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Evaluation of the physiological alterations and cardiotoxicity induced by doxorubicin chemotherapy in women cancer patients in El-Oued region and Study of the protective effect of *Ocimum basilicum* L. and MgNPs from these changes on Wistar rats

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إهداء

تأمل قوله تعالى (فنادى في الظلمات) . . حتى في بطن الحوت كان هناك أمل . .

الأمل هو تلك النقطة البيضاء التي لا ترى بالعين المجردة . . النقطة البيضاء التي يجب على وهجها

أن لا يختفي من حياتنا

الأمل هو أول خطوات النجاح . . فالنجاح ليس قفزة إنما خطوة تليها خطوة . . نحو قمة لا يرتقي سلمها

إلا أصحاب الهمم العالية لأن همتهم تقودهم إلى المواصلة وإن تعثرت خطاهم

واليوم بعد مسيرة مليئة بالأمل دامت سبعة عشر عام . . أهدي نجاحي وتخرجي وفرحتي...

إلى من علمتني أن الحب ليس له عمر وأن العطاء ليس له حدود . . أمي الغالية

إلى الشمعة التي احترقت لتنير لي طريق حياتي . . أبي الغالي

إلى إخوتي عزوتي . . خالد ومحمد الأمين وسيف الدين ومحمد الهادي وصبري

إلى أخواتي زينة حياتي . . رميصاء وهديل وجمعة

إلى من أنار لي دروب العلم والمعرفة . . قدوتي وأبي الثاني . . درويش سمير

إلى كل صديق كان قريب من قلبي وكل روح شاركتني بدعائها

إسلام

إهداء

«كن عالما، فإن لم تستطع فكن متعلما، فإن لم تستطع فأحب العلم والعلماء، فإن لم تستطع فلا تبغضهم. . .»

مسيرتي بدأت بخطوة، وها أنا اليوم أقطف ثمار مسيرة أعوام كان هدفي فيها واضحا، وكنت أسعى في كل يوم لتحقيقه والوصول إليه مهما كان صعبا .

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Abstract

This investigation was aimed to determine the side effects of doxorubicin in cancer patients and study the effect of leaves aqueous extract of *Ocimum basilicum* L. and magnesium oxide nanoparticles for prevention of cardiotoxicity and heart failure induced by doxorubicin in rats. For patient study, 22 women with cancer and control were chosen, blood samples were collected from them and some biochemical, enzymatic and hematological markers were measured. For in-vivo rats study, twenty five female albino Wistar rats, weighing between 184.84 ± 8.48, were divided into 5 groups (n = 5); control group, doxorubicin treated rats group (Doxo), rats co-treated with doxorubicin and aqueous extract of *O. basilicum* group (Doxo + *O. basilicum*), rats co-treated with doxorubicin and magnesium nanoparticles group (Doxo + MgNPs) and rats co-treated with doxorubicin, aqueous extract of *O. basilicum* and magnesium nanoparticles group (Doxo + *O. basilicum* + MgNPs). Doxorubicin induced cardiotoxicity using intraperitoneally single dose 1.5 ml/kg/week. *O. basilicum* treated orally at a dose 200 mg/kg/day and MgNPs was supplemented intraperitoneally at one dose/week, 50 mg/kg for 4 weeks. Various hematological, biochemical, electrolyte, enzymatic, and oxidative stress markers were estimated. Histopathology of heart tissues was observed. Results of patients study show that doxorubicin induced change in biochemical and enzymatic markers of heart function in women cancer patients compared to women control. Results of in vitro phytochemical and HPL test results revealed that *O. basilicum* contains most of active compounds, especially flavonoids of various kinds with high antioxidant activity and without toxicity in rats. But, the MgNPs has a toxicity in rate up a dose (500mg/kg). Results of in-vivo rats study show that doxorubicin treated rats induced elevation in doxorubicin level in heart tissue, alteration in hematological and biochemical parameters compared to control group. In addition, doxorubicin treated rats induced oxidative stress and histological alteration in heart cells compared to control rats. Co-treatment of doxorubicin with leaves aqueous extract of *O. basilicum* and/or MgNPs were partially reversed all of previous parameters. This study indicated that the anti-inflammatory and antioxidant property of leaves aqueous extract of *O. basilicum* and MgNPs allowed to use them to protect organs from the side effects of drugs or from the destructive effects of various diseases. Other studies are necessary to delve into these findings and to determine the factors responsible for the beneficial effects.

Keywords: *Ocimum basilicum* L., MgNPs, cardiotoxicity, doxorubicin, oxidative stress, Wistar rats.

الملخص

يهدف هذا البحث إلى تحديد الآثار الجانبية لاستخدام دواء الدوكسوروبيسين عند مرضى السرطان ودراسة تأثير المستخلص المائي لأوراق نبات الحبق وجزيئات النانو مغنيزيوم للوقاية من التسمم القلبي وفشل القلب الناجم عن استخدام دواء الدوكسوروبيسين عند الفئران. في دراسة المرضى، تم اختيار 22 امرأة مصابة بالسرطان، وتم جمع عينات الدم منها وقياس بعض المؤشرات البيوكيميائية والإنزيمية والدموية. ولدراسة الفئران في الجسم الحي، تم تقسيم خمس وعشرون أنثى من الفئران من نوع ألبينو ويستار، يتراوح وزنها بين 184.84 ± 8.48، إلى 5 مجموعات (العدد = 5) ؛ المجموعة الشاهدة ، مجموعة الفئران المعالجة بدواء الدوكسوروبيسين (Doxo)، مجموعة الفئران المعالجة بدواء الدوكسوروبيسين والمستخلص المائي لنبات الحبق (Doxo + *O. basilicum*) ، مجموعة الفئران المعالجة بدواء الدوكسوروبيسين و جزيئات النانو مغنيزيوم (Doxo + MgNPs) و مجموعة الفئران التي تمت معالجتها بدواء الدوكسوروبيسين والمستخلص المائي لنبات الحبق و جزيئات النانو مغنيزيوم (Doxo + *O. basilicum* + MgNPs). التسمم القلبي الناجم عن دواء الدوكسوروبيسين باستخدام جرعة واحدة 1.5 مل / كغ في الأسبوع. يستعمل الحبق عن طريق الفم بجرعة 200 ملغ / كغ يوميا ويتم استعمال جزيئات النانو مغنيزيوم بجرعة 50 ملغ / كغ مرة وحدة أسبوعيا، لمدة 4 أسابيع. تم تقدير العديد من العلامات الدموية والبيوكيميائية والأنزيمات والشوارد والإجهاد التأكسدي. مع الملاحظة الضوئية لأنسجة القلب. أظهرت نتائج دراسة المرضى أن دواء الدوكسوروبيسين يسبب تغيرًا في المؤشرات البيوكيميائية والإنزيمية لوظائف القلب لدى مرضى السرطان من النساء مقارنة بالمجموعة الشاهدة. أوضحت نتائج اختبار المواد الكيميائية النباتية المختبرية ونتائج اختبارHPL أن نبات الحبق يحتوي على معظم المركبات النشطة، وخاصة مركبات الفلافونويد من أنواع مختلفة ذات نشاط مضاد للأكسدة مرتفع وبدون سمية في الفئران. ولكن جزيئات النانو مغنيزيوم لديها سمية في معدل يصل إلى جرعة (500 مغ / كغ). أظهرت نتائج دراسة الفئران، أن الفئران المعالجة بدواء الدوكوروبيسين تعرضت إلى ارتفاع مستوى الدوكسوروبيسين في أنسجة القلب، وتغيير في المؤشرات الدموية والبيوكيميائية مقارنة بالمجموعة الشاهدة. بالإضافة إلى ذلك، تعرضت الفئران المعالجة بالدوكسوروبيسين إلى الإجهاد التأكسدي والتغيير النسيجي في خلايا القلب مقارنة بالفئران الشاهدة. العلاج المشترك للدوكسوروبيسين بأوراق المستخلص المائي لنبات الحبق و / أو جزيئات النانو مغنيزيوم يعكس جزئيًا جميع المؤشرات السابقة. أشارت هذه الدراسة إلى أن الخاصية المضادة للالتهابات والمضادة للأكسدة لنبات الحبق وجزيئات النانو مغنيزيوم سمحت باستخدامهم لحماية الأعضاء من الآثار الجانبية للأدوية أو من الآثار المدمرة للأمراض المختلفة. دراسات أخرى ضرورية للخوض في هذه النتائج وتحديد العوامل المسؤولة عن الآثار المفيدة.

الكلمات الرئيسية: نبات الحبق، جزيئات النانو مغنيزيوم، التسمم القلبي، دوكسوروبيسين، الإجهاد التأكسدي، فئرانWistar .

Figures list

Tables list

Abbreviation list

1XPBS :	Phosphate-Buffered Saline
AI :	Atherogenic index
$AlCl_3$:	Aluminum chloride
ALT :	Alanine aminotransferase
AST :	Aspartate aminotransferase
BHT :	Butylated Hydroxy Toluene
BSA :	Bovine Serum Albumin
Ca^{+2}:	Calcium
CAT :	Catalase
CCL2/MCP1 :	Chemokine ligand 2/ monocyte chemoattractant protein 1
CDNB :	l – chloro 2,4 –dinitrobenzene
CHD :	Coronary heart disease
Cl^- :	Chloride
COX-2 :	Cyclooxygenase 2
CPK :	Creatine phosphokinase
CVD :	Cardiovascular disease
DNA :	Deoxyribonucleic acid
Doxo :	Doxorubicin
DPB :	Diastolic blood pressures
DPPH :	1,1-diphenyl-2-picryhydrazyl
DTNB :	-5'-dithiobis2-nitrobenzoic acid
EDTA :	Ethylenediaminetetraacetate
FCR :	Folin-Ciocalteu Reagent
$FeCl_3$:	Ferric chloride
FNS :	Numeration of the Blood Formula
FTIR :	Fourier Transform Infrared spectroscopy
GAE :	Gallic acid equivalents
GGT :	Gamma-glutamyltranspeptidase
GOT :	Glutamic-oxaloacetic transaminase
GPT :	Glutamate pyruvate transaminase
GPx :	Glutathione peroxidase
GRx :	Glutathione reductase
GSH :	Reduced Glutathion

GST :	Glutathione-S-transferase
H_2O_2 :	Hydrogen peroxide
H_2SO_4 :	Sulfuric acid
H_3PO_4 :	Phosphoric Acid
HCl :	Chloride hydrogen
HDL :	High-density lipoproteins
HGB	Haemoglobin
HPLC :	High performance liquid chromatography
HTC :	Haematocrit
IFN-γ :	Interferon gamma
IL-1 :	Interleukins 1
IL-10 :	Interleukins 10
IL-1β :	Interleukins 1 β
IL-2 :	Interleukins 2
IL-4 :	Interleukins 4
Il-6 :	Interleukins 6
IL-8 :	Interleukins 8
IR :	Infrared spectroscopy
K^+ :	Potassium
LC-Q-TOF-MS:	Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry
LDH :	Lactate dehydrogenase
LDL :	Low density lipoprotein
MDA :	Malondialdehyde
Mg :	Magnesium
MgNPs :	Magnesium nanoparticles
MgO :	Magnesium oxide
Na^+ :	Sodium
Na_2CO_3 :	Sodium carbonate
NaCl :	Sodium Chloride
NADPH :	Nicotinamide adenine dinucleotide phosphate
NaOH :	Sodium hydroxide
NBT :	Nitro blue Tetrazolium Chloride
NF-κB :	Nuclear factor-kappa B

NH_2 :	Amino groups
NK :	Natural killer cells
NO :	Nitric oxide
$O^{2\bullet-}$:	Superoxide
OH :	Hydroxyl free radical
$ONOO^-$:	Peroxynitrite
RA :	Rosmarinic acid
RBC :	Red blood cell
RNA :	Ribonucleic acid
ROS :	Reactive oxygen species
SBP :	Systolic blood pressures
SDS :	sodium dodecyl sulfate
SEM :	Scanning electron microscopy
SOD :	Superoxide dismutase
TBA :	Thiobarbituric Acid
TBS :	Tert-Butyldimethyl Silyl Ethers
TCA :	Trichloroacetic Acid
TG :	Triglyceride
TGF β :	Transforming growth factor- β
TNF-α :	Tumor necrosis factor
TOFMS :	Time-of-flight mass spectrometry
UHPLC :	Ultra-Performance Liquid Chromatography
UV-Vis :	Ultraviolet–visible
VLDL :	Very Low Density Lipoprotein
WBC :	White blood cell

Summary

INTRODUCTION

Introduction

Cancer is a group of diseases causing change in body cells and proliferate beyond of control (Erica et al., 2018). Cancer is a major public health problem worldwide (Siegel et al., 2020), and the second leading cause of death after cardiovascular diseases (Lodi et al., 2019). There are many cancer treatments, the most important of which are chemotherapy that considered the most effective and extensively used modality in most types of cancers (Abbas & Rehman, 2018).

Chemotherapy literally means the use of chemicals in order to inhibit malignant cell or to the infectious agents of a disease such as microorganism without much affecting the host cells. However, chemotherapy always followed by side effects, causing treatment failure such as Doxo chemotherapy (Lei et al., 2019). Among the available cancer drugs, doxorubicin (Adriamycin) is considered as one of the most effective (Wakharde et al., 2018).

Doxorubicin is an anthracycline glycoside antitumor antibiotic used as a first-line drug in combination with other chemotherapy drugs for various types of cancers (Choi et al., 2020). Unfortunately, Doxo also can induce toxic and side effects in many organs, compromising its usage and efficacy (Lei et al., 2019). Cardiotoxicity is the major side effect of this drug and limits its use (Baniahmad et al., 2020), leading to a significant damage of the heart with consequent cardiac failure in treated patients (Adamcova et al., 2019).

The traditional use of plants for the treatment of diseases by the public and the confirmation of their biological effect have stimulated their therapeutic use and their active ingredients may relieve symptoms and even cure diseases. Nevertheless, the advance of research about the components in such plants demonstrates that the use of these plants is beyond its folk medicine use (Ferreira et al., 2014). Among the medicinal plants which locally known as sweet basil (*Ocimum basilicum* L.). The genus *Ocimum* has a number of species that are used to treat different types of disorders and diseases from ancient times. In this genus, *O. basilicum* plays a vital role due to its various medicinal properties (Purushothaman et al., 2018).

Nanotechnology is an area of emerging interest in the field of science and technology due to its wide variety of applications in the field of biomedicine, optics, and electronics (Mubarak et al., 2019). Nowadays, nanotechnology involving green synthesis of nanoparticles has become an eye-catching idea (Chartarrayawadee et al., 2020). Recently, plant-mediated route or green approach for preparing metal and metal oxide nanoparticles has received enormous attention due to the ease of preparation and environmental friendliness when compared to physical and chemical methods (Enobong et al., 2019). Biosynthesis of nanoparticles using plant extracts is gaining importance in biomedical applications because of their unique properties (Poka et al., 2019).

The objective of the present study was undertaken to assess the level of some biochemical and hematological markers and their relation to cardiotoxicity in women cancer patients treated by doxorubicin. Also, to assess the toxic effects of doxorubicin and to study the protective effects of therapeutic systems based on phytotherapy with Ocimum basilicum L. and on nanotherapy with biosynthesized magnesium oxide nanoparticles based on Ocimum basilicum L. in female Wistar albino rats.

In light of these data, the present study was carried out to investigate the three following complementary aspects:

The first part: based on the evaluation of the physiological alterations and cardiotoxicity induced by doxorubicin as chemotherapy in women cancer patients.

The second part: based on the in-vitro study; extraction of plant extract, preparation of nanoparticles of magnesium, quantitative and qualitative characterization of these compounds and evaluation of their biological activity.

The third part: based on the in-vivo study for evaluation of the protective effect of *Ocimum basilicum* L. leaves extract and MgNPs biosynthesized by plant extract against cardiotoxicity induced by doxorubicin in rat.

Bibliographic Part

Chapter I

Cardiovascular System

I.1. Cardiovascular system

Cardiovascular system is primarily considered as the human body's transport system. Oxygen, carbon dioxide, nutrients and other vital substances to the various tissues of human body are carried by the blood which circulates in a closed circulation. The cardiovascular system has been comprised of a combination of several basic compartments, which are structurally connected to and functionally interact with each other (Lefta Mossa, 2008). The circulation of the heart and lungs (central circulation) and the rest of the body (peripheral circulation) form a single closed system with two components: an arterial system and a venous system (Figure 01). The arterial system carries blood from the heart and the venous system returns blood to the heart (Melillo, 2011). The cardiovascular system keeps life pumping through the body. Understanding the functions of the cardiovascular system, along with its various pathways of veins, arteries and capillaries, is essential in the provision of safe and effective care (Peate, 2018). The system consists primarily of the heart, which serves as the pump, the blood, which serves as the conducting medium, and the vasculature, which serves as the conduit through which the blood flows (Tringelova et al., 2007).

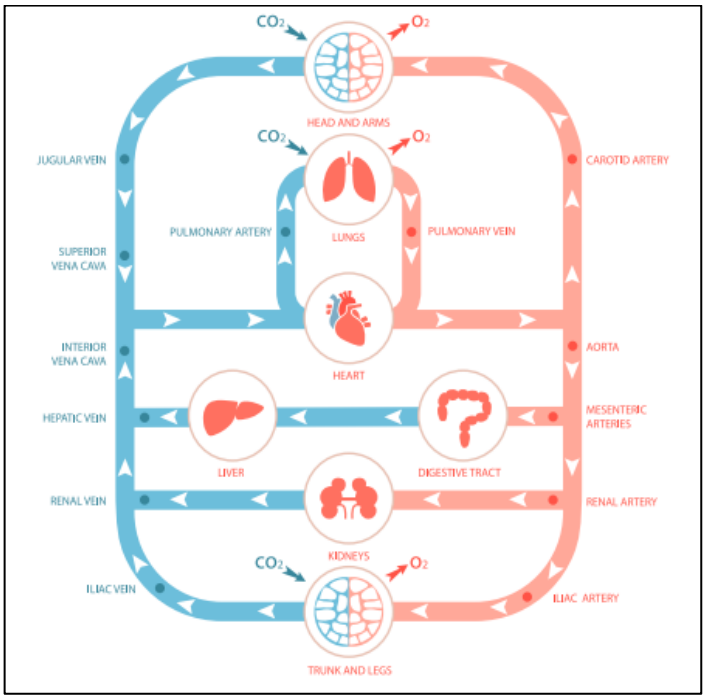


Figure 01: Cardiovascular system (Peate, 2018)

I.1.1. Heart

I.1.1.1. Definition and situation

The heart is a thick, muscular, rhythmically contracting organ of the vascular system (Mesotten, et al., 1998). With every heartbeat, the heart pumps blood that carries oxygen and nutrients to all parts of the body (Yousef et al., 2013). The muscular heart consists of two atria and two ventricles. The atria are upper receiving chambers for returning venous blood. The ventricles comprise most of the heart's volume, lie below the atria, and pump blood from the heart into the lungs and arteries (Shaffer et al., 2014). The heart is a hollow located in the chest between the lungs behind the sternum and above the diaphragm. It is surrounded by the pericardium (Hussein, 2017). The heart is usually positioned within the mediastinum with one-third of its mass to the right of the midline, and with its own long axis directed from the right shoulder towards the left hypochondrium (Anderson et al., 2004) (Figure 02).

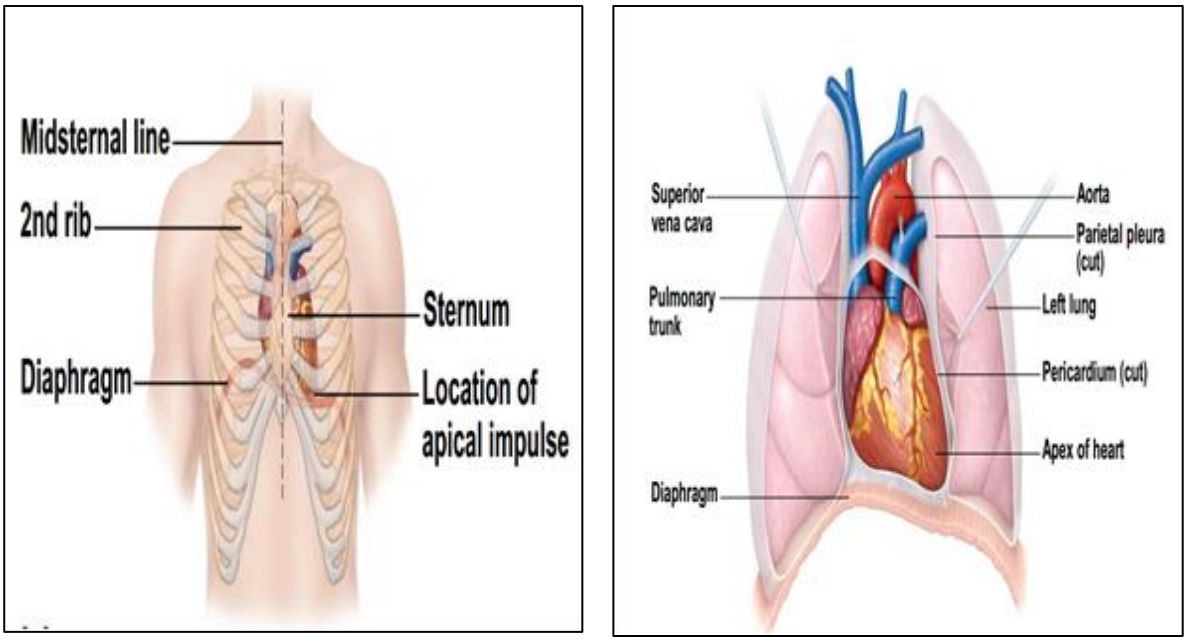


Figure 02: Situation of heart (Heard, 2013)

I.1.1.2. Anatomical description

The heart is primarily a muscular pump and possesses many muscle fibers (Peate, 2018). Atria and ventricles have a different microstructural organization; also, heart valves and ventricle walls possess a different microstructure (Tringelova et al., 2007). Heart is a fibroserous sac comprising three concentric layers (Mahadevan, 2018) with diverse structure and function (Figure 03). The inner layer endocardium and the outer layer epicardium are both very thin, each being about 100 µm thick; the middle layer, called myocardium, constitutes the bulk of the cardiac tissue and endows it with the ability to pump blood (Tringelova et al., 2007).

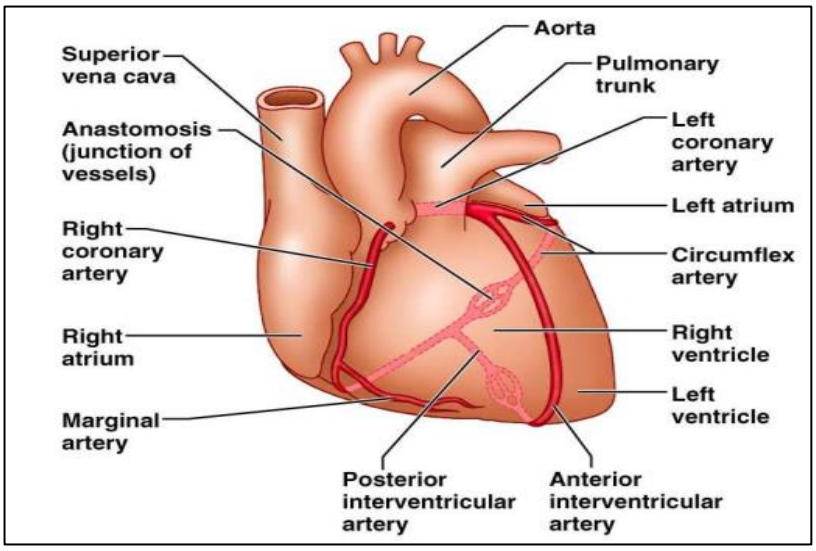


Figure 03: Anatomy of heart (Ebneshahidi, 2006)

I.2. Cardiovascular disease

Cardiovascular disease (CVD) remains to be the leading cause of premature deaths across the globe (Kumar, 2014), (Yousef et al., 2013) and (Nystoriak & Bhatnagar, 2018). Cardiovascular disease (CVD) is an umbrella term for a number of linked pathologies, commonly defined as coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease, rheumatic and congenital heart diseases and venous thromboembolism (Stewart et al., 2017). As a harrowing statistic, every 39 seconds someone dies due to cardiovascular disease, claiming more lives than cancer in the United States (Ruiz-Esparza et al., 2013).

I.3. Cardiovascular disease and oxidative stress

Cardiovascular diseases (CVD) are complex entities with heterogeneous pathophysiologic mechanisms and increased oxidative stress has been viewed as one of the potential common etiologies. Increased oxidative stress has been viewed as one of the potential common etiologies in various CVD (Senoner & Dichtl, 2019). Oxidative stress in cardiovascular system may produce various cardiovascular diseases such as atherosclerosis, ischemic heart disease, hypertension, congestive heart failure, cardiac hypertrophy and cardiomyopathies by producing cell injury to cardiovascular tissue (Qazi & Molvi, 2018).

Chapter II

Doxorubicin

&

Oxidative Stress

II.1. Doxorubicin

II.1.1. Definition

Doxorubicin (Doxo), or Adriamycin, an anthracycline antibiotic discovered serendipitously as a chemotherapeutic drug several decades ago (Kalyanaraman, 2020). Doxo was first isolated from *Streptomyces peucetius* in the 1960s and approved for medical use in the United States in 1974 (Smitha et al., 2020), is one of the important chemotherapeutic agents for different types of cancers (Alanazi et al., 2020) such as acute myeloid leukemia, breast and ovarian cancers (Tomlinson et al., 2019), lymphoma, lung cancer, multiple myeloma, and sarcoma (Sandamali et al., 2019), gastric cancer, and Hodgkin's and non-Hodgkin's lymphomas (Moreira Romao et al., 2019).

II.1.2. Chemical Structure

Doxorubicin,or-(7S,9S)-7-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione (Alghorabi et al., 2019). From a chemical viewpoint, doxorubicin contains a sugar moiety bonded to a tetracycline ring (Quryshi et al., 2018) and aminosugar part, which are connected through an ester oxygen. Substituents and quinone-hydroquinone system sharply determinate a rigid structure of Doxo (Ďurica & Breier, 1998). The functional groups that are thought to be critical to the Doxo mechanism of action are marked (Kalyanaraman, 2020). Doxorubicin has both hydrophilic and hydrophobic regions, allowing it to bind to plasma proteins as well as cell membranes, is also amphoteric, in having both acidic and basic functions (Mitry & Edwards, 2016) (Figure 04).

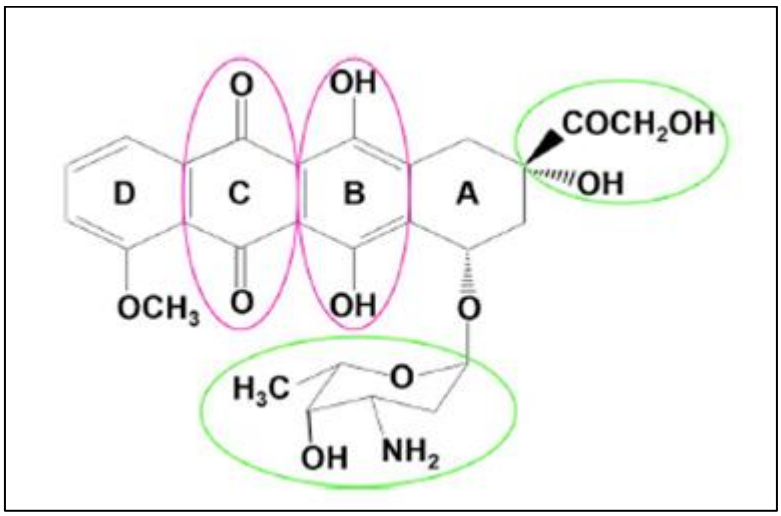


Figure 04: Chemical structure of doxorubicin (Kalyanaraman, 2020)

II.1.3. Pharmacological effect

Doxorubicin (Doxo) is an effective antineoplastic drug indicated to treat many cancerous diseases (AlQahtani et al., 2019)**.** However, its clinical utility is thus limited (Bin Jardan et al., 2020), due to its dose-dependent and cumulative cardiotoxicity (Wu et al., 2019). The clinical use of doxorubicin is hampered by its serious cardiotoxicity, which often leads to irreversible degenerative cardiomyopathy and heart failure (Sandamali et al., 2019).

II.1.4. Side effect of doxorubicin

One of the most dose-limiting side effects of anthracycline therapy is the development of a characteristic cardiomyopathy (Wallace et al., 2020). It is true that most notably observed is cardiotoxicity, but other organ systems are also degraded by doxorubicin use (Mitry & Edwards, 2016). It can produce many side effects such as hepatotoxicity, testicular toxicity and hematological toxicity (Anber, 2018). Doxo induced cellular toxicity occurs as a result of increased oxidative damage, resulting in apoptosis and cell death (Smuder, 2019). A variety of hypotheses have been suggested to explain the damaging effects of doxorubicin on the heart (Doroshow et al., 2020) (Figure 05).

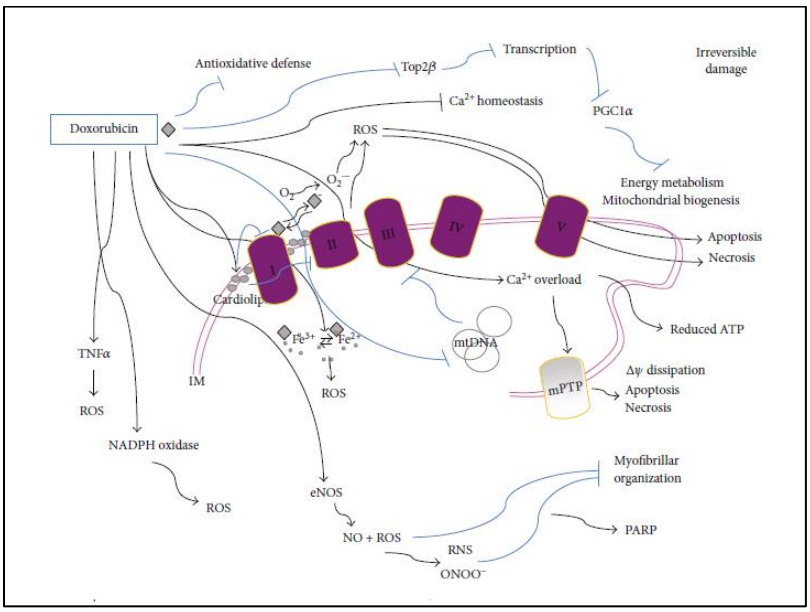


Figure 05: Basic pathophysiological molecular mechanisms underlying Doxo-induced cytotoxicity (Gorini et al., 2018)

II.2. Oxidative stress

II.2.1. Definition

Oxidative stress is a phenomenon that reflects an imbalance between the production of reactive oxygen species, so-called oxidants (Al-Dalaen & Al-Qtaitat, 2014) and antioxidants that play a major role in protecting against molecular oxidative damage (Derouiche et al., 2018) (Figure 06). Oxidative stress is implicated as a pathophysiological mechanism of different diseases and is a topic of growing interest (Derouiche et al., 2018), is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids (Young & Woodside, 2001).

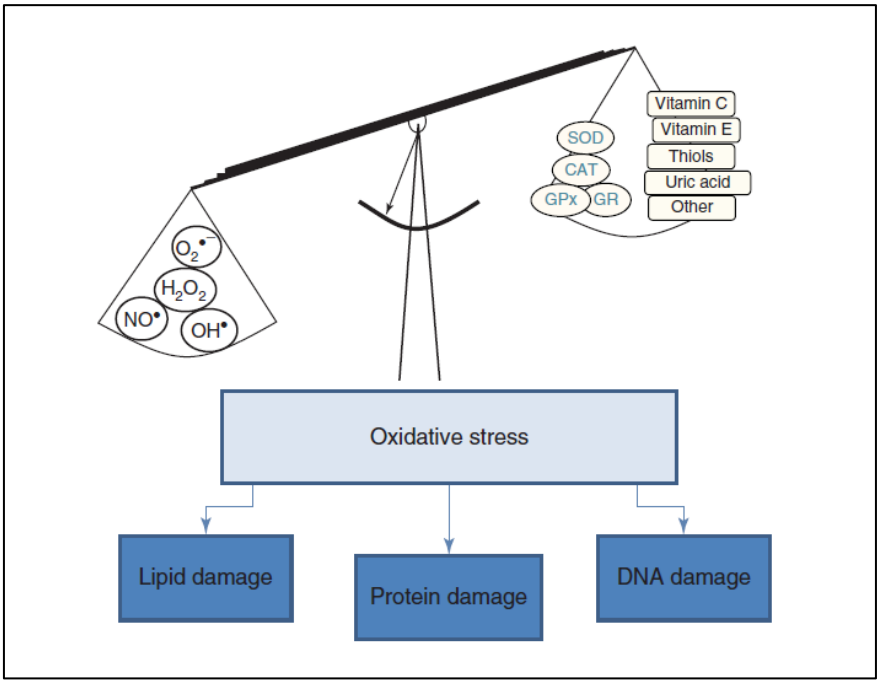


Figure 06: Predominance of oxidation processes caused by oxidative stress (Armstrong & Stratton, 2016)

II.2.2. Free radicals

II.2.2.1. Free radical definition

Free radicals can be defined as atoms or molecules or molecular fragments (Qazi & Molvi, 2018), and are highly reactive because (Ifeanyi, 2018) they contains an unpaired electron in an atomic orbital (Young & Woodside, 2001). ROS can damage DNA leading to mutations, single, or double-strand breaks (Gašparovic, 2020). Therefore, in biological research, the term "free radicals" is frequently replaced by "reactive oxygen species" (ROS), which is a more general term and includes both free radical and non-radical species (Lushchak, 2014). Common examples of free radicals include the hydroxyl radical anion ($OH^{\cdot}$), superoxide ($O^{2\cdot-}$), transition metals such as iron and copper, nitric oxide (NO), and peroxynitrite ($ONOO^{-}$). (Betteridge, 2000).

II.2.2.2. Sources of free radical

There are three types of free radical which are internal sources **(**mitochondria, reactions involving iron and other transition metals, inflammation…etc**)**, external sources **(**ultraviolet light, ozone, certain drugs, pesticides, anesthetics, industrial solvents (Kumar, 2011) and environmental factors, such as air pollutants or cigarette smoke (Birben et al., 2012), and physiological factors **(**Mental status like stress, emotion…etc**)** (Kumar, 2011) (Figure 07).

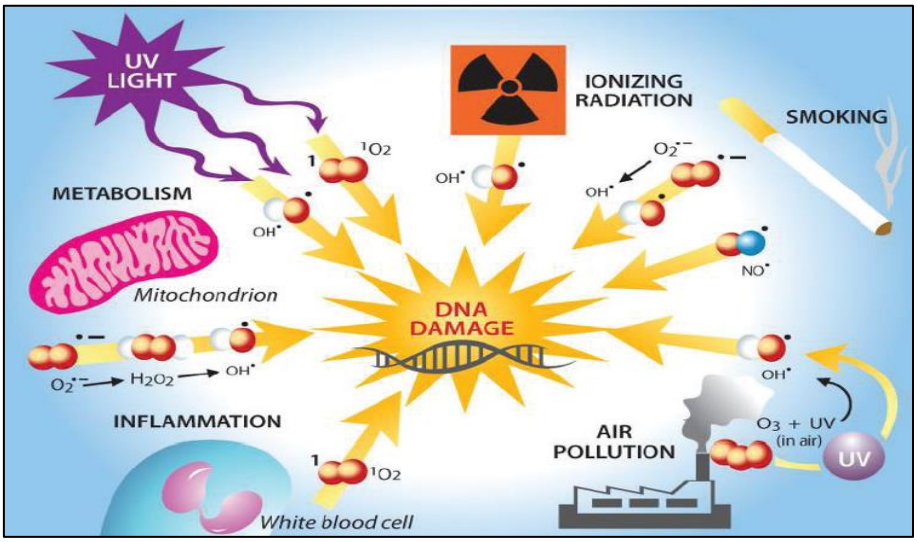


Figure 07: Sources of free radicals that arise oxidative stress (Zamora-Perez et al., 2017)

II.2.2.3. Damage of free radical

An imbalance between free radicals and antioxidants leads to oxidative damage of proteins, fat, nucleic acids, and carbohydrates (Adwas et al., 2019) (Figure 08).

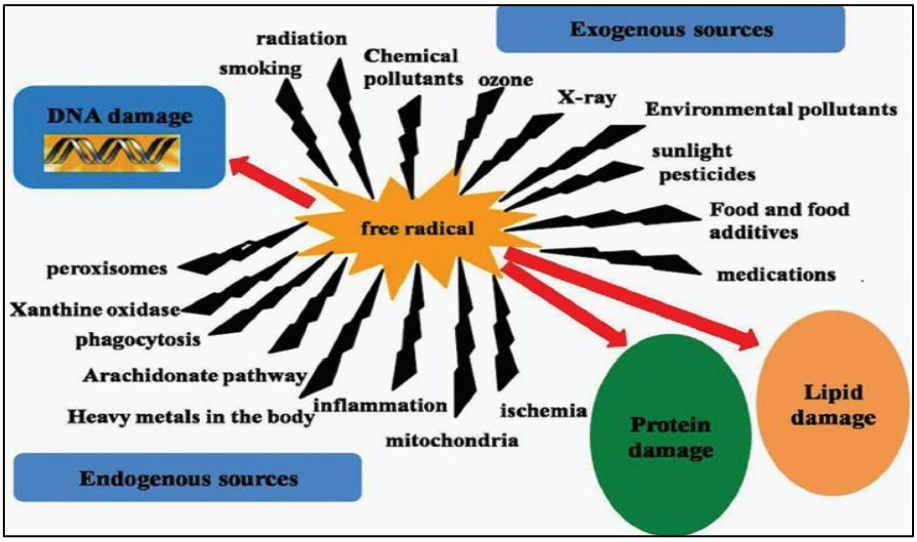


Figure 08: Exogenous and endogenous sources of free radicals causing damages to macromolecules (Ahmad et al., 2017)

II.2.3. Antioxidant

II.2.3.1. Definition

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause diseases (Elmasry et al., 2015). Antioxidant molecules are in fact nucleophilic and reductant molecules able to react with oxidants, which are generally electrophiles, giving them one or two electrons (Espinosa-Diez et al., 2015).

II.2.3.2. Classification

There are two types of antioxidants, enzymatic and non-enzymatic. The major antioxidant enzymes directly involved in the neutralization of ROS and RNS are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) (Pham-Huy et al., 2008).

The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants, belonging to endogenous antioxidants, are produced by metabolism in the body, while nutrient antioxidants, belonging to exogenous antioxidants, are compounds that cannot be produced in the body and must be provided through foods or supplements (Gupta et al., 2014)

They are mainly polyphenolic compounds, which inhibit free radical reaction by stabilizing free radicals. They are classified into mineral (selenium, copper, iron, zinc and manganese), vitamins (A, E and C) and phytochemicals (flavonoids, tannins, terpenoids, catechin, caretonpids etc) (Qazi & Molvi, 2018).

Chapter III

Ocimum basilicum L.

III.1. Definition and distribution of *Ocimum basilicum* L.

Ocimum basilicum L. named commonly as sweet basil (Ahmed et al., 2019), is one of the most important aromatic plants belonging to the Lamiaceae family, it is very important for flavor foods (Taha et al., 2020) and well-known as a medicinal plant and culinary herb because of its phytochemical contents (Rumengan et al., 2019). *Ocimum basilicum* L. herb grown in field conditions **(**Onofrei et al., 2018) (Figure 09).

Basil (*Ocimum basilicum* L.) a well-known herb, has been planted and used form ancient times (Georgiadou et al., 2018) which grows in several regions around the world (Murali & Prabakaran, 2018) in some regions of Asia (Iran, India, and Turkey), warm and subtropical regions of Africa, and central South America (Choi et al., 2019).

Figure 09: Leaves of *Ocimum basilicum* L. (Nadeem et al., 2020)

III.2. Botanical and taxonomy description

Ocimum basilicum L. is a branched herbaceous plant with an average height ranging from 0.6 to 0.9 m, having square, glabrous stems and branches, generally green to somewhat light purple in color. Leaves of basil are simple and oppositely arranged on stem with average length ranging from 2.5 to 5 cm or more. These longer leaves are ovate, having acute tip with lobed or toothed margins (Nadeem et al., 2020).

The taxonomy of basil is considered to be vast and complex. This taxonomic complexity is believed to be due to genetic diversity influenced by crosspollination and several environmental factors (Chowdhury et al., 2017).

Ocimum basilicum L. belongs to the mint family (Lamiaceae) in the order Lamiales (Idowu & OzIegbe, 2017) often called Labiatae; the traditional name (Azoz et al., 2016). Basil is one of

the most important species from the genus of *Ocimum* (Zahedifar et al., 2018) that comprising more than 60 species (Murali &Prabakaran, 2018).

Kingdom : Plantae

Phylum : Magnoliophyta

Class : Magnoliopsida

Order : Lamiales

Family : Lamiaceae

Genus : *Ocimum*

Species : *Ocimum basilicum* (Purushothaman et al., 2018)

III.3. Chemical composition

Basil plant has moderate macro and micro nutritional values including protein, fat, energy, Vitamin C, Vitamin E, Vitamin A, Vitamin K, Calcium, Iron, Potassium, Magnesium and Sodium (Falowo et al., 2019). As well as, the chemical constituents which have been isolated from the plant include terpenoids, alkaloids, flavonoids, tannins, saponin glycosides and ascorbic acid (Rumengan et al., 2019).

III.4. Therapeutic effect and economic value of sweet basil

Sweet basil (*Ocimum basilicum* L.) is deemed as one of the important medicinal and aromatic plants cultivated commercially in many parts of the world for their economic and culinary importance (Bahcesular et al., 2020), because it contains important components (Kalamartzis et al., 2020). The basil leaves are used in folk medicine as a remedy for a large number of diseases (Ahmed et al., 2019) due to their high antioxidant and antimicrobial properties, caused by a high content of secondary metabolites (Dörr et al., 2020). There are many evidences that show the basil essential oil has fungistatic and insecticidal activities (Akbari et al., 2019) also can be used as flavouring agents in food, medicine and cosmetics (Rezzoug et al., 2019). Traditionally, every part of the plant is used as medicine to treat headaches, coughs, diarrhea, constipation, warts, worms, kidney malfunction, and digestive problems (Falowo et al., 2019).

Chapter IV

Nanotechnology

IV.1. Definition of nanotechnology

Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization, in at least one dimension, is on the nanometer scale or one billionth of a meter (Saini et al., 2010). Nanotechnology is one of the most promising technologies of the 21st century. It is the ability to convert the nanoscience theory to useful applications by observing, measuring, manipulating, assembling, controlling and manufacturing matter at the nanometer scale (Bayda et al., 2020). Nanotechnology has opened up by rapid advances in science and technology, creating new opportunities for advances in the fields of medicine, electronics, foods, and the environment (Morais et al., 2014). Nanotechnology has emerged as one of the leading fields of the science having tremendous application in diverse disciplines (Gatoo et al., 2014). It employs knowledge from the fields of physics, chemistry, biology, materials science, health sciences, and engineering (Godwin et al., 2015). Researchers use bionanotechnology techniques as eco-friendly and cost-effective routes to fabricate nanoparticles and nanomaterials (Linhai et al., 2018). In recent years, there has been a rapid increase in nanotechnology applications to medicine in order to prevent and treat diseases in the human body (Gopal et al., 2010).

IV.2. Nanoparticles

IV.2.1. Definition

The prefix ‘nano’ is referred to a Greek prefix meaning ‘dwarf’ or something very small and depicts one thousand millionth of a meter (10^{-9} m) (Bayda et al., 2020) ,(Kirdar , 2015). Where nanoparticles have been shown to have utility in various fields of science (Sharma et al., 2017) and possess unique physical and chemical properties due to their high surface area and nanoscale size (Khan et al., 2019).

IV.2.2. Classification of nanoparticles

Nanoparticles can be classified based on the following criteria

- ✔ Origin: natural and anthropogenic.
- ✔ Size: 1-10 nm, 10-100 nm and over 100 nm.
- ✔ Chemical composition: inorganic substances, organic substances and elements of the living kingdom (Khan et al., 2019).

IV.2.3. Magnesium oxide nanoparticles

The metal elements are able to form a large diversity of oxide compounds (Meenakshi et al., 2012). MgO NPs are one such metal oxide NP, exhibiting increased utilization in recent time (Verma et al., 2020). Nanoscale MgO possesses unique optical, electronic, magnetic, thermal,

mechanical and chemical properties due to its characteristic structures (Narendhran et al., 2019). Magnesium oxide (MgO) is a category of the practical semiconductor metal oxides, which is extensively used as catalyst and optical material (Nemade & Waghuley, 2014). MgO is an important inorganic oxide (Tang et al., 2014) and has many applications (Camtakan et al., 2012). Magnesium oxide (MgO) is widely used in the chemical industry (Meenakshi et al., 2012).

IV.3. Application of nanotechnology

Recently, nanotechnology has been a subject of great interest, offering considerable advantages in many areas, an increased interest in nanotechnology applications can be observed in various fields medicine, materials science, pharmacy, environmental protection, agriculture (Baranowska-Wójcik et al., 2019), nutrition, and energy (Elumalai et al., 2019). Their reactivity, toughness and other properties are also dependent on their unique size, shape and structure. Due to these characteristics, they are suitable candidates for various commercial and domestic applications, which include catalysis, imaging, medical applications, energy-based research, and environmental applications (Khan et al., 2019) (Figure10).

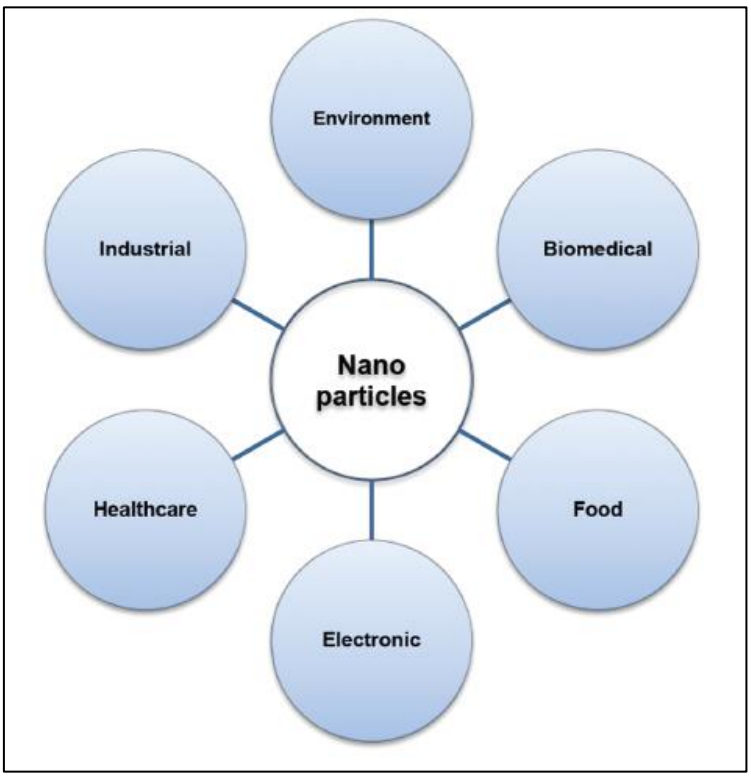


Figure 10: Application of nanoparticles in various fields (Alshammari et al., 2016)

IV.4. Green synthesis of nanoparticles

Nowadays, nanotechnology involving green synthesis of nanoparticles has become an eye-catching idea and has gained much importance and significance in recent years due to its great facility, clean processing, non-toxic chemicals used, cost- effectiveness, and being environmental and ecofriendly (Chartarrayawadee et al., 2020). The conventional methods of developing NPs are costly and produce very toxic product, so the need of hour is to reduce the risk of toxicity in the environment from the different chemicals used in the physical and chemical methods. The alternate approaches found to develop NPs is "green synthesis" (Gour & Jain, 2019). Green synthesis method, provides a faster metallic nanoparticle production by offering an environmentally friendly, simple, economical and reproducible approach. Given the wide range of applications of metallic nanoparticles produced, biological methods play a major role in the synthesis of metallic nanoparticles (Nadaroğlu et al., 2017).

Synthesis of metal nanoparticles using plant extracts is very cost effective, and therefore can be used as an economical and valuable alternative to the large-scale production of metal nanoparticles. In addition, the full utilization of plant waste is a sustainable path for development (Linhai et al., 2018)

Experimental Part

Chapter I

Materials & Methods

I.1. Patients and biological study

❖ Study subject

Ethical approval was requested and approved by the Ethics Committee of the Department of Cellular and Molecular Biology, Faculty of Natural Sciences and Life, University of El-Oued. Our study was conducted on 22 volunteers, their age between 23 and 50 years, which were divided into two groups; a group of 11 volunteers healthy controls their mean age is 25.82±1.32 years old, the other group of 11 patients diagnosed for cancer and undergoing treatment with doxorubicin, their mean age is 47.27±2.83 years old. All the volunteers (controls and patients) in this study live in the El-Oued area located in the southeast of Algeria.

❖ Inclusion criteria

Inclusion criteria for cancer, clinical diagnosis shows cancer suffering for three months confirmed by specialist doctors, also patients receiving doxorubicin chemotherapy but no other type of chronic disease treatment for 30 days. Regarding the controls are healthy peoples do not suffer from a chronic or acute diseases and consume no drug for 30 days.

❖ Exclusion criteria

Were to eliminate the factors which might affect the parameter's study, we excluded all cancer patients receiving other kind of chemotherapy, diabetics, arterial hypertension or any evidence of endocrine disorders in their medical history from patient groups and healthy controls.

❖ Laboratory Investigations and methods

Blood samples were collected and placed into containing tubes. Blood was transferred into EDTA tubes for hematological markers and the serum were obtained after centrifugation at 3000× g for 5 min, removed and retained for assay of biochemical parameters. Serum samples were stored at -20°c until analysis. Hematological analysis (FNS) is performed by the hematology autoanalyzer (Sysmex).

I.2. laboratory animal study

I.2.1. Material

I.2.1.1. Plant materials

The plant studied in this work; *Ocimum basilicum* L. was harvested from the region of EL-Oued (El-Gharbia, Guemar) in August, the leaves are cleaned and then allowed to dry out of direct sunlight and room temperature. The dry leaves were powdered by mechanical grinder until a fine powder was obtained. *Ocimum basilicum* L. powder is stored in the room temperature in airtight containers until start of the experiment.

The botanist Youcef Hellis asserted that the plant used was actually *Ocimum basilicum* L.

I.2.1.2. Animal materials

I.2.1.2.1. Animal and husbandry condition

Our study carried out on 25 female Wistar rats, from the Pasteur Institute of Algiers, aged 8 weeks with a weight of 184.84 ± 8.48. The animals are bred at the pet store in the Faculty of Natural and Life Sciences, at Echahid University Hamma Lakhdar-El-Oued. The animals were carried under the same conditions, a period of adaptation, photoperiod (12h of light/12h of black), at room temperature. The rats are housed in plastic cages and have free access to water and food by a standard diet (Southon et al., 1984). The experiment was conducted over a period of 30 days.

Table 01: Standard diet composition (Southon et al., 1984)

Raw materials	Quantity (g / kg)	Percentage (%)
Maize	326	32.6
Sacrose	326	32.6
Protein	168	16.8
Cellulose	40	4
Minerals	20	2
Vitamin	20	2
Oil	40	4

I.2.1.2.2. Cardiotoxicity induced

Cardiotoxicity was induced by intraperitoneal injection of doxoribicin for 4 weeks (one dose/week 1.5ml/kg) (Al Qahtani et al., 2019).

I.2.1.2.3. Treatment animals

After a period of adaptation, the animals were divided into 5 experimental groups of 5 animals each as follows:

Group 1 (Control): were normal rats inoculated with physiological saline (one dose/week, 1.5 ml/kg) for 4 weeks.

Group 2 (Doxo): Drug control was inoculated with Doxo (one dose/week, 1.5 ml/kg) for 4 weeks.

Group 3 (Doxo + *O. basilicum*): Rats were inoculated with Doxo (one dose/week, 1.5 ml/kg) and received oral dose of aqueous extract of *O. basilicum* (200 mg/kg/day) for 4 weeks.

Group 4 (Doxo + MgNPs): Rats were inoculated with Doxo (one dose/week, 1.5 ml/kg) and MgNPs (one dose/week, 50 mg/kg) for 4 weeks.

Group 5 (Doxo+ *O. basilicum* + MgNPs): Rats were inoculated with Doxo (one dose/week, 1.5 ml/kg), MgNPs (one dose/week, 50 mg/kg) and received oral dose of aqueous extract of (200 mg/kg/day) for 4 weeks.

Regarding the absence of previous studies, the doses of *Ocimum basilicum* L. extract and MgNPs used in this study are according to our selection.

I.2.1.2.4. Sacrifice, blood sampling and tissues collection

After 16 h of fasting these animals were sacrificed under slight anesthesia by chloroform (94%) by inhalation; blood samples were collected during the slaughter of animals into EDTA tube to carried FNS and dry tubes. The serum was obtained by centrifugation for 10 min at 3000 tour/min and used for biochemical analysis; blood sugar is measured during sacrifice period by a glucometer. The liver and heart and are carefully sampled, washed with normal saline (NaCl) then weighted and stored at -20°c for oxidative stress. On the other hand, the heart is placed in 10 % formaldehyde for histological analysis.

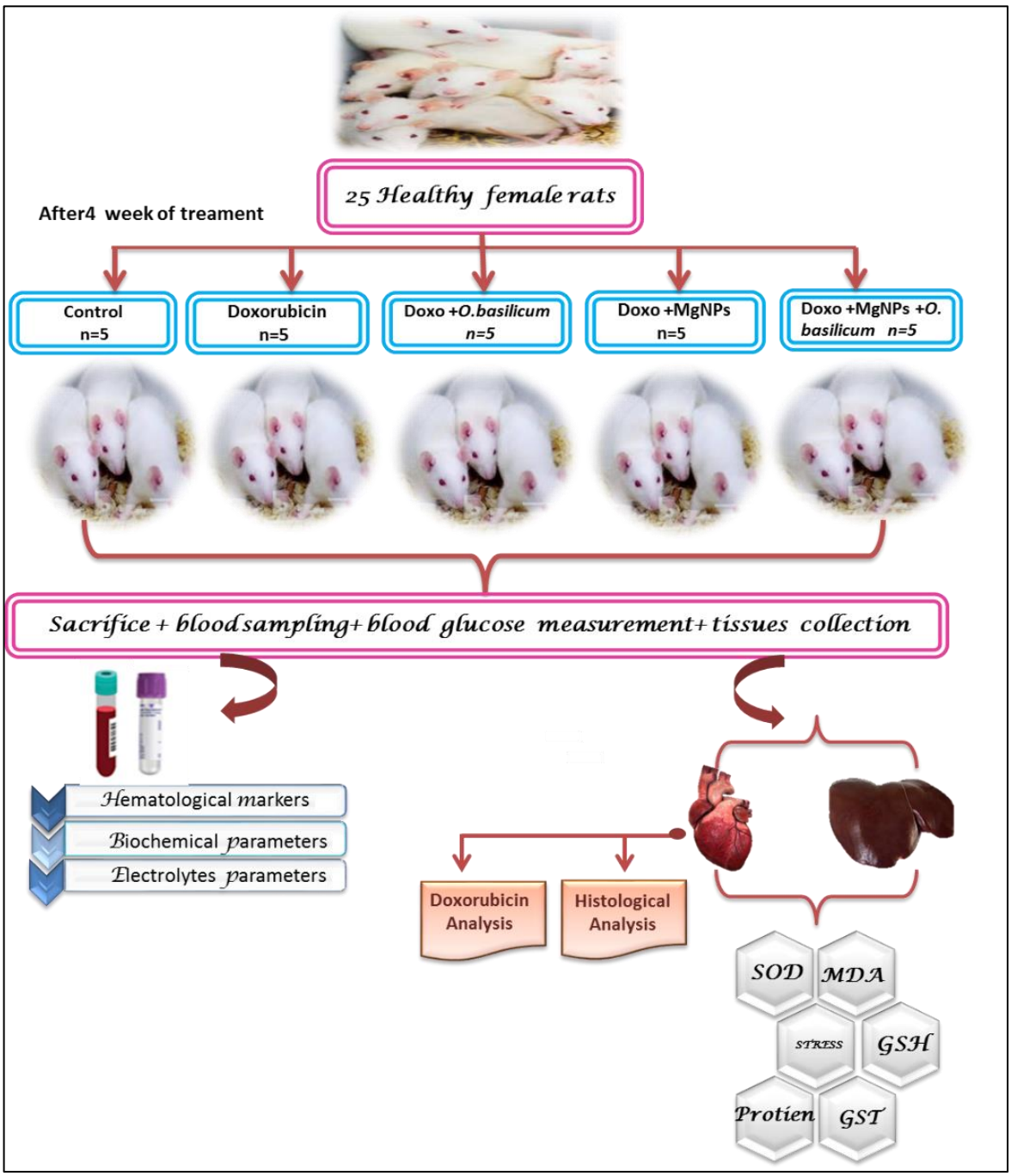


Figure 11: Experimental design of study

I.2.1.3. Reagents and products

Magnesium nitrate, NaOH, Chloroform, Chloride hydrogen (HCl), Sodium Chloride (Nacl), Phosphoric Acid (H_3PO_4), Methanol, Bovine Serum Albumin (BSA), Comassie Blue, Tris, Salicylic acid, DTNB (5-5'-dithiobis2-nitrobenzoic acid), Gallic Acid, GSH, ferric chloride $FeCl_3$, Magnesium (Mg), Fehling liquor, Sulfuric acid H_2SO_4, Aluminum chloride ($AlCl_3$), Riboflavin, EDTA, Methionon, Folin-Ciocalteu (FCR), Sodium carbonate (Na_2CO_3), 1-chloro 2,4-dinitrobenzene (CDNB), Acetic acid, Drajendrof, Trichloroacetic Acid (TCA), Thiobarbituric Acid (TBA), Butylated Hydroxy Toluene (BHT), Phosphate buffer, NBT.

I.2.2. Methods

I.2.2.1. In vitro study

I.2.2.1.1. Method of Aqueous extract preparation

Aqueous extract was preparing by putting 10 g of dried Leaves powder of *Ocimum basilicum* L. with 100 ml of distilled water was boiled over low heat (50°c) for 2 hours. After cooled and macerated to room temperature for 24 hours, then filtered through Whatman filter paper, the extract was then evaporated using a rotary evaporator and was drying using oven. The product resulting from this process was used in this study (Majhenic et al., 2007).

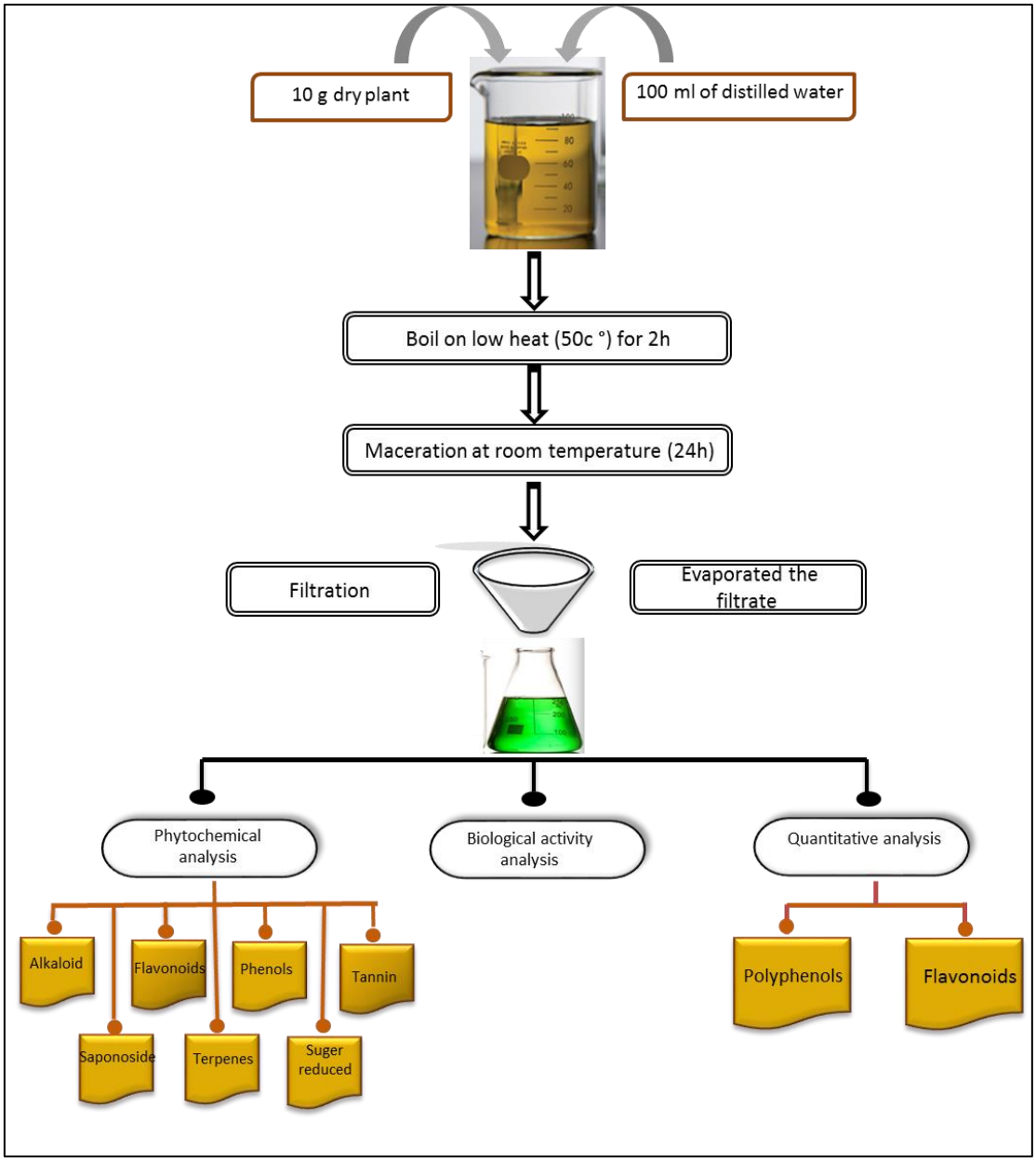


Figure 12: Method of preparation of the aqueous extract of *Ocimum basilicum* L.

I.2.2.1.2. Method of MgNPs preparation

In the experiment, 5 ml of fresh leaves extract and 20 ml of distilled water was added to a 250 ml beaker and heated. Magnesium Nitrate is added to the solution. The Magnesium nitrate ions were reduced to Magnesia or Magnesium Oxide nanoparticles by using plant leaves extract. The formation of Magnesium oxide nanoparticles (MgNPs) have been observed by color change of the solution from yellow to yellowish-brown color (Moorthy et al., 2015).

I.2.2.1.3. Characterization of MgNPs

The MgO nanoparticles prepared by the above method was characterized using UV-Vis spectrophotometer in the UV-Vis range of 200-800 nm. Furthermore, the morphology of the nanoparticles (NPs) was determined using scanning electron microscopy (SEM). The Fourier transform infrared spectroscopy (FTIR) analysis of biosynthesized magnesium oxide nanoparticles was recorded in the range 400-4000 cm^{-1}.

I.2.2.1.4. Phytochemical analysis

Phytochemical tests were carried out on the aqueous extracts by techniques of qualitative characteristics.

- **Alkaloids test:** 2ml was acidified with a few drops of dilute hydrochloric acid. Then 1ml of Dragendorff's reagent was added. The appearance of orange to red precipitate indicates the presence of alkaloids (Thilagavathi et al., 2013).
- **Flavonoids test:** To the test solution, few fragments of Magnesium ribbon are added and concentrated HCl was added drop wise, pink scarlet, crimson red or occasionally green to blue color appears after a few minutes (Parihar et al., 2015).
- **Phenol test:** To the 5 ml of extract, a few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compounds (Parihar et al., 2015).
- **Terpenoids test:** 2 ml of chloroform were mixed in extract of selected plant sample and 3 ml of sulphuric acid were added in selected sample extract. Formation of reddish brown color indicates the presence of terpenoids in the selected plants (Wadood et al., 2013).
- **Tannins test:** Ferric chloride ($FeCl_3$) reagent (3 drops) was added to the extract. A blue-black or green precipitate confirmed the presence of gallic tannins or catechic tannins, respectively (Karumi et al., 2004).
- **Reducing sugars test:** To carry out the Fehling test, 5 ml of extract raw are added 5 ml of Fehling liquor.

The formation of a brick-red precipitate after 2-3 min heating in a water bath at 70°c indicates a positive reaction (Bekro et al., 2007).

- **Saponins test**: To highlight the saponins, we introduced 10 ml of each extract aqueous in a test tube. The tube is agitated while 15 seconds then left to stand while 15 min. A height of foam persistent, greater than 1 cm indicates the presence of saponosides (Bidie et al., 2011).

I.2.2.1.5. Total phenolic and flavonoids compounds

I.2.2.1.5.1. Total phenolic

Determination of the total polyphenols was carried out according to the Folin-Ciocalteu (FC) method (Boizot & Charpentier, 2006): 100 µl of plant extract are mixed with 500 µl of the FC reagent and 400 µl of Na_2CO_3 at 7.5% (w / v). The mixture is stirred and incubated in the dark and at room temperature for ten minutes and the absorbance is measured at 760 nm by a UV spectrophotometer. The results are expressed in mg gallic acid equivalent / g of dry vegetable material with reference to the calibration curve of gallic acid.

Calibration curve is carried out by gallic acid at different concentrations (50-100-150-200 g /ml) under the same conditions and the same steps of the assay. The results are thus expressed in milligrams of gallic acid per gram of dry extract (mg of EAG / g). All measurements are repeated 3 times.

I.2.2.1.5.2. Total Flavonoids

A quantity of 1 ml of each sample and standard (prepared in methanol) is added to 1 ml of the $AlCl_3$ solution (2% dissolved in methanol). After 10 minutes, absorbance was measured relative to the reagent blank prepared at λ max = 430 nanometers (Ahn et al., 2007). The flavonoid concentrations have been deducted from the range of the calibration curve established with quercetin (0.01 - 0.03 - 0.05 - 0.07 - 0.09 mg / ml). Performed by the same dosing procedure, all the measurements are repeated 3 times, the results are expressed in mg equivalent to quercetin / g of extract.

I.2.2.1.6. DPPH antioxidant test

In vitro antioxidant activity was evaluated by measuring the scavenging power of the DPPH (1,1-diphenyl-2-picryhydrazyl) radical.

A DPPH radical scavenging assay was employed to determine, by a spectroscopic method, relative plant antioxidant ability. Anti-radical activities of plant extracts were estimated, according to the method of Nwidu et al. 2017. Stock solutions of extracts (5 mg/ml) were prepared and diluted to final concentrations of 200, 100, 50, 25, 12.5 and 6.25 µg/ml in ethanol. 160 µL of 0.1 mM DPPH in ethanol solution was added to 20 µl of the extracts or standard, and then mixed with 20 µl of H_2O. β-tocopherol (as a control solution) over the concentration range of 1.56, 0.78 0.39, 0.195, and 0.0975 µg/ml was assayed under similar conditions. The mixtures were incubated at

37°c for 40 min in the dark. Sample absorbance was read at 517 nm, as described in Nwidu et al. 2017.

I.2.2.1.7. Hemolysis assay

The Hemolysis assay was done as described by Henkelman et al., 2009. 5ml of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 40°c. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5 min. Washed erythrocytes were stored at 4°c and used within 6 h for the hemolysis assay. 50 µl of 10 dilutions (100 µl erythrocytes suspension: 900 µl 1XPBS) of erythrocytes suspension was mixed with 100 µl of test samples (extract of *Ocimum basilicum* L.) (20-80 µg/ml), 100 µl of 1XPBS was used as negative control and 100 µl of 1% SDS as positive controls. Reaction mixture was incubated at 37°c water bath for 60 min. The volume of reaction mixture was made up to 1 ml by adding 850 µl of 1XPB. Finally, it was centrifuged at 300 rpm for 3min and the resulting hemoglobin in supernatant was measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin. The percentage hemolysis was calculated as follows.

$$\text{Hemolysis inhibition \%} = 100\text{-}[\text{Sample} / \text{Control}] \times 100$$

I.2.2.2.In vivo study

I.2.2.2.1. Acute toxicity test of extract of *Ocimum basilicum* L.

The test was performed using healthy albino rats of Wistar strain weighing between 204.5±0.29 g. The animals were divided into three groups of two rats each and administered 0, 2000 and 5000mg/kg of aqueous extract of *O. basiluim* orally. Animals were observed after dosing at least once during the first 30 min, periodically during 24 H (Derouiche & Kaouachi, 2018).

I.2.2.2.2. Acute toxicity test of biosynthesized MgNPs

The test was performed using six healthy albino rats of Wistar strain weighing 213.5 ± 9.31g. The animals were divided into three groups of two rats in each and administered 0, 250 and 500 mg/kg of Magnesium Oxide nanoparticles by injection. Animals were observed after dosing at least once during the first 30 min, periodically during the first 24 h.

I.2.2.2.3. Biochemical parameters analysis

The determination parameters were measured using commercial kits (Spinreact), (refs: TG-1001311, TC-1001090, and total proteins-1001291). Serum enzymatic activities of Lactate dehydrogenase (LDH), Gammaglutamyltranspeptidase (γ-GT), Alanine aminotransferase (GPT),

and aspartate aminotransferase (GOT) were measured using commercial kits (Spinreact) (refs: (refs: GOT-1001161, GPT-1001171 LDH-1001260 and γ -GT-MD41288)

I.2.2.2.4. Method of electrolytes analysis

Determination of the ionogram parameter (sodium, potassium and chloride) by Automatic electrolyte analyzer (Easylute).

I.2.2.2.5. Oxidative stress parameters

I.2.2.2.5.1. Preparation of homogenates

About 1g of tissue (liver and heart) was homogenized in 9 ml of buffer solution of Tris buffer saline (TBS, pH=7.4). Homogenates were centrifuged at 4000xg for 20 min and the obtained supernatant was used for the determination of antioxidant activity.

I.2.2.2.5.2. Determination of protein concentration

- **Principle**

Protein concentration is determined according to the method of Bradford M.M., 1976 which uses Comassie blue as a reagent. The latter reacts with the amino groups (NH2) of proteins to form a blue complex. (The appearance of the blue color reflects the degree of ionization of the acid medium and the intensity corresponds to the concentration of proteins).

- **Operating mode**

✔ Take 40 µl of the homogenate (Solution p).

✔ Add 2 ml of Comassie blue.

✔ Shake and let stand for 5 minutes.

✔ Read the optical densities against the blank at 595 nm.

✔ Compare the concentration of proteins in the tissues studied

The protein concentration is determined by comparison with a standard range of bovine serum albumin (0.1,0.2, 0.4, 0.6, 0.8, 1 mg / ml) previously carried out under the same conditions.

I.2.2.2.5.3. Determination of malondialdehyde (MDA) level

MDA was measured according to the method described by SASTRE et al. (2000). In brief, Pipette 100 µl of sample, 400 µl of TBA reagent into the glass and screw test tubes and seal.

Heat the mixture in the Marie bath at 100 ° C for 15 minutes. Then cool in a cold-water bath for 30 minutes leaving the tubes open to allow evacuation of the gases formed during the reaction. Centrifuge at 3000 rpm for 5 minutes and read the absorbance of the supernatant at 532 nm using a spectrophotometer. TBARS concentration was determined using the MDA molecular extinction coefficient (å = $1.53\times10^{5}M^{-1}.cm^{-1}$). The results were expressed in µmol / mg proteins.

I.2.2.2.5.4. Determination of Reduced glutathione (GSH) level

The level of reduced Glutathion is determined according the WEAK and CORY (1988).by measuring the optical density results from the formation of 2-nitro-5-mercocapturic acid from the reduction of dithio-bis-2-nitrobenzoic acid, which is called Ellman reagent with SH groups exist in GSH briefly.

- 800µl of homogenate samples are add to 200µl of salicylic acid (0.25%).
- The mixture was centrifuge at 1000 rpm for 5 min.
- Take 500 ml of supernatant and mixed with 1000µl of tris buffer (tris 0.4 mol, 0.02 mol NaCl, pH =8.9) and 25 µl of DTNB (0.01 mol/l).
- Read the absorbance at 412 nm after 5 min of incubation.

$$GSH\ (nM/mg\ of\ Hb) = \frac{(OD \times 1 \times 1.525)}{13133 \times 0.8 \times 0.5 \times mg\ of\ pr}$$

- **OD:** Optical Density.
- **1.525:** total volume of blend an ml.
- **13133:** Absorption constant of SH groups at 412 nm.
- **0.5:** volume of solution float an ml.
- **1:** volume of protein mixture.
- **0.8:** volume of homogeneous solution without protein exists in 1 ml.
- **GSH:** concentration of glutathione.

I.2.2.2.5.5. Determination of superoxide dismutase activity assay

The assay method of SOD activity using the NBT by the superoxide anion ($O_2^{\cdot}$), is used as a basis for detecting of presence of SOD by measuring the spectrophotometrically absorbance at 560 nm (Beauchamp &Fridovich.,1971).

I.2.2.2.5.6. Determination of the enzyme activity of glutathione-S-transferase (GSTs) levels

The method used in this study to measure the GSTs is that of Habig et al (1974); this consists in causing the GSTs contained in the homogenate to act on a mixture (GSH + CDNB) at a temperature of 37°c and at a pH of 6.5. The variation in optical density, due to the appearance of the GSH-CDNB complex, is measured for 1 minute for 5 minutes at a wavelength of 340 nm.

Table 02: Procedure of GST activity assay

Reagents	Blank (µl)	Sample (µl)
Phosphate buffer(0.1 M) pH=6.5	850	830
CDNB (0.02 M)	50	50
GSH (0.1 M)	100	100
Homogenate	-	20

Expression of results

The activity of the GSTs expressed in nanomoles of CDNB per minute per milligram of proteins (nmol CDNB / min / mg prot) according to the following formula:

$$\mathbf{GSTs(nM/min/mgdeprot)} = \frac{\mathbf{DO\ échant/min - DO\ blank/min}}{\mathbf{9.6 \times} \boldsymbol{mg\ de\ prot}}$$

I.2.2.2.6. Method of histopathological study of heart tissues

The anatomopathology technique used in the laboratory of the Faculty of Natural Sciences and Life and laboratory of the BEN AMOR DJILANI hospital.

- **Fixation and incubation**: The dehydration, clarification and inclusion.
- **Coating and making cuts**: The samples were carefully removed from the cassettes with forceps and then put in stainless steel molds before filling with liquid paraffin.
- **Coloring and mounting of sections:** The staining of the slides was done with two dyes which make it possible to highlight the cell and tissue morphology.
- **Microscopic observation**: The slides were observed using a microscope lens with camera. Images obtained by this camera were transferred to the computer screen.

I.2.2.2.7. Statistical analysis

The values of the results were expressed as a percentage or an average ± ES (standard deviation). Student's t-test of independent samples was used. All data in this study were examined by Minitab 13.0 software. $P < 0.05$ indicates statistically significant difference.

Chapter II

Results

II.1. Part one: Patients study

II.1.1. Description of study population

Characteristics of the study population are shown in table 03. Women volunteers for this study are recruited in hospitals by the specialist doctors of the Center for the fight against cancer Rezki Elbachir wilaya of El-Oued. After a women's agreement to participate in this study, the selected population reaches 11 control and 11 cancerous women treated by doxorubicin. The results obtained showed the difference concerning mean age, body weight, systolic blood pressures (SBP) and diastolic blood pressures (DB).

Table 03: Description of study population

Parameters	Control group	Patient group
Age (year)	25.82±1.32	47.27±2.83
Body weight (kg)	60.82±2.55	72.64±3.69
Systolic blood pressures	11.18±0.22	11.63±0.24
Diastolic blood pressures	6.81±0.26	7.36±0.33

Data are expressed as mean± SD (n=11)

II.1.2. Biochemical markers

Our results obtained concerning biochemical parameters revealed that a significant increase ($P < 0.05$) in serum lipid profile (Cholesterol and Triglyceride) and a very highly significant difference increase ($P < 0.001$) in creatinine level in women with cancer who are treated with doxorubicin as compared to controls. Also, our results show that there is no significant change ($P > 0.05$) concerning the urea level in the groups studied.

Regarding the enzymatic activity, the results obtained show that the activities of LDH and aspartate aminotransferase (GOT) is a very highly significant augmentation ($P < 0.001$) in women with cancer who are treated with doxorubicin as compared to the control, in the other hand, we observed a significant raise for the mean of gamma-glutamyltranspeptidase (GGT) and alanine aminotransferase (GPT) in patients compared to the control (Table 04, Figure 13).

Table 04: Level of biochemical markers in control and patient groups

Parameters	Control groups	Patient groups	P value
Cholesterol (g/l)	1.46±0.030	1.85±0.13	0.017*
Triglyceride(g/l)	0.57±0.04	1.29±0.26	0.022*
Urea (g/l)	0.23±0.01	0.21±0.02	0.52 NS
Creatinine(mg/l)	5.90±0.25	4.72±0.27	0.001***
LDH (UI/l)	238.99±7.31	361.7±18.5	0.000 ***

Data are expressed as mean± SD (n=11). **NS p>0.05**: no significant difference,*** p<0.05**: significant difference,****p<0.01**: highly significant difference,*****p<0.001**: very highly significant difference.

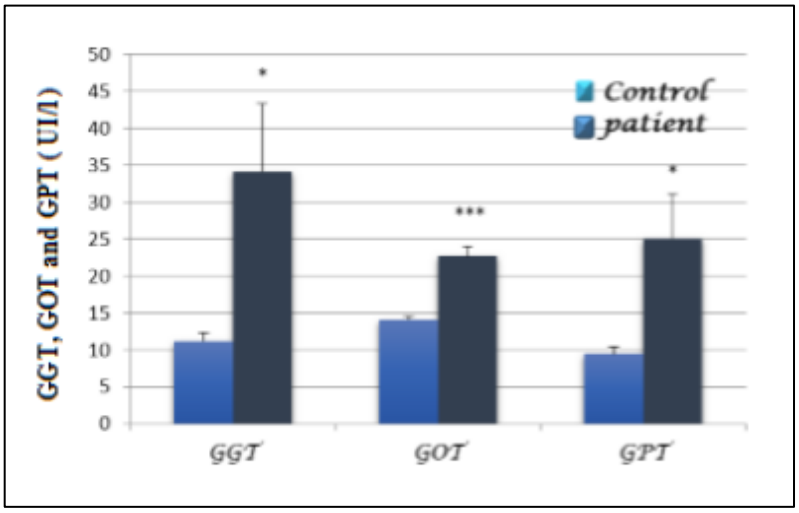


Figure 13: Variation of gamma-glutamyl-transpeptidase (GGT), alanine aminotransferase (GPT) and aspartate aminotransferase (GOT) activities in control and patient groups. **NS p>0.05**: no significant difference,*** p<0.05**: significant difference,****p<0.01**: highly significant difference,*****p<0.001**: very highly significant difference.

II.1.3. Hematological markers

Our results obtained concerning hematological markers revealed that there was a very highly significant decrease (p <0.001) in lymphocyte level in women with cancer compared to controls. On the other hand there is no significant change (P> 0.05) concerning white blood cell, neutrophil, monocyte, eosinophil and basophile in patients compared to control (Table 05).

Table 05: Leukocyte line markers level in control and patient groups

Parameters	Control groups	Patient groups	P value
White blood cell (10^3/ul)	6,92 ± 0,47	7,12 ± 0,88	0,822 NS
Neutrophyle (10^3/ul)	3,94 ± 0,39	5,49 ± 0,85	0,101 NS
Lymphocyte (10^3/ul)	2,34 ± 0,12	1,12 ± 0,13	0,000 ***
Monocyte (10^3/ul)	0,49 ± 0,05	0,41 ± 0,07	0,366 NS
Eosinophyle (10^3/ul)	0,10 ± 0,02	0,06 ± 0,04	0,387 NS
Basophyle (10^3/ul)	0,02 ± 0,00	0,02 ± 0,00	0,999 NS

Data are expressed as mean± SD (n=11). **NS p>0.05**: no significant difference,*** p<0.05**: significant difference,****p<0.01**: highly significant difference,*****p<0.001**: very highly significant difference.

The result of hematological analysis (Table 06) showed that there is a significant decrease (p <0.05) in red blood cell, hematocrit and hemoglobin levels in all patients with cancer as compared to control. Simultaneously with no significant difference (P> 0.05) in platelet level in the patient group against the control.

Table 06: Erythrocyte line markers level in control and patient groups

Parameters	Control groups	Patient groups	P value
Red blood cell (10^6/ul)	4,52 ± 0,08	4,07 ± 0,20	0,048 *
Hemoglobin (g/dl)	12,60 ± 0,21	11,20 ± 0,37	0,004 **
Hematocrit (%)	39,17 ± 0,65	35,37 ± 0,95	0,003 **
Platelet (10^3/ul)	282,9 ± 18,0	315,9 ± 31,3	0,317 NS

Data are expressed as mean± SD (n=11). **NS p>0.05**: no significant difference,*** p<0.05**: significant difference,****p<0.01**: highly significant difference,*****p<0.001**: very highly significant difference.

II.2. Part two: In vitro study

II.2.1. In vitro essays of *Ocimum basilicum* L

II.2.1.1. Phytochemical study

II.2.1.1.1. Qualitative phytochemical analysis of *Ocimum basilicum* L.

Results of phytochemical essays showed that aqueous extract of *Ocimum basilicum* L. very rich on different important chemical compounds such as flavonoids, phenols, catechic tannin, saponoside, carbohydrate and terpnes (Table 07).

Table 07: Phytochemical essays of aqueous extract *Ocimum basilicum* L.

Phytochemical composition	Aquatic extract of *Ocimum basilicum* L.
Flavonoids	+++
Phenols	+++
Catechic Tannin	+++
Saponoside	+++
Carbohydrate	+++
Alkaloid	+++
Terpenes	+++

(+): Present, (-): Absent

II.2.1.1.2. Total phenols and flavonoids concentration

The total phenolic and flavonoids compounds was expressed in terms of gallic acid equivalents (mg of GAE/g sample) and of Quercetin equivalents (mg of QE/g sample) respectively, using the following equation based on the calibration curve: $Y = 0.0045x + 0.009$, $R^2 = 0.995$ for phenolic compounds and $Y = 0.0096x + 0.0521$, $R^2 = 0.994$ for flavonoids compounds. Total phenolic and flavonoids contents of *Ocimum basilicum* L. obtained from water solvent is 77,66 ± 3,27 mg GAE/g and 16,527 ± 0,061 mg of QE/g respectively (Table 08).

Table 08: Total phenols and flavonoids concentration in *Ocimum basilicum* aqueous extract

Compounds	Total phenols (mg of GAE/g of extract)	Total flavonoids (mg QE/g of extract)
Aqueous Extract of *Ocimum basilicum* L.	77,66 ± 3,27	16,527 ± 0,061

II.2.1.2. Infrared analysis of aqueous extract of *Ocimum basilicum* L.

Infrared (IR) analyze of *Ocimum basilicum* L. revealed the existence of hydroxyl group at 3201.07 cm^{-1}. The sharp peak at 1599.49 cm^{-1} is ascribed to the stretching vibration of C=C. The tow peaks of at 2886.41 cm^{-1} and 2830.41 cm^{-1} indicate the existence of C-H function in our plant (Figure 14).

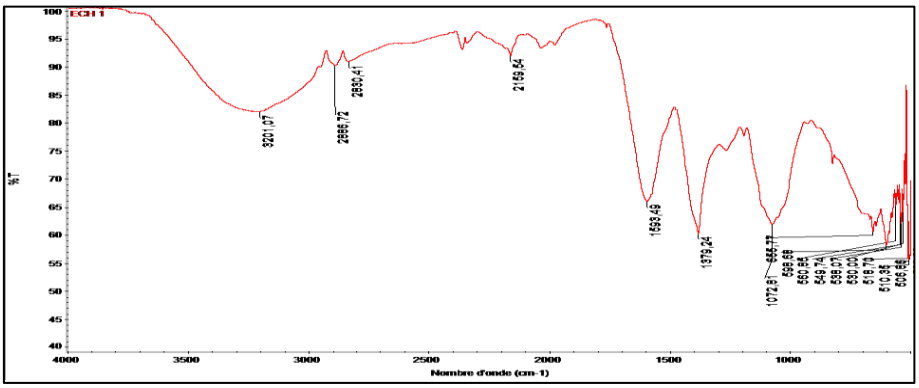


Figure 14: Infrared spectrum of *Ocimum basilicum* L.

II.2.1.3. Antioxidant activity

The antioxidant activity of *O. basilicum* appeared in (Figure 15) show that, IC_{50} values in the DPPH radical scavenging activity assay of the extracts aqueous of *Ocimum basilicum* L. obtained by a percentage inhibition curve as a function of the concentrations of the extract of *O.basilicum*. Also, the percentage inhibition of ascorbic acid, which is calculated by the equation y=0.6232x +48.111 with R^2 = 0.9607, obtained by a percentage inhibition curve as a function of ascorbic acid concentrations.

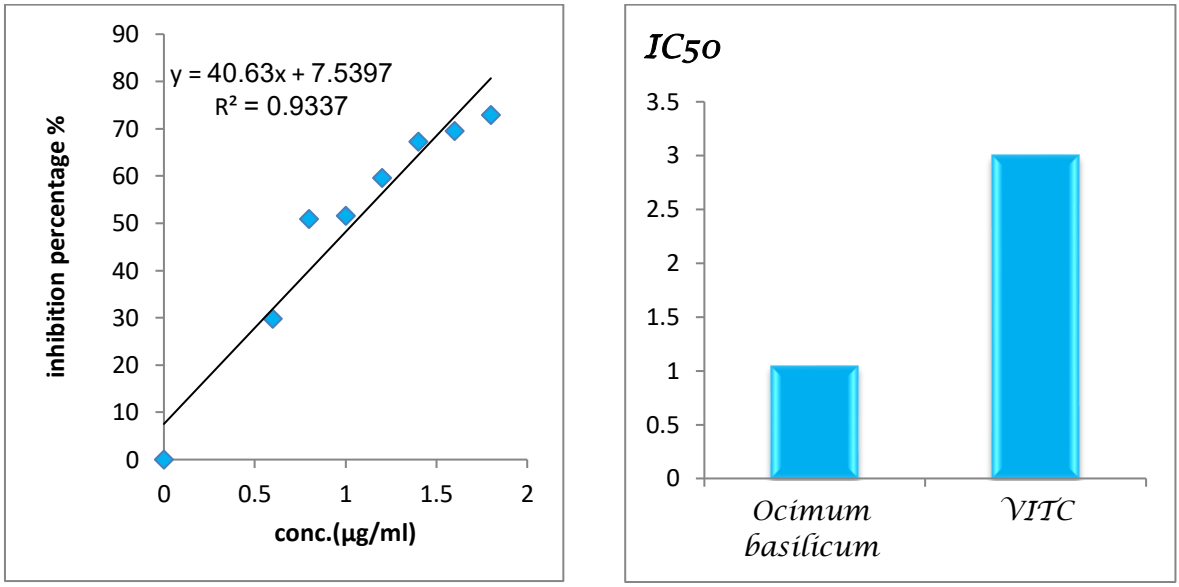


Figure 15: Inhibition percentage and IC_{50} values of *Ocimum basilicum* L. aqueous extract

II.2.1.4. Membrane stabilizing (Hemolysis assay)

As shown in (Figure 16), the results of variability membrane stabilization according to deferent concentrations of aqueous extract of *O. basilicum* which have a high effect in membrane stabilization, increase of *O. basilicum* concentration (80- 100- 120µg/ml) correlated positively with the membrane stabilization.

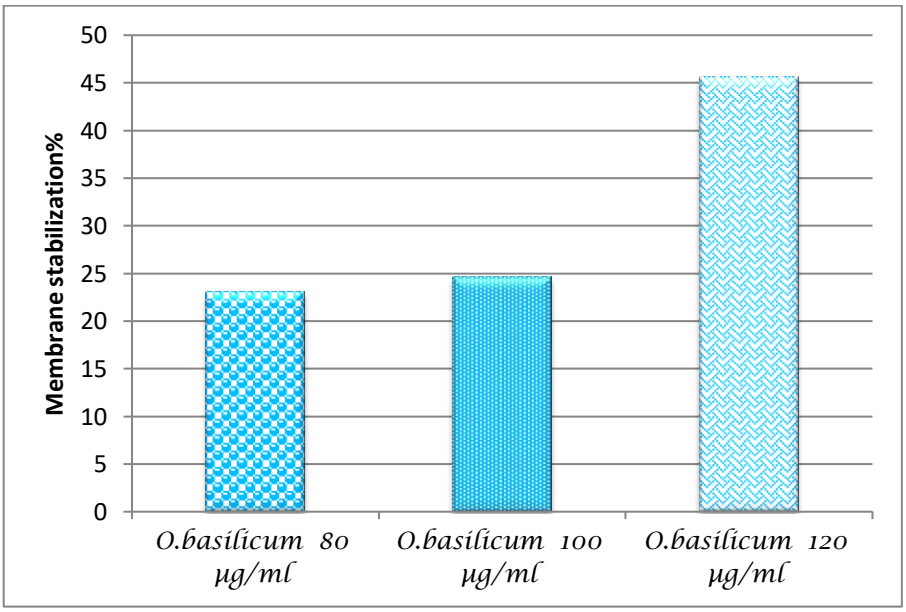


Figure 16: Membrane stabilizing effect of aqueous extract of *Ocimum basilicum* L.

II.2.2. Characterization of magnesium oxide nanoparticles

II.2.2.1. UV-Vis analysis

UV-Vis absorption spectrum of MgNPs is shown in (Figure 17). Broad bell-shaped spectrum band was obtained at the wavelength 300-320 nm from UV-Vis analysis, confirming the formation of MgO.

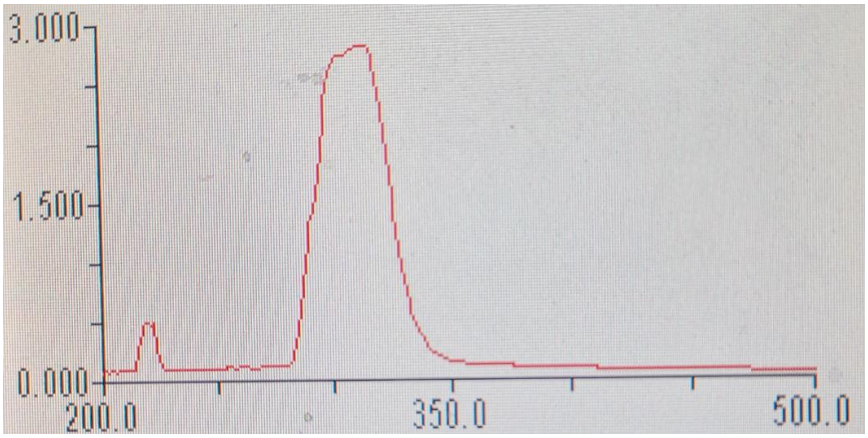


Figure 17: UV-Vis spectrum of MgNPs

II.2.2.2. Infrared spectroscopy analysis

The functional group of MgO powder was analyzed by FTIR spectrophotometer in the range 400-4000 cm^{-1} (Figure 18). The absorption bands for MgO powder were 3353, 1644.8, 1362.06, 818.40, and 602.61 cm^{-1}. The wide band at 3353 cm^{-1} signifies the presence of O–H bond resulting from bending vibrational mode in an hydroxyl while the band at 1644.80 cm^{-1} is assigned to the stretching of C=C. The band at 602.61 cm^{-1} is the characteristic bands of the manganese dioxide nanoparticles MgO_2. The small peak located at 818.40 cm^{-1} are due to C–H stretch of aromatics. An sharp peak on the wave number 1352.06 cm^{-1} due to the vibration of the Mg─O bond indicate the presence of magnesium oxide nanoparticles (MgO)

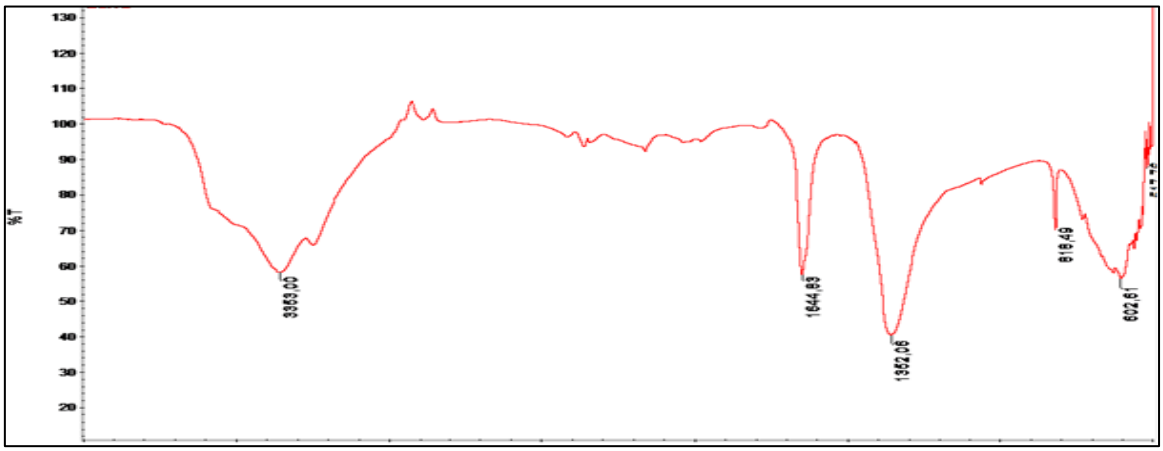


Figure 18: Infrared spectrum of MgNPs

II.2.2.3. Scanning electron microscopy (SEM) analysis

The size of the MgNPs was analyzed through scanning electron microscopy (SEM) images (Figure 19). The size of some selected biosynthesized nanoparticles was 439.2-629.3 nm according to SEM images.

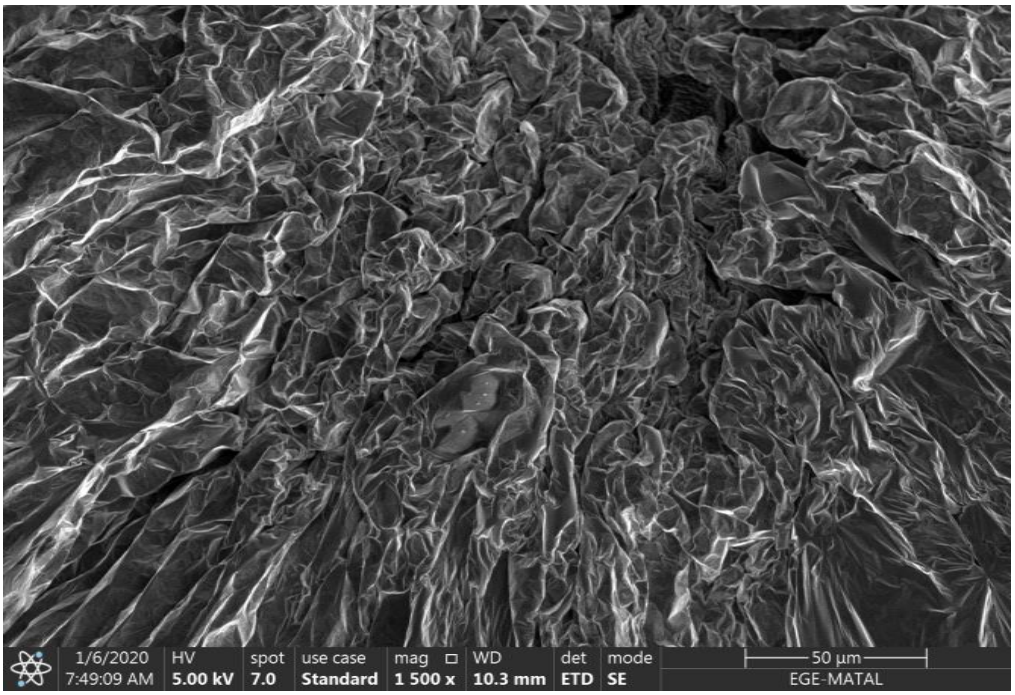


Figure 19: Scanning electron microscopy (SEM) of MgNPs

II.3. Part three: In vivo study

II.3.1. Acute toxicity essays of *Ocimum basilicum* L.

In this experiment, the acute toxicity test was performed on Wistar albino rats for 24 hours. Our plant is used with dose of 2000 mg to 5000 mg /kg weight/rats. The results obtained during this test showed that no mortality was observed before 24 hours, which suggests the non-toxic nature of the aqueous extract of *Ocimum basilicum* L. The other physiological parameters of the rats were also determined during the experimental period and showed that treatment with the aqueous extract of *Ocimum basilicum* L. caused no symptoms or complications and also no adverse effects in the rats during the treatment period (Table 09)

Table 09: Effect of aqueous extract of *Ocimum basilicum* L. on physiological parameters of Wistar albino rats

	0 mg/ kg					**2000 mg/ kg**					**5000 mg/ kg**				
Parameters	**0h**	**3h**	**7h**	**14h**	**24h**	**0h**	**3h**	**7h**	**14h**	**24h**	**0h**	**3h**	**7h**	**14h**	**24h**
Death rats	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eyes	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N, Normal

II.3.2. Acute toxicity test of biosynthesized MgNPs

In this experiment, the acute toxicity test was performed on albino Wister rats for 24 hours. Our magnesium nanoparticles are used with dose of 250 mg to 500 mg per kg of weight of rats. The results obtained during this test showed that no mortality was observed before 24 hours, which suggests the non-toxic nature of the magnesium nanoparticles in the law dose. The other physiological parameters of the rats were also determined during the experimental period and showed that treatment with the magnesium nanoparticles caused no symptoms or complications, also no adverse effects in the rats during the treatment period in control group at dose (0 mg/ kg) and in the group at dose (250 mg/ kg), but in the group at dose (500mg/ kg) we observed death rats in rate of 50% (Table 10).

Table 10: Effect of magnesium nanoparticles on physiological parameters of Wister albino rats

	0 mg/ kg					**250 mg/ kg**					**500 mg/ kg**				
Parameters	**0h**	**3h**	**7h**	**14h**	**24h**	**0h**	**3h**	**7h**	**14h**	**24h**	**0h**	**3h**	**7h**	**14h**	**24h**
Death rats	0	0	0	0	0	0	0	0	0	0	0	50%	/	/	/
Eyes	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N, Normal

Concerning the distribution of drug between the groups studied, we observed that the highest concentration of doxorubicin in drug group then MgNPs group, *Ocimum basilicum* L. group and *Ocimum basilicum* L. + MgNPs group successively (Table 10)

II.3.3. Hematological markers

According to the result of (Table 11), the results of the hematological analysis for the Doxo treated group compared with the control group showed that WBC, lymphocyte, eosinophil and basophil are high decreased, but neutophil and monocyte are showed that no significant differences (P>0.05). As well, our results demonstrated that there was significant variation in the means of WBC, neutrophil and lymphocyte in experimental groups than the drug group. In this study, findings demonstrated that there was a reducing of monocyte in *O. basilicum* and MgNPs groups ($p<0.001$, $p<0.01$) respectively but no significant change in the last group than the control. Furtheremore, there is no significant (P>0.05) variation in the rate of eosinophil in *O. basilicum* L and *O. basilicum* + MgNPs groups with a very high significant incidence in MgNPs groups compared to control. Also, no difference in the levels of basophil in MgNPs and *O. basilicum* + MgNPs groups with a significant raising (P>0.05) in *O. basilicum* than the control.

Table 11: Leukocyte line markers level in control and experimental groups

Paramaters	Control	Doxo	Doxo+ *O.basilicum*	Doxo +MgNPs	Doxo+ *O.basilicum* + MgNPs
White blood cell(10^3/ul)	3.43±0.27	2.04±0.44 **	4.24±0.39 NS c	2.88±0.26 NS b	4.64±0.92 NS b
Neutophil (10^3/ul)	0.78±0.11	0.55±0.09 NS	1.35±0.16 ** c	1.19±0.09 ** c	1.288±0.23 NS a
Lymphocyte (10^3/ul)	1.98±0.18	1.03±0.14 *	2.52±0.26 NS c	1.44±0.17 * a	2.214±0.34 NS b
Monocyte (10^3/ul)	0.04±0.00	0.18±0.06 NS	0.05±0.01 NS c	0.09±0.02 * b	0.968±0.61 NS
Eosinophil (10^3/ul)	0.10±0.00	0.06±0.009 **	0.06±0.01 * NS	0.02±0.003 *** c	0.048±0.009 *** NS
Basophil (10^3/ul)	0.20±0.02	0.04±0.003 ***	0.22±0.06 NS a	0.06±0.01 *** NS	0.12±0.04 NS

Data are expressed as mean± SD (n= 5). * p<0.05, **p<0.01, ***p<0.001: significantly different from control group.[a] p<0.05, [b]p<0.01, [c]p<0.001: significantly different from Doxo group.

Concerning erythrocyte line markers, our results in (Table 12)showed no significant change (P>0.05) in RBC and PLT, significant decrease (P<0.05) in HGB and very high significant decrease (P<0.001) in HTC percentage in drug group as compared to that in the controls. On the other hand, there is a very highly significant ($p<0.001$) decrease in RBC values of *O.basilicum* group with high significant low of *O. basilicum* + MgNPs when compared to Doxo group (Table 12). Results obtained show that HGB ($p<0.01$) and HTC ($p<0.001$) rates are significantly decreasing simultaneously with a significant elevate ($p <0.05$) in the levels of PLT, results reveals also a very high signification decrease in HGB and HCT level ($p < 0.001$) in MgNPs group and

signification decrease (P<0.05) *O. basilicum* + MgNPs with no significant change (P>0.05) as compared to Doxo group.

Table 12: Erythrocyte line markers level in control and experimental groups

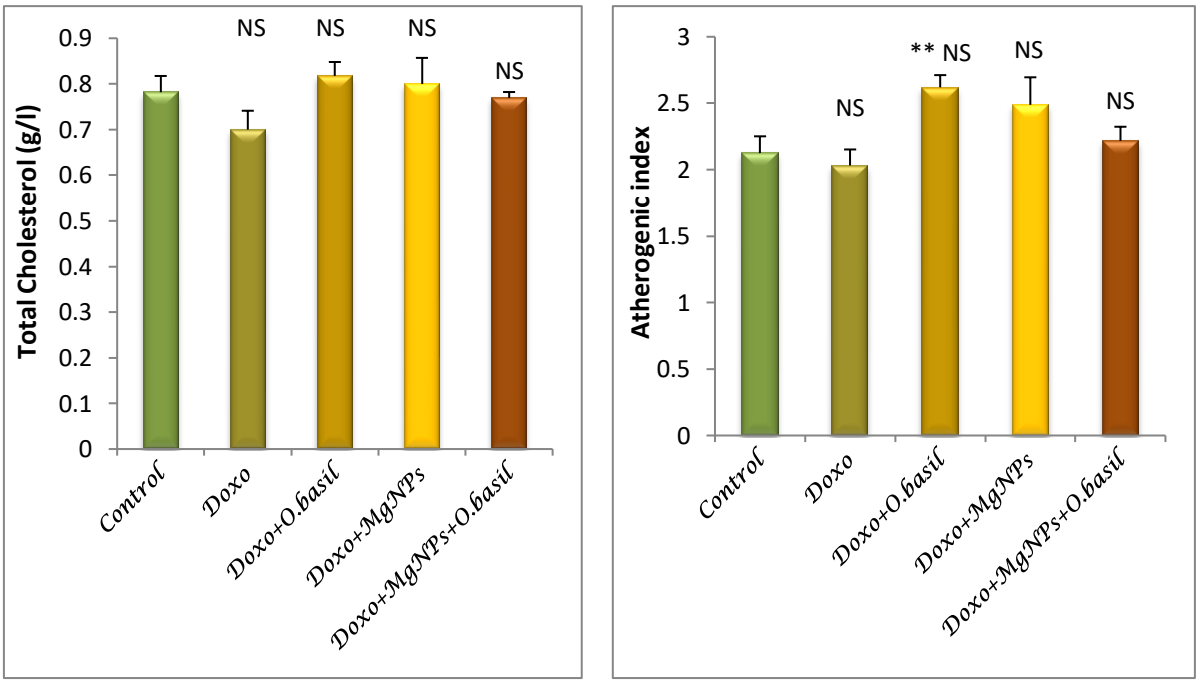
Paramaters	Control	Doxo	Doxo+ *O.basilicum*	Doxo +MgNPs	Doxo+ *O.basilicum* + MgNPs
Red blood cell (10^6/ul)	8.37±0.061	8.20±0.166 NS	7.69±0.09 *** c	7.37±0.192 *** b	7.59±0.17 ** b
Hemoglobin (g/dl)	14.32±0.03	13.9±0.132 *	13.2±0.17 *** b	12.84±0.14 *** c	12.86±0.37 ** a
Hematocrit (%)	49.14±2.59	43.93±0.36 ***	41.65±0.74 *** a	41.12±0.432 *** c	40.7±1.16 *** a
Platelet (10^3/ul)	896.8±67.4	1232±135 NS	1362.5±48.3 *** a	1259.5±15.4 *** NS	1150±105 * NS

Data are expressed as mean± SD (n= 5). * p<0.05, **p<0.01, ***p<0.001: significantly different from control group.[a] p<0.05, [b]p<0.01, [c]p<0.001: significantly different from Doxo group.

II.3.4. Biochemical parameters

II.3.4.1. Lipid profil

The results of lipid profile show in (Figure 20). No significant change in all the lipid parameters excluding TG and VLDL show a significant (p>0.05) decrease in toxic group as compared to the control group. When we compare between Doxo with the others groups we note no significant change (p>0.05).



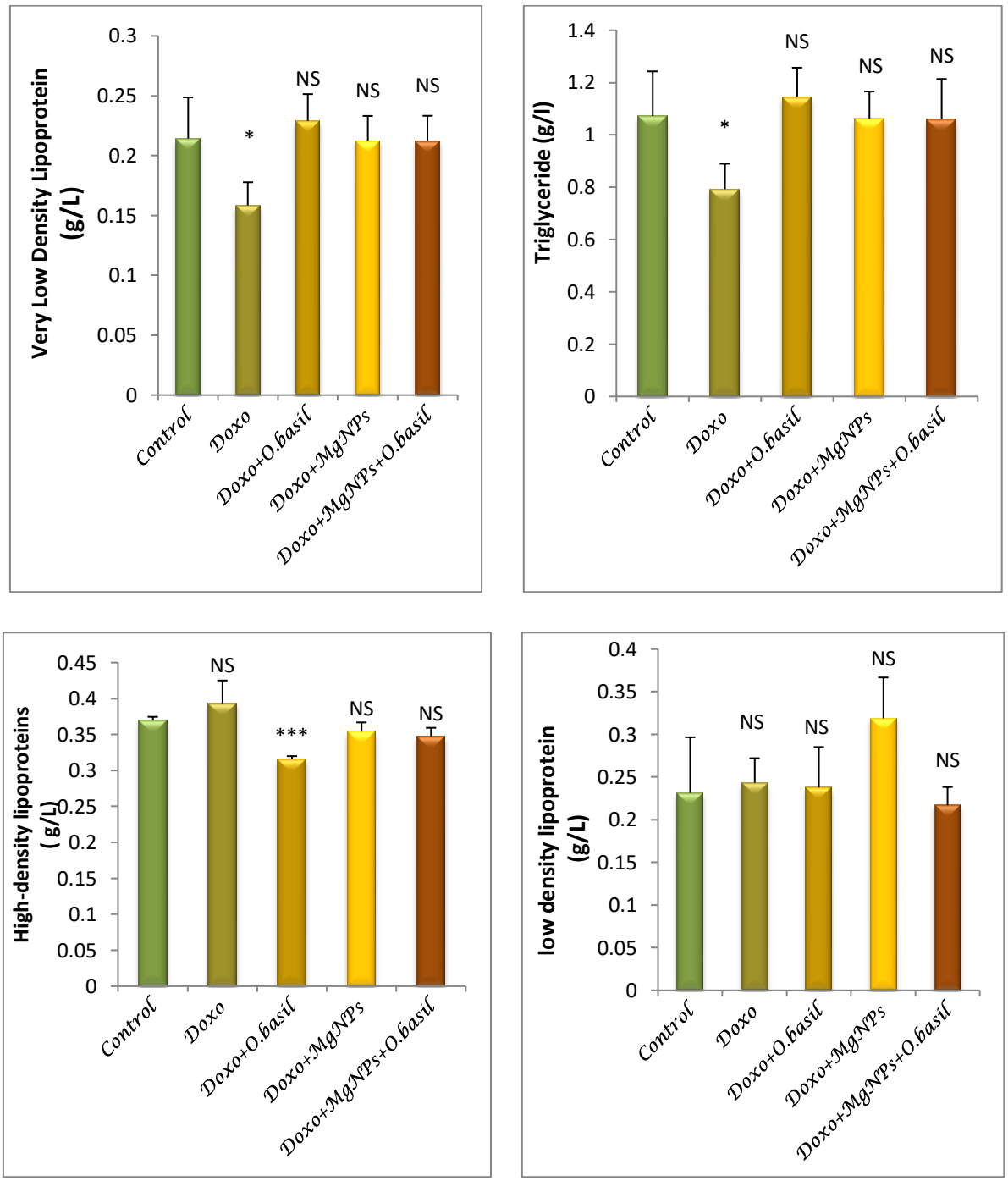


Figure 20: lipid profile levels of control and experimental groups. * p<0.05, **p<0.01, ***p<0.001: significantly different from control group. [a] p<0.05, [b]p<0.01, [c]p<0.001: significantly different from Doxo group.

II.3.4.2. Blood glucose level

As for which level illustrated in (Figure 21). Below, the results of blood glucose parameters showed a high significant increase (p<0.01) in Doxo group compared to the control. In the other side, (Figure 21) shown that blood glucose is significant decrease (p<0.05) in rats treated with *O. basilicum*, also no significant difference (p>0.05) in MgNPs and *O. basilicum* + MgNPs groups compared to the Doxo group.

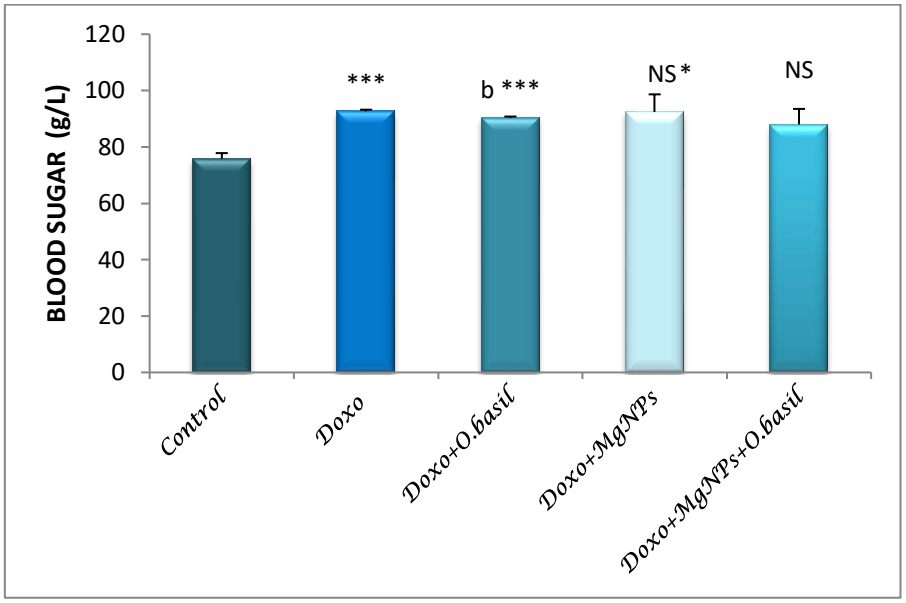


Figure 21: blood sugar levels of control and experimental groups. * p<0.05, **p<0.01, ***p<0.001: significantly different from control group. [a] p<0.05, [b]p<0.01, [c]p<0.001: significantly different from Doxo group.

II.3.4.3. Urea and creatinine levels

In (Figure 22), the results showed a high increase in creatinine and urea levels in Doxo group compared to control. Moreover, different variations in experimental groups compared to drug group.

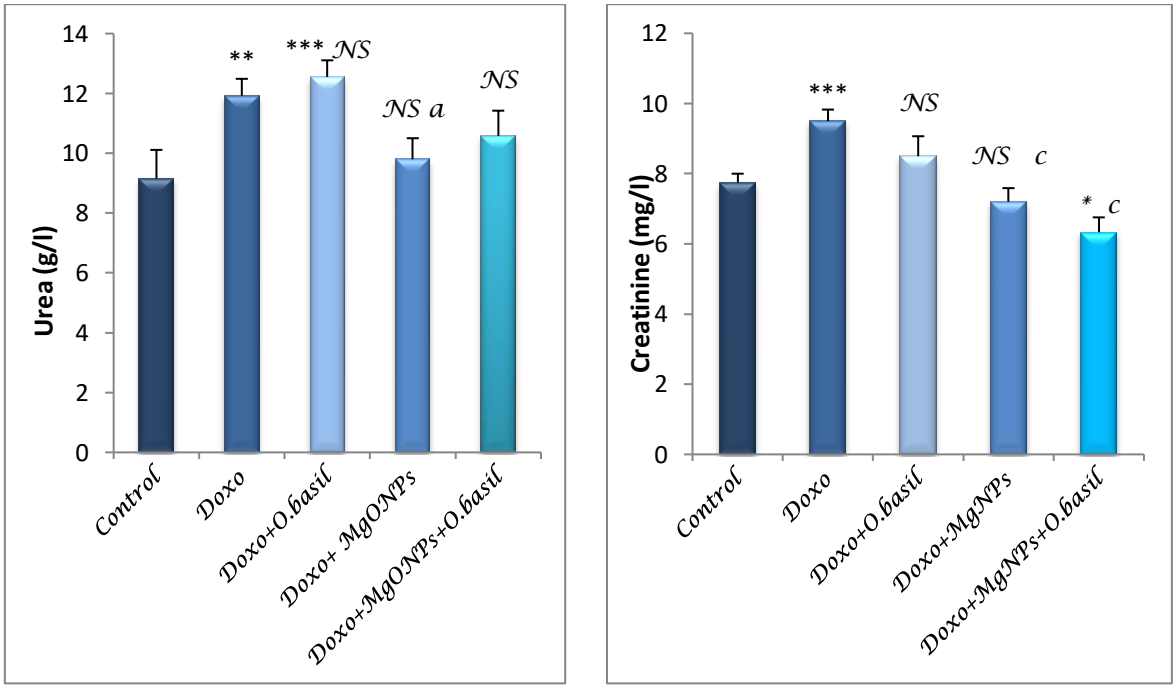


Figure 22: Creatinine and urea levels of control and experimental groups. * p<0.05, **p<0.01, ***p<0.001: significantly different from control group. [a] p<0.05, [b]p<0.01, [c]p<0.001: significantly different from Doxo group.

II.3.5. Enzymatic activities

As shown in (Figure 23), the results of transaminases enzymes activities appeared an increase of GPT but not significantly, counter to diminution highly significant ($p<0.01$) in GOT activities in Doxo group as compared to the control one. In addition, a significant decrease in the mean of GPT, increase no significant in *O.basilicum* and *O.basilicum* + MgNPs and lowering highly signification ($p<0.01$) in MgNPs as compared to the Doxo one.

Regarding the Enzymatic activity activity, the results obtained in (Figure 24) represent a no significant increase in serum of LDH and a very highly significant increase ($p<0.001$)in serum of CPK activity in toxic group as compared to the control one.

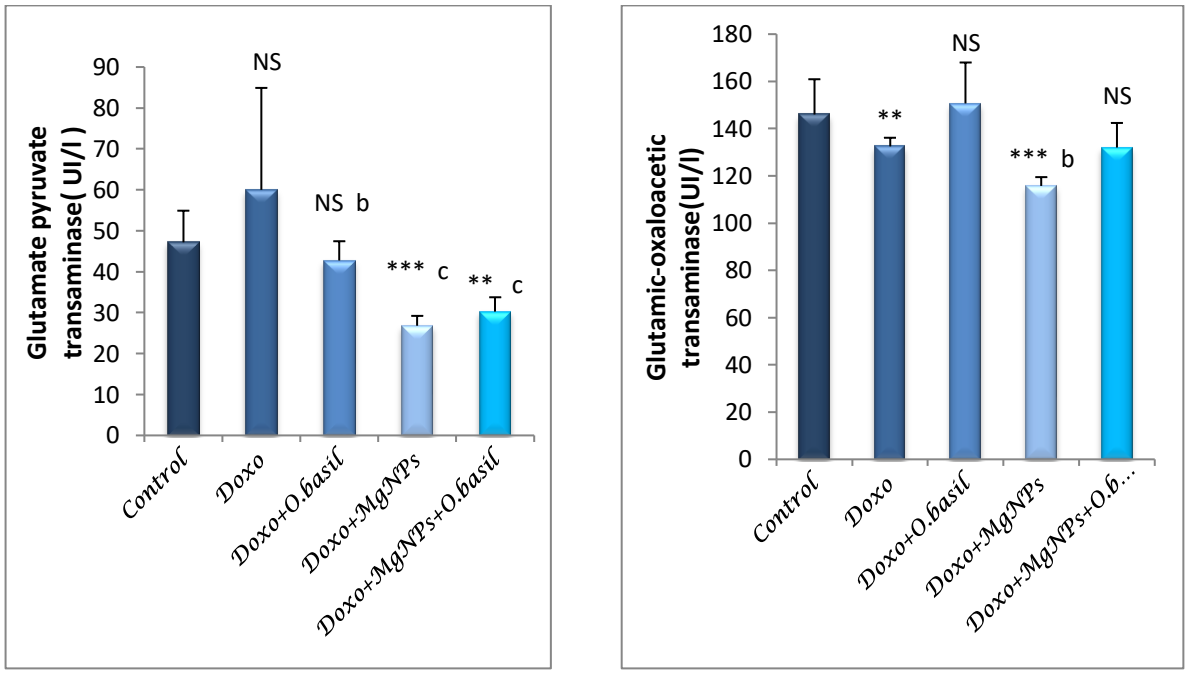


Figure 23: GPT and GOT activities of control and experimental groups. * $p<0.05$, ** $p<0.01$, *** $p<0.001$: significantly different from control group. [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$: significantly different from Doxo group.

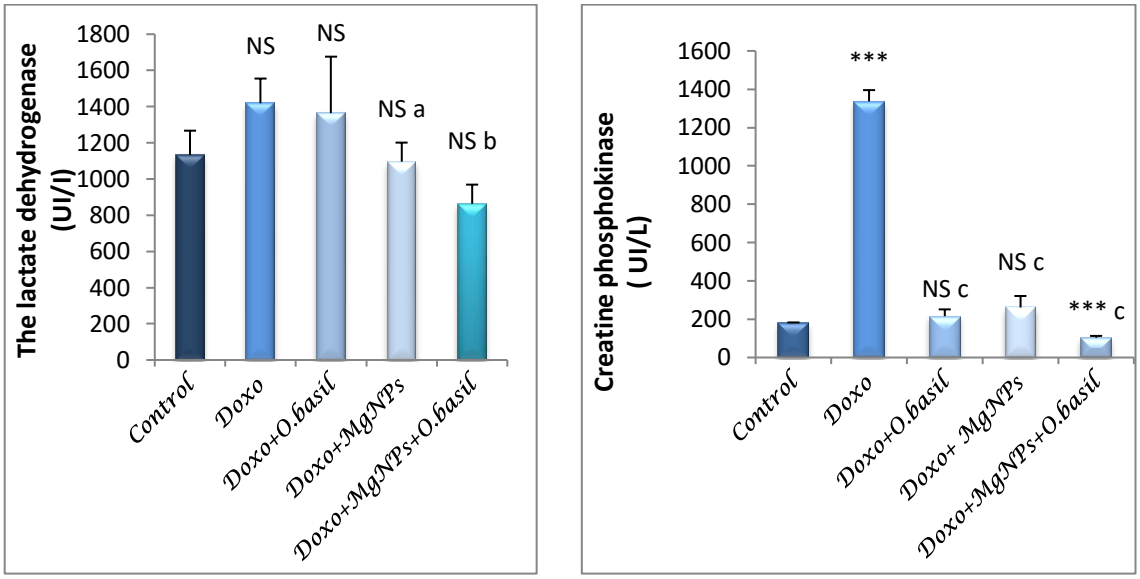
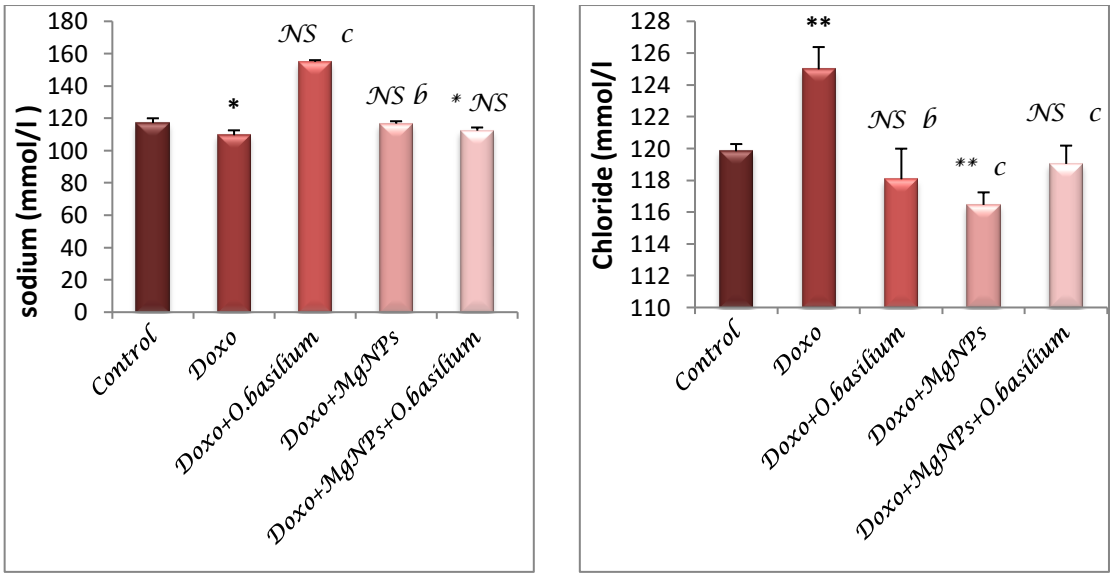


Figure 24: CPK and LDH activities of control and experimental groups. * p<0.05, **p<0.01, ***p<0.001: significantly different from control group. [a] p<0.05, [b]p<0.01, [c]p<0.001: significantly different from Doxo group.

II.3.6. Electrolyte levels

Electrolytes parameters illustrated in (Figure 25) showed that there was significant decrease in Na^+ and Ca^+ levels (p<0.05, p<0.01) respectively in Doxo group compared to the control. Also treatment with *Ocimum basilicum* L. showed a very highly signification (p<0.001) increase, as well rise in MgNPs group compared to the Doxo group. Furthermore, a raising in Cl^- and K^+ levels in drug group compared to the control one. *O. basilicum*, MgNPs and *O. basilicum* + MgNPs treatment decrease the levels of Cl^- and K^+ compared in Doxo induced heart failure animals.



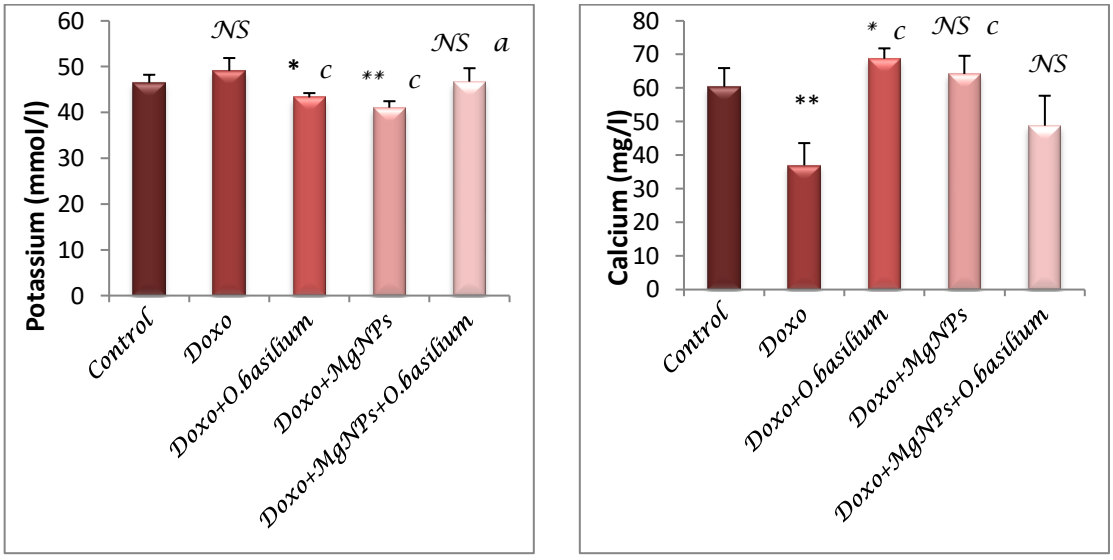


Figure 25: Na^{+2}, Ca^{+2}, Cl^{-} and K^{+} levels of control and experimental groups. * p<0.05, **p<0.01, ***p<0.001: significantly different from control group. [a] p<0.05, [b] p<0.01, [c] p<0.001: significantly different from Doxo group.

II.3.7.Oxidative stress parameters

II.3.7.1. Reduced glutathione (GSH) levels

Our results (Figure 26) showed that a significant decrease (p< 0.01) of GSH level of liver and heart in Doxo group as compared to the control one. In liver, GSH levels showed a significant decrease in experimental group treated by *O. basilicum* extract, *O. basilicum* + MgNPs (p<0.001) and a significant augmentation (p<0.0 1) in MgNPs compared to the Doxo, however, the results showed an increase significant in GSH level of heart in MgNPs group (p<0.05), but on the contrary, *O. basilicum* + MgNPs group showed a very highly significant diminution (p<0.001).

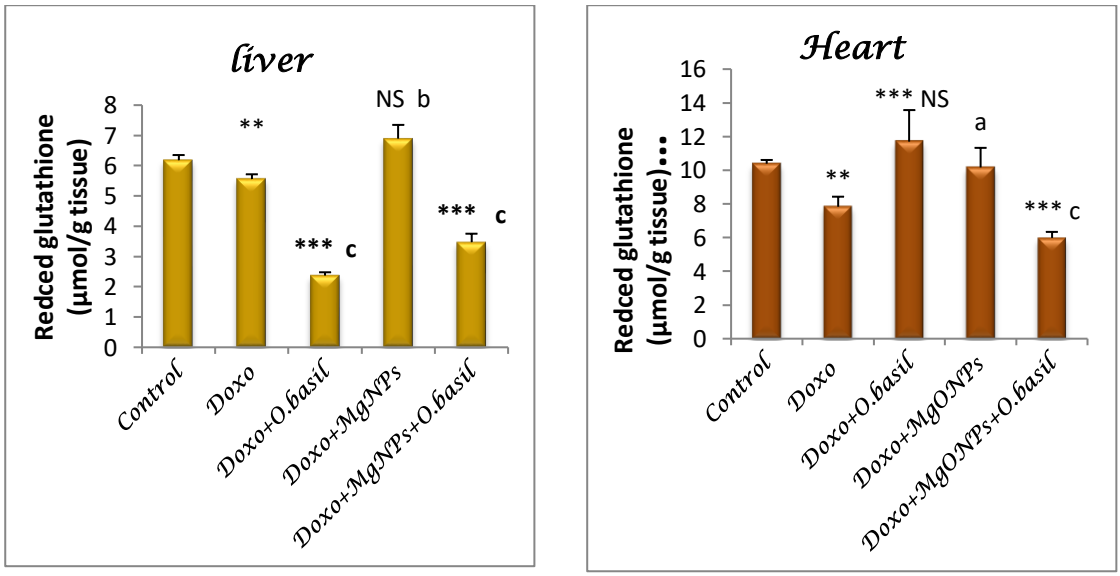


Figure 26: GSH levels in liver and heart in control and experimental groups. * p<0.05, **p<0.01, ***p<0.001: significantly different from control group. [a] p<0.05, [b] p<0.01, [c] p<0.001: significantly different from Doxo group.

II.3.7.2. Malondialdehyde (MDA) levels

Heart and liver MDA levels showed in (Figure 27). A very highly significant ($p<0.001$) raise of the MDA level of heart and highly significant ($p<0.01$) increase of liver in Doxo group compared to the control, but treatment *O. basilicum* , MgNPs and *O. basilicum* + MgNPs signification decrease ($p<0.001$, $p<0.01$) in MDA level in heart and liver compared to the DOXO group.

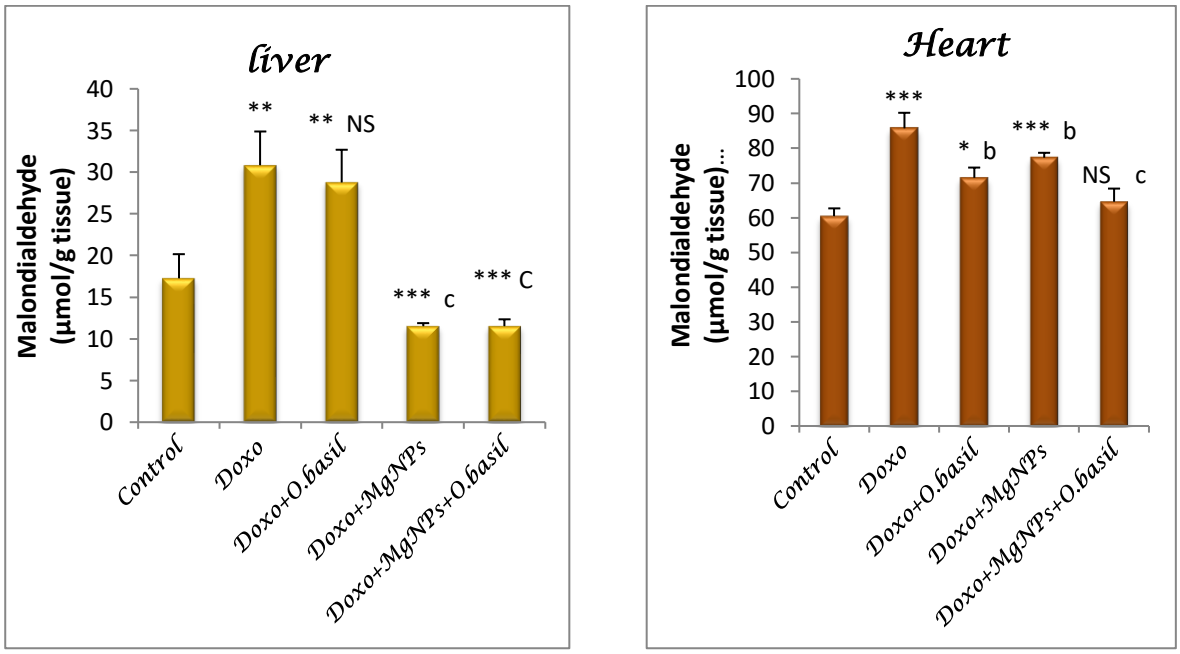


Figure 27: MDA levels in liver and heart in control and experimental groups. * $p<0.05$, ** $p<0.01$, *** $p<0.001$: significantly different from control group. [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$: significantly different from Doxo group.

II.3.7.3. Superoxide dismutase (SOD) activity

Results of SOD enzyme activity is illustrated in (Figure 28) appeared a high significant increase of SOD activity of heart and a very high significan t increase of liver in Doxo group compared to the control. On the other hand, the treatment rats by *O. basilicum* extract alone or combined with MgNPs and MgNPs groups showed a very high significant diminution ($p<0.001$) in SOD of liver. For heart SOD activity, *O. basilicum* and *O. basilicum* + MgNPs significantly augmentation ($p<0.01$ and $p<0.001$) the SOD activity respectively compared to drug one. Also treatment with MgNPs showed a very high signification decrease ($p<0.001$).

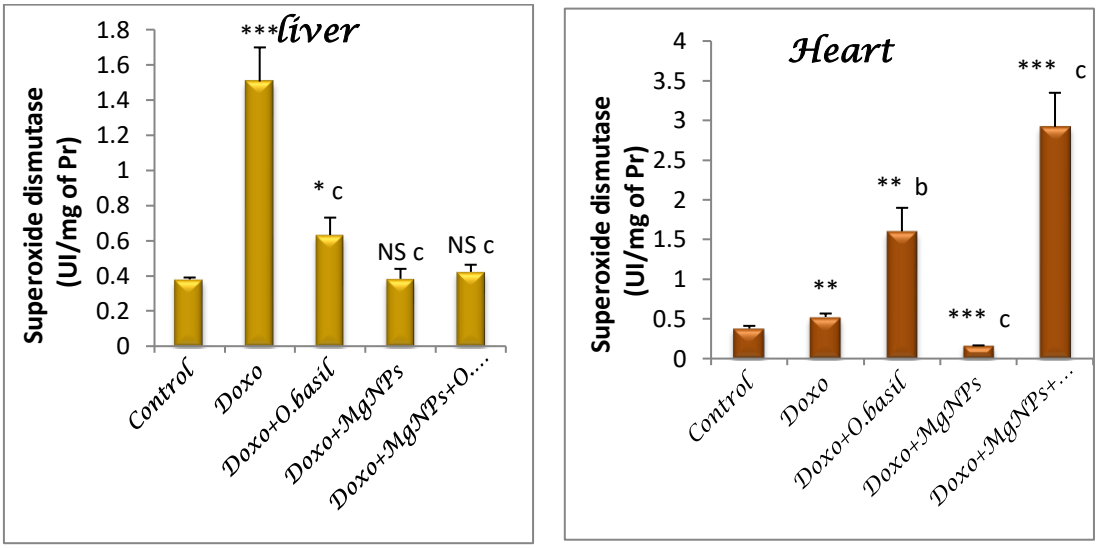


Figure 28: SOD activity in liver and heart in control and experimental groups. * p<0.05, **p<0.01, ***p<0.001: significantly different from control group. [a]p<0.05, [b]p<0.01, [c]p<0.001: significantly different from Doxo group.

II.3.7.4. Glutathione s transferase (GsT) activity

Liver and heart GST activity shows in (Figure 29). For liver, GST activity had a high significant increase (p<0.01). For heart, our results indicated a significant augmentation (p<0.05) in experimental cardiotoxic group compared to the control. But treatment *O. basilicum*, MgNPs and *O. basilicum* + MgNPs significant decreased (p<0.001) in the GST activity in liver compared to the Doxo group. On the other hand, GST activity in heart demonstrated a very high significant increase (p<0.001) in *O. basilicum* and *O. basilicum* + MgNPs groups but in the MgNPs group we observed a very high significant decrease (p<0.001).

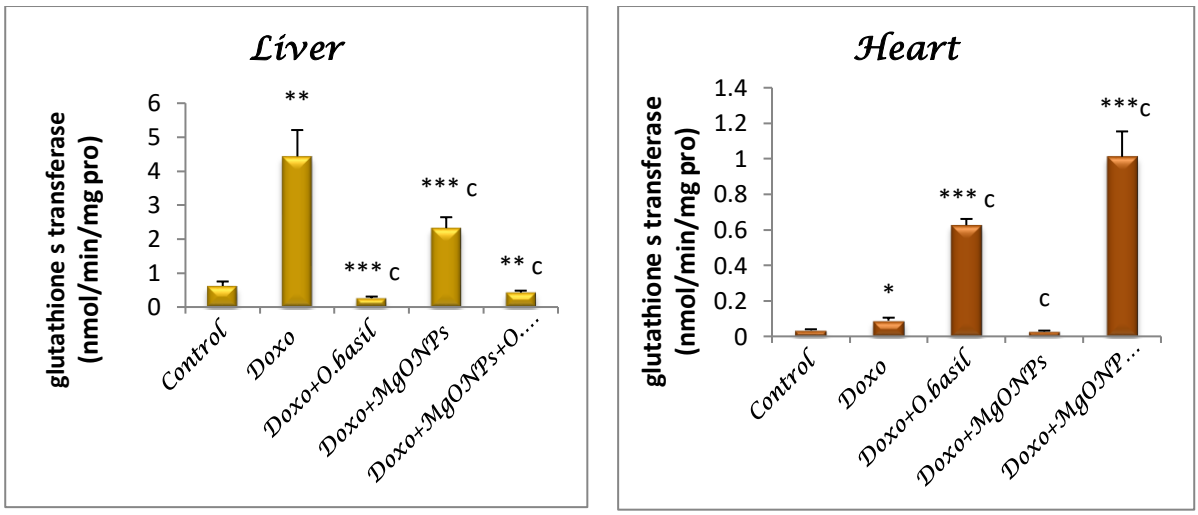


Figure 29: GST activity in liver and heart in control and experimental groups. * p<0.05, **p<0.01, ***p<0.001: significantly different from control group. [a]p<0.05, [b]p<0.01, [c]p<0.001: significantly different from Doxo group.

II.3.8. Correlation between biological markers

Our results present a significant negative correlation between the redced glutathione and white cell blood level also significant negative correlation between the redced glutathione and monocyte level (Figure 30).

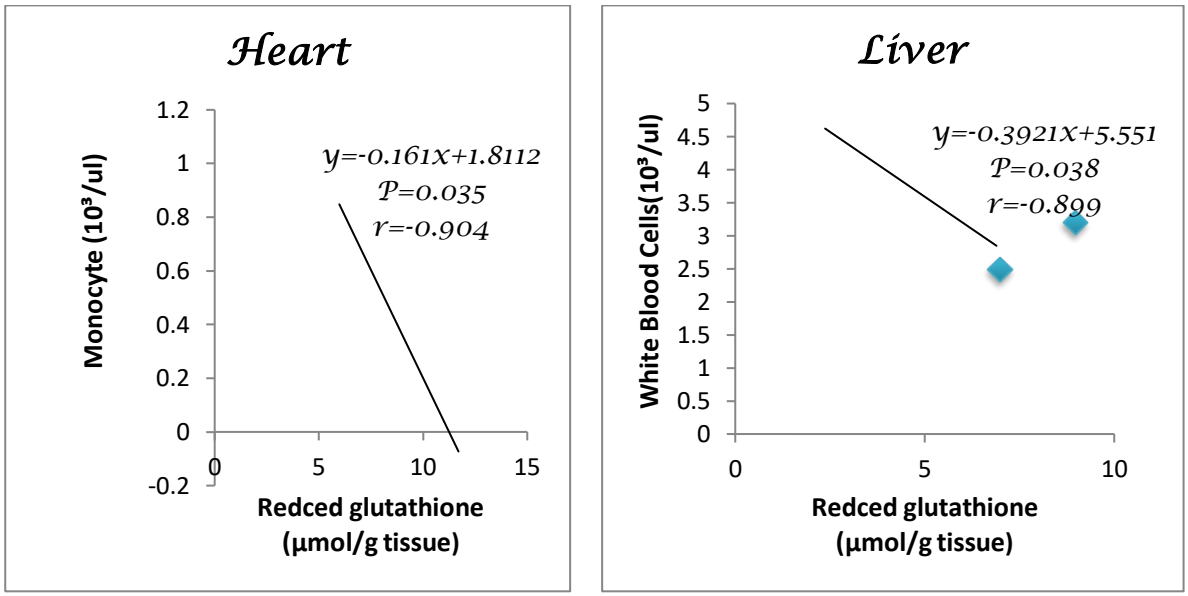


Figure 30: Variation of WBC and Monocyte level on function of variation of GSH of liver & heart in the experimental rats

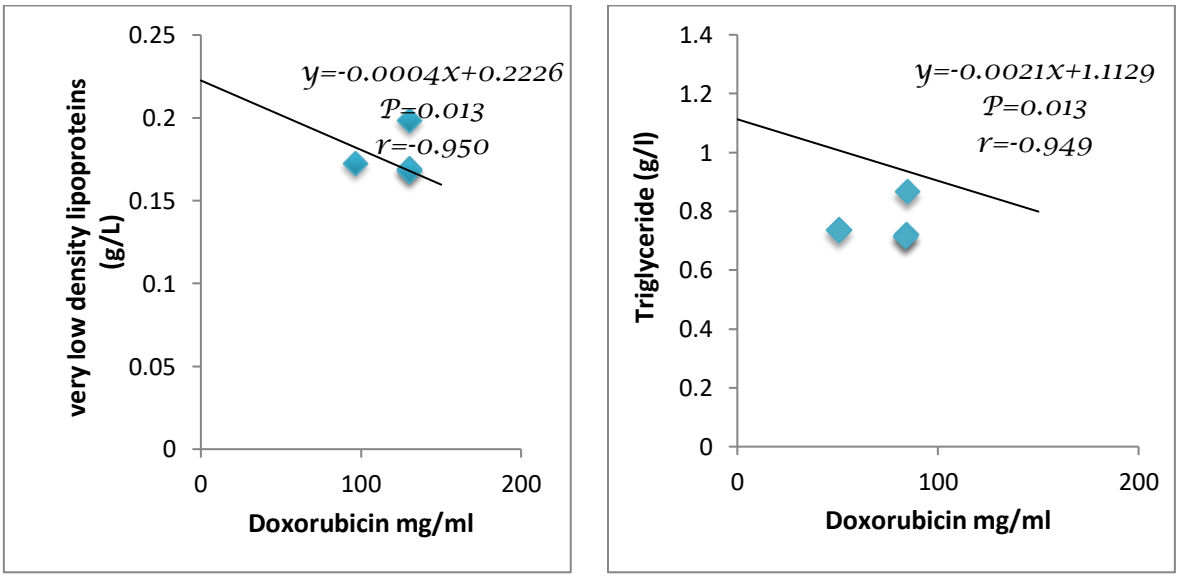
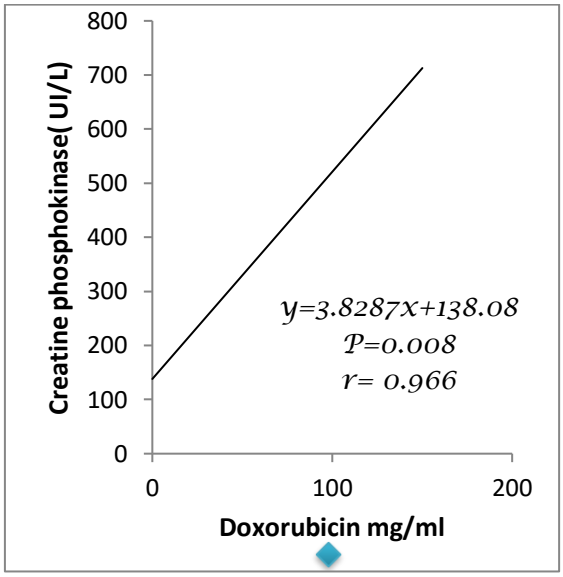


Figure 31: Variation of VLDL-c and TG level on function of variation of doxorobucin in the experimental rats

Our results present a significant negative correlation between the doxorubicin and VLDL level significant negative correlation between the doxorubicin and TG level (Figure 31).



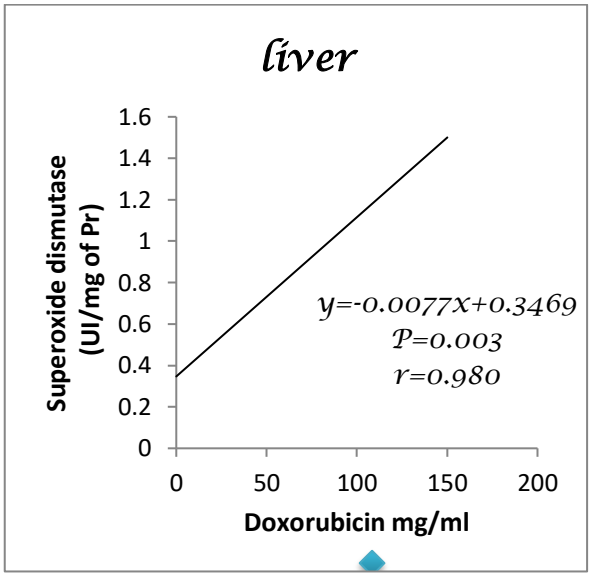


Figure 32: Variation of CPK and SOD of liver level on function of variation of doxorobucin in the experimental rats

Our results present a high significant positive correlation ($r = 0.966$; $p < 0.01$) between the doxorubicin and CPK activity. Also significant positive correlation ($r = 0.980$; $p < 0.01$) between the doxorubicin and SOD of liver level (Figure 32).

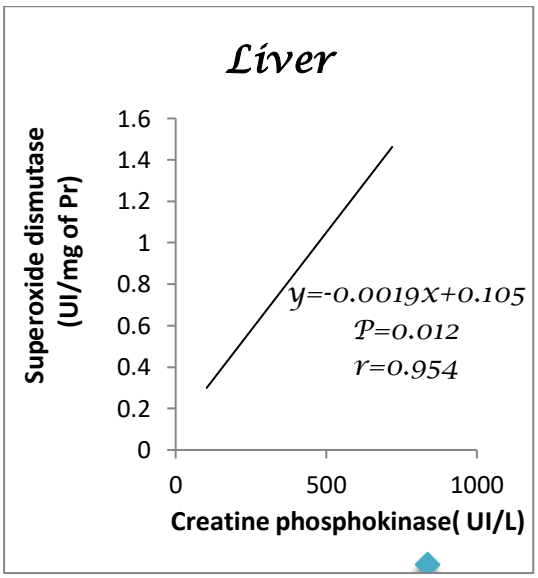


Figure 33: Variation of sod level on function of variation of CPK in the experimental rats

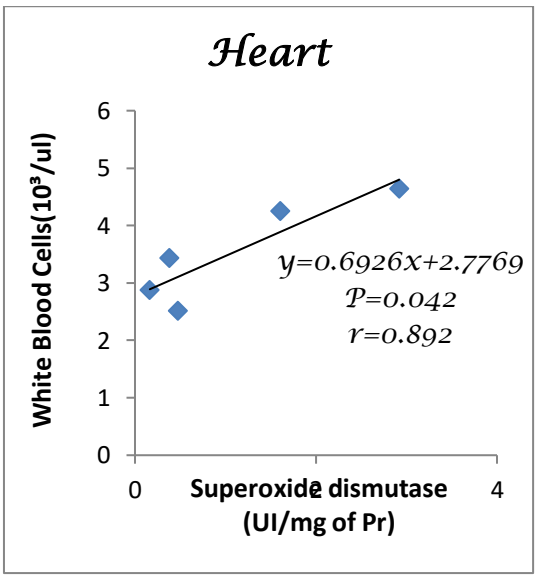


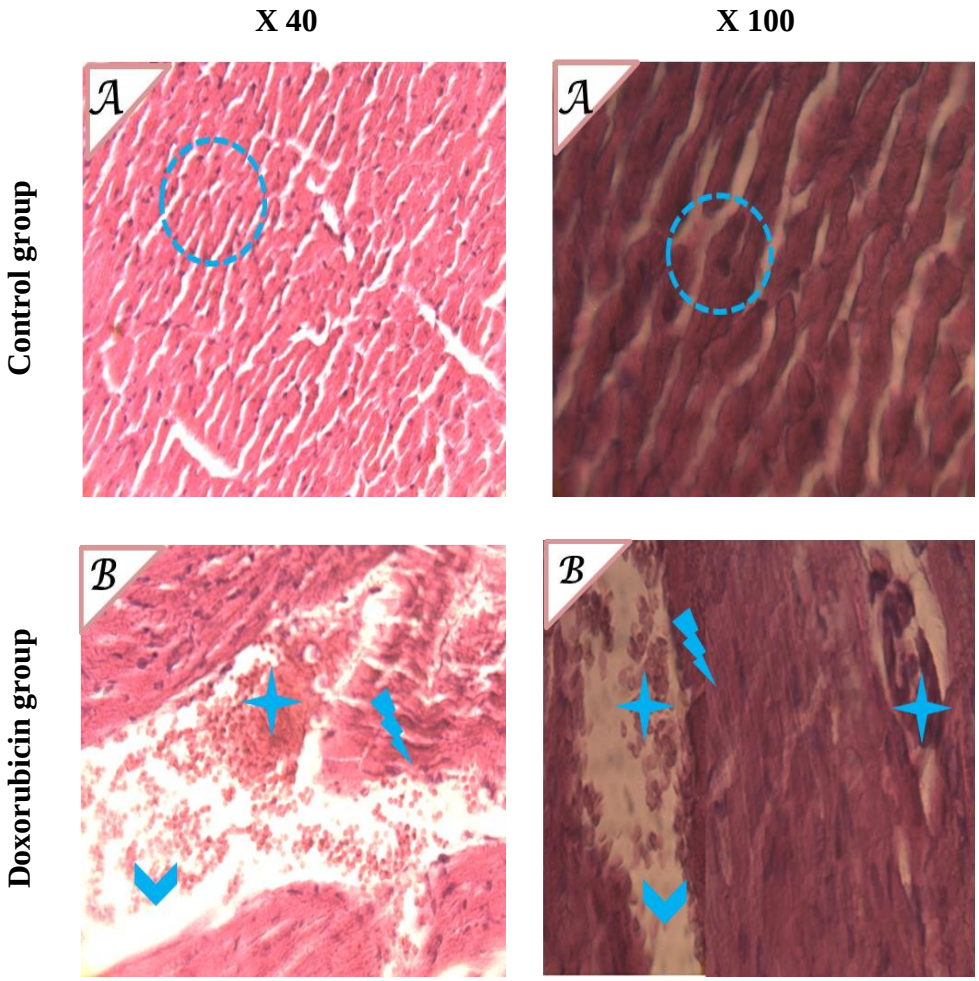
Figure 34: Variation of WBC level on function of variation of SOD of heart in the experimental rats

Our results show a significant positive correlation between the mean of SOD and CPK activities (Figure 33).

Our results indicate a significant positive correlation between white cell blood level and SOD activity (Figure 34).

II.3.9. Heart histological results

Photomicrograph of the heart tissues of control showed the normal myofibrillar structure with striations and branched appearance and normal cells (Figure 35 A). Heart tissues of Doxo-treated rats also showed deformations of muscle fibers with focal hemorrhage, inflammatory cell infiltrations and vacuolization of cardiomyocytes (Figure 35 B). Microscopic section of *O. basilicum* treated group, rat heart showed less infiltration of inflammatory cells, normal appearance of heart cells (Figure 35 C). Heart sections of the rats administered MgNPs dose (50mg/Kg) showed moderate degree of heart damage, less focal hemorrhage, less vacuolization of cardiomyocytes (Figure 35 D). The results of *O. basilicum* combined with MgNPs showed lesion of heart sections, hemorrhage and inflammatory cell infiltrations less than heart tissues of Doxo-treated rats" B" (Figure 35 E) .



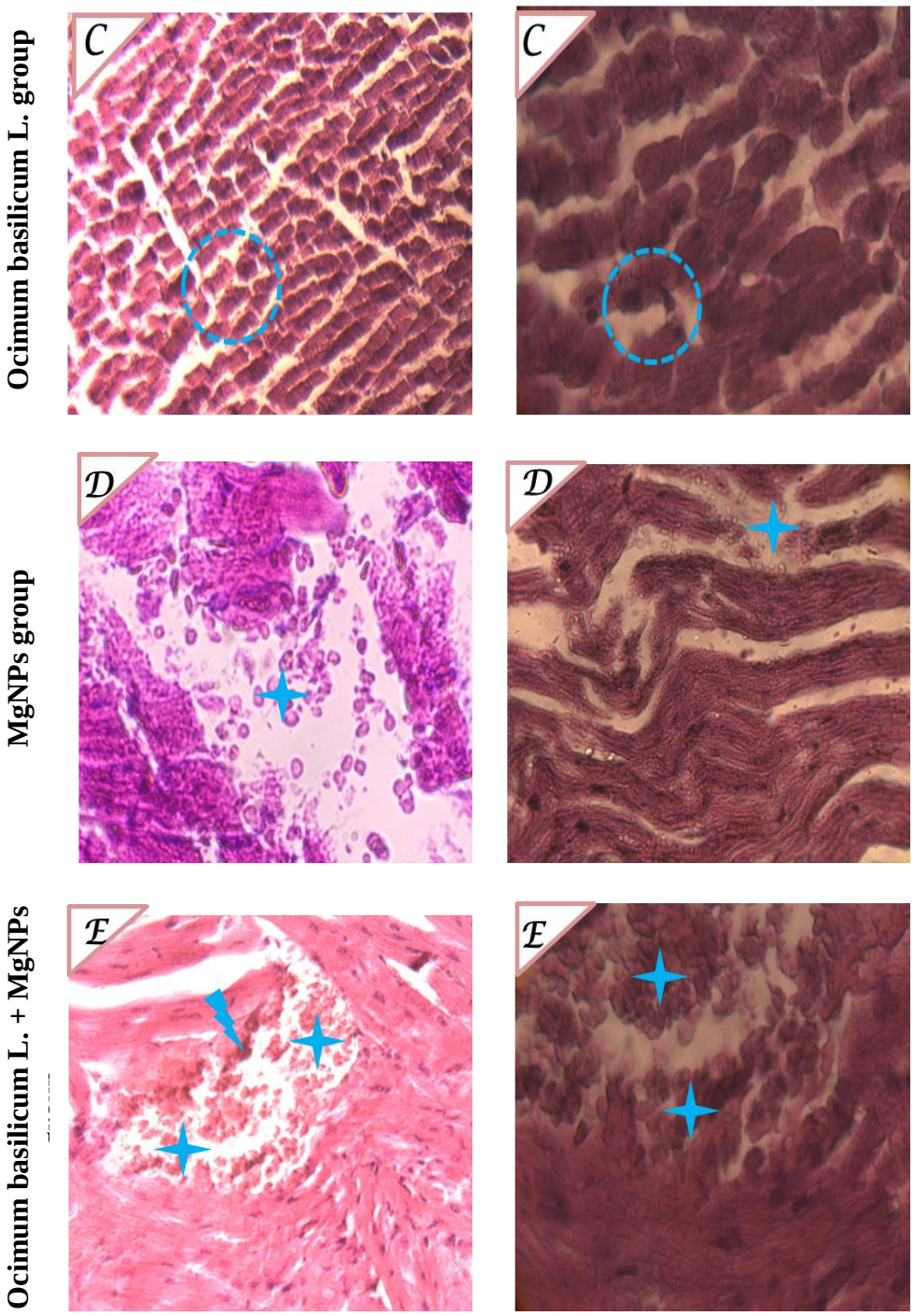


Figure 35: Photomicrographs of heart of all the experimental groups after treatement. Haematoxylin and Eosin X40 and X100.

Table 13: Photomicrographs of heart of all the experimental groups after treatments

Groups	Hemorrhage	Inflammation	Vacuolization
Control	- - - -	- - - -	- - - -
Doxorubicin	+ + + +	+ + + +	+ + + +
Doxorubicin + *Ocimum basilicum* L.	+	-	- -
Doxorubicin + MgNPs	+ +	- - -	+
Doxorubicin + MgNPs + *Ocimum basilicum* L.	+ + +	- - -	- - -

Chapter III

Discussion

Doxorubicin (Doxo) (Adriamycin) is an anthracycline chemotherapeutic drug that is used alone or with other treatments/medications to treat several different types of cancer (Ajzashokouhi et al., 2019), so doxorubicin (Doxo) is a broad-spectrum anticancer drug with high efficacy in numerous solid tumors and hematological malignancies (Abboud et al., 2018), but its clinical using is limited because of its cardiotoxicity (Hosseini & Rajabian, 2016). A variety of hypotheses has been suggested to explain the damaging effects of doxorubicin on the heart (Doroshow et al., 2020).

III.1. Part one : patient study

III.1.1.Biochemical markers

Anthracycline (doxorubicin) is widely used to treat very kind of cancer including breast cancer. Although it can produce many side effects such as hepatotoxicity (Zainab, 2018) and renal insufisance (Dorsaf et al., 2017). Doxorubicin is able to enter the outer mitochondrial membrane and enter the cytosol (Kamal et al., 2012). Result of our study revealed that there is an elevation in the levels of GOT, GPT and GGT activities which explained by accumulation in liver cell (Alciona et al., 2016) also this alteration may be due to a cardiac hepatopathy that is the term that describes liver damage due to cardiac diseases (Teodora et al., 2018). We can explain this liver or cardiac damage by oxidative stress induced by doxorubicin, which has been confirmed in many experimental models (Metra et al., 2012). The mechanism of this oxidative stress is based on the release of free radicals following a reduction of the doxorubicin molecule by the enzyme NADPH oxidase and cytochrome P-450 reductase which increases the level of malondialdehyde (MDA) and decreases the activity of superoxide dismutase (SOD) and catalase (Damodar et al., 2014).

Doxorubicin induces cardiotoxicity through upregulation of death receptors (TNFR1, Fas, DR4 and DR5) mediated apoptosis in cardiomyocytes (Zhao & Zhang et al., 2017), so an elevated serum LDH enzyme activity is the consequence of increased anaerobic glycolysis. Also, the toxicity of doxorubicin induced a cellular lysis which makes it possible to leave certain mitochondrial enzyme what explains the increase in the activity of serum LDH (Basnyat et al., 2017).

In this study, we demonstrated that the incidence of dyslipidemia in cancer patients was significantly higher than that in control group and the total serum levels of cholesterol and triglycerides are significantly higher in cancer patients under doxorubicin treatment than those in normal controls and this due to that many chemotherapy agents are cardio-toxic and some appear to also alter lipid profiles (Sharma al., 2016). Which can cause obesity which can trigger the inflammatory pathways by activating the necrosis factor (Humaira et al., 2018) which can damage the myocardium. Also during chemotherapy, up to 85% of patients develop hepatic steatosis that

points to a malfunctioning lipid metabolism by altering the level of hepatocyte lipoprotein synthesis (Jiang et al., 2013) and may cause necrosis due to lipid accumulation (Derouiche & Zeghib, 2019). Hypercholesterolemia, hypertriglyceridemia is a well-known risk factor in cardiovascular disease (Velli et al., 2016).

Our results showed that non-significant change ($p>0.05$) was noticed in serum urea level in patients compared to controls. Opposite findings were observed in result of (Refaie et al., 2016) which found the elevation of serum urea in rats treated with doxorubicin compared to control.

III.1.2.Hematological markers

About hematological markers which designed to measure hemoglobin concentration, hematocrit and red blood cell count for the diagnosis of anemia in volunteer's women, according to our result, we found that doxorubicin intoxication might lead to anemia as a result of either altered activity of hematopoietic tissues, impaired erythropoiesis, and/or defective iron metabolism (Afsar et al., 2017). Hemoglobin is a protein with the property of fixing transporting and delivering oxygen (Derouiche et al., 2019). Therefore, anemia occurs when the number of red blood cells (or the Hb in them) fall below normal and the body gets less oxygen and therefore has less energy than it needs to function properly (Khan et al., 2013). The explanation of hemoglobin result is based on erythropoietin which is a natural glycopeptide hormone developed by the kidneys also by the liver which stimulates erythropoiesis, the process of production of erythrocytes (red blood cells) (Derouiche et al., 2018).

Our outcomes demonstrated a decrease in rate of lymphocyte of patient compared to control. The lymphocyte count is an important auxiliary diagnostic test as changes occur with systemic inflammation and other disease (Atoussi et al., 2018). A decrease in lymphocyte levels due to certain syndrome such as acute coronary syndrome, which explains the low percentage of lymphocytes following cardiovascular disease (Turfan et al., 2014). Other, the levels of white blood cell, neutrophil , monocyte ,eosinophil ,basophil and platelet were non-significant different in patient group as compared to the control, these findings are similar to the study of (Molyneux et al., 2010) which found that the doxorubicin at a dose 1.6 mg/kg have no effect on leucocyte line in female mice.

III.2. Part two: in vitro study

The objectives of the present study is the evaluation of the protection effect of aqueous extract of *Ocimum basilicum* L. alone or associated with magnesium oxide nanoparticles against cardiotoxic induced by doxorubicin drugs in rats.

III.2.1.Qualitative and quantitative study of *Ocimum basilicum* L.

The result of phytochemical screening confirmed the presence of flavonoids, phenols, catechic tannin, saponoside, carbohydrate and alkaloid in aqueous extracts of the leaves of *Ocimum basilicum* L. In agreement with (Tariq et al., 2016), *O. basilicum* is a rich source of secondary metabolites such as glycosides, tannins, phenolic compounds, saponins, flavonoids and terpenes. Terpenes are found to exhibit anti-inflammatory activity. Flavonoids exert anti-oxidative effects as free radical scavengers and possess therapeutic potential against osteoporosis and even cancer. Moreover, consumption of phenolic compounds reduces the risk of coronary heart disease (Tariq et al., 2016).

Antioxidants are compounds which restrain oxidative damage through variable mechanisms such as reacting with free radicals, and acting as oxygen scavengers (Rameshrad et al., 2015). Regarding the antioxidant DPPH test, our results recorded that the aqueous extracts of *O. basilicum* have an antioxidant power. Also, the results show that the aqueous extract of plant is rich on polyphenols and flavonoids. These findings are in concurrence with study of (El-Dakaret al., 2015). Besides, HPLC analysis and quantification of total phenolics, tannin and flavonoid contents in the aqueous sweet basil extract confirmed the demonstrating that the phenolics are the major polar compounds of *O. basilicum* and their data strongly suggest the predominance of phenolic compounds such as flavonoids and tannins (Harnafi et al., 2009). *O. basilicum* has shown antioxidant activities due to its phenolic and aromatic compounds (Gebrehiwot et al., 2016) and (El-Dakaret al., 2015). Antioxidant activity of *O. basilicum* aqueous extracts showed very strong free radical scavenging activity. Is a widely used medicinal plant (Constantinescu & Adriana, 2019). Another study was conducted in 2020 by Pistelli et al. found that basil contains phenolic antioxidant compounds.

Also, basil was frequently reported as a common anti-inflammatory. Aiming to confirm this activity and evaluate the mechanism involved, we performed a series of tests in human erythrocytes cell exposed to a pro-inflammatory agent. The results shows the importance anti-inflammatory activity of *O. basilicum* leaves with deferent concentration (Ahmad et al., 2015), (Fathiazad et al., 2012) and (Rameshrad et al., 2015). Cytokines are subdivided in pro-inflammatory (initiate defense against pathogens) and anti-inflammatory (regulate the inflammatory process helping to balance the inflammatory response) acting with an important role in inflammatory response. The proinflammatory cytokines includes IL-1, IL-2, IL-6, IL-8, and TNF-α, and the anti-inflammatory cytokines includes IL-1 antagonist receptor, IL-4, IL-10, and IL-13 (Güez et al., 2017). The anti-inflammatory activity can be ascribed chiefly to the inhibition of prostaglandin biosynthesis by inhibition of the enzymes cyclooxygenase and lipoxygenase of the arachidonic acid metabolism (Barbalho et al., 2011).

III.2.2.Analysis for flavonoids compounds

In this study, the phenolic compounds have been investigated by HPLC-MS, in aqueous extracts of *Ocimum basilicum* L. After analysis, almost 30 phenolic compounds were identified in the extracts, such as rutin, the phytosphingosine, caffeic acid and the phenolic compounds was based on the retention time. It has been proved by the presence of peaks in various retention time. The extracts of *Ocimum basilicum* L. contain a large amount of total polyphenolic compounds, including flavonoids (rutin and isoquercitrin) and caffeic acid derivatives (Benedec et al., 2012). The antioxidant properties of basil can be attributed to rosmarinic acid, one of the esters of caffeic acid (Barbalho et al., 2011). According to (Purushothaman et al., 2018) the aqueous extract of basil has been separated to specifc compound. Rosmarinic acid and caffeic acid were quantifed in all basil samples using a dual pump waters HPLC system.

III.2.3.Magnesium nanoparticles study

This study confirmed that *Ocimum basilicum* L. has a capability for the biosynthesis of MgNPs. The characteristics of the biosynthesized MgNPs were measured by different experiment technic. The optical properties of metal nanoparticles strongly depend on the size, shape and interaction between the particles present on the surface of the nanoparticles (Essien et al., 2020). The UV-Vis absorption spectrum of the MgNPs solution shows an absorption band between 300-320 nm. MgNPs is reported to exhibit a broad absorption peak in between 260-330 nm (Almontasser et al., 2019). Scanning electron microscopy observation provided further insight into the shape and size of the synthesized nanoparticles (Abdallah et al., 2019). Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm (Mohanraj & Chen, 2006). FTIR spectra of the biosynthesized MgNPs is shown a band 1644.80 cm^{-1} is ascribed to the stretching vibration of C=C in according with Ogunyemi et al., 2019 that they found a band at 1633 cm^{-1}. The peaks observed below 800 cm^{-1} confirmed the bond between magnesium and oxygen (Imani & Safaei, 2019). Also, the stretching vibration mode ~600–850 cm^{-1} indicating Mg–O–Mg bonds (Balakrishnana et al., 2020). Noori & Kareem et al. found in their study that bands at 581 cm^{-1}, 850 cm^{-1}, and 890 cm^{-1} corresponded to stretching vibrations of the metal-oxygen bond, which corresponded to the presence of the MgO nanoparticles (Noori & Kareem et al., 2019). A broad band is observed ~3353 cm^{-1} due to O–H stretching vibration of water molecule in agreement with Balakrishnana et al., 2020. The prominent peak at 1382cm^{-1} is assigned to Mg–O vibration (Essien et al., 2020) almost the same result that we get, a sharp peak on the wave number 1352.06 cm^{-1}.

Moreover, the outcome in this research determine the concentration of MgO effect and be appropriate for various applications, we found that treatment with the magnesium nanoparticles

cause no toxic effect at low dose but may be no safe at great dose, in agreement with the result of study of (Mazaheri et al. 2019) .

III.3. Part three : In vivo study

III.3.1.Study of tissular concentration of doxorubicin

Concerning the quantitative analysis of heart doxorubicin, the results obtained show a significant increase in the heart doxorubicin concentration in rats treated with doxorubicin.

Existing evidence points to a multitude of molecular mechanisms involved in Doxo-induced cardiac dysfunction (Iqbal et al., 2008). The mitochondria are reported to be the main target of cationic drugs like Doxo at the subcellular level because Doxo forms an irreversible complex with cardiolipin (Ajzashokouhi et al., 2019), an anionic phospholipid in the inner mitochondrial membrane (Iqbal et al., 2008) and is known for maintaining mitochondrial structure, function, cardiac energy metabolism, and cell survival (Wenningmann et al., 2019), resulting in the accumulation of Doxo inside cardiac cell (Mohammad et al., 2013). This formed doxorubicin-cardiolipin complex is a principle component for chemotherapy-induced inhibition of various enzymes and tissue (Quryshi et al., 2018).

Treatment with *Ocimum basilicum* L. caused a decrease in the heart doxorubicin concentration, due to presence of secondary metabolites such as tannins, phenols and flavonoids, *Ocimum* species have been known for their healthful properties for long time and have been used in traditional folk medicine (Filip, 2017). Number of studies has demonstrated that consumption of polyphenols limits the incidence of coronary heart diseases (Pandey & Rizvi, 2009). Our results showed that *Ocimum basilicum* L. consist of various types of flavonoids such as rutin, caffeic acid and rosmarinate that contain in their structure a hydroquinone ring as well as in doxorubicin, and this can be the raison for the competition between flavonoids and doxorubicin on heart cells.

Nanomaterials such as nanostructured surfaces, nanoparticles, and nanocomposites represent new viable sources for future therapeutics for cardiovascular diseases (Jiang et al., 2017). Nanoparticles can easily cross the cell membranes and interact with intracellular metabolism (Kumar et al., 2017). Due to their small size, therefore accumulate in organs (Staroń et al., 2020). Magnesium oxide nanoparticles (MgO) are considered a promising alternative to heavy metal based nanoparticles, because (MgO) contains metal ion Mg^{2+} (mineral) that is essential for human health (El-Shaer et al., 2019). Magnesium is involved in several essential physiological, biochemical, and cellular processes regulating cardiovascular function (Kolte et al., 2014). This explains the reason for the low concentration of the doxorubicin in the heart in MgNPs treatment group.

The co-administration of *O. basilicum* extract with MgNPs contribute to preventing the accumulation of Doxo in the heart. This shows the critical importance of plant extract with the compounds of magnesium oxide nanoparticles in protecting the heart.

III.3.2.Study of hematological analysis

Our data for the hematological analysis in this study shown that the levels of WBC, lymphocyte, eosinophil and basophil are high reduce in the Doxo treated group compared with the control group. According to (Laoui et al., 2013) chemotherapy has been a standard treatment for cancer for the past several decades and has long been suspected to cause systemic immune suppression. Also, used of doxorubicin as chemotherapy treatment possesses several side effects including cardiotoxic, hepatotoxic (Sutejo et al., 2016). The use of the drug affected the immune functions (Ali et al., 2016). Doxorubicin has also inflammatory effects (Abou El Hassan et al., 2003). Doxorubicin suppress the immune system by decreasing the expression level of IL-2, production of the γ-interferon, natural killer (NK) cells, proliferation of lymphocytes, and ratio CD^{4+}/CD^{8+} (Sutejo et al. 2016). The levels of proinflammatory cytokines, such as IL-1 and TNFα, were significantly increased following doxorubicin administration (Wang et al., 2016). For erythrocyte line markers, our results showed a significant decrease in HGB and HTC levels in drug group as compared to that in the controls. Anemia occurs when the number of red blood cells (or the Hb in them) fall below normal (Khan et al. 2013). Doxo intoxication might lead to anemia as a result of either altered activity of hematopoietic tissues, impaired erythropoiesis, and/or defective iron metabolism (Afsar et al. 2017).

III.3.3.Study of blood glucose and serum biochemical parameters

Biochemical parameters have a significant role in determining toxicity because changes in their levels are considered as one of the best general indicator of the toxicity (Khorrami et al., 2019).

As for blood glucose level the results appeared a very high increase in doxorubicin group compared to control, our results corresponds to the results of (Arunachalam et al., 2013) who found doxorubicin treatment also showed an increase in blood glucose and glycogen levels. The effects of Adriamycin on islet β cells has been investigated for insulin secretion, Ca^{+2} movements and glucose oxidation in order to determine other toxic effect of the new antineoplastic drug than cardiotoxic effects. Results suggest that therapeutic doses of Adriamycin could be highly toxic for endocrine secretory function (Deleers & Goormaghtigh, 1985). The effects of doxorubicin on rat pancreatic islets of Langerhans found that doxorubicin inhibited insulin secretion (Heart et al., 2016).

In treatment with aqueous extract of *O. basilicum* showed a significant decrease in blood glucose level compared to doxorubicin group. *O. basilicum* extract was stated as an herbal plant that contains antidiabetic active substances, can reduce blood glucose. The extract of *Ocimum basilicum* L. could inhibit the enzymes of α-glucosidase and α-amylase with minimum side effects. It was reported that dietary inhibitors α-glucosidase and α-amylase can modulate decreased glucose absorption in hyperglycemia (Syafrina et al., 2020). The extract of aerial parts of *O. basilicum* possesses antidiabetic effects, possibly mediated by limiting glucose absorption through inhibition of carbohydrate metabolizing enzymes and enhancement of hepatic glucose mobilization (Ezeaniet al., 2017). The chemical constituents that have been isolated from the plant include terpenoids, alkaloids, flavonoids, tannins, saponin and glycosides. These compounds have been related to reduce blood glucose level (Rumenganet al., 2019).

Our study in renal function showed increased serum urea levels and creatinine in doxorubicin-treated rats as compared to control group. Nephrotoxicity is one of the limiting factors for using doxorubicin (Refaie et al., 2016). Also, Doxo-induced changes in the kidneys of rats include increased glomerular capillary permeability and tubular atrophy (Ayla et al., 2011). Various mechanisms have been suggested to explain Doxo-induced nephropathy (Heravi et al., 2018), mainly consists of two aspects: one is direct damage. The general toxicity of doxorubicin on cells directly damaged renal cells, which produced inflammatory response in renal tissues and released such inflammatory factors as TNF-α and interleukin, hence strengthening the growth of renal matrix and glomerular sclerosis, the other is indirect damage. Under the action of multiple reductase, doxorubicin produced free radical, induced lipid peroxidation in glomerular epithelial cell and caused abnormal glucose and protein metabolism; moreover, it brought about elevated urinary albumin through altering and breaking the structure and filterability of renal filtration membrane. Most Researches come to the conclusion that indirect damage predominates (Liu et al., 2016). On the other hand, renal function is an underappreciated prognostic factor in heart failure. A number of studies have reported that renal insufficiency is associated with adverse cardiovascular outcomes (Alister et al., 2004).

Regarding the protective effects of the *Ocimum basilicum* L. We notice decreased in the creatinine levels by compounds endowed with activity antioxidant such as flavonoids, polyphenols. According to (Abdel-Sattar et al., 2012), probably the most important natural phenolics are flavonoids because of their broad spectrum of chemical and biological activities including radical scavenging properties as such could possess antioxidant activity and antiinflammatory effects (Vargas et al., 2018). With an increase in urea level, where it is considered blood urea nitrogen (BUN) a sensitive indicator of renal abnormalities. The level of

creatinine in serum is considered more sensitive kidney function test than BUN by way of kidney injury is the only cause of elevated creatinine (Abou Zaid et al., 2017).

In the other side, there is a decreasing serum creatinine level in MgNPs-treated rats as to Doxo group. Magnesium is an essential cation playing a crucial role in many physiological functions (Laires et al., 2004). It is a key element for cell metabolism and the deficiency of it leads to inactivities in critical metabolic points (Esfandiari et al., 2010). Mg deficiency is believed to indirectly enhance oxidative damage of biomolecules by inducing a stress response (Zheltova et al., 2016). In addition, this might be associated with the antioxidant function of Mg increased antioxidase activities, stabilized biological membranes and avoided cell damage (ZHANG et al., 2016).

The combined treatment of both plant extract of *O. basilicum* and magnesium oxide nanoparticles led to resulted in improvement in the level of renal function against the drug used. This confirms the effective effect of magnesium molecules and the antioxidant properties of the plant extract on the kidney.

III.3.4.Study of enzymatic parameters

In the present study, Doxo induced cardio-toxicity presented by increase of LDH, CPK and GPT activities which are known as diagnostic marker of myocardial function and cardiotoxicity induced by doxorubicin was also manifested by increasing of these enzymes as well (Aniss et al., 1997). Doxorubicin treatment induced free radical generation, triggers membrane degradation and disruption of cardiac myocytes, which can lead to elevations of LDH and CPK (Barman et al., 2013). Our results are in good agreement with findings of (Swamy et al., 2012), because the heart muscle is rich in transaminases such as ALT and AST both are liberated into the serum after extensive tissue injury (Swamy et al., 2012). In addition ALT and AST are found in kidney, liver, muscle but ALT greater concentration in liver compared with other tissues of the body and AST is found in highest concentration in heart (Gowda et al .,2009). The results of (Amin et al., 2012) realized in patients after treatment with a combination of the anticancer chemotherapy drug of 5-fluorouracil, doxorubicin and cyclophosphamide (FAC) revealed decrease in activity of GOT as compared to control which are in accordance with our study. Based on our previous information, oxidative stress causes cells damage and this explains why these enzymes are released from the cells to the blood and increase their concentrations.

Treatment with *Ocimum basilicum* L. and MgNPs caused a decrease of LDH, CPK and GPT activities as compared to doxorubicin exposed rats, this may explain by the presence of bioactive phytochemical compounds have been efficacious as serving in a cardio-protective capacity (Luis, 2013). Flavonoids inhibited the Doxo induced increment of CPK activity. Therefore, it is shown

that the combination of flavonoids with Doxo reduce the cardiotoxicity of Doxo (Sadzukaet al., 1997). The best-described property of almost every group of flavonoids is their capacity to acts as antioxidants (Hozayen & Abou Seif, 2011).

After entering the body, regardless of the routes to exposure, nanoparticles are transported through the central circulatory system to various organs and tissues, including the liver, spleen, heart, kidneys and brain (Staron et al., 2020). Due to their small size, nanoparticles easily enter the blood (Gabbett et al., 2010). Magnesium has been recognized as a cofactor for more than 300 enzymatic reactions (Gröber et al., 2015), such as those responsible for regulating blood pressure and lipid peroxidation (DiNicolantonio et al., 2018).

The blend between *O. basilicum* extract and MgNPs showed a significant improvement in the enzymatic activity against the antibiotic doxorubicin that causes cardiac toxicity. This is by the secondary metabolites present in the aqueous extract, the properties of magnesium at the heart.

III.3.5.Study of electrolyte parameters

Results obtained of electrolytes parameters showed that Na^{+2} and Ca^{+2} levels were significantly decreased simultaneously with an increase in the level of Cl^{-} in Doxo group compared to the control. The careful monitoring of the serum electrolytes plays very important role in prognosis of disease (Yadav & Khodke, 2015). Electrolyte disorders are specifically associated with chemotherapeutic regimens (Rosner & Dalkin, 2014). So, electrolyte derangement has been documented during cancer chemotherapy leading to the electrolyte imbalance (Kumari et al., 2018). Several studies have proved that reactive oxygen species resulted from Doxo treatment has either direct or indirect effect to calcium regulatory proteins (Agustini et al., 2016). In other side, the use of Doxo has been limited by the occurrence of dose-dependent toxicities to vital organs, as the kidney (El-Sheikh et al., 2012). With progressive loss of kidney function, derangements in electrolytes inevitably occur (Dhondup & Qian, 2017). The primary function of the kidney is to maintain electrolyte homeostasis (Thomson & Macnab, 2009) which is the prerequisite for numerous metabolic processes and organ functions (Qian & Dhondup, 2017). The kidney is the primary mechanism for rapid release or absorption of calcium through the filtration and urine excretion functions (Beto et al., 2015). Chloride (Cl^{-}) is a major anion responsible for the maintenance of cation/anion balance between intra and extra cellular fluids (Omoyajowo et al., 2018), this may be the reason for the rise in Cl^{-}, in order to try to adjust the imbalance electrolytes.

Furthermore, there was a significant amelioration against electrolytes disorders in *Ocimum basilicum* L. MgNPs and combined groups may because of the improvement in the renal function due to the effect of magnesium molecules and the antioxidant properties of the plant extract on the kidney.

III.3.6. Study of oxidative stress markers

The therapeutic application of anthracycline antibiotics are limited by side‑effects mainly, chronic cardiotoxicity and hepatotoxicity (Damodar et al., 2014). Several studies have shown that Doxo-induced cardiotoxicity is also related to increases in oxidative stress and production of reactive oxygen species (ROS) (Gasser et al., 2019). The major types of cardiotoxicity ROS are superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl free radical (HO) (Zhu, 2018).

In our study, the level of reduced glutathione (GSH) in heart and liver, was significantly decreased in Doxo treated rats compared to control. These findings are in concurrence with (Haque et al., 2014) who stated that Doxo generates free radicals in heart and decreases its ability to detoxify ROS (Khan et al., 2014). Also, our results indicate augmentation of MDA levels in heart and liver of Doxo group. The underlying mechanism is that doxorubicin causes an increase in the malonaldehyde levels through the one-electron reduction of nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome P-450 reductase enzyme (Anber, 2018). The level of lipid peroxidation was estimated by measuring MDA, which is the end product of lipid peroxidation (Hosseini & Rajabian, 2016). Doxo-induced oxidative stress is confrmed by the elevation of oxidized lipids (MDA) (Dogan et al., 2014). The toxicity is started at imbalance the free radical production by two pathways: enzymatic pathway using a respiratory chain of the mitochondrial and a non-enzymatic pathway occurred by utilizing iron (Sahib et al., 2019). The first way implicates the formation of a semiquinone free radical by the action of several NADPH-dependent reductases that produce a one electron reduction of the Doxo to the corresponding Doxo semiquinone. In the presence of oxygen, redox cycling of Doxo derived quinone–semiquinone yields superoxide radicals. In the second way, Doxo free radicals come from a non-enzymatic mechanism that involves reactions with iron. Doxo not only increases free radical production in the tissue, but also decreases its ability to detoxify reactive oxygen species (Dogan et al., 2014). Doxo is likely to have toxic effects on liver (Bengaied et al., 2017). These free radicals can damage the hepatic membranes, by phospholipid activation and lipid peroxidation, that increases the intracellular Ca^{2+} and the release of ALT, followed by apoptotic cell death (Sasu et al., 2016). Superoxide dismutase (SOD) and glutathione s transferase (GsT) are among the enzymatic antioxidants, and its high activity in our study, this is because the body is trying to repair by raising the activity of these enzymes as a result of increasing the free radicals which are the substrate of SOD and GsT. The hearts had a lot of mitochondria and relative lower levels of antioxidant enzymes when compared with other organs, rendering the heart particularly vulnerable to free radical damage and Doxo-induced cardiotoxicity (Li et al., 2019). In this respect, mitochondria may not only be a target of direct Doxo toxicity but also contribute to the overall cell damage by inducing oxidative stress (Adamcova et al., 2019).

Our findings of aqueous extract of *O. basilicum* and MgNPs treatment appeared a protective effect against oxidative stress. Using antioxidants could be an important strategy for partially protecting cardiac cells from Doxo-induced oxidative damage and cardiotoxicity (Wu et al., 2019). The reduction effect of doxorubicin toxicity by flavonoids was partly caused by antioxidative action (Wang et al., 2013). The result of the effect of aqueous extract of O. *basilicum* on oxidative stress parameters appeared a high decrease of MDA in heart homogenate compared to Doxo group. The plant extract can decrease the MDA by contains high level of phenolic and flavonoid compounds, responsible for its high antioxidant activities (Hosseini et al., 2014). In the other hand, flavonoids suppress Doxo-induced lipid peroxidation in the heart and liver (Sadzuka et al., 1997). Our earlier findings of *O. basilicum* analyzed by LC-Q-TOF-MS indicate the presence of differents types of flavonoids such as rutin. Rutin as a polyphenolic flavonoid has been illustrated to protect hearts from diverse cardiovascular diseases. Its function is known to be related to its antioxidant and antiinflammatory activity which may regulate multiple cellular signal pathways (Ma et al., 2017). Also, Rosmarinic acid (RA), one of the main phenolic compounds in *Ocimum basilicum* L. has antioxidant activity and pharmacological properties (Shiga et al., 2009). RA was found to inhibit these apoptotic characteristics by reducing intracellular reactive oxygen species (ROS) generation and by recovering the mitochondria membrane potential (Ferreira et al., 2013).

Magnesium (Mg^{2+}) is involved in several essential physiological, biochemical, and cellular processes regulating cardiovascular function (Kolte et al., 2014). Deficiency of magnesium may result in many disorders, including cardiac arrhythmias (Cieoelewicz et al., 2013). Where it is considered, Mg^{2+} is the most abundant divalent cation in living cells and it plays a vital role in many cellular processes (Chakrabort et al., 2002).Also, magnesium status is closely linked with liver function where magnesium deficiency is commonly associated with liver diseases (Liu et al ., 2019). Magnesium is essential for the stability of cell function, RNA and DNA synthesis, and cell repair, as well as maintaining the antioxidant status of the cell ,and it is an important cofactor for the activation of a wide range of transporters and enzymes (Uwitonze et al ., 2019). Mg^{2+} was found to reduce the production of free radicals by inhibiting NADPH oxidase, which could stimulate the production of reactive oxygen species (Zhang et al., 2016). Also, Magnesium ions are also essential to glutathione synthesis (Pasternak et al., 2010). MgNPs is an important functional metal oxide that has been widely used in various fields. Due to their smaller size, nanomaterials have novel properties, which is different from others (NARENDHRAN et al., 2019).According to the study of Sushma et al, who stated that the biologically synthesized MgNPs exhibit good antioxidant activity (Sushma et al., 2015).

The combination between *O. basilicum* extract and MgNPs show in improvement the protection in oxidative stress markers versus Doxo caused cardiac and hepatotoxicity, this ameliorate probably due to the activity antioxidant of flavonoids and MgNPs.

III.3.7.Histopathological study

Four weeks of Doxo injection in rats caused severe histopathological lesions, which were characterized by myocardial swelling, hemorrhage, inflammation and vacuolization in the tissue. These structural and cellular changes are consistent with other results on Doxo induced cardiomyopathy in rat.

Several mechanistic pathways are probable to be implicated in Doxo-induced structural change including increased inflammatory reactions within the myocardium (Hijazi et al., 2019). Doxo induces a significant increase in the levels of specific inflammatory cytokines and chemokines, including IL-1β, IL-6, TNF-α, COX-2 and CCL2/MCP-1(GUO et al., 2013). Cytokines are important mediators' proteins, essential in networking communication for immune system. Cytokines can be produced by lymphocytes, or monocytes with pro-inflammatory and anti-inflammatory effects. Cytokines with chemotactic activities are termed chemokines. The equilibrum between pro-inflammatory cytokines (IL-1β, IL-2, TNFα, Il-6, IL-8, IFN-γ…) and anti-inflammatory cytokines (IL-10, IL-4, TGFβ) (Yahfoufi et al., 2018). Inflammation is also an early event in cardiac stress. Elevated levels of endothelial adhesion molecules and increased inflammatory cytokine and chemokine production and release are observed in affected cardiac tissues. The innate immune system is the primary cardiac defense against pathogens and tissue damage (Chenet al., 2018). Doxo induces apoptosis, which contributes to cardiotoxicity. Doxo stimulates ROS generation and produces oxidative stress (Zhu, 2018) this apoptosis may be caused vacuolization in the tissue. Numerous clinical studies have implicated that several chemotherapies cause direct vascular injury, yet the mechanism and characteristics of this toxicity remains to be elucidated (Bar-Joseph, 2015). So, the hemorrhage is a loss blood from a blood vessel that may be due to either a vascular toxicity induced by Doxo or free radicals released by Doxo.

In the other side, histological results of *O. basilicum* treatment in rats shows a high protection against tissues lysis induced by doxorubicin. Phytochemical analysis of the extract of *Ocimum basilicum* L. revealed that it is rich in phenolic and flavonoid active constituents. Flavonoids naturally occurring secondary plant metabolites because of their claimed antioxidant and anti-inflammatory. These proposed positive pharmacological activities have been mostly attributed to their free radical scavenging (Mladěnka et al, 2009). *O. basilicum* has the effect of anti-oxidative stress (Syafrina et al., 2020).

Basil extract presented anti-inflammatory properties, the mechanism of which is a composed interaction between the inhibition of pro-inflammatory mediator and the stimulation of anti-inflammatory cytokines (Güez et al., 2017**).** Numerous studies have attributed to polyphenols a broad range of biological activities including but not limited to anti-inflammatory, immune-modulatory, antioxidant, cardiovascular protective, anti-cancer actions, modulate cytokines production and pro-inflammatory genes expression (Yahfoufi et al., 2018). Many types of natural substances have been investigated for their cardioprotective effects (Zhang et al., 2020). Rosmarinic acid was one of the most abundant caffeic acid esters present in *O. basilicum* .It has been related that this compound has antioxidant and anti-inflammatory activities (Güez et al., 2017**)**.

The histological section of the heart appeared almost normal in the MgNPs group compared to doxo group and also a complete cure of inflammation because, magnesium plays a critical regulatory role in NF-κB activation, cytokine production, and disease pathogenesis (Sugimoto et al., 2012). Magnesium defciency induces an inflammatory response that results in leukocyte and macrophage activation, release of inflammatory cytokines and acute-phase proteins, and excessive production of free radicals (Nielsen, 2018). It is also an antihypertensive, anti-inflammatory and anticoagulant agent can be of benefit in the prevention and treatment of cardiovascular diseases (Gröber et al., 2015). So Nano-MgO induces greater analgesic and anti-inflammatory effects (Jahangiri et al., 2015).

The co-treatment of *O. basilicum* extract with MgNPs ameliorate the protection of cardiac cell against doxo induced cardiotoxicity. Which gives the impression of the importance of increasing the positive effect of the plant extract with magnesium Nano form in preserving the structure and function of the heart from the negative effect of doxorubicin anticancer treatment .

Conclusion & Perspectives

Conclusion

Doxorubicin (Doxo) is a powerful anti-cancer chemotherapy drug that is widely used in the therapy of a broad range. However, the clinical use of Doxo can also trigger dilated cardiomyopathy and heart failure in a dose dependent manner. As the risk of Doxo induced cardiotoxicity in patients with cancer is increasing.

In patient study, our results show that cancerous women treated by doxorubicin reveal a change in biochemical enzymatic and hematological markers which indicate that doxorubicin chemotherapy induce some physiological and metabolic disorders as side effect on the cancerous patients.

The presence of many bioactive molecules in the aqueous extract of plant, both qualitatively and quantitatively through various analyzes, especially LC-Q-TOF-MS, also with positive results in in-vitro tests, especially anti-oxidants and anti-inflammatory activity proves that the plant has a high biological effects in reducing many diseases related to these mechanisms.

Through the results of the concentrations of Doxo in heart tissues and histological part, this proves the efficiency of the proposed treatments *O. basilicum* and MgNPs in protection of limiting the spread of this drug in healthy tissues and thus reducing its toxicity, especially on the heart.

Partial improvements to the plant *O. basilicum* or MgNPs through improvement in biochemical, enzymatic, hematological markers or through reducing the oxidative stress confirm the effectiveness of these compounds in protecting the body of patients from the side effects of Doxorubicin in heart and other organ in the body.

Perspectives

Given the importance of these results, they open other perspectives, including:

- ✔ Evaluation of the protective effect of plant and MgNPs against doxorubicin in rats with cancer disease.
- ✔ Carrying out other in-depth analyzes to know the effect of Doxo and the treatments on the DNA and the factors involved in it.
- ✔ Evaluation of anticancer effect of plant and MgNPs alone or combination.
- ✔ Exploration of new drugs and strategies to reduce Doxo induced cardiomyopathy.

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Annex

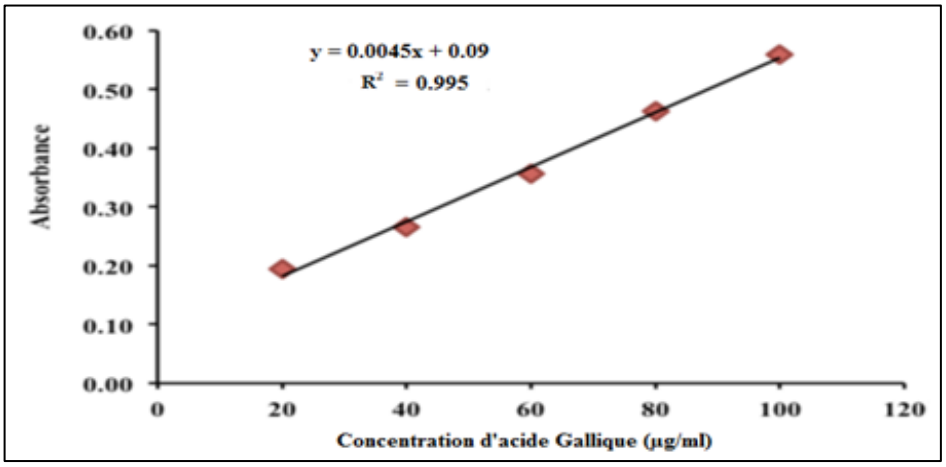

Figure: Gallic acid calibration curve for the determination of polyphenols

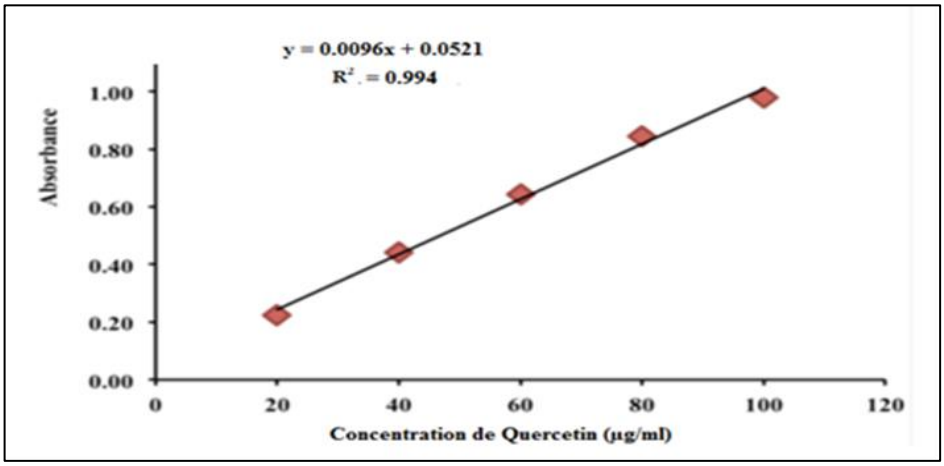

Figure: Quercetin calibration curve for flavonoid assays

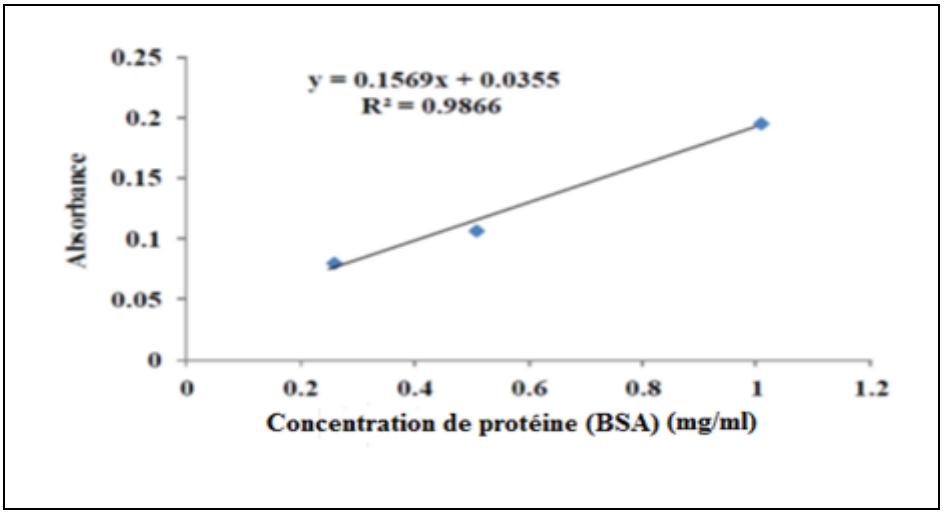

Figure: BSA calibration curve for protein assay