble mediator with a pleiotropic effect on inflammation, immune response, and hematopoiesis(Tanaka, *et al*, 2014), was expressed by configuration of cells,involving mononuclear phagocytes, T cells, B cells, fibroblasts, endothelial cells, keratinocytes, hepatocytes, and bone marrow cells. IL-6 is including at haematopoiesis, and is pivotal in the last maturation of B-cells into antibody-producing plasma cells, T cell activation, division and regulation of Th2 (Turner *et al.*, 2014). Interleukin-6 have specific role in inflammatory responses of autoimmune diseases, and cancers through the evolution and progression. Many viral infections, including Theiler's murine encephalomyelitis virus (TMEV), contribute in production of IL-6, inaddition to cancer cells, growth and survival of different cells promoted by IL6(Hou *et al.*, 2014)

1.11.3:Tumor Necrosis Factor- alfa (TNF-α):

TNF-α produced mainly by activated macrophages, lymphocytes and other cell types. It could be distinguished in two forms : soluble TNF-α (sTNF-α) and transmembrane TNF-α(mTNF-α). mTNF-α, consider the precursor of sTNF-α, that by the TNF-α converting enzyme (TACE) can be cleaved and is secreation as sTNF-α. which linked primarily to TNF receptor 1 (TNFR1) and have a significant function in the inflammatory immune response, while mTNF-α bind primarily with TNF receptor 2 (TNFR2) which motivate of cellular reproduction ,duration and other vital effects(Qu, *et al*, 2017). TNF-α is a major cytokine for the inflammatory

response, sharing at various inflammatory cases, and therapeutics resistance, whereas TNF-α signaling are confirmed factor for rheumatoid arthritis or Crohn's disease therapy(Barberà-Cremades *et al.*, 2017). TNF-a is a potent pro-inflammatory cytokine result pleiotropic effects on different cell types(Horiuchi *et al.*, 2010).

1.12: Role cytokines in regulation and activation of osteoclast:

Osteoclast are affected by adifferent of proinflammatory osteoclastogenic and anti-osteoclastogenic cytokines, that can be either motivate or inhibition their activity (Zupan, *et al* , 2012) Amongst these, proinflammatory cytokines function in booster of osteoclast division TNF-α induces osteoclast differentiation directly and independent manner by stimulating NF-κB and JNK (c-Jun N-terminal kinases) in a RANKL- and indirectly by activation the osteoblasts to express RANKL, inaddition IL-1, IL-6 and IL-11 are efficient osteoclastogenic agents in the same way, In other side, cytokines such us (IL-3, IL-4, IL-10 and IL-12)suppress osteoclast division and bone resorption. (Maurizi and Rucci, 2018)

Many reports was confirmed potential mechanisms of action of zoledronic acid, involving suppression of osteoclast maturation, inhibition of mature osteoclast function, decreasing of IL–6 cytokine production in addition to direct antitumor activity, having anti-angiogenic activity and inhibition of tumor-cell extended , invasion, and adhesion to the bone matrix (Korkmaz, 2018)

1.13: Cell culture:

In an artificial environment, the removal of cells from a tissue prior to their growth is referred to as cell culture. The cells can be directly isolated from the tissue and disaggregated by mechanical means or enzymatic methods prior to culture, or before a cell line or cell strain can be established from there. The base nutrients (amino acids, carbohydrates, vitamins, and minerals), growth factors, hormones, gases (O2, CO2), and a medium that controls the physicochemical environment can all be found in an artificial environment where cells can proliferate (pH, osmotic pressure, temperature). (Segeritz and Vallier, 2017)

The maintenance of disaggregated cells in vitro, refers to "Cell culture" whereas "organ culture" mention to a culture of a non-separated tissue. The term "tissue culture" including both terms. "primary culture" refer to initial culture that is undergoes multiple sub-cultures or passages to result a "cell line". Cell lines may be continuous (capable of uncontrolled growth) or fixed, characterized by senescence after a finite number of population doublings. Continuous cell lines are "transformed" – this seems to modulate to their gaining of telomerase activity in most instance.(Cree, 2011)

1.14: Cell line:

The selected of a cell line for cell culture base vigorously on the functional characteristic and particular readouts needed of the cell model. Choosing cell lines will also required to stratified with the available tools and requirements of their specific hazard group. Have three different types of

Cells cultured in the laboratory : primary cells, transformed cells, and self-renewing cells. Primary cell represent the fibroblasts is isolated from in biopsies and hepatocytes isolated from liver. Often depend on using for these cell types in Biomedical and translational studies because they are consider a good representatives of their tissue of origin. primary cells are in general characterized as "finite" and therefore depended on a continuous provided of stocks since often of impossible their proliferation stopping after a fixed amount of cell divisions and cell expansion. Transformed cells can be created either naturally or by genetic manipulation. Commercially Cell lines can be obtained, where certain quality control measures are in place that undertaking genomic constancy and lack of contaminants. Cell banks or other cell culture laboratories consider the places to source cell lines. To ensure clean cultures should always be the introduction of new cell lines in a laboratory accompanied by a Mycoplasma PCR test. (Segeritz and Vallier, 2017).

- **LNCaP cell line** derive from a human lymph node metastatic lesion of prostatic adenocarcinoma that was considered as a universally . LNCaP cells we established a PCa progression model using Previously(Yu *et al.*, 2017)
- **MG-63cell line** is Osteosarcoma are derived form malignant bone tumors. osteosarcoma-derived cells are commonly used as osteoblastic models,These cells share some osteoblastic properties ,but their chromosomal alterations lead to abnormal molecular and cellular functions. MG-63 cells revealed both mature and immature osteoblastic characteristic by labelling profile and most

heterogeneous of the investigated osteosarcoma cell lines(Pautke *et al*., 2004)

1.14.1 Advantage of cell line:

Cell lines have several characteristic, they are cost-effective, work-friendly, and can work for more passages than primary cells. Moreover they are easy to manipulate and expand, making it favored for multiple screening owing to an unlimited material provision advantage. Cell line is immortal ,widely used model in cell culture literature, drug studies, biochemical assays, bioactive production, (Cree, 2011)

1.14.2: Disadvantage of cell line:

The functional levels of cell line are frequently various from those found in primary cells and some main functions are loosed due to modification. furthermore cell lines are originated from a single donor, as a result genotypic and phonotypic differences in response to drugs in human they cannot be used for estimation(Cherbas and Gong, 2014)

Chapter two
Materials and Methods

2.1 Materials:

All The experiments of this Study were done in Cell Culture Labratory/ College of Medicine, University of Babylon, through the period from (1/112020- 1/11/2022)

2.1.1 Chemicals:

Chemicals used in this study are listed in **(Table 2.1)** with their company and the manufacturing country.

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Table 2.1: List of chemicals used in the study as shown in below:

Chemicals	Company	Country
Alcohol spray (ethanol 70%)	Ameya Fze	UAE
Dimethyl sulfoxide (DMSO)	Roth	Germany
Fetal bovine serum (FBS)	Gibco	UK
MTT(3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) dye powder	Roth	Germany
Phosphate buffer saline tablet	Gibco	UK
Roswell Park Memorial Institute-1640 (RPMI-1640) powder medium	Gibco	UK
Sodium bicarbonate powder	Ludeco	Belgium
Trypsin-Ethylenediamine-tetraacetic acid (EDTA) powder	USA Bilogy	USA

2.1.2 Drugs:

Drugs used in the study are listed in **(Table 2.2)** with their company and the manufacturing country.

Table 2.2: List of drugs used in the study as shown below :

Drugs	Company	Country
Zoledronic acid (vial)	Cipla	India
Doxorubicin (amp)	Pfizer	US
Gentamicin (vial)	The Arab pharm	Jordan

2.1.3 Instruments and Tools:

The instruments and tools used in the study are listed in **(Table 2.3)** with their company and the manufacturing country.

Table 2.3: List of Instruments and Tools used in the study as shown below:

Items	Company	Country
Automatic micropipettes (different sizes)	Human	Germany
Cell culture flask (25ml)	SPL	Korea
Cell culture plate (96- wells)	SPL	Korea
Distiller	ROWA	Germany
Double distillation water stills	GFL	Germany
ELISA Reader	Human	Germany

Sterile freezing vial (1.5 ml)	Biofil	Australia
The Inverted microscope	T.C Meiji techno	Japan
Laminar air flow cabinet	Labtech	Korea
Liquid nitrogen container GT38	Air Liquide	France
Magnetic stirrer	Labinco	Netherland
Microcentrifuge	Memmert	Germany
Millipore filter (0.45, 0.22µm)	Biofil	Australia
pH meter	WTW	Germany
Sensitive balance	Labtech	Korea
Vortex	Kottermann	Germany
Water bath	Memmert	Germany

2.1.4 Equipment:

The equipment used in the study are listed in **(Table 2.4)** with their company and the manufacturing country.

Table 2.4: List of equipments used in the Study as shown below:

Items	Company	Country
Autoclave	Jeiotech	Korea
Electric oven	Memmert	Germany
The Incubator	Memmert	Germany
Refrigerator	Arcelik	Turkey

2.1.5 Assay kit:

the assay kits used in this study include:

Table 2.5 list of ELISA assay kits used in the present study as shown below:

Item	Company	Country
ELISA kit Interleukin-6	Elabscience	USA
ELISA kit Tumor Necrosis factor-α	Elabscience	USA

Kit contents include the following:

Table 2.6 List of contents of the ELISA assay kit

Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips
Reference Standard	96T: 2 vials 48T: 1 vial
Concentrated Biotinylated Detection Ab (100×)	96T: 1 vial, 120 µL 48T: 1 vial, 60 µL
Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 µL 48T: 1 vial, 60 µL
Reference Standard & Sample Diluent	1 vial, 20 mL
Biotinylated Detection Ab Diluent	1 vial, 14 mL
HRP Conjugate Diluent	1 vial, 14 mL
Concentrated Wash Buffer (25×)	

Substrate Reagent	1 vial, 10 mL
Stop Solution	1 vial, 10 mL
Plate Sealer	5 pieces
Manual	1 copy
Certificate of Analysis	1 copy

2.1.6 Cell lines:

Frozen vials of human prostate cancer LNCaP and osteosarcoma MG-63 cell lines were obtained from Tissue Culture laboratory in the College of Medicine / University of Babylon.

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2.2. methods:

2.2.1. Preparation of drugs:

2.2.1.1. Preparation Zoledronic acid :

Zoledronic acid monohydrate (4 mg vial) was dissolved in 4ml of the complete media to give the first dilution (1000 µg/4ml), then, the other serial dilutions of zoledronic acid (500, 250, 125, 62.5 ,31.25, 15µg/ml) were prepare using the same medium.

2.2.1.2. Doxorubicin :

Doxorubicin ampule (50 mg/25 ml) was used to prepare the stock solution (2000 µg/ml) using complete medium . Other serial dilutions (100,50,25,12.5,6.25,3µg/ml) were prepared by the same way using the same medium

2.2.2. Preparation of Reagents and Solutions :

2.2.2.1. Phosphate Buffer Saline (PBS):

According to Gibco manufacturer manual. The PBS was prepared by dissolving one tablet of PBS in 500 ml deionized distilled water (DDW) with stirring constantly on a magnetic stirrer at room temperature, the pH should be (7.45). Autoclaving is required for complete sterilization and stored in a closed bottle until use to keep sterile(Thermofisher, 2019).

2.2.2.2. Gentamycin Stock Solution:

A gentamycin vial (40 mg/ml) solution was used as antibacterial agents in complete medium. About 100 µl was added to 250 ml of the medium.

2.2.2.3. Trypsin-(EDTA) Solution:

As indicated by US Biological headings, a weight of 10.1 gm of trypsin-EDTA (ethylenediaminetetraacetate) powder and dissolving in 900ml of deionized distilled water (DDW) with continuous mixing at room temperature, with PH value 7.2 should be reached and complete the

volume to 1000ml by DDW, the solution was sterilized through using Millipore filters of 0.45 and 0.22 μm respectively, after that, the solution was kept at (- 20C˚) of temperature.

2.2.2.4. Preparing MTT assay :

The MTT dye 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) powder (0.5 gm) was dissolved in PBS (100 ml) to achieve 5 mg/ml concentration, (0.2 μm) Millipore filter was utilized to sterilize the MTT solution and stored in a sterile and light-protected bottle. The solution was stored at (4°C) of temperature for multiple uses or at (-20°C) of temperature for long storage (J. van 2011, Meerloo, 2011).

A. Principle:

The principle of the MTT assay is to detect the cellular mitochondrial activity of the viable and thereby elevating or lowering in the viability percentage is linearly binding to the mitochondrial activity. activity of mitochondrial is reverse by the transformation of the pale-yellow tetrazolium salt (MTT dye) into dark purple formazan crystals by NADH (Figure 2.1). any elevation or lowering in viability percentage can be reveal by measuring formazan concentration utilizing a plate

reader at 570 nm. The darker the solution, the number of viable and metabolically active cells is increasing (Meerloo, 2011).

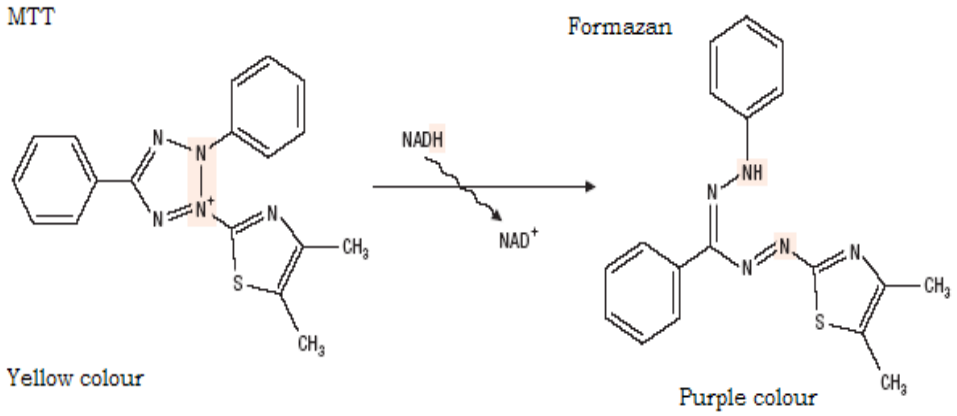

Figure 2.1: Principle of MTT assay.

B. Procedure:

1- At the end of the drug exposure period, the medium was removed from the wells and then the cells were washed with PBS

2- A volume of 1.2 ml of MTT solution (5 mg/ ml) was added to 10.8 ml medium to obtain final concentration of 0.5 mg/ml. Then, 200 µl of the resulting solution was added in each well. A blank control was carried to assess unspecific formazan conversion

3- the plate was incubated for (2-4)hr at 37°C at this stage, the intracellular purple formazan crystals were apparent down the inverted microscope.

4- The supernatant was removed and 100 µl DMSO was added in each well to dissolve the resultant formazan crystals.

1- 5- The plate is left for 30 min incubation period at room temperature until purple crystals dissolved.

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2- 6- Measure the absorbance using microplate reader at 570 nm.

7.To calculate the percentage of cell viability or proliferation, absorbance readings from test samples must first be divided by those from the control sample and multiplied by 100. Higher than the control absorbance values denote cell growth, while lower values denote cell death or suppression of proliferation The following formula was used to calculate the percentage of cell viability or the percentage of inhibition.

% viability = (AT – AB) / (AC – AB) × 100%

AT = Absorbance of treated cells (drug).

AB = Absorbance of blank (only medium).

AC = Absorbance of control (untreated).

% Inhibition = 100 – % viability(Meerloo, 2011).

2.2.3 Preparation of Tissue Culture Media:

2.2.3.1.Preparation of Serum-Free Medium:

RPMI-1640 medium was prepared according to the Gibco product manual from RPMI-1640 medium powder as the following: 10.43 gm of RPMI-1640 medium powder was dissolved in 900ml of DDW in a volumetric flask. Other constituents added include: 2 gm sodium bicarbonate powder as needed and 400 ug of gentamycin solution were added with continuous stirring. The solution was completed to 1 liter by DDW with adjusting the PH at 7.4. Using 0.45 and 0.22 μm Millipore filters respectively to sterilize the solution under the airflow cabinet(Phelan and May, 2017).

2.2.3.2. Preparation of Serum-Medium:

Medium with serum was prepared by adding 10 percent of fetal bovine serum(FBS) to serum free medium prepared as described in (2.2.3.1).

2.2.3.3. Preparation of Freezing Medium:

The freezing medium was prepared from the following compositions: 6 ml serum-free medium, 3 ml FBS, and 1 ml DMSO. The solution was stored at (- 20) C° temperature between uses. (Meleady and O'Connor, 2006)

2.2.4.Preparation of cells lines:

2.2.4.1.Thawing of prostate LNCaP, MG-63 osteosarcoma cell lines:

The frozen cell line vial was removed from a liquid nitrogen container and placed into a beaker filled with pre-warmed (37°C) sterile DDW With caution. Before the ice floccule completely disintegrated, the vial was removed from the water and cleaned with 70% ethanol. The vials of cell suspension material was immediately pipetted into a 15ml sterile plastic container under a laminar stream cabinet. 10ml of pre-warmed serum-free media in a centrifuge tube. The supernatant was aspirated and decanted after five minutes of centrifugation at a rate of 1000 rotations per minute. The cell pellet was re-suspended in 5 ml of warm (37C) serum-medium and placed in a 25ml cell culture flask. After incubation at this temperature, the serum medium was changed(Phelan and May 2017).

2.2.4.2. sub-culturing of prostate LNCaP and MG-63 osteosarcoma cells lines:

1. The inverted microscope was utilized to analyze that the cells are healthy and sub-confluent without contamination.
2. The medium was removed by the pipette and add a sufficient amount of PBS to wash the monolayer to ensure the removal of all medium from the cell culture flask.

3. Convenient volume of trypsin- (EDTA) solution was added to the flask (1-2 ml per 25-cm^2 flask). Flask was rotated to completely cover the monolayer with trypsin
4. To detach the cells from the surface of the flask, the flask was returned to the incubator at 37°C. Usually, the cells detaching period depends on a cell line which could take 2 to 10 minutes.
5. An inverted microscope could be used to evaluate the cells whether they are detached and floating or not. The flask might be tapped gently on its side to detach any remaining cells.
6. To deactivate trypsin in the flask, an equal volume of serum containing media was added.
7. An aliquot of cells are transferred to another flask containing pre-warmed serum-containing medium (5–7ml for a 25-cm2 flask), labeled with cell line name
8. The flask was incubated at 37C° temperature.
9. This process has been repeated according to the characteristics of the growth for each cell line.(Meleady and O'Connor, 2006)

2.2.4.3:Harvesting of cell culture

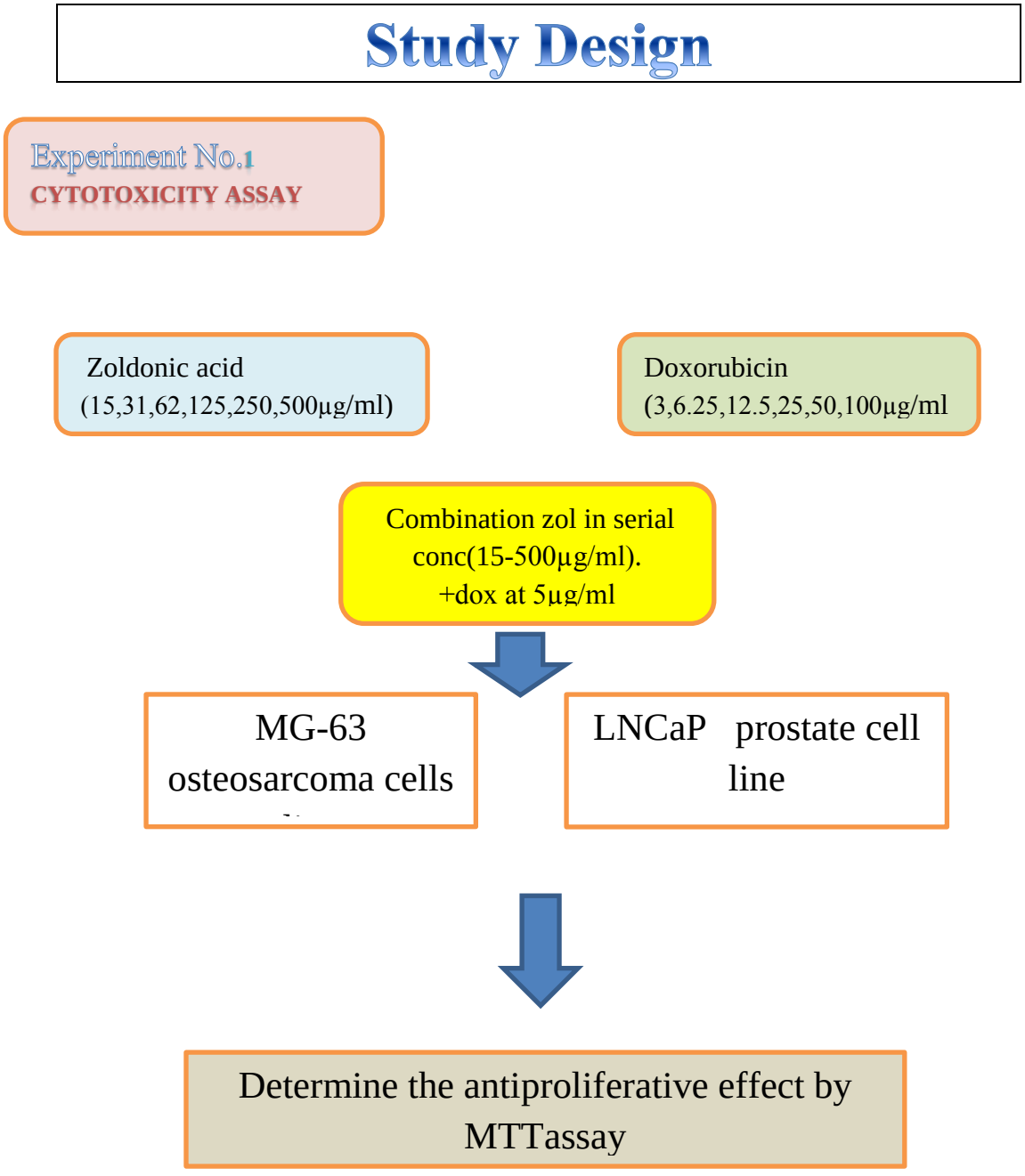
Harvesting is a technique that uses proteolytic enzymes to detach adherent cells from the surface of a cell culture flask. First, the growth medium in the vessel was aspirated and discarded. PBS was used to wash the cells twice. Afterward, the enzymatic harvesting solution was added to the vessel. After 15 minutes, the proteolytic reaction was neutralized by adding the serum-containing culture medium. The cells in the tissue culture flasks were harvested by using different enzymatic solutions composed of different concentrations of trypsin and Ethylenediaminetetraacetic acid (EDTA)(Viazzi *et al.*, 2015)

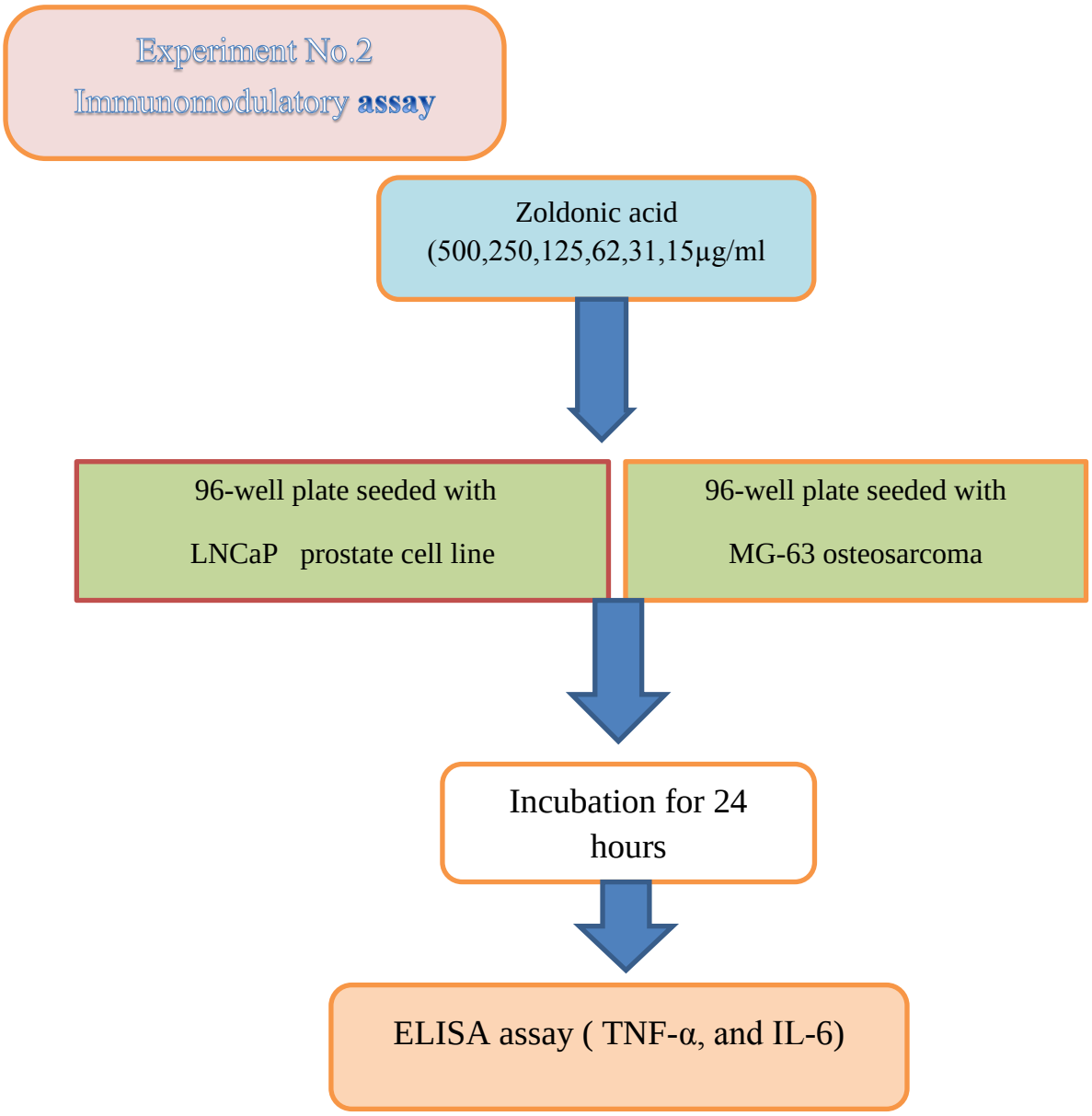
2.2.4.4:Freezing of the cell line

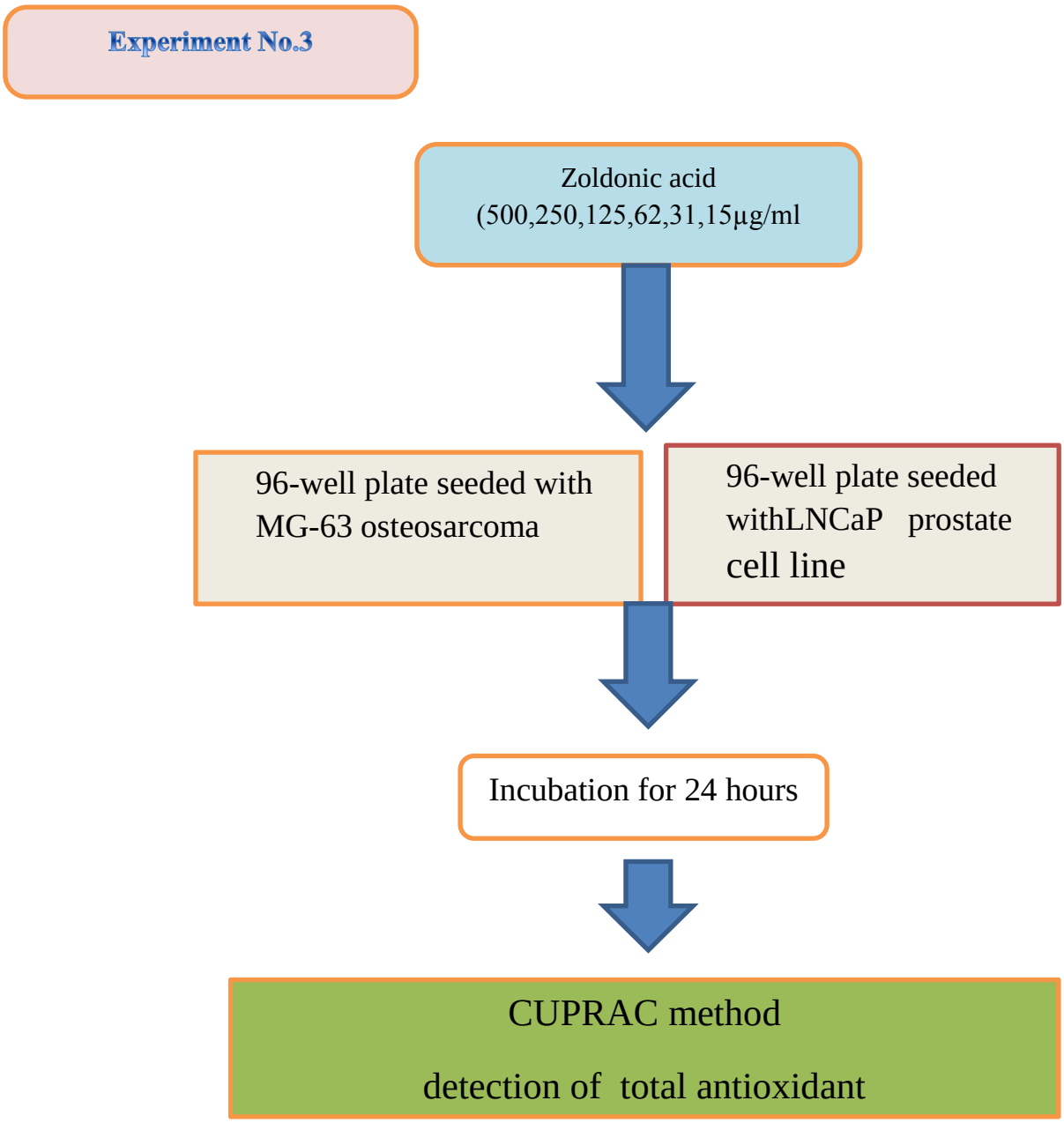
Cells lines source were kept frozen at -196 C^0 in liquid nitrogen freezer according to the following protocol

Tissue culture flask with a monolayer near the exponential phase was taken and washed twice with 5 ml of PBS, and then 3 ml of warm trypsin EDTA was added Then incubated at 37C° until the cell layer detached and the cells was aided to disaggregate into single cells by gentle rocking on the flask sides.The flask content was transferred into 15 ml sterile plastic centrifuge tube; rotate800 rpm for 10 minutes.The supernatant was decanted and the cell pellet was re-suspended with 1 ml of the freezing media and transferred into 1.5

ml sterile freezing vial and then stored for a long time in the liquid nitrogen freezer.(Yang *et al.*, 2019).




Experiment No.2
Immunomodulatory assay
Zoldonic acid
(500,250,125,62,31,15µg/ml
96-well plate seeded with
LNCaP prostate cell line
96-well plate seeded with
MG-63 osteosarcoma
Incubation for 24
hours
ELISA assay (TNF-α, and IL-6)


Experiment No.3
Zoldonic acid
(500,250,125,62,31,15µg/ml
96-well plate seeded with
MG-63 osteosarcoma
96-well plate seeded
withLNCaP prostate
cell line
Incubation for 24 hours
CUPRAC method
detection of total antioxidant

2.2.5. The Experiments parts:

2.2.5.1:Cytotoxicity assays

A. A. Study the cytotoxicity of zoledronic acid on MG-63cell line:

For cytotoxicity measurement of zoledronci acid on osteo MG-63 cell line, cells were exposed to serial dilutions of zoledronic acid (15, 31.25,62.5,125,250,500 µg/ml) in addition to the control group. each concentration having four duplicates. After that, the plate underwent a 24hour incubation cycle while being covered with a self-plastic lid. The wells were rinsed with 200µl of sterile PBS following the completion of the incubation time. The MTT assay was used to evaluate how zoledronic acid affected the proliferation of the osteo MG-63 cell line.

B. Study the effect of (zoledronic acid+ doxorubicin) combination on MG-63 cells viability:

For cytotoxicity measurement of zoledronic acid with doxorubcin (5 µg/ml) togather on MG-63 cell line with zoledronic acid a serial dilutions (500, 250, 125, 62.5, 31.25, 15 µg/ml). A group of doxorubicin (5 µg/ml) was used as a positive control. Four duplicates were used for each concentration as well as with the control group. After that, the plate underwent a 24-hour incubation while being covered with a self-plastic lid. The wells were rinsed with 200µl of sterile PBS following the completion of the incubation time. The MTT assay was used to evaluate how doxorubicin and zoledronic acid affected the proliferation of the MG-63 cell line.

C.Study the effect of zoledronic acid on LNCaP cell line:

For cytotoxicity measurement of zoledronci acid on LNCaP cell line, cells were exposed to serial dilutions of zoledronic acid (15,31.25,62.5,125,250,500 µg/ml) in addition to the control group ,four replicates were used for each concentration. After that, a self-plastic lid was placed on the plate, and it was incubated once for 24 hours. Following the incubation period, 200 µl of sterile PBS was used to wash the wells. By using the MTT assay, the impact of zoledronic acid on LNCaP cell line proliferation was evaluated.

D. Study the cytotoxicity of doxorubicin on LNCaP cell line:

For cytotoxicity measurement of doxorubicin on L.NCaP cell line, cells were exposed to serial dilutions of doxorubicin (3,6.25,12.5,25,50,100 µg/ml) in addition to the control group.for each concentration, with four replicates. The plate was then placed in an incubator for 24 hours once, with the lid made of self-adhesive plastic. The wells were cleaned with 200 µl of sterile PBS when the incubation period was complete. MTT assay was used to determine doxorubicin's impact on LNCaP cell line proliferation.

E. Study the effect of (zoledronic acid - doxorubicin) combination on cells viability of LNCaP cell line:

For cytotoxicity evaluation of zoledronic acid with doxorubicin together on LNCaP cell line, zoledronic acid in a serial dilutions (500, 250, 125, 62.5, 31.25, 15 µg/ml) was added to the cells with a constant

concentration of doxorubicin (5 µg/ml) as positive control. In addition to the control group, group of doxorubicin (5 µg/ml) was used as a positive control,four replicates for each concentration was used . Then the plate was covered with a self-plastic lid and incubated once for 24 hours. After the end of the incubation period, the wells were washed with 200 µl of a sterile PBS. The effect of doxorubicin with zoledronic acid on LNCaP cell line growth was assessed by MTT assay. (Figure2.2) in below shown addition MTT for cells:

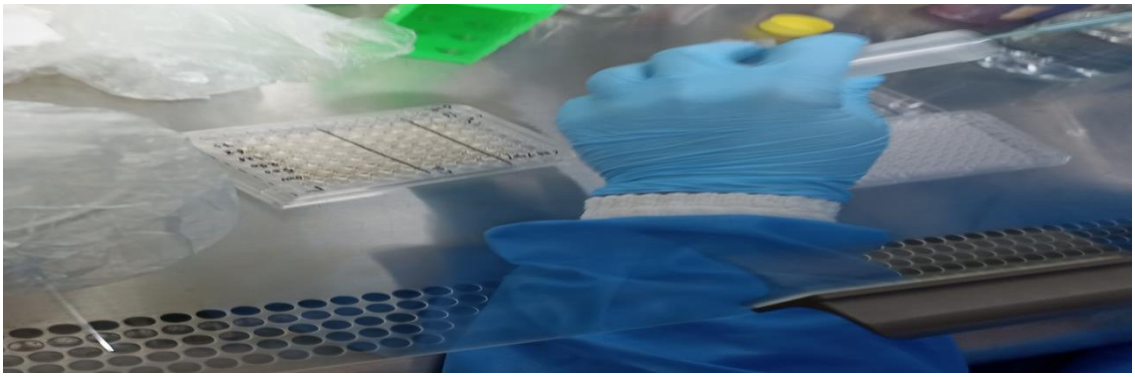


Figure 2.2 shown addition MTTto treated cells.

In (Figure 2.3) shown transformation yellow color of tetrazolium salt to dark purple color of formazan

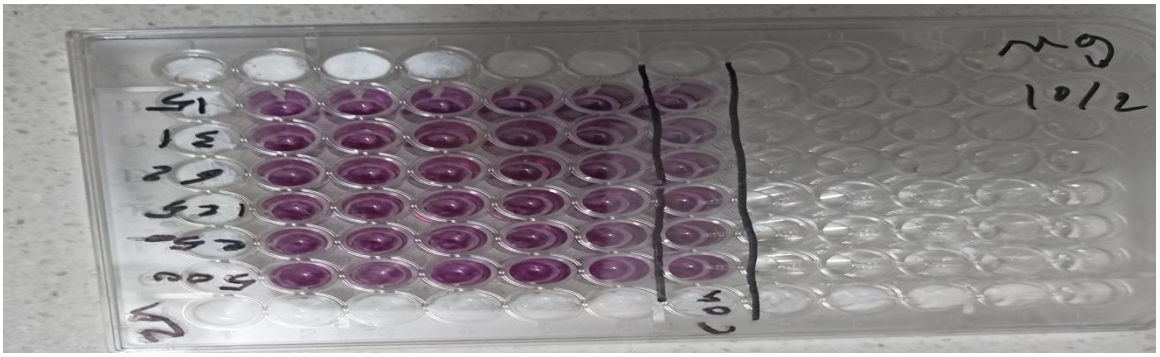


Figure 2.3 shown transforming tetrazolium salt to formazan in MTT dye after incubation 4 hr

2.2.5.2 Immunomodulatory effect assays

I.Study the anti-inflammatory effect of zoledronic acid on both LNCap and MG-63 cell line:

Cells line of MG-63 osteosarcoma and LNCaP prostate cancer were seeded in 96tissue culture plates. Then cells will treated by diluted concentrations of zoledronic acid rang from (15-500µg/ml) with four replicates for each concentration of zoledronic acid. Then the plate was covered with a self-plastic lid and incubated once for 24 hours. After 24 hours of incubation period, each well is withdrawn by a micropipette the supernated after centrifuge ,then selected serial concentration of zoledronic acid, taken for immunoassay by ELISA method using IL-6, and TNF-α according to the protocol mentioned below:

II.Test principle:

This ELISA kit uses the sandwich-ELISA principle in which the plate is precoated with an antibody specific to the human cytokine of interest. Samples or standards are added to the plate and combined with the antibody. When the avidin-HRP conjugate and biotinylated detection antibody are applied, the plate turns blue. A stop solution is added to cease the enzyme substrate reaction, which causes the color to turn yellow. At 450 nm in wavelength, the 68 optical density is calculated. By comparing the sample's OD to the standard

curve, the concentration of an interest cytokine is determined (Alhajj and Farhana, 2022).

III.Assay procedure : Prior to usage, all reagents and samples are maintained at room temperature. The material should be shaken once more after thawing before the test. Prior to pipetting, all the reagents must be properly mixed by gently swirling; foaming is not desired. It is advised that every sample and standard be analyzed twice.

1. Addition of Sample: Each well receives 100 mL of Standard, Blank, or Sample. The Reference Standard and A Sample diluent is applied to the blank well. The bottom of the microELISA plate is filled with solutions; care is taken to prevent inside wall contacting and foaming. Stir it slowly. Apply the sealant we supplied to the plate. 90 minutes of incubation at 37 °C.

2. Biotinylated Detection Ab: then the liquid of each well removed, without washing. Addition of 100µl of Biotinylated Detection Ab working solution to each well. Then the plate is covered with the Plate sealer. Gently tap the plate to ensure through mixing, incubate for 1 hour at 37°C.

3. Wash:. Three times each well is drained and rinsed; during the third wash, approximately 350µl of wash buffer are delivered to each well (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). It's

crucial to entirely drain each step of all liquid. The residual wash buffer is aspirated or decanted away after the last wash. After that, the dish is placed upside-down on a large sheet of spotless absorbent paper.

4. HRP Conjugate: Each well receives 100 mL of the HRP Conjugate substrate solution, followed by incubated at 37 °C for 30 minutes.

5. Substrate: Add a fresh Plate sealer on top. At 37°C, incubate for approximately 15 minutes. shield the plate from the light. Depending on the actual color shift, the reaction time may be reduced or prolonged, but not beyond 30 minutes. The operator should stop the reaction when an apparent gradient forms in conventional wells.

6. Stop: To each well, 50µl of Stop Solution are put. The color then abruptly changes to yellow, substrate solution and stop solution should be applied in the same order.

7. OD Measurement: Using a micro-plate reader set to 450 nm, the optical density (OD value) of each well is calculated simultaneously. The operator should pre-heat the instrument, unlock the micro-plate reader, and establish the testing parameters beforehand. (Alhajj and Farhana, 2022). in (figure 2.4) and (2.5) demonstrated addition reagent and converted blue color to yellow color after addition stop solution.

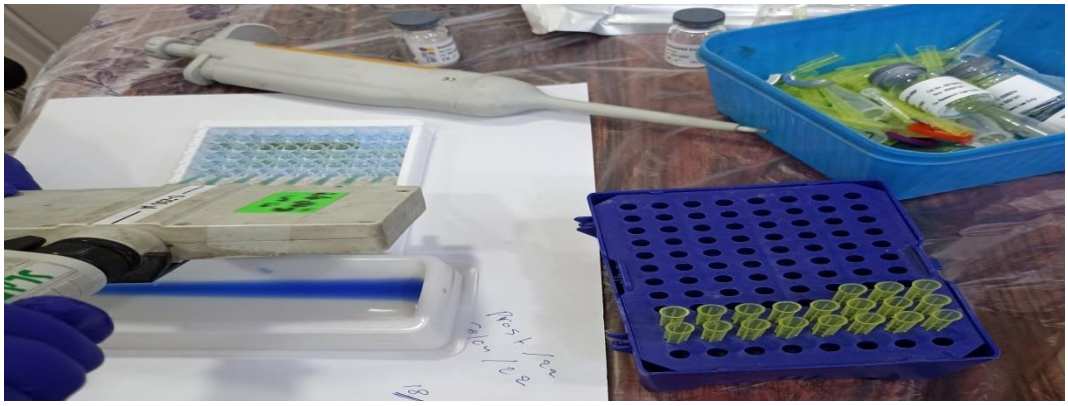


Figure 2.4 shown addition reagents and sample to plate at room tempreture .

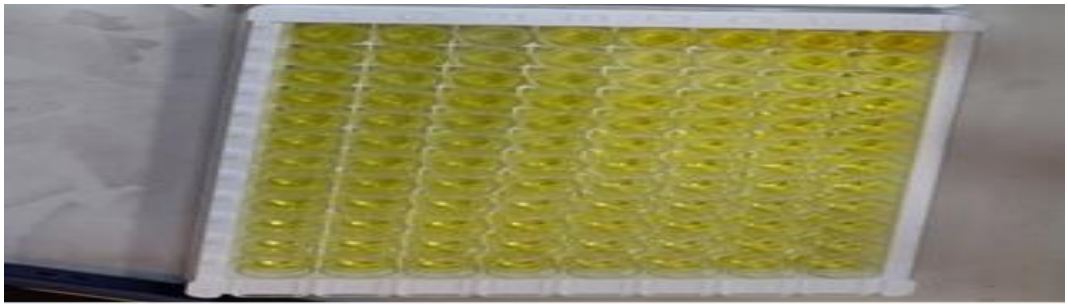


Figure 2.5 shown addition stop solution to plate .

2.2.5.3: Measurement of total antioxidant activity in LNCaP and MG-63 cells lines treated with zoledronic acid:

Cells lines, LNCaP prostate cancer and MG-63 osteosarcoma were seeded in 96 tissue culture plate ,then exposed to serial diluted concentrations of zoledronic acid ranging from(15-500µg/ml). with four replicates for each concentration of zoledronic acid. Then the plate was covered with a self-plastic

lid and incubated once for 24 hours. After 24 hours of incubation period, each well is withdrawn by a micropipette and centrifuge ,then selected serial concentration of zoledronic acid, then taken for total antioxidant capacity assay.

2.2.6 The CUPRAC Method for The Measurment of Total Antioxidants Capacity:

I.Principle:

The CUPRAC method involves combining the antioxidant solution (either directly or after acid hydrolysis) with a copper(II) chloride solution, a neocuproine (2,9-dimethyl-1,10-phenanthroline) alcoholic solution, and an ammonium acetate aqueous buffer at pH 7, and then measuring the developed absorbance at 450 nm after 30 min (normal measurement). Naringin and naringenin were assayed after incubation at 50 °C on a water bath for 20 min (after Cu(II)- Nc reagent addition) in order to enable complete oxidation because the colour change is fast for substances like ascorbic acid, gallic acid, and quercetin but slow for naringin and naringenin (incubated measurement). To have the greatest inhibition possible against Cu(II)-Nc, the flavonoid glycosides were hydrolyzed to their equivalent aglycones by reacting in 1.2 M HCl-containing 50% MeOH (hydrolyzed measurement). order to achieve the greatest absorbance at 450 nm, it is therefore necessary to assess the total CUPRAC antioxidant capacity of a mixture including several antioxidants after a proper combination of hydrolysis and incubation methods (Apak *et al.*, 2007)

Total antioxidants + Cu^{+2} ⟶ Cu^{+}

Cu^{+} + 2,9-dimethyl-1,10-phenanthroline ⟶ complex (λ_{max} at 450 nm

II.Reagents preparation

1.Copper chloride (CuCl2•2 H2O)weighing 0.4262 g was dissolved in H2O, and 250 ml of water was added to make the Copper(II) chloride solution, which has a concentration of 10-2M.

2. To create an ammonium acetate (NH4Ac) buffer with a pH of 7.0, 19.27 g of NH4Ac was dissolved in water, and the amount was then increased to 250 ml.

3. Neocuproine (Nc) 2,9-dimethyl-1,10-phenanthroline solution at a concentration of 7.5 * 10-3M was made by combining 0.039 g of Nc with 96% EtOH, then adding ethanol to make a volume of 25 ml.

4. Standard antioxidant solution preparation was done at 1.0 * 10-3M Torolox.

III-Reagents mixtures

Table 2.7: represent reagent mixtures

Reagents	Test	STD	Blank
Copper(II) chloride solution	1ml	1ml	1ml
Sample	50 µl	------	-------
Working standard solution	-------	50 µl	-------
D.W	-------	-------	50 µl
Neocuproine (Nc) solution	1ml	1ml	1ml
Ammonium acetate (NH_4Ac) buffer	1ml	1ml	1ml

Test tubes were mixed by vortex and incubated for 30 minutes at 37°C, after that the absorbance was read on a spectrophotometer at 450 nm.

IV-Calculation:

$$\text{Total antioxidants levels} = \frac{\text{A.test}}{\text{A.STD}} * Conc.of \quad STD \ (\text{mmol/l})$$

2.2.5 Statistical Analysis :

All data were collected and analyzed by Microsoft Office Excel 2016 and Sigma plot version 12.5 software. ANOVA one-way test was used to assess significant differences among the means of data. the *p*-value ($p \leq 0.001$, $p \leq 0.05$) were considered statistically significant

Chapter three

The results

3.1 Introduction

In this chapter, the results yielded from the experimental part of this study are illustrated. Results are displayed in figures after statistical processing using mean and standard deviation.

3.2 The effect of zoledronic acid on cell viability of osteosarcoma cell line:

The result showed there are a highly significant decrease ($p \leq 0.001$) in cell viability at zol concentrations(500, 250, 125µg/ml) but at concentrations (62.5, 31.25, 15 µg/ml) showed significant decrease($p \leq 0.050$)comparison with control group after incubation for 24 hour after evaluated by MTTassay . as shown in figure 3.1

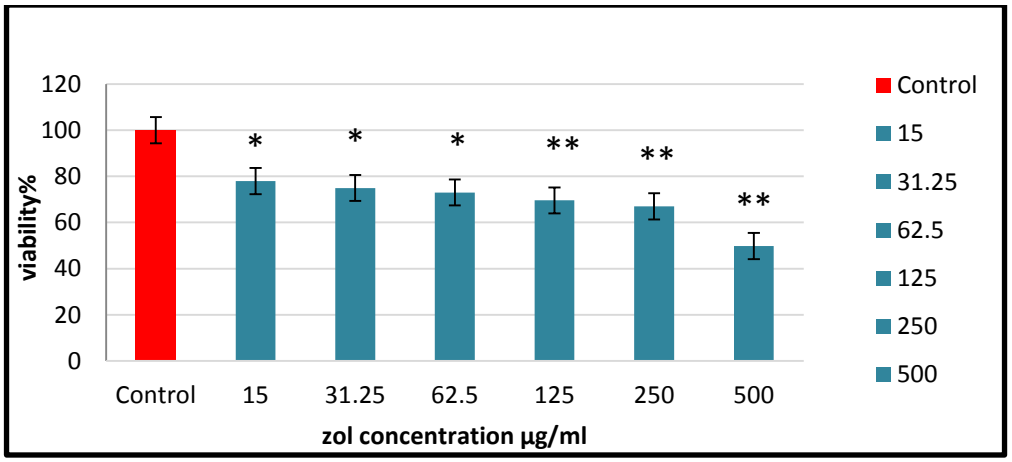


Figure 3.1: Represent Cell viability Percentage of MG-63 cell line treated with zoledronic acid . after incubation for 24hr by MTT assay . high significant p≤0.001 *significant p≤0.050**

3.3 The effect of combination zoledronic acid with doxorubicin on cells viability of osteosarcoma cell line:

The result showed there were a highly significant decrease $p \leq 0.001$ in cell viability of at zol concentrations(15,31.25,62.5,125,250,500µg/ml) comparison with control group after incubation for 24hr. also noted that doxorubicin at constant concentration 5µg/ml versus control group showed there were a highly significant decrease $p \leq 0.001$at same incubation time. As shown in figure3.2

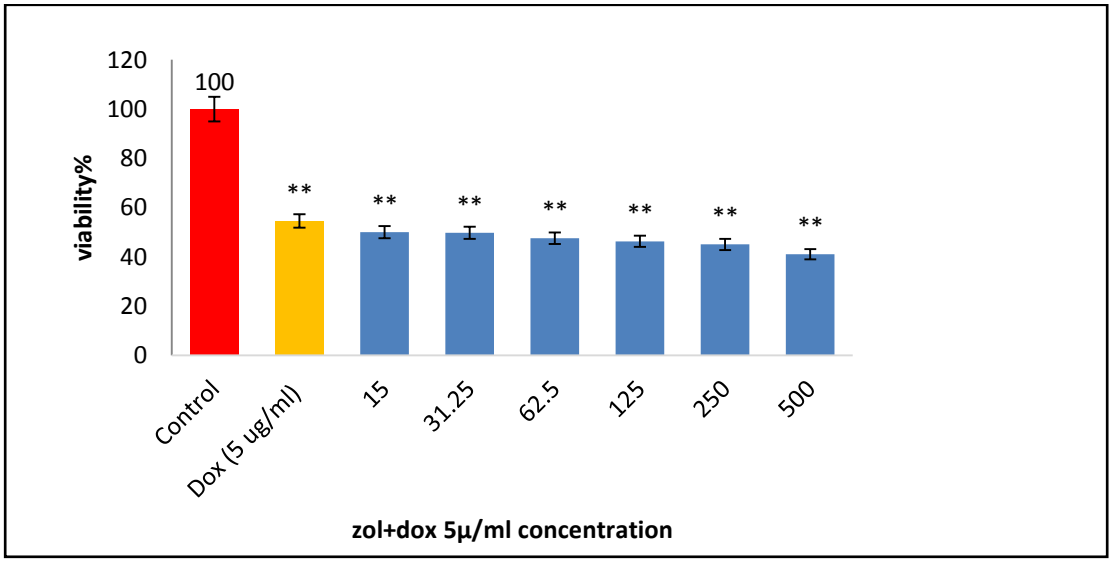


Figure 3.2 : Represent Cell viability Percentage of MG-63 cell line treated with . zoledronic acid with doxorubicin at constant conc. after incubation for 24hr by MTT assay. ** high significant $p \leq 0.001$

While when comparison zoledronic acid at serial concentrations with positive control(doxorubicin 5µg/ml) the result showed there were a high significant decrease $p \leq 0.001$ in cell viability of the zol concentrations (500, 250, µg/ml) while at concentrations(125, 62.5µg/ml) it showed

significant decrease $p \leq 0.05$ comparison with positive control after incubation for 24 hr. while there were no significant differences between the positive group and zoledronic acid at concentration (31, 15µg/ml) at same the incubation time. As shown in (figure 3.3)

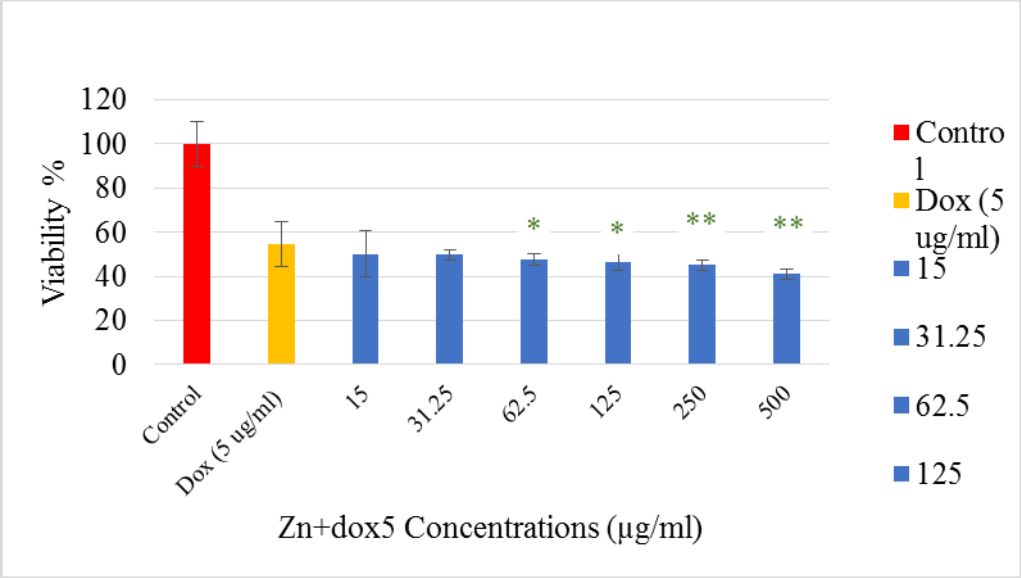


Figure 3.3 Represent comparison between dox: (positive control) and treatmen groups(zo+dox at con. 5µg/ml) in osteosarcoma cells line

****high significant** $p \leq 0.001$ ***significant** $p \leq 0.050$

3.4 Effect of zoledronic acid at serial concentration on TNF-α conc. for osteosarcoma cell line:

The result showed there were a significant decrease $p \leq 0.050$ at zol concentration (500µg/ml) comparison with control group, while at zol concentration (15µg/ml) showed a significant increase $p \leq 0.050$

comparison with control group after 24hr incubation period. after evaluated by ELISA method. As shown in(figure 3.4)

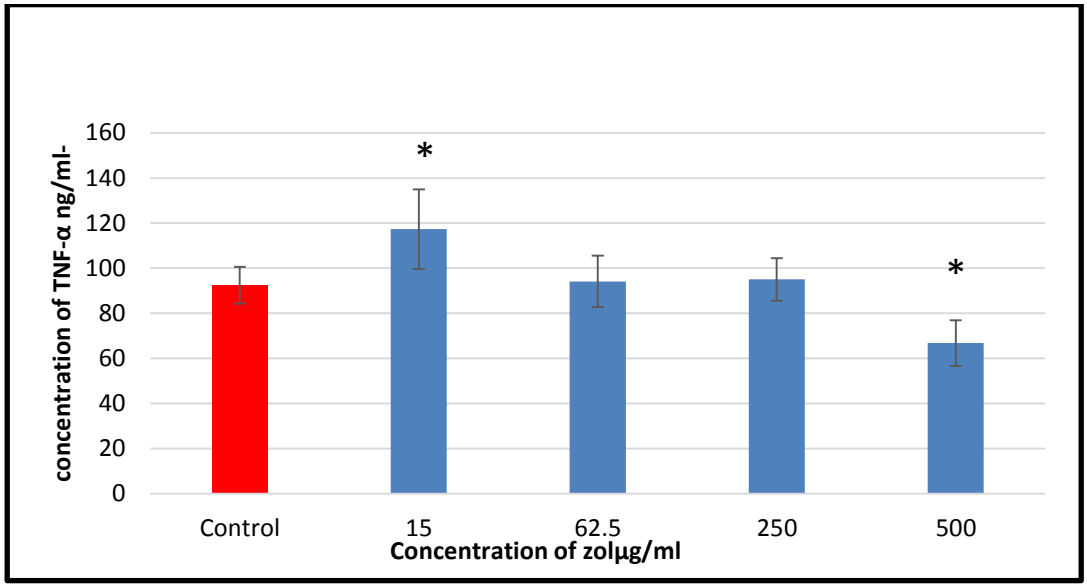


Figure 3.4: Represent effect of zoledronic acid on TNF-α level in osteosarcoma cell line.*significant $p \leq 0.050$

3.5 Effect of zoledronic acid on IL-6 level on osteosarcoma cell line:

The result showed there were a highly significant decrease ($p \leq 0.001$) of IL-6 level at concentration of zoledronic acid (500µg/ml) comparison with control group while no significant difference showed at concentrations (62.5, 250, 125, 31.25, 15µg/ml) comparison with control

group after incubation 24hr after evaluated by ELISA method.as shown in(figure 3.5)

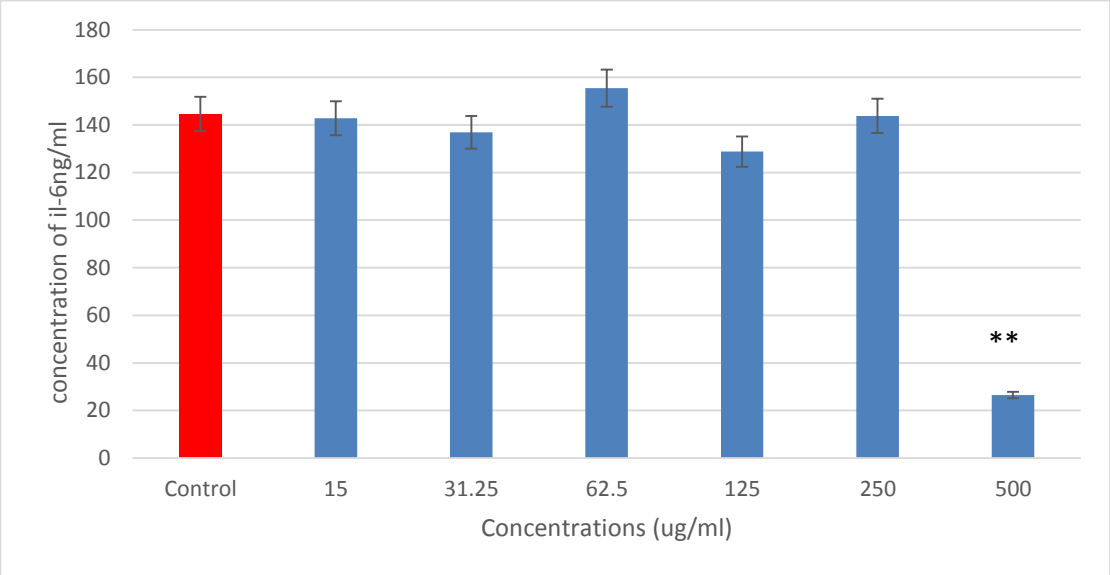


Figure 3.5: Represent effect of zoledronic acid on IL-6 level in osteosarcoma cell line. high significant $p \leq 0.001$**

3.6 study antioxidant effect of zoledronic acid in serial concentration on MG-63 cell line:

The result showed there were a significant decrease($p \leq 0.050$) in antioxidant level at concentration(500 ,125µg/ml) comparison with control group after incubation 24hr. as shown in (figure 3.6)

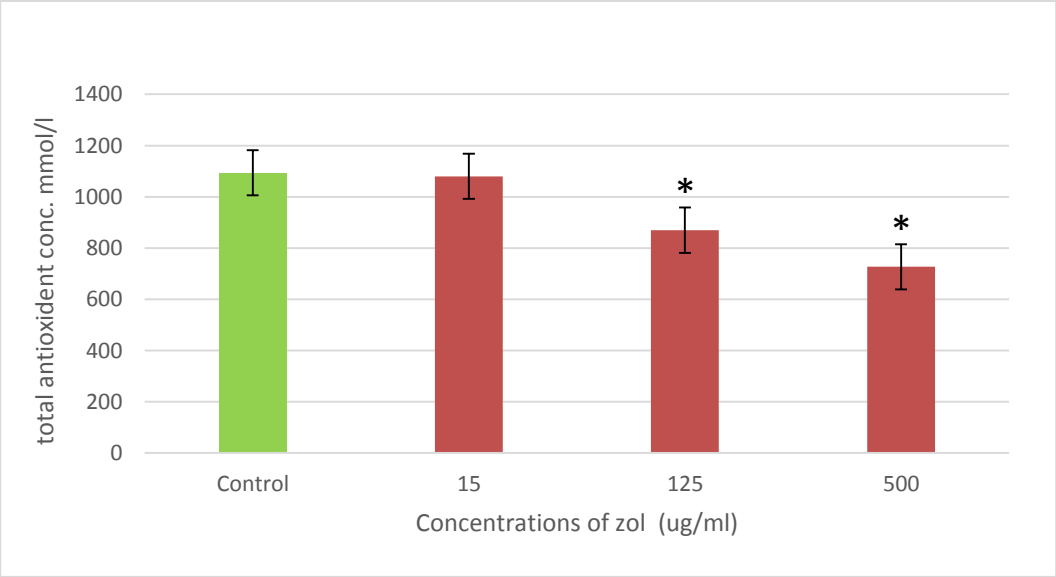


Figure 3.6: Represent antioxidant effect of zoledronic acid in osteosarcoma cell line. * significant $p \leq 0.050$

3.7 :The effect of zoledronic acid on cell viability of LNCaP cell line:

The results showed there were a highly significant decrease($p \leq 0.001$) in cell viability at concentrations(500 µg/ml) of zoledronic acid in comparison with the control group after incubation for 24hr. While there were no significant differences between the control group and concentrations of zoledronic acid(15,31.25,62.5,125,250,µg/ml) for the same time of incubation. When evaluated by MTTassay. As shown in (figure 3.7)

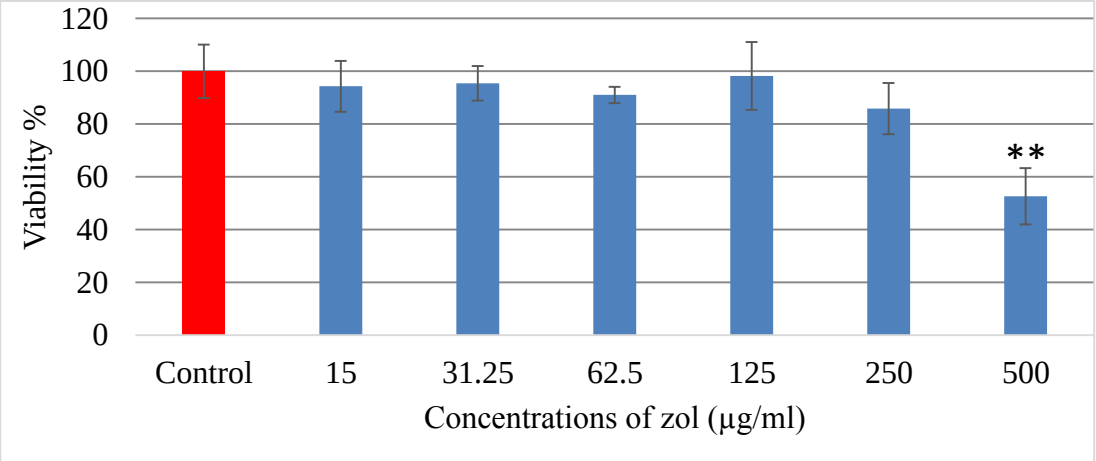


Figure 3.7: Represent Cell viability Percentage of LNCaP cell linetreated with zoledronic acid after incubation for 24 hours by MTT assay.highly significant $p \leq 0.001$**

3.8 :Effect of doxorubicin alone on cell viability of LNCaP:

The result showed there were a highly significant decrease($P \leq 0.001$) in cell viability at concentrations(3,6.25,12.5,25,50,100µg/ml) of doxorubicin in comparison with the control group after incubation for 24hr. as shown in(figure 3.8)

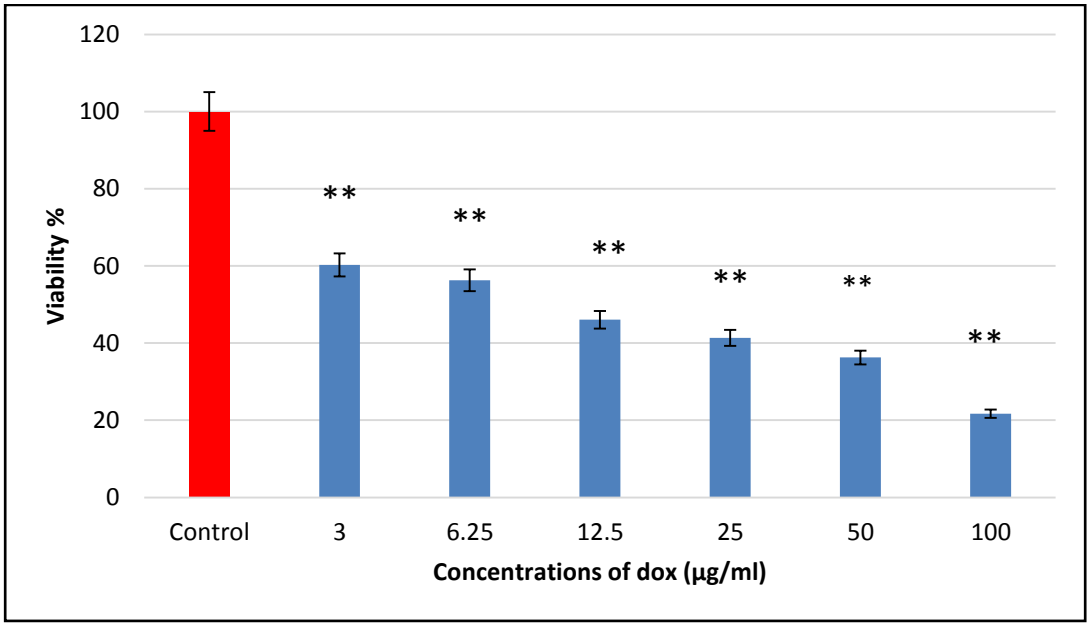


Figure 3.8: Represent Cell viability Percentage of LNCaP cell line treated with doxirubicin after incubation for 24 hours by MTT assay.highly significant p<0.001**

3.9: The effect of combination zoledronic acid with doxorubicin on cells viability of LNCaP cell line.

The result showed there were a highly significant decrease($p \leq 0.001$) in cell viability at zol concentrations (500, 250, 125, 62.5, 31.5, 15 µg/ml) comparison with control group after incubation for 24hr , in addition the constant concentration of doxorubicin (5µg/ml) positive control group showed high significant decrease ($p \leq 0.001$) in cell viability comparison with control group after incubation for 24hr. as shown in (figure 3.9)

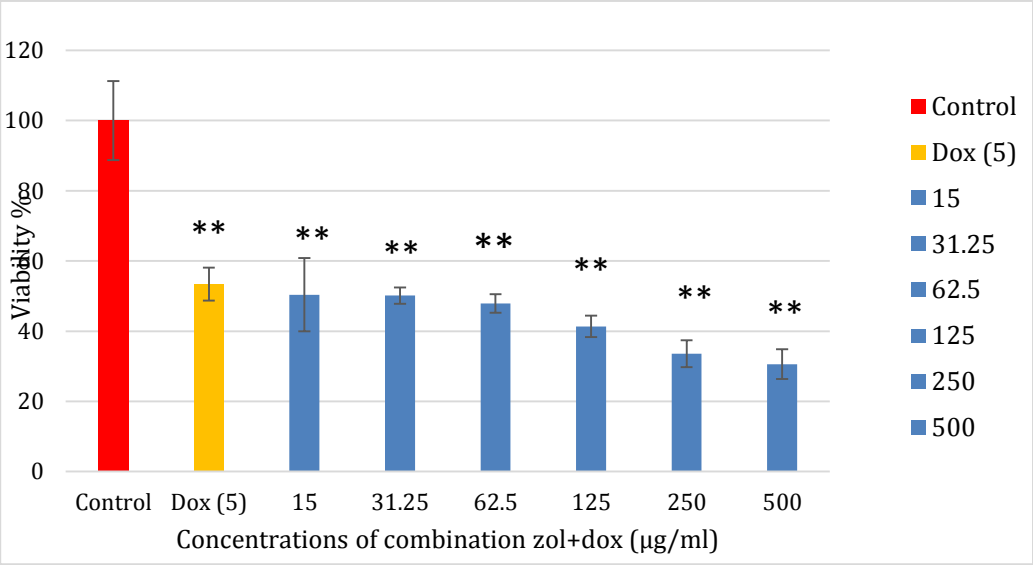


Figure 3.9 : Represent Cell viability Percentage of LNCaPcell line treated with zoledronic acid with constant conc. of doxorubicin comparison with control after incubation for 24 hours by MTT assay.

****highly significant. *P*≤0.001**

While the comparison between serial concentration of zoledronic acid and positive control group(doxorubicin at 5µg/ml) the result showed there were a highly significant decrease*(p*≤0.001) in cell viability of concentration zoledronic acid (500, 250, µg/ml) ,and significant decrease(*p*≤0.050) at concentration (125µg/ml) where no significant difference in cell viability at concentration (62.5, 31.25, 15 µg/ml) after incubation for 24 hr. as shown in(figure 3.10)

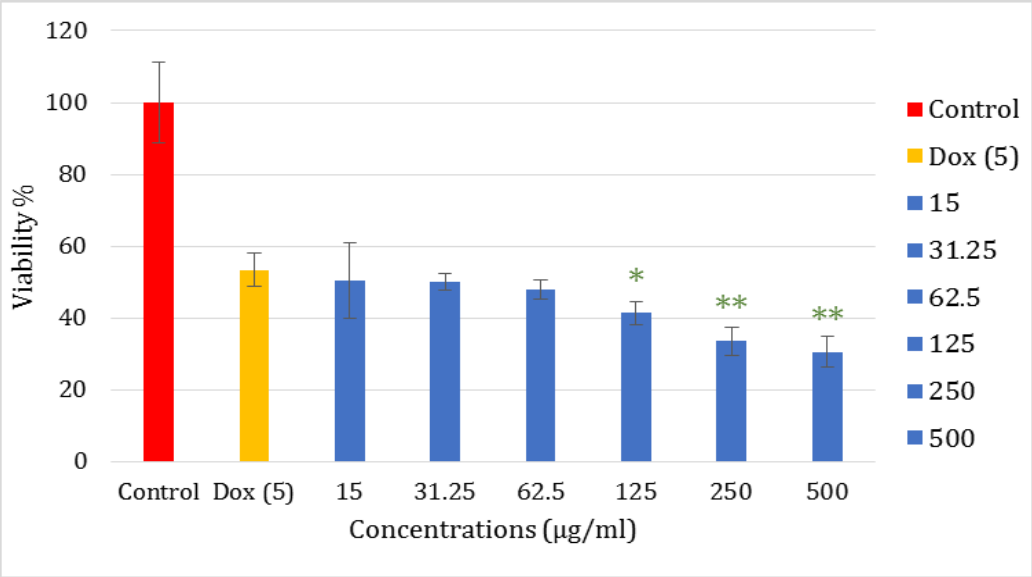


Figure 3.10: Represent cell viability percentage on LNcap cell line and comparison between positive control and(zol-dox5µg/ml) concentrations

****high significant** $p \leq 0.001$ ***significant** $p < 0.050$

3.10 : Effect zoledronic acid on TNF-α. level in LNCaP cell line:

The result showed there were a significant decrease $p \leq 0.05$ in concentration of TNF-α at zol concentration(500µg/ml) comparison with control group , while at zol concentrations (250,62.5,15µg/ml) showed a significant change in TNF-α comparison with control group ,for 24hr incubation period. after evaluated by ELISA method. As shown in (figure 3.11)

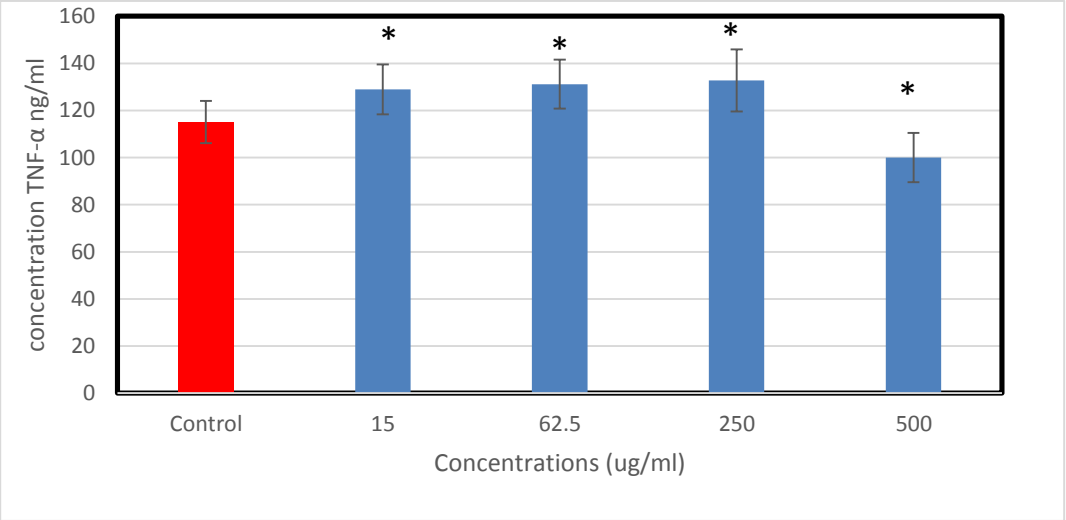


Figure 3.11 :Represent the effect of zoledronic acid on TNF-α for LNcap cell line. *significant $p \leq 0.050$

3.11 :Effect of zoledronic acid on IL-6 in LNCaP cell line:

The result showed there were a highly significant decrease ($p \leq 0.001$) in IL-6 level at concentrations (500, 250 µg/ml) Comparison with control group after incubation for 24hr. while at concentration 62.5µg/ml showed significant decrease ($p<0.050$) Comparison with control group at same condition. In concentrations(15,31.25,125µg/ml)

of zoledronic acid showed no significant difference comparison with control group.as shown in (figure 3.12)

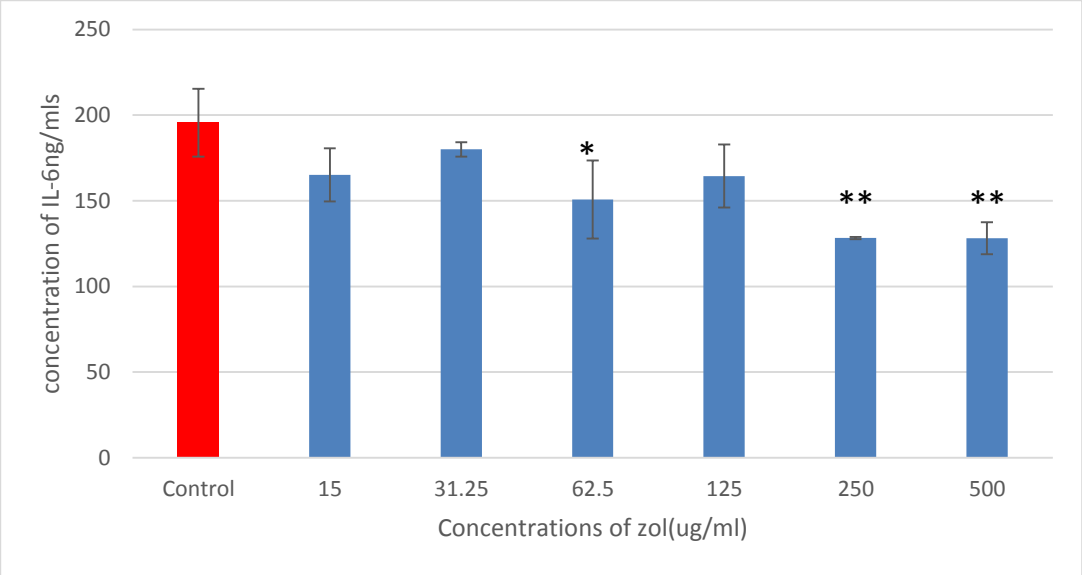


Figure 3.12: Represent effect of zoledronic acid on IL-6 in LNCaP. Cell line. **high significant *significant

3.12 :Effect antioxidant effect of zoledronic acid in serial concentration in LNcap cell line:

The result showed there are statically no significant difference for concentrations of zoledronic acid on antioxidant level comparison with control group. After evaluated by total antioxidant capacity assay. As shown in figure 3.13

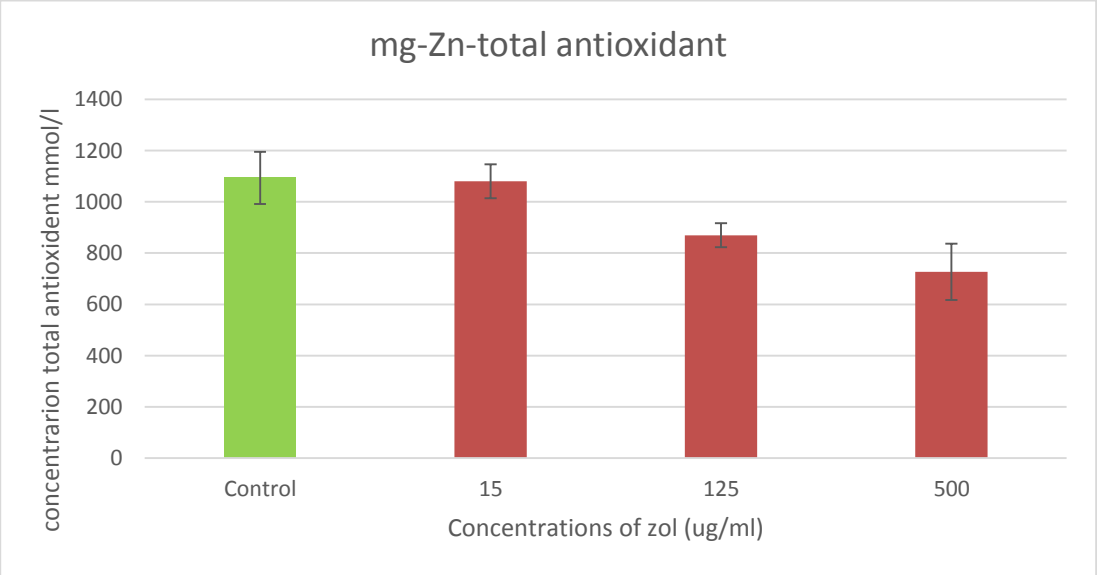


Figure 3.13: Represent Effect antioxidant effect of zoledronic acid in LNCaP cell line.

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability**; there is not a statistically significant difference**
(P = 0.737).

Chapter Four

Discussion

4.1Discussion:

In order to prevent skeletal-related complications in individuals with bone metastases from any solid tumor or bone lesions from multiple myeloma, zol has been clinically and broadly certified for prevent(SREs) (Zekria, Mansour and Karim, 2014), Human malignancies that spread to the bones most frequently are those that occuring in the breast, prostate, multiple myeloma, and kidney (Polascik and Mouraviev, 2008)

Many studies have shown that zol has effect as antitumor, not only treating osteoporosis, whereas they are found zol have action not just against osteoclast cells in bone , but moreover its inhibition proliferation tumor cells, according to Liu *et al.*, which was showed that zoledronic acid, is most potent nitrogen-containing BPs, enhance anti proliferative and apoptotic effects on multiple myeloma cell lines in vitro,(L. Liu *et al.*, 2021). In this study will discusses zoledronic acid activity, as antitumor.

4.2 The effect of zoledronic acid alone on cell viability on osteosarcoma cell line:

The results in (figure 3.1) has demonstrated, zol have antiproliferative effect in all concentrations .These activity of zoledronic acid in decreasing viability percentage on MG-63 cell line may be belong to .

In the study by Bosch-Barrera **,** which has demonstrated zol has an anticancer impact through the suppression of a crucial enzyme called farnesyl diphosphonate (FPP) synthase in the mevalonate pathway leading to suppressed prenylation of signaling GTPases like Ras, Rho, and Rac, which correlated with cell growth and survival, , or accumulated of isopentenyl pyrophosphate (IPP), which may be transformed to ApppI via aminoacyltRNA-synthetases. By ApppI, the mitochondrial adenine nucleotide translocase (ANT) will be suppressed, and apoptosis induction (Bosch-Barrera *et al.*, 2011)

The study is agree with Ouyang *et al*.studies was demonestrated zoledronic acid can been blocking for cancer cell growth through cell cycl arrest in the S phase inducing blocking of OS cell proliferation, and was enhancig of apoptosis, this inhibitory effect for zoledronic acid is mediated at high doses(Ouyang *et al.*, 2018)**.**

Conry, Rodriguez studies were demonstrated zoledronic acid reducing skeletal complication and increase survival through exert direct anti-proliferative effect in osteosarcoma, and apoptosis in vitro, moreover have immune activation and anti-angiogenic activity(Conry, *et al*, 2016)

4.3.The effect of combination zoledronic acid with doxorubicin on cells viability of osteosarcoma cell line:

The results in (figure 3.2) and (figure 3.3)which demonstrated, zol has induced synergestic effect with doxorubicin by augmentation anticancer effect of dox.

The results was confirmed by many studies that are approved, zoledronic acid increase potential cytotoxic effect of many of anticancer agent, when combination with their, according to Ottewell et al studies has been shown that zoledronic acid synergistically increase cancer cell death when combined with a variety of anticancer agents in vitro (Ottewell *et al.*, 2008)**,** such doxorubicin , cisplatin, and etoposide on various types of cancer cells. (Fukai, *et al*, 2014)**.**

Additionally, agree with a study by Zekri, Mansour, that combining zol with other systemic cancer medications may leading increased antitumor and anti-metastatic activity (Zekri, *et al*, 2014)The main mechanism of zoledronic acid involves, suppression of the protein prenylation method, which occurs as a result of a deficiency in the FPP and GGPP enzymes. The function of many proteins, including Ras and Rho, is dependent largely on the prenylation process; these proteins play a crucial role in cellular processes like adhesion, proliferation, differentiation, and carcinogenesis (Haga and Ridley, 2016). On the other hand, it was demonstrated that doxorubicin, which is very cytotoxic for tumors,

gave a high significant decrease, with a $p \leq 0.001$, when used alone at a modest dose of 5 µg/ml.

Its agree with Micallef and Baron, 2020 ; that have been shown doxorubicin is greatest activity in suppressing rapidly dividing cells and delaying the developing for solid and liquid tumors, used mainly in breast cancer, sarcoma and multiple myeloma (Micallef and Baron, 2020) , by intercalation into DNA and inhibition topoisomerase type II (Thorn *et al.*, 2011).

Moreover Goldsby et al ,report that was demonestrated, zol, can be safely added to the backbone of chemotherapy agent which used to treatement metastatic osteosarcoma (Goldsby *et al.*, 2013).

In osteosarcoma , osteoid and immature bone is offspring ,which they have containing focal calcium hydroxyapatite crystals, whereas zoledronic acid is N-BP analog of endogenous, pyrophosphate that potent calcium-containing hydroxyapatite binding of bone mineral make it attractive concentration in primary osteosarcoma (Tawara, Oxford and Jorcyk, 2011) .

According to Wang et al which hypothesized, zol was combind with other anticancer drugs leading to reducing in its dosage with same effects, low toxic effects and adverse effect (Wang *et al.*, 2020) .

Zoledronic acid can be consider good choice as synergistic agent; because having antitumor efficacy; leading to decrease of many anticancer drugs limitation, through decreasing of dosage, immune resistance , and increasing respond rate.

4.4 Effect of zoledronic acid on TNF-α level for osteosarcoma cell line:

The result in (figure 3.4) showed zol have anti-inflammatory effect in higher concentrations by decreasing proinflammatory cytokin;

Many of studies will demonstrated that TNF-α is one of potent proinflamatory cytokines that releasing through osteolysis , whereas its play role in induces osteoclast differentiation directly and independent manner by stimulating NF-κB and JNK (c-Jun N-terminal kinases) in a RANKL- and indirectly by activation the osteoblasts to express RANKL (Maurizi and Rucci, 2018), then next to the RANK on the surface of osteoclast precursor cells was activation . Macrophage colony-stimulating factor and receptor activator of NF-κB ligand have critical role in osteoclastogenesis(Akiyama, *et al* , 2008)**.** Whereas, zol suppress bone destruction mediated by inhibition of differentiation and function of osteoclasts through breaking RANKL/RANK pathway. reduce the expression of RANK , leading to inhibition TNF-α and RANKL(Wang *et al.*, 2020)**.** its agreement with this study**.**

Also agree with Kimachi *et al* which suggested zoledronic acid inhibited the mevalonat pathway for several types of cells, such monocytes/macrophages, osteoblasts, and various cancer cells which they are responsible for production of TNF-α, through inflammatory tissue destruction, bone resorption, and this in final leading to inhibition of TNF-α (Kimachi *et al.*, 2011).

4.5 Effect of zoledronic acid on IL-6 level on Osteosarcoma cell line:

The results showed in(figure3.5) zol have anti-inflammatory activity at high concentrations by decreasing IL-6.

Many reports confirmed, IL-6 is potent osteoclastogenic factor (Maurizi and Rucci, 2018) through causing upregulation and modulating for cancer induce bone destruction(Tawara, *et al*, 2011)**.** According to Hou *et al* study have been shown cancer cells growth and survival, and different cells promoted by IL-6 (Hou *et al.*, 2014), IL-6 through activation of Janus kinase (Jak)- signal transducer and activators of transcription (STAT) factor 3 mitogen-activated protein (MAP) kinase and phosphoinositol 3-kinase (PI3-K)-Akt signalling pathways, has induction for cell proliferation, apoptosis and angiogenesis(Malinowska *et al.*, 2009)**.**

Korkmaz, suggest of zoledronic acid on osteoclast cells causing inhibition of osteoclast maturation, blocking of osteoclast induction to the site of bone resorption ,inhibition of mature osteoclast function, decreasing of IL–6 cytokine releasing(Korkmaz, 2018)**.**

In this study, hypothesis, zoledronic acid at high concentration have anti -inflammatory effect by decreasing IL-6, TNF-α concentration,

4.6 effect of zoledronic acid in serial concentration as antioxidant in osteosarcoma cell line:

In(figure 3.6) the result showed zol contributed in release ROS in higher concentration by decreasing antioxidant concentration .

This result may be belong to zol induce oxidative stress when induction apoptosis through inhibition for mevalonat pathway of tumor cells, in Tai *et al.*,study have been found zoledronic acid induce apoptosis acts in a manner similar to chemotherapy agents. It triggers ROS generation followed by ROS-mediated cell apoptosis in osteoclast precursors and mature osteoclast-like cells(Tai *et al.*, 2017) . In another study Ge *et al.* that he was found zol can enhance apoptotic cell death alone and inhibit colony formation associated by an increase of ROS. suggestion that ROS are involved in apoptosis and inhibition of colony formation in zol treated SACC-83 cells(Ge *et al.*, 2014)

Zoledronic acid have potential effect on osteoclast cell rather than another cell by inhibiton for its mevalonat pathway and it was exert antiproliferation action and induce apoptosis specially in high dose , zol have different mechanism to exert antitumor effect but we cannot which mechanism of action zol in this line.

4.7 Effect of zoledronic acid alone on viability percentage of prostate cancer LNCaP cell line:

In (figure 3.7) was showed which zol have antiproliferative effect in high concentration in LNCaP prostate cancer.

These result may be belong to, zoledronic acid have antiproliferation effect through was blocked protein isoprenylation , that represent a key step in many survival and proliferation pathways , it acting on both farnesyl diphosphonate synthase, but in case geranyl- geranylpyrophosphate synthase in lesser degree and causing inhibition. Both their were consider a vital enzymes in the mevalonate pathway(Steinman, *et al* , 2012)**,** in fact, zol have high activity in bone disease and malignant tumor metastasis to bone to reducing skeletal related event and improve survival ; because zol has high affinity for bone its pyrophosphate analog .

The study agreement with Bosch-Barrera *et al;* studies which found direct cytotoxic impact of zol is that generally a high concentration of zol is needed for enhancing apoptosis when utilized alone or in mixing togaether with chemotherapy (Bosch-Barrera *et al.*, 2011)

In prostate cancer a metastasis to bone has high potential. (Fan, 2007) 80% of men with prostate cancer have been with advanced develop bone metastases and there are often correlated with skeletal complications(Ullén *et al.*, 2005)**.** Metastasis to bone as result from activation of osteoclasts by RANKL, which it is secrete by cancer cells, play role in mechanism of bone metastases and bone destruction(Bi *et al.*, 2017a)**,** According to preclinical studies have been demonstrate zoledronic acid exerted direct anti-tumor effects, activate cytotoxic T-cells, that leading to decreasing metastatic potential of prostate cancer cells (Finianos and Aragon-Ching, 2019)**.** Another study demonstrate that zoledronic acid has

been show as anti angiogenic effect through inhibition macrophage MMP-9 expression in models of prostate , MMP-9 is a matrix metalloproteinase secreted as result as response for energizing tumour- derived factors, from macrophage, that it is involved in angiogenesis and tumour cell invasion(Rogers and Holen, 2011)

4.8 Effect of doxorubicin alone on cell viability of LNCaP:

The results that showed in (figure 3.8) doxorubicin have potent antiproliferative effect may be belong.

doxorubicin an anthracyline drug used in the treatment of many types of cancers, through it acting in the cancer cell, by intercalation DNA, inhibits topoisomerase typeII and is accompanied byG2/M phase cell cycle arrest(Kazantseva, *et al*, 2022). also this result may be belong , doxorubicin has potential toxicity action on DNA of tumor cell, many studies have been demonstrate great activity in resiste quickly dividing cells and delaying the progression for solid and liquid tumors used mainly in breast cancer, sarcoma and multiple myeloma (Tacar, *et al*, 2012)

According to Kazantseva,; Doxorubicin is using alone mainly or in combination with methotrexate and cisplatin in high-dose for osteosarcoma treatment, In spite of cardiotoxicity, that is associated with dox in cumulative dosing and can lead to congestive heart failure later in life(Kazantseva, *et al*, 2022)**.**

4.9 The effect of combination zoledronic acid with doxorubicin on cells viability of LNCaP cell line:

The results in figures (3.9) (3.10) showed zol have synergestic effect by increasing antiproliferative effect of doxorubicin when combination togather comparison with control and positive control group may be belong to many studied that had improved cytotoxicity of both drugs.

This results are agree with several studies have been shown zoledronic acid increase antitumor activity when combination with chemotherapy drugs, Ottewell *et al* studies has been shown that zoledronic acid synergistically increase cancer cell death when combined with a variety of anticancer agents in vitro. (Ottewell *et al.*, 2008)**.** Also zoledronic acid had showing synergetic effect when combination with anticancer agents such doxorubicin, cisplatin, and etoposide on various types of cancer cells. (Fukai, K, *et al.* 2014)**,** Another study combining zol with other systemic cancer therapies may give additional antitumor and anti-metastatic activity(Zekri, Mansour and Karim, 2014)**;**

In previose study ,combination of zol and cisplatin have abiliyy to suppress osteoclast division , survival, and activation, leading to stopping the "vicious circle" to suppress tumor growth and osteolysis destruction(L. Liu *et al.*, 2021)

In many studies that approveded, zoledronic acid, is a potent of current bisphosphonates, therefor its using in controlling bone

metastasis in various solid tumors in vitro and vivo because its high bioavailability in bone.

The function of several proteins, such as (Ras, Rho), is dependent primarily on prenylation method. These proteins are involved in important cellular processes such as proliferation, division, adhesion, and carcinogenesis(Haga and Ridley, 2016) One basic mechanism of that causes this inhibition of protein prenylation process is the absence of FPP and GGPP enzymes mediated by zol(Clyburn *et al.*, 2010). in fact doxorubicin is potent cytotoxic actionthrough irreversible disrupt of tumor cell DNA. In basic mechanism which involving intercalation DNA, causing suppress of micromolecule synthesis, (Mobaraki *et al.*, 2017)The potential benefit of combination therapy for reduce toxicity when used alone and using lower dose especially with doxorubicin that consider cardio toxicity (Thorn *et al.*, 2011) .

doxorubicin have limitation specially when used in cumulative dosage, its consider cardiotoxicity, if using for long period in high dose, therefor , using in combination given a better response, because they have reducing it dosage by same effect.

4.10 Effect zoledronic acid on level of IL-6. in LNCaP cell line:

The results in (figure 3.11) have been showed zoledronic have anti-inflammatory effect by decreasing IL-6 depending on dose .

According to the result ,which it agree with many of studies, which they are confirm, zoledronic acid have potent effect on osteoclast cells by inhibition of osteoclast maturation, blocking of osteoclast induction to the site of bone resorption ,inhibition of mature osteoclast function ,decreasing of IL–6 cytokine releasing(Korkmaz, 2018). According to **Hou *et al*** study have been shown cancer cells growth and survival of different cells promoted by IL-6.(Hou *et al.*, 2014), also according to Maurizi and Rucci, report that were demonstrated, pro -inflammatory cytokines play a critical role in the boost of osteoclast differentiation such IL-6 is consider potent osteoclastogenic factor (Maurizi and Rucci, 2018). There has lately been an increase in interest in evaluating serum IL-6 as a particular predictive factor for prostate cancer and breast cancer, due to potential in upregulating and modulating cancer-mediated bone destruction(Tawara, *et al.*, 2011). In this study have been showed, zoledronic acid induce reduction in IL-6 concentration in higher concentration.

4.11 Effect zoledronic acid on level of TNF-α. in LNCaP cell line:

In(figure 3.12) The result showed zol have anti-inflammatory effect by decreasing TNF-α dependent on dose and cell species .

As according to many studies which have been showed, TNF-α is one of factors that releasing through osteolysis , whereas play role in induces osteoclast differentiation directly and independent manner by stimulating NF-κB and JNK (c-Jun N-terminal kinases)

in a RANKL (Maurizi and Rucci, 2018)According to Wang et al study's zoledronic acid has several potential mechanisms for preventing osteoclast differentiation in vitro, particularly blocking the RANKL/RANK pathway, which is a receptor stimulator of nuclear factor κB (Wang *et al.*, 2020) indirectly causing the levels of TNF-α to decline.

Zol could be lowering TNF-α concentration at high concentration above than 250µg/ml, it's may be enough to destruction macrophage cell within tumor cell, which responsible for cytokines production.

In this study have been showed zoledronic acid at high concentration followed as anti-inflamatory effect by decreasing for IL-6, TNF-α concentration.

3.12 Effect zoledronic acid in serial concentration as antioxidant in LNCaP cell line:

In figure 3.13 showed zoledronic acid have no effect on antioxidant concentration .

The result disagree with, reported by Karabulut et al. which finding, zol require the use with other antioxidant supplements. If zoledronic acid was discovered to enhance rabbit liver oxidative stress and decrease antioxidant peaks in liver tissue, this would be a problem (Karabulut *et al.*, 2010) .Zoledronic acid induces osteoclast

apoptosis by inhibiting the mevalonat pathway when cell death is caused by reactive oxygen species (ROS) (Tai *et al.*, 2017).

Also disagree with study by Wang et al**.,**found elevated ROS levels when zoledronic acid was used to treat prostate cancer and salivary adenoid cystic carcinoma cell models. This supported the theory that zoledronic acid generates free radicals as a result of its ability to induce apoptosis(Wang *et al.*, 2020),

may be belonged to zoledronic acid appear variable effect on anti-oxidant level depending on species of cell line .or may be belong to environment correlated by concentration or exposure duration.

The effecctivity of zoledronic acid to enhance apoptosis change with the experimental conditions. the specific cell line utilized , the zol concentration as well as the period of zol exposure, all influenced on the ability of zol to enhanced tumour cell apoptosis (Bosch-Barrera *et al.*, 2011).

Zoledronic acid have different pathway to suppress tumor cells and many research warrented this activity, but cannot know which mechanism was followedin this study

Conclusions and Recommendations

Conclusion

1. The antiproliferative effect of zoledronic acid on MG-63 osteosarcoma cell line is more effective than on LNCaP prostate cancer cell line.

2. Zoledronic acid show anti-inflammatory effect at higher concentrations by decreasing cytokines IL-6,TNF-α in both cell lines.
3. Zoledronic acid antioxidant effect show dose and cell type dependent when evaluated using total antioxidantt capacity.

Recommendations

1. study effect zoledronic acid as antiproliferation and induce apoptosis in normal cells.
2. Study the antitumor activity of combination of zoledronic acid with other anticancer agents.
3. Study the sequential treatment with zoledronic acid before and after the treatment with chemotherapeutic agents.
4. Study the effect of zoledronic acid in vivo (on animal model) alone or in combination with other anti-inflammatory or anticancer agent.

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الخلاصة:

يعد السرطان أحد الأسباب الرئيسية للوفاة في جميع أنحاء العالم . تعتبر أورام العظام الخبيثة من بين الأمراض التي ترتبط في أغلب الأحيان بمعدل الوفيات المرتفع .يمكن أن تكون أورام عظام أولية مثل الساركوما العظمية اوثانوية نتيجة لانتشار الورم الى العظم عندما يكون الورم في مراحل متقدم مثل سرطان الثدي والبروستات وغيرها الي تكون غالبا مقترنه بالانتشار الى العظم.
البسفوسفنات التي تحتوي على النايتروجين في تركيبها مثل زولدرونيك اسيد تعتبر مركب فعال جدا ضد ارتشاف العظم بسبب الفعالية العالية في قمع نشاط خلايا ناقضة العظم ، تستخدم بشكل رئيسيفي هشاشة العظام كخيار أساسي ، بالإضافة إلى وجود نشاط مباشر ضد الاورام السرطانية، بعيدا عن تأثيراته المستقلة على خلايا ناقضات العظم.

تم في هذه الدراسة استخدام نوعين من الخلايا هما خلايا الساركوماو MG-63 وخلايا سرطان البروستاتLNCaP
ثم تعريض الخلايا لتراكيز متسلسلة من حمض الزوليدرونيك500 (،250،125،62،31،15 ميكروغرام / مل)وتراكيز متسلسلة للدوكسوروبيسين كدواء سام للخلايا 100 (، 50،25،12.30،6.25، 3ميكروغرام / مل)، كل على حدة أو معًا ، وكانت فترة الحضانة 24 ساعة

تضمنت هذه الدراسة ثلاثة اجزاء:
في الجزء الاول تم اجراء فحوصات السمية الخلوية لتقييم التأثير المضاد لنمو الخلايا لحمض الزوليدرونيك والدوكسوروبيسين ، كل على حدة او بعد مزجهما معًا ، ثم قياس حيوية الخلايا في كلا خطي الخلايا عن طريق اختبارالحيوية الخلوية .في الجزء الثاني من الدراسة تم تقييم التأثير المضاد للالتهابات لحمض الزوليدرونيك عن طريق قياس تراكيز انترلوكين 6 -وعامل نخر الورم-الفا بتقنية الالايزا في كلا خطي الخلايا .اما في الجزء الاخير فتم تقييم التأثير المضاد للأكسدة لحمض الزوليدرونيك بتراكيز مختلفة عن طريق قياس قدرة مضادات الأكسدة الكلية في كلا خطي الخلايا.

قبل اجراء التعريضن تم زراعة الخلايا لكلا النوعين في صفائح الزرع الخلوي ذات 96 حفرة وحضنت لمدة 24 ساعة .بعد ذلك تم تعريض الخلايا لتخافيف متسلسلة من مادة الزلدرونك اسد (500، 250 ،
125، 62.5 ، 31.25 ، 15.5 ميكروغرام / مل .)وعرضت صفائح اخرى لتخافيف متسلسلة من مادة الدوكسوروبسين 100,50,25,12.5,6.25,3 (ميكروغرام / مل)ومن ثم حضانت الصفائح مرة اخرى لمدة 24 ساعة .بعد نهاية فترة الحضانة ، تم إجراء MTT لتقيم السمية الخلوية لكل دواء.

لتقييم التأثير المشترك لكلا الدوائين ، تم معالجة الخلايا بحمض الزوليدرونيك بنفس التخفيفات التسلسلية المذكورة أعلاه بوجود تركيز ثابت من دوكسوروبيسين 5 (ميكروغرام / مل .)وتم استخدام مجموعة من الخلايا غير المعالجة كسيطرة سالبة واخرى معالجة بالدوكسوروبيسين 5 (ميكروغرام/ مل)كمجموعة تحكم إيجابية ويتم قياس السمية الخلوية باستخدام. MTT

لتقييم التأثير المضاد للالتهاب والفعالية الكلية المضادة للأكسدة لحمض الزوليدرونيك ، تم معاملة خلايا MG-63 و LNCaP بتراكيز متسلسلة لحمض الزوليدرونيك وحضانت لمدة 24 ساعة ومن ثم تم سحب الرائق وتخزينها لقياس الاستجابة المناعية بواسطة طريقة الالايزا بقياس الانترلوكين6 - وعامل نخر العظم- الفا ولقياس الفعالية الكلية المضادة للاكسدة باستخدام طريقةCUPRAC
أظهرت النتائج انخفاضًا كبيرًا في عدد الخلايا عند التركيز العالي لحمض الزوليدرونيك عند استخدامه منفردا في خط خلايا البروستات بينما اظهرت خلايا الساركوما انخفاضا معنويا عند جميع التراكيز المستخدمة .ولكن عند المعاملة الخلايا بمزيج حمض الزوليدرونيك + دوكسوروبيسين ، اظهرت النتائج تآزر ساما كبيرا على الخلايا في جميع التراكيز المستخدمة في كلا الخطين الخلويين.
أظهرت نتائج الالايزا أن حمض الزوليدرونيك بتركيز عال يسبب انخفاضا معنويًا في تراكيز،انترلوكين-6 و عامل نخر الورم-الفا في كلا خطي الخلايا .بينما اظهرت نتائج اختبار الاكسدة ان زولدرونيك اسيد عند التركيز العالي يسبب انخفاضا معنويا في تركيز مضادات الاكسدة في خلايا الساركوما, اما خلايا سرطان البروستات فلم تظهر اي تاثر.

يستنتج من هذه الدراسة ان الزوليدرونك اسيد باتراكيز عالية يظهر تاثير مضاد لنمو الخلايا اعتمادا

على الجرعة و نوع الخلية . كذلك فان حمض زولدرونيك اظهرتـأثيرا تآزريـا مـع الدوكسوروبيسين ضد
كلا خطي الخلايا ،ووجد أيضًا أن حمض الزوليدرونيك لـه تـأثير مضـاد للالتهـاب بتركيـز عـال عن طريق تقليل السايتوكينات المسببة للالتهاب(انترلوكين 6 -وعامل نخر
الورم-الفا

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية الطب

تاثير حامض الزولدرونك لوحده وبعد مزجه مع الدوكسروبيسين في خطي خلايا سرطان العظام وسرطان البروستات

رسالة

مقدمة الى مجلس كلية الطب / جامعة بابل

كجزء من متطلبات نيل شهادة الماجستير في علوم الادوية والسموم

من قبل

الطالبة / **منار عمران علي**

بكالوريوس صيدلة (٢٠٠٧-٢٠٠٨)

اشراف

أ.م .ماجد كاظم عباس

ماجستير ادوية

أ.م.د قيصر نعمة مظلوم

دكتوراه علم الخلية

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