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Department of Chemistry

Study the Cytotoxicity, Antioxidant Activity and Nanoparticles Influences Of Ginger Extracts in Patients with Thyroid Cancer

A Thesis

Submitted to the college of science / Al-Nahrain University as partial fulfillment of the requirements for the Degree of Master of Science in chemistry

By

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وَيُسْقَوْنَ فِيهَا كَأْسًا كَانَ مِزَاجُهَا

زَنجَبِيلًا {17}

And they will be given to drink there of a Cup (of Wine) mixed with Zanjabil

صدق الله العظيم

سورة الإنسان

Dedication

To the Moon and the Sun of my life my parents …

To Planets of my life my brothers and sisters…

To all my friends…

To my Fiancé …

To my best friend Whose always support me Ala 'a Bader and Rasha Hussain.

Doa 'a

Supervisor Certification

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Doaa

Summary

Background

Thyroid gland is an endocrine gland, and consist of two connected lobes. It is found at the front of the neck, below the Adam's apple. The thyroid gland release thyroid hormones, which influence the metabolic rate, protein synthesis, and have a wide range of other effects. The thyroid hormones T3 and T4 are synthesized from iodine and tyrosine. The thyroid also produces calcitonin, which plays a role in calcium homeostasis.

Ginger (*Zingiber officinale*) has many medicinal properties besides being highly used as a flavouring agent all over the world. It can be widely used in alleviating many diseases of humans and animals.

Ginger has many medical properties like a used it as anti-bacterial, anti-inflammatory, anti-cancer and anti-angiogenic addition to effect it on arthritis, diabetes, Alzheimer's disease.

Recently, nanoparticle synthesis is among the most interesting scientific areas of inquiry and there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry), Silver nanoparticles are of interest because of the unique properties.

Aims of study:

Trying to treatment the patients with thyroid cancer by herb (ginger) and knowledge the effect of thyroid cancer on kidney, liver function and level of glucose in blood by doing this steps:

1- Study the changes in levels of thyroid hormones (T3, T4, and TSH), kidney function (Urea, Creatinine), liver function (ALT, AST) and random blood Glucose RBG in patients with thyroid cancer

2- Study the effect of ginger *in vitro* in papillary thyroid cancer cells line (PTC-1) and explore the antioxidant activity *in vivo* in mice.

3- Prepare the ginger nanoparticles and study the properties of GNPs and its effect on papillary thyroid cancer cells line.

Subjects and methods:

This study included fifty thyroid patients female their age less than 45year (group 1) and fifty thyroid patient's male with all ages (Group 3). Fifty female subjects were apparently healthy and their age less than 45year chosen as control group (group2) and Fifty male subjects were apparently healthy chosen as control group(group 4) . This study was conducted during the period from November 2015 to January 2016.

Part one: The study was designed to investigate the change of the following parameters in serum of thyroid cancer patients:

1. Biochemical markers: Kidney Function (Urea, Creatinine), Liver Function (AST, ALT) and Random Blood Glucose (RBG) were measured by enzymatic colorimetric methods.
2. Hormones: thyroid hormones (Total triiodothyrionin T3, Total thyroxine T4, Thyrotropin (TSH)). The measurements done by use Biotek (ELISA).

Part two: the study was planned to investigate the ginger properties through study:

1. Extracted ginger with six organic solvent and once with water .the extracts were analyzed by FT-IR.
2. Determined the biological activity (antibacterial and antifungal).

3. Determined cytotoxicity for the best extract with papillary thyroid cancer (PTC-1).
4. Study the antioxidant in vivo in mice with three parameters malondialdehyde (MDA), SOD activity and catalase enzyme.

Part three: in this study ginger nanoparticles were synthesized from aqueous extract of ginger and were studied ginger nanoparticle (GNPs) properties:

1. The ginger nanoparticles were measured by UV-Vis spectra and compared with aqueous extract.
2. The ginger nanoparticles were analyzed by FT-IR, AFM, SEM, XRD and TEM.
3. Biological activity (antibacterial, antifungal) for ginger nanoparticles were measured.
4. Cytotoxic activity of ginger nanoparticles was determined.

Results:

Part one:

The results showed that the T3, T4, Urea, Creatinine, AST, ALT and Glucose in serum of were significantly higher ($p<0.001$) than that controls, while Creatinine in male patients did not show significant difference.

The results of T3 showed positive correlation with (Urea, Creatinine, AST, ALT, and Glucose) in all patients groups.

The results of T4 showed positive correlation with (Urea, Creatinine, AST, ALT, and Glucose) in all patients groups.

The time of treatment showed negative correlation with (Urea, Creatinine, Glucose, AST, ALT) while there was positive coloration with AST in male patients group.

Part two:

The results of FT-IR ginger extracts showed that many active group appeared in spectrum that refer to presence antioxidant compounds of ginger.

The results of biological activity of ginger extracts showed that presence inhibition zone (x-) of ethanol, hexane and methanol extracts on Staphylococcus aureus and presence inhibition zone (x-) of ethanol, hexane and methanol extracts on E. coli while there is not significant inhibition zone for acetone, aqueous and chloroform extracts.

The results of antifungal activity of ginger extracts showed that no significant inhibition zone for all ginger extracts.

Cytotoxic effect of ethanol ginger extract in papillary thyroid cancer cells line (PTC-1) was showed that high concentration of extract gave treatment compared with normal cells line.

The antioxidant activity in mice showed that antioxidant activity increase with increasing the concentration of dose that have been vaccinated.

Part three:

The results of ginger nanoparticles (GNPs) showed that size of particles was 25-30 nm by TEM technique and 1-75nm by AFM technique. FT-IR spectrum result showed many active group appeared that mean active compounds remained after synthesis of GNPs, SEM was showed synthesized ginger nanoparticles (GNPs) have a high yield of Ag element and showed shape of GNPs.

X-ray diffraction analysis the dry powders of the silver-ginger nanoparticles was showed the obtained results illustrate that silver ions had indeed been reduced to Ag^0 by ginger plant extract under reaction conditions.

The biological activity of ginger nanoparticles (GNPs) had the highest zone inhibition for E.coli and S.aureus compared with another extracts with less concentration of another extracts, cytotoxic activity of GNPs was more significant than ethanol ginger extracts.

Conclusions

Based on the findings of present study, it is possible to reach the following most important conclusions:

1. The results showed that thyroid cancers patients had high T3, T4 and light rise in kidney function (Urea, Creatinine), liver function (AST, ALT) and glucose levels, and reveal a positive and strong correlation between T3, T4 and (Urea, Creatinine, AST, ALT, Glucose) .the papillary thyroid carcinoma was popular more than another type of thyroid cancer (follicular and medullary) in our study.
2. In this study ginger was extracted with water and with five organic solvent (ethanol, methanol, acetone, hexane, chloroform), then it has been tested for biological activity (antibacterial activity and antifungal activity) and FT-IR spectrum for all extracts. Hexane extract was most powerful biological activity.
3. The results showed significant antioxidant activity (MDA, Catalase, SOD) for aqueous extract of ginger *in vivo* in mice.
4. Preparation of Ag nanoparticles from aqueous ginger extract and check nanoparticle size by TEM, AFM, SEM and XRD and was 25-30nm and that silver ions had indeed been reduced to Ag0 by ginger plant extract under reaction conditions.
5. Ethanol ginger extract and synthesized ginger nanoparticles GNPs have cytotoxic effect against papillary thyroid carcinoma PTC-1 determined *in vitro* by MTT assay. Cytotoxic activity of GNPs was more than ethanol ginger extract.

List Of Abbreviations

Abbreviations

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
ADME	absorption, distribution, metabolism, and excretion
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ALT(ALT)	Alanine Aminotransferase
AST(AST)	Aspartate Aminotransferase
AFM	Atomic Force Microscope
AFM	atomic force microscopy
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CNS	Central nervous system
TNM	Classification of Malignant Tumours
cAMP	cyclic adenosine monophosphate
DNA	Deoxyribonucleic acid
DTC	differentiated thyroid cancer
DMSO	Dimethyl sulfate oxide
EDS	energy dispersive spectroscopy
E.coli	Escherichia coli
FNAB	Fine-needle aspiration biopsy
FTC	Follicular thyroid cancer
FBS	fetal bovine serum
FT-IR	Fourier transform infrared spectroscopy
GNPs	Ginger nanoparticles

MDA-MB-231	Human breast cancer cell line
H2O2	hydrogen peroxide
IC50	Inhibition concentration 50
IFCC	International Federation of Clinical Chemistry
LDH	lactate dehydrogenase
MDA	malondialdehyde
MIC	Minimum inhibitory concentration
NTRK1	neurotrophic Receptor Tyrosine Kinase 1
NADH	Nicotinamide adenine dinucleotide hydrogen
WRL	Normal cells line
OD	Optical density
PTC	Papillary thyroid carcinoma
KBR	Potassium bromide
XRD	powder X-ray diffraction
RAI	Radioactive Iodine Therapy
ROS	reactive oxygen species
ROS	reactive oxygen species
RET	rearranged during transfection
RXR	retinoid X receptor
SEM	scanning electron microscopy
AgCl	Silver chloride
S.Aureus	Staphylococcus Aureus
SOD	Superoxide dismutase
3D	Three dimension
TSH	Thyroid - stimulating hormone

TPC-1	Thyroid Papillary Cancer Cell Line
TRH	thyrotropin releasing hormone
T4	thyroxine
TEM	transmission electron microscopy
TCA	trichloroacetic acid
T3	triiodothyronine
UV-vis	Ultra violate – visible
VEGF	vascular endothelial growth factor
VEGF	Vascular endothelial growth factor

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CHAPTER ONE

INTRODUCTION

1.1 The thyroid gland

The thyroid gland is under the thyroid cartilage in the facade part of the neck in most people, the thyroid cannot be recognized or sensed. It is butterfly designed, with 2 lobes, the left lobe and the right lobe merged by a slim isthmus. [1]

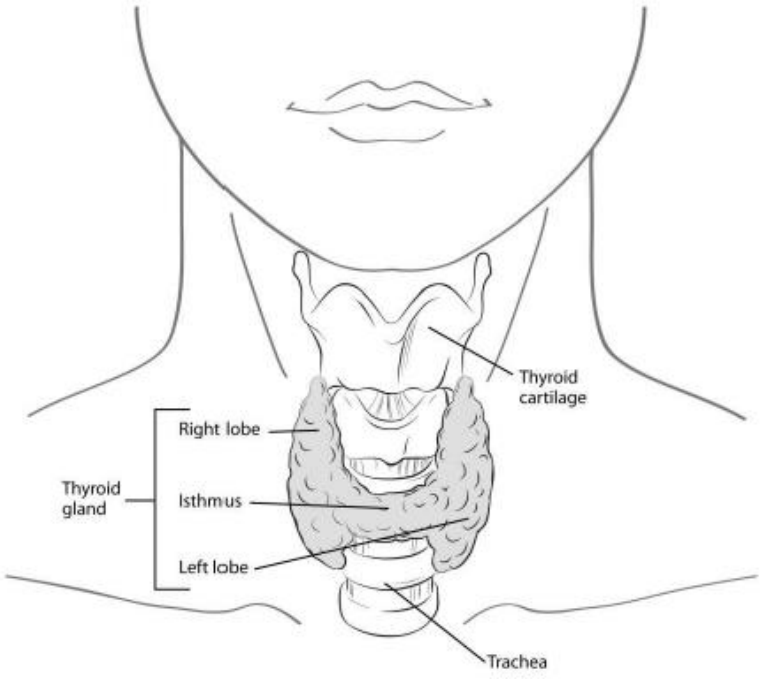


Figure (1-1): Thyroid gland [1].

The thyroid gland has 2 main types of cells:

1. **Follicular cells** use iodine from the blood to create thyroid hormones, which help control the metabolism. High level of thyroid hormone (a condition called hyperthyroidism) can lead to irregular or fast or heartbeat, nervousness, trouble sleeping, weight loss, hunger, and a feeling of being too warm. Having too little hormone (called hypothyroidism) causes a person to slow down, feel tired, and gain weight. The amount of thyroid hormone released by the thyroid is regulated by the pituitary gland at the baseof the brain, which makes a substance called thyroid-stimulating hormone (TSH).

2**. C cells** (also called parafollicular cells) release calcitonin, a hormone that aids control the uses of body from calcium. Other, fewer common cells in the thyroid gland contain supportive (stromal) cells and immune system cells (lymphocytes). Different cancers develop from each kind of cell. The differences are important because they affect how serious the cancer is and what type of treatment is needed.

Many types of growths and tumors can progress in the thyroid gland. Most of these are benign (non-cancerous) but others are malignant (cancerous), which means they can extent into neighboring tissues and to other parts of the body.[1]

1.1.1 Thyroid Hormones

Thyroid hormones perform their action practically on each cell in the body. The three main hormones released by the thyroid gland. The first two hormone, thyroxine (T4) and triiodothyronine (TRIIDOTHERONINE), are mainly controlled by thyroid stimulating hormone (TSH) released by the anterior pituitary gland. TSH manufacture is modulated by thyrotropin releasing hormone (TRH) secretion from the hypothalamus. The third hormone secreted by the thyroid gland, calcitonin, is not controlled by hypothalamic/pituitary. Calcitonin is partipicitated in responds to calcium levels and calcium homeostasis in the blood.[2]

Thyroid hormones secreted from the thyroid gland, about 80% are in the form of T4 and 20% in the form of TRIIDOTHERONINE. About 80% of the T4 secreted from the thyroid gland is altered to the more potent hormone triidotheronine by peripheral organs for example the renal and liver. triidotheronine is a more potent hormone (approximately four times as strong), but Thyroxine is have high T-half than triidotheronine . At a given time, most of the Thyroxin and Triidotheronine in the body are curried by transporter proteins; it is only the small, “unbound” or “free” fraction of the hormones that is biologically active.[3]

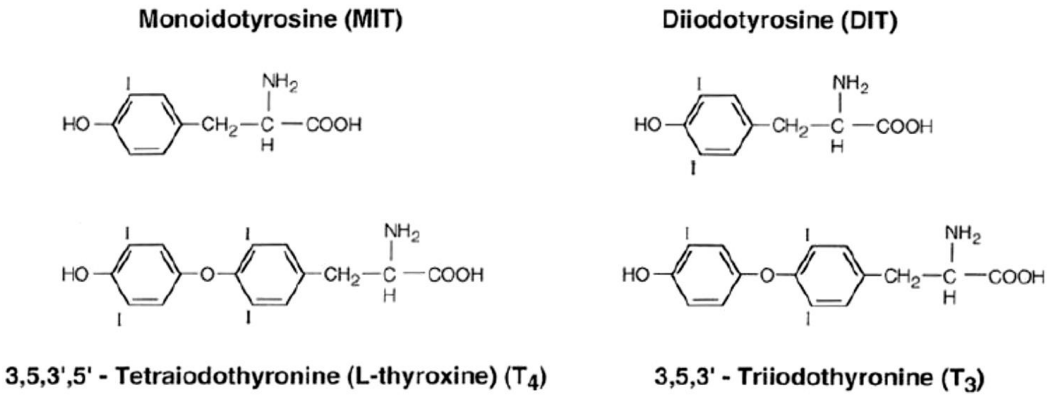


Figure (1-2) thyroid hormone structure. [3]

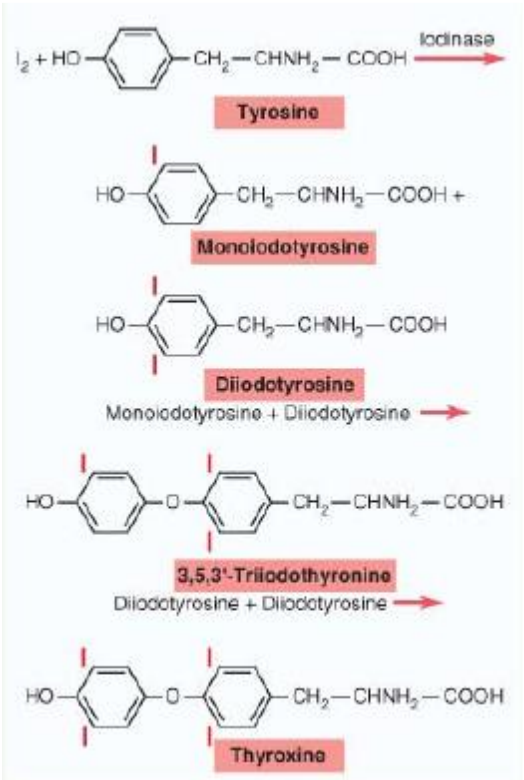


Figure (1-3) Chemistry of thyroxineand triiodothyronine formation.[4]

The thyroid gland required about 1/5000th of a gram from iodine every day to for body usage to the produce these two hormones. Tyrosine is also essential to produce thyroid hormones.

1.1.2 Thyroid Hormone Release and Transport

About 93 per cent of the thyroid hormone secreted from the thyroid gland is usually thyroxine hormone and only 7 per cent is triiodothyronine. Nevertheless, during the following few days, about one half of the thyroxineis gradually deiodinated to form extra triiodothyronine. Thyroxin and Triidotheronine syndicate mostly with thyroxine-binding globulinand much fewer so with thyroxine-binding prealbumin(transthyretin) and albumin. Binding to carrier proteins permits for uniform delivery of hydrophobic entities all over the circulation. [5]

The free fraction is the major determinant of:

- Accessible Quantity to the cells
- Turnover rate of hormone
- The individual Metabolic state
- Thyroid axis regulation.[6]

1.1.3 Thyroid Hormone Concentration

The thyroid hormones concentration inside the cell is 2-3 bend that seen in the plasma. The hormone curried into cells is intermediated by a family of energy-dependent transporters and many of them are cell-type specific. Triiodothyronine and Thyroxin generally do not contest for approval. Ones arriving the tissue cells, both thyroxine and triiodothyronine yet again bind with intracellular proteins. Proteins that bind Thyroxin and Triiodothyronine are existing in the cytosol and nucleus. [7]

1.1.4 Thyroid Hormone Receptor

Receptors for thyroid hormones are members of a huge family of nuclear receptors that contain those of the steroid hormones. They purpose as hormone-activated transcription factors and in that way act by modifying gene expression. In compare to steroid hormone receptors, thyroid hormone receptors bind DNA in the absence of hormone, typically principal to transcriptional suppression. Hormone

binding is related to a conformational alteration in the receptor that lead to function as a transcriptional activator. [8]

Mammalian thyroid hormone receptors are fixed by two genes, nominated alpha and beta. Additional, the chief transcript for each gene can be alternatively joined, producing different alpha and beta receptor isoforms. Presently, four different thyroid hormone receptors are recognized: alpha-1, alpha-2, beta-1 and beta-2. Like other members of the nuclear receptor superfamily, thyroid hormone receptors summarize in three functional domains.[9]

- **A *transactivation domain*** at the amino terminus that interrelates to other transcription factors to procedure complexes that suppress or trigger transcription. There is significant deviation in categorization of the transactivation domains of alpha and beta isoforms and among the two beta isoforms of the receptor.
- **A *DNA-binding domain*** that binds to series of promoter DNA known as hormone response elements.
- **A *ligand-binding and dimerization domain*** at the carboxy-terminus.[10]

The DNA-binding domains of the diverse receptor isoforms are very similar, but there is extensive departure between transactivation and ligand-binding domains. Supreme particularly, the alpha-2 isoform has a irreplaceable carboxy-terminus and does not bind triiodothyronine (Triiodothyronine) .[11]

1.1.5 Thyroid Hormone Actions

The broad-spectrum influence of thyroid hormone is to stimulate nuclear transcription of huge numbers of genes, in almost all cells of the body, countless numbers of structural proteins, transport proteins, protein enzymes, and other materials are created.[12]

The thyroid hormone receptor regularly formulae a heterodimer with retinoid X receptor (RXR) and binds at precise thyroid hormone response elements on the DNA. After that the hormone receptor complex initiate's the transcription of many genes. The thyroid hormones surge the metabolic activities of practically in each body tissues. [13]

1.1.6 Physiological Effects of Thyroid Hormones

• Increase foods utilization rate to make energy.

• Significant increase in Protein synthesis also increase the catabolism of protein.

• Increase the number and size of the mitochondria in all animal's body cells. What's more, increases the total membrane surface area of the mitochondria which elevated straight in percentage to the amplified the rate of metabolic of the entire animal. [14]

• Growth and development of the brain throughout the fetal life time and for the first few years of

Postnatal life require thyroid hormones.

- Inspiration the Metabolism of Carbohydrate

• Incentive the Metabolism of the Fat

– Reduce cholesterol concentrations, triglycerides, and phospholipids.

– Surges the low-density lipoprotein receptors numbers on the liver cells, as a results increase the removal of low-density lipoproteins from the plasma.

– Upturns expressively the cholesterol secretion rate in release in the feces and bile.

• Amplified tissues metabolism in the causes more quick use of oxygen than normal and relief of superior than normal amounts of metabolic end yields from the tissues. These effects effect vasodilation in most body tissues, therefore increasing blood flow. [15]

• surges Cardiac output.

• Nonstop influence on the nervousness of the heart, which in sequence rises the heart rate.

• Upturn the power of the heart when only a small surplus of thyroid hormone is secreted.

• Nevertheless, when thyroid hormone is amplified noticeably, the heart muscle strength develops dejected because of long-term extreme catabolism of the protein.

• Amplified metabolism rate of rises the use of oxygen and creation of carbon dioxide; these properties trigger all the mechanisms that upsurge the degree and penetration of respiration. [16]

1.1.7 CNS Effects of Thyroid Hormones

• Excitatory properties on the central nervous system, including cognition.

• Small growth in thyroid hormone habitually brands the muscles respond with potency, but when the quantity of hormone develops extreme, the muscles convert enfeebled because of additional protein catabolism.

• One of the most distinguishing ciphers of hyperthyroidism is a reasonable muscle shock.

• Because of the impulsive possessions of thyroid hormone on the synapses, it is hard to sleep, in case of surplus.[17]

1.1.8 Regulation of Thyroid Hormone Secretion

To uphold normal levels of metabolic action in the body, exactly the accurate quantity of thyroid hormone necessity be veiled at all times. TSH, also known as thyrotropin, is an anterior pituitary hormone, a glycoprotein with a molecular weight of about 28,000. The most significant initial influence after administration of TSH is to recruit proteolysis of the thyroglobulin, which causes freedom of thyroxineand triiodothyronine into the blood within 30 minutes. TSH binds with precise TSH receptors on the basal membrane surfaces of the thyroid cell. Furthermost, if not all, of its special effects result from activation of the "second messenger" cyclic adenosine monophosphate(cAMP)system of the cell.[18]

1.1.9 Thyroid Axis

Anterior pituitary secretion of TSH is ordered by a hypothalamic hormone, thyrotropin-releasing hormone (TRH), which is released by nerve endings in the median eminence of the hypothalamus. From the median eminence, the TRH is then transported to the anterior pituitary by way of the hypothalamic-hypophysialportal blood. TRH directly have emotional impact the anterior pituitary gland cells to upsurge their output of TSH. [19]

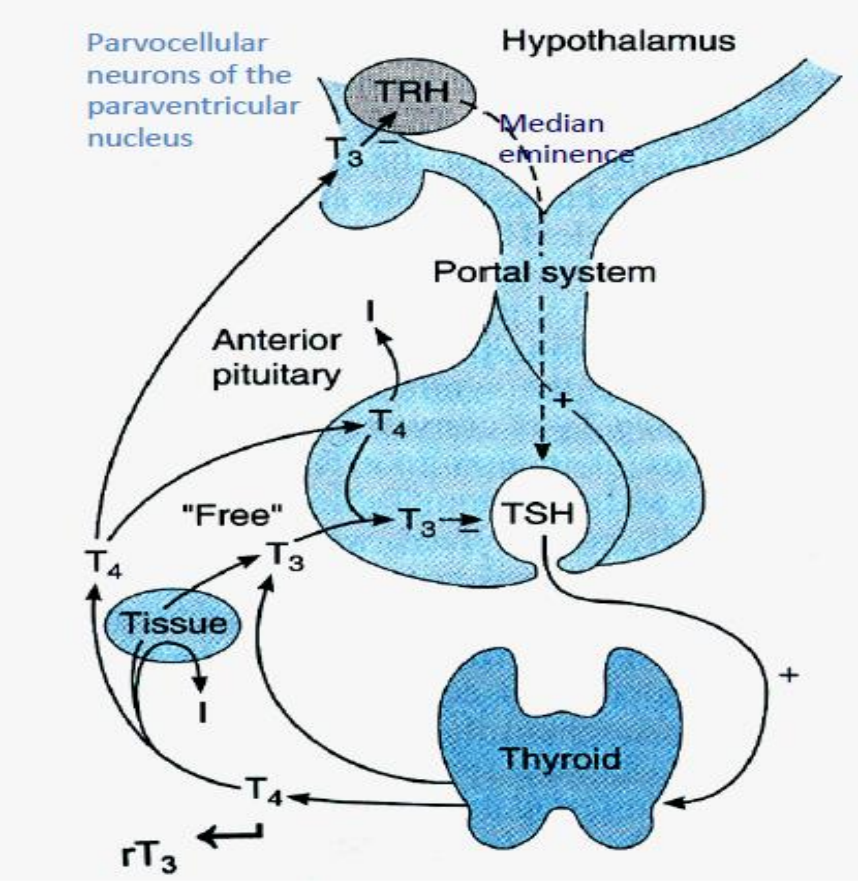


Figure (1-4): Thyroid Axis. [19]

1.2 Cancer

Cancer is a disease which take place when there are a fluctuations in an assembly of normal cells inside the body indication to abandoned growth producing a lump called a tumor. If left untreated, tumor can develop and extent into the nearby normal tissue. The explanations for a cell to convert cancerous are genetic modifications which are guilty for the directive of cell separation and cell death. Oncogenes and tumor suppressor genes are controlling genes that play significant part in cancer. A mutation in proto oncogene, which is a normal gene originate in many organisms as well as humans, taking part in signal transduction and implementation of mitotic signals, may turn it into a tumor prompting agent by inspiring the expression levels of oncogenes and their protein products. Alternatively, tumor suppressor genes are the genes that avoid a cell to progress to cancer. They code for proteins that regulate cell cycle, repair DNA impairment or stimulate apoptosis. Mutations in those genes surge the danger of cancer. [20]

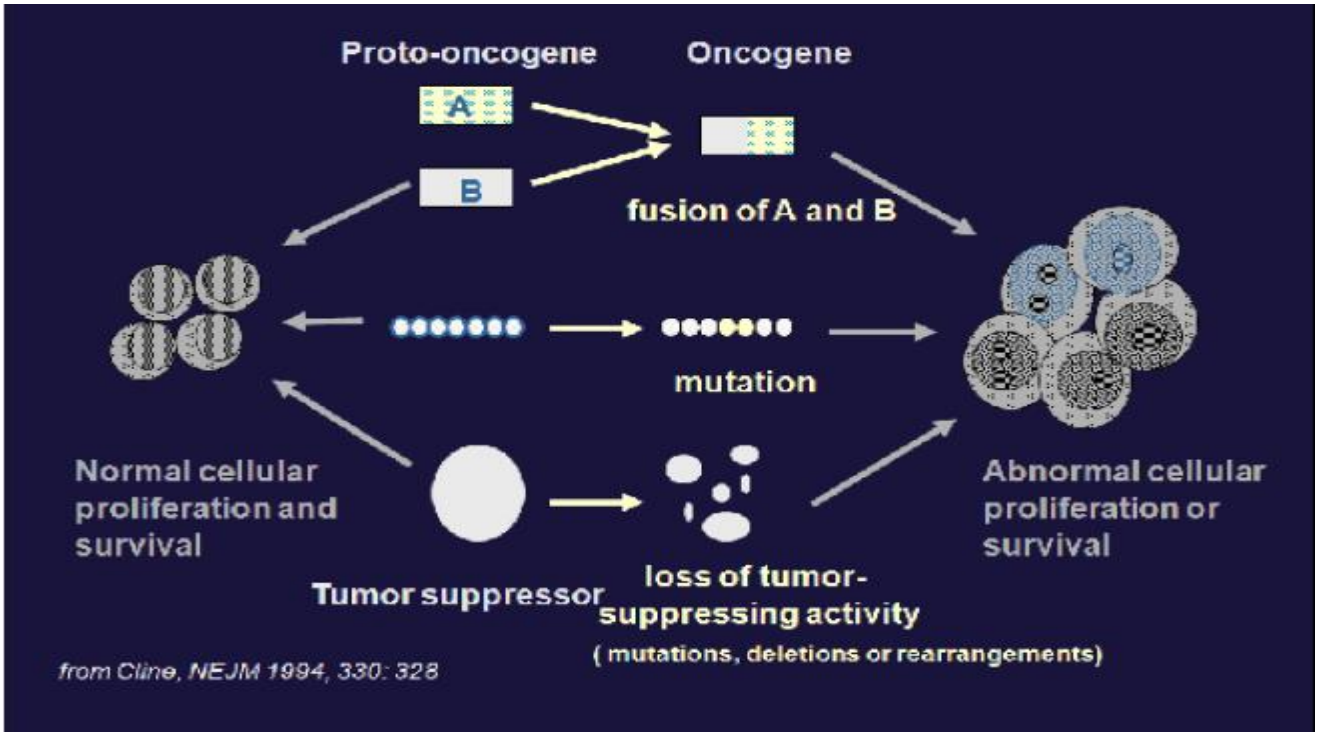


Figure (1-5): Oncogenes and Tumor Suppressor Genes. [20]

Tumors (lumps) can be benign or malignant.

• **Benign tumors** are not cancerous and seldom creep up life. They incline to grow quite Unhurriedly, do not spread to other parts of the body and are generally complete up of cells quite similar to normal / healthy cells. They will lone origin a tricky if they cultivate very large, becoming painful or press on other organs - for example a brain tumor inside the skull.

• **Malignant tumors** are more rapidly growing than benign tumours and have the capability to supper and terminate adjacent tissue. Cells of malignant tumours can disruption off from the main (primary) tumour and extent to other parts of the body through a process known as metastasis. Upon entering healthy tissue at the new site they endure to divide and grow. These secondary sites are known as metastases and the condition is referred to as metastatic cancer.[21]

Cancer can be classified according to the following categories:

• **Carcinoma** – A cancer that arises from the epithelial cells (the lining of cells that helpsprotect or enclose organs). Carcinomas may invade the nearby tissues and organs and metastasis to the lymph nodes and other areas of the body. The most common forms of cancer in this group are colon, lung, breast and prostate cancer.

Causes There are about 200 known types of cancer. As with most illnesses cancer is multifactorial, denotation there is no single cause for any one type of cancer, Causes by Cancer-causing substances (carcinogens), Genetics, Age and the immune system.

Accretion of mass of tissue established from abandoned cell divisions form tumor. Benign tumors are not cancerous, do not extent to other parts of the body and often can be detached without harm. On the conflicting malignant tumors are cancerous; they attack nearby tissues, mobile through blood and metastasize to other organs.

Cancer expansion process called carcinogenesis has 3 stages containing of initiation, promotion and progression. Initiation includes damage to DNA, chromosome or to the epigenome that controls the gene expression. Oxidative stress is the chief

driving force for initiation. Promotion is a long term stage of cellular growth of genomically uneven cells assisted by inflammation. Throughout the progression stage, while increasing, cells add more damage to their genome, evolving themselves into an aggressive malignant tumor.[21]

There are many types of cancer, but approximately all have similar abnormal physiology that demonstrations malignant growth. Self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, boundless replicative potential, sustained angiogenesis, and evading apoptosis are the developed abilities of cancer.[21]

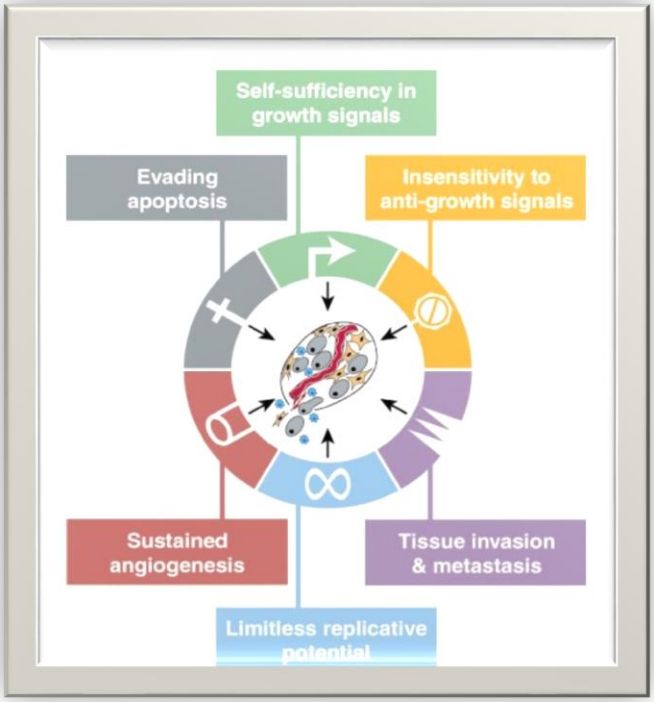


Figure (1-6): Acquired Capabilities of Cancer. [20]

Thyroid cancer starts in the thyroid gland. To understand thyroid cancer, it helps to know about the normal structure and function of the thyroid gland.

1.2.1 Medicinal Plants and Cancer Chemoprevention

Since 1960s, after National Cancer Institute (United States) ongoing to screen antitumor activity of plant extracts, interest on chemoprevention of medicinal plants and their extracts augmented. It is shown that dietary plants like fruits, vegetables, spices, cereals, and edible roots prevent cancer by inducing cellular defense systems such as cumulative detoxification and motivating antioxidant enzymes and constraining inflammation. Plant metabolites named phytochemicals have anticarcinogenic and antimutagenic properties. They affect with tumor promotion and progression. Among phytochemicals, phenolic compounds are under great investigation since they have wide variety of bioactivities. For instance, phenolic acids, ellagic acid and resveratrol, improved the expression of apoptotic genes, thus inhibiting cell proliferation in prostate cancer. In another study, when MDA-MB-231 cell intercardiac vaccinated mouse was treated with curcumin, reticence of matrix metalloproteinase which damages basement membrane and extracellular matrix, producing cancer cell movement making the cell invasive and metastatic was experiential.[20]

1.2.2 Drug Metabolizing Enzymes

Drug metabolism is the biochemical process in which the living organism alters the xenobiotics by particular enzyme systems to detoxify or activate the substances. After occupied into body, a pharmaceutical compound follows those 4 steps which determine the drug levels and kinetics of drug exposure to the tissue; absorption, distribution, metabolism and finally excretion (ADME).[20]

Drug metabolism consists of metabolic pathways which biotransform the molecules in a way that detoxifies and deactivates the poisonous compounds or occasionally activates the inactive prod rugs. Drug metabolism is divided into 2 phases. Figure (1-7) Phase I enzymes are Cytochrome P450 oxidases which carry out oxidation, hydrolysis or reduction reactions. They add reactive groups such as hydroxyl radical to the xenobiotic by using oxygen and NADH. These reactive intermediates are then further metabolized by Phase II enzymes, which carry out conjugation reactions

such as glucuronidation, sulfation, acetylation, methylation and glutathione and amino acid conjugations that transform the xenobiotic into water soluble compounds so that they can be excreted through urine or bile. [20]

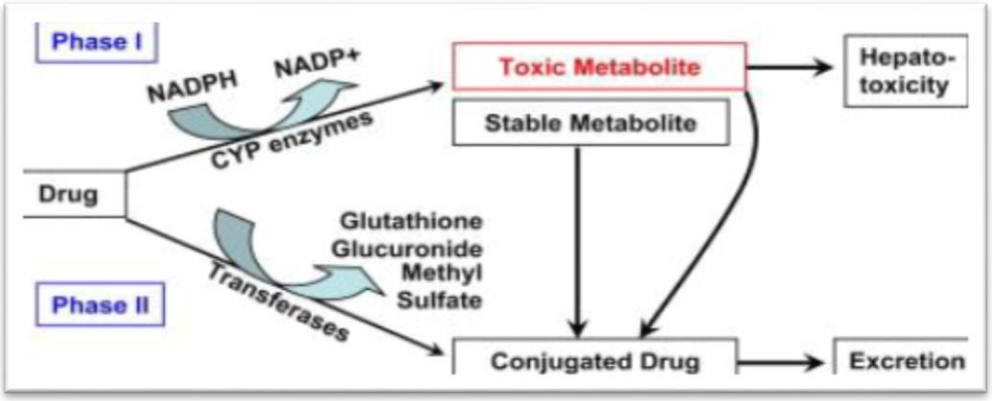


Figure (1-7): Phase I and Phase II reactions. [20]

Inhibition or induction of drug metabolizing enzymes may affect the efficacy of drug or may cause drug mediated toxicity. Nutrients can also have inspiration on drug disposition by inhibiting or persuading a number of enzymes. [20]

1.2.3 Benign Thyroid Enlargement and Nodules

Fluctuations in the thyroid gland's size and shape can often be sensed or even realized by patients or by their doctor. The medical term for an unusually big thyroid gland is goiter. Some goiters are diffuse, meaning that the whole gland is large. Other goiters are nodular, implication that the gland is large and has one or more nodules (bumps) in it. There are numerous details the thyroid gland might be larger than normal, and most of the time it is not cancer. Both diffuse and nodular goiters are regularly caused by an imbalance in certain hormones. For example, not getting sufficient iodine in the diet can lead to fluctuations in hormone levels and cause a goiter. [21]

Lumps or bumps in the thyroid gland are named thyroid nodules. Maximum thyroid nodules are benign, but about 2 or 3 in 20 are cancerous. Occasionally these nodules create too much thyroid hormone and lead to hyperthyroidism. Nodules that produce amplified thyroid hormone are practically always benign. [21]

People can progress thyroid nodules at any age, but they happen most commonly in older adults. Fewer than 1 in 10 adults have thyroid nodules that can be touched by a doctor. But when the thyroid is watched at with ultrasound, many more people are originate to have nodules that are too slight to sense. Furthermost evidence proposes that they are benign. [21]

Most nodules are cysts occupied with fluid or with a stored form of thyroid hormone called colloid. Solid nodules have little fluid or colloid. These nodules are extra likely to be cancerous than are fluid-filled nodules. Still, most solid nodules are not cancer. Some types of solid nodules, such as hyperplastic nodules and adenomas, have too several cells, but the cells are not cancer cells. [22]

Benign thyroid nodules occasionally can be left alone (not treated) as long as they’re not growing or causing symptoms. Others may need some form of treatment.

Types of malignant (cancerous) thyroid tumors

The main types of thyroid cancer are:

● Differentiated (including papillary, follicular and Hrthle cell)

● Medullary

● Anaplastic (an aggressive undifferentiated tumor)

A tumor involves new blood vessel development. The most significant factor associated with introduction and conservation of the new vasculature in human tumors

is a component known as the vascular endothelial growth factor (VEGF). If a component is tumor preventive. [22]

1.2.4 Papillary Carcinoma

Papillary carcinoma (PTC) is the most communal form of well-differentiated thyroid cancer, and the most common form of thyroid cancer to consequence from contact to radiation. Papillary carcinoma looks as an irregular solid or cystic mass or nodule in a usual thyroid parenchyma. Papillary/follicular carcinoma must be reflected a irregular of papillary thyroid carcinoma.[23]

Notwithstanding its well-differentiated characteristics, papillary carcinoma may be overtly or minimally aggressive. [24] Actually, these tumors may spread easily to other organs. Papillary tumors have a tendency to invade lymphatic but are less likely to invade blood vessels. [25]The life expectation of patients with this cancer is related to their age. The prognosis is improved for younger patients than for patients who are older than 45 years. [26]Of patients with papillary cancers, about 11% present with metastases external the neck and mediastinum. Some years ago, lymph node metastases in the cervical area were thought to be abnormal (supernumerary) thyroids because they contained well-distinguished papillary thyroid cancer, [27, 28] but occult cervical lymph node metastases are now known to be a common discovery in this disease.[29]

Fine-needle aspiration biopsy (FNAB) is deliberated the best first-line diagnostic procedure for a thyroid nodule. Surgery is the final management of papillary thyroid cancer. Approximately 4-6 weeks after surgical thyroid removal, patients may have radioiodine therapy to sense and destroy any metastasis and residual tissue in the thyroid.[23]

The papillary thyroid microcarcinomas are generally associated with an excellent prognosis that was find; however, 0.5% of patients may die. Risk factors for overall survival include the following:

- Age older than 45 years
- Male gender
- Minority strain
- Metastases nodes
- Extra thyroidal invasion
- Distant metastases

If two or more risk factors are existent, patients should be considered for more aggressive management.[30]

The serum thyroglobulin doubling time was a significant prognostic predictor in patients with papillary thyroid carcinoma was find. The authors concluded that this finding was superior to classical prognostic factors, including TNM age, stage, and gender.[31]

In a study comparing the behavior of 63 cases of encapsulated follicular-variant PTC with 43 cases of encapsulated classical papillary thyroid carcinoma (PTC), Rivera et al reported that the papillary form had a lower rate of vascular invasion (5% versus 25%; P = 0.007) but a higher frequency of capsular invasion (65% vs 38%; P = 0.01) and a significantly higher lymph node metastatic rate (26% vs 3%; P = 0.0014). According to the authors, even a meticulous search for vascular and capsular invasion cannot reliably predict the metastatic potential of encapsulated classical CPTC, so those cases can be treated like unencapsulated classical PTC.[32]

In a study of 39,562 patients with papillary thyroid carcinoma from the National Cancer Data Base, risk factors for central lymph node metastasis included age ≤45 years, male gender, Asian strain, and larger tumors.[33] A family history of papillary thyroid

carcinoma is an independent danger factor for disease duplication in patients with papillary thyroid microcarcinoma.[34]

1.2.9 Treatment

Treatment options for people with thyroid cancer

- Surgery
- treatment of Thyroid hormone
- Radioactive iodine therapy
- External radiation therapy
- Chemotherapy.[35]

1.3 Ginger (*Zingiber officinale*)

Since the beginning of recorded history, the use of herbs as medicines has played an important role in every culture including Asia, Africa, Europe and the Americas[36], the change from the use of herbal home remedies to medication prescription, medicinal plants are used as primary health care aid among 80% of the world's population in the form of plant extracts or their active components[37], Due to the safe status of herbal medicine then manufactured drugs , a need to be in control of their health and medical needs, a trend toward mistrust of the medical establishment, the high cost of medications and doctor visits, concern with preventive medicine, and a desire for a natural approach toward medical care. They are in great order in the developing as well as developed countries for daily health care. [38]

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Medicinal plants are of great importance to the health of individuals and communities. Herbal medicines serve the health needs of about 80% of the world's population. Ginger rhizome is typically consumed as a fresh paste, dried powder, slices preserved in syrup, candy (crystallized ginger) or for favoring tea. In many countries, especially in India and China, fresh ginger is used to prepare vegetable and meat dishes and as a flavouring agent in beverages and many other food preparations [39]

Therefore, ginger has many medicinal properties besides being highly used as a flavouring agent all over the world. It can be widely used in alleviating many diseases of humans and animals. Numerous experimental studies regarding the therapeutic activity of turmeric reported a plethora of pharmacologicalproperties of this vegetal extract, including: antioxidant, anti-inflammatory, anti-angiogenic, antibacterial, analgesic, immunomodulatory, proapoptotic, anti-human

immunodeficiency virus properties, being also studied in arthritis, diabetes, Alzheimer's disease.[40]

1.3.1 Botany

Botanical family: Zingiberaceae. Ginger is closely related to two other cooking spices, turmeric and cardamom Common names: Ginger, African ginger, Black ginger, Cochin ginger, Gan jiang , Gegibre, Ingwer, Jamaican ginger, Race ginger, Ginger Power, Zingiberis rhizoma, Gingerall, Cayenne Ginger, Ginger Peppermint Combo, Gingembre, Ginger root, and Ginger Trips.

Plant description: The plant produces an orchid like flower with petals that are greenish yellow streaked with purple colour. Ginger is an approximately 2-3 cm. in tall perennial with grass like leaves up to a foot in length. It is the underground root or rhizome.

Where it's grown: Ginger is cultivated in areas of abundant rainfall Indigenous to warm tropical climates, ginger is widely grown in Asia, India, Mexico, Jamaica, Africa, and Hawaii. Medicinal and culinary parts: The rhizomes (underground root)[41,42]

Figure (1-8): Ginger plant and roots.[43]

1.3.2. History

Plants have been used in a number of systems of medicines in our country likewise in other countries. India is well known as the 'Emporium of Medicinal Plants'. The use of plants to treat diverse diseases in India dates back to the times of RigVeda (3500 to 1800 B.C.). Later, the monumental Ayurvedic works like Charaksamhita and Sushrutasamhita followed by other Ayurveda and Siddha treatises have incorporated nearly 700 plant drugs entering into several medicinal preparations used in the management of health care. In fact these systems have been in practice even in distant areas of our country for centuries. Zingiber,is the genus name, came from a Sanskrit word denoting "horn-shaped", ginger is known as Sringavera In Sanskrit, which has given way to Zingiber in Latin and to the Greek Zingiber, from Vedic period Ginger has been used as medicine and is called "maha aushadhi", means the great medicine. [40] Ginger has been used by Indian medicine and traditional Chinese for over 25 centuries, it was also known from the 9th century in Europe and from the 10th century in England. For ginger medicinal properties Native Americans have also used wild ginger rhizome to heartbeat and regulate menstruation. [44]

By the Spaniards Ginger was brought to Mexico and introduced to Jamaica later, the latter currently being one of the world's essential producers of this species. Ginger is used mainly for gastrointestinal complaints in Mexican traditional medicine.[45]

It is a rhizomatous plant grown throughout South-eastern Asia, China and in parts of Japan, Austria, Latin America, Jamaica and Africa. It has been used as a spice and medicine in India and China since ancient times. It was known in Germany and France in the ninth century and in England in 10th century for its medicinal properties. [46]

The ginger family is a tropical group especially abundant in Indo-Malaysia, consisting of more 1200 plant species in 53 genera. The genus Zingiber includes about 85 species of aromatic herbs from tropical Australia and East Asia.[45]

1.3.3 Nutrient Composition

Practically, Ginger has 34.13% crude protein, 4.02% crude fat, 4.07% Ether Extract, 4.02% crude fibre content, 13.75% moisture content, 7.64% Ash content and 1.036% vitamin C. Furthermore, ginger contains major minerals like: Zn 64.0 mg/l , Mn 5.90 mg/l, Fe 279.7 mg/l, Cu 8.80 mg/l, Ca 280.0 mg/l and P 8068.0 mg/l. Ginger has been reported to contain usually 1-3% of volatile oil. The presence of high amount of fiber content, fat/oil, protein and essential minerals coupled with the therapeutic value of ginger root also contain a potent proteolytic enzyme called zingibain. It has a wide application in the area of food bio–fortification and for the development of bio-fortified foods. Although ginger root has been used several years as medicinal herb for the treatment of cancer cells and several other therapeutic purposes, it has however been found to be without side effect.[46]

Ginger exists with a numeral of commercial variety. Nigerian Ginger is more pungent taste, minute size and darker in color. Cochin Ginger is contains more starch, habitually larger, breaks with a shorter fracture, and well scraped. African Ginger is darker in color, less flavor and more pungent in taste than Jamaica Ginger. Ginger plant is proliferated by rhizome cuttings each carrying a bud. The pieces of rhizome are planted in a well- drained clayey soil in holes during March and April. In December or January rhizomes are unruffled. Ginger requires a humid and warm atmosphere. Required for its cultivation a well distributed rainfall. If the area is getting fewer rainfalls, the harvest needs usual irrigation.[40]

1.3.4 Chemistry

Present in ginger more than 60 active constituents are known, which have been approximately divided into volatile and nonvolatile compounds. Hydrocarbons mostly monoterpenoid hydrocarbons and sesquiterpene include the volatile component of

ginger and impart distinct aroma and taste to ginger. On the other hand, nonvolatile compounds include gingerols, paradols, shogaols, and also zingerone. Zingerone is produced by thermal degradation of shogaols or gingerols and also during drying of ginger directly. [47]

1.3.4 .1. Phenolic compounds: in shogaols and gingerols

Gingerol can be remain in two form depends upon its structure (the position of the methoxy group) known as [6]-gingerol and [10] - Gingerol. Both are the active constituent of fresh ginger which is chemically related with capsaicin and piperine, gives spiciness. It is normally found as pungent yellow oil, but also can form a low-melting crystalline solid [24]. In the fresh ginger rhizome, the gingerols were identified as the major active components and [6] gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one is the most abundant constituent in the gingerol series. In dried ginger powder, shogaol a dehydrated product of gingerol, is a predominant pungent constituent upto biosynthesis. In which [6]-Gingerol, [10]-Gingerol Zerumbone and Shogaols inhibit thyroid Cancer But various evidence are reported on [6]- Gingerol and [10]-Gingerol. Several experiment suggest that [6]-gingerol is effective in the suppression of the transformation, hyperproliferation of cells, and inflammation that initiate and promote carcinogenesis angiogenesis and metastasis. The gingerols, a series length of their unbranched alkyl chains, were identified as the major active components in the fresh rhizome, the leukotrienes are derived from the oxidative metabolism of arachidonic acid and are implicated in chronic inflammation and human cancer.[39]

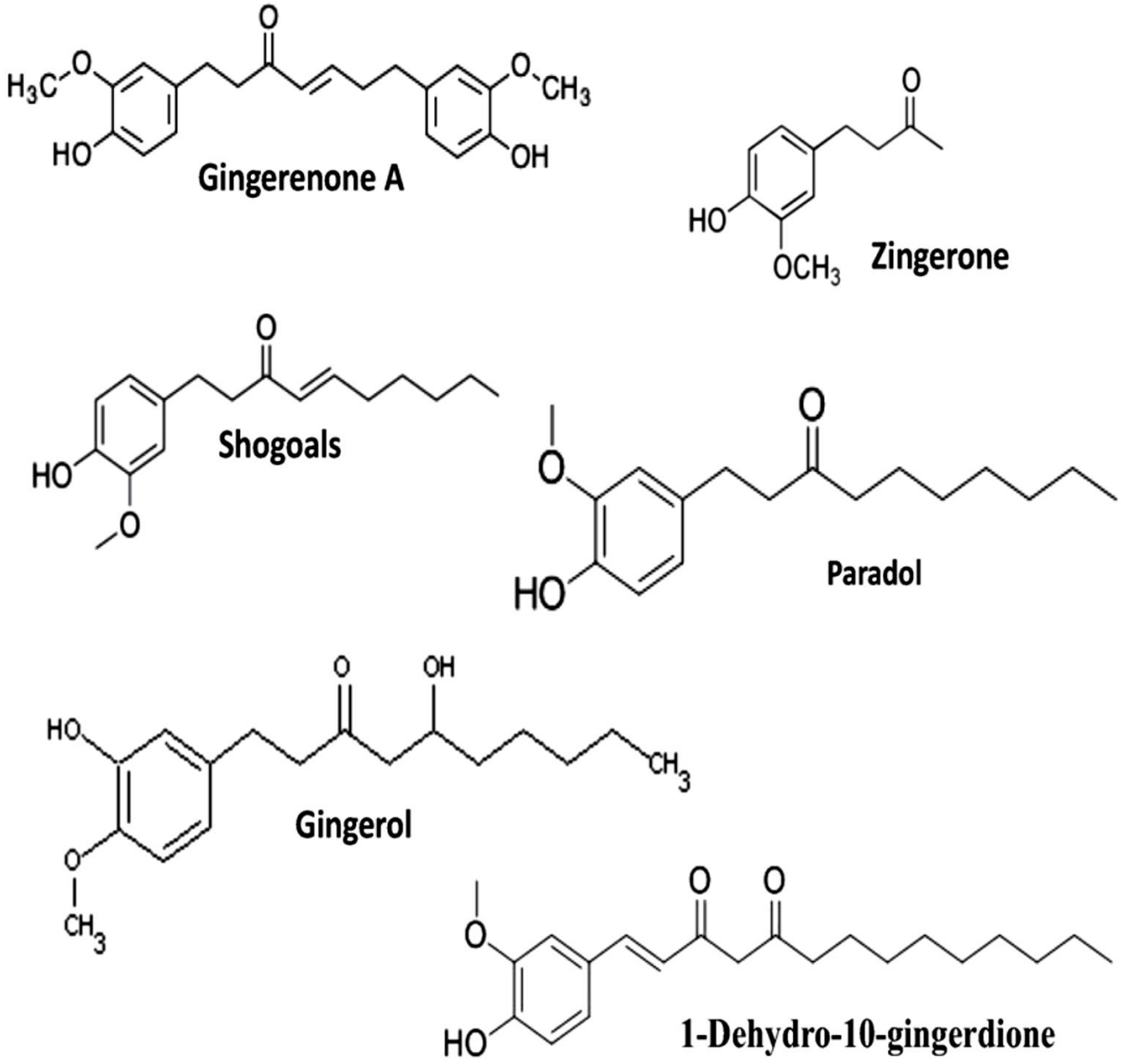


Figure (1-9): Chemical of structure of active ingredients of Ginger [45]

[6]-gingerol, the major pharmacologically active component of ginger which have antioxidant and anti-inflammatory properties and essential antimutagenic and anticarcinogenic activities.[39]

[10]-gingerol effect on[Ca^{+2}] and cell death in human colorectal cancer cells [6]-gingerol also induced[Ca^{+2}] elevation and which was cytotoxic to canine renal cells, But [10]-gingerol inhibits the human promyelocytic leukemia (HL-60) cells better than [6]-gingerol's and the activity of sarcoplasmic reticulum of Ca^{2+}-ATPase could be

stimulated by [10]-gingerol. The major Ca^{2+} storage in the majority of cells is endoplasmic reticulum. Various proteins and lipids are synthesized and modified in the endoplasmic reticulum. [40]

1.3.4.2. Sesquiterpenes: bisapolene, zingiberene, zingiberol, sesquiphellandrene, curcurmene

Numerous plants emit volatile compounds from their flowers. These volatiles belong primarily to three major groups of compounds: phenylpropanoids, fatty acid derivatives and terpenes.

1.3.4.3. 6-dehydrogingerdione, galanolactone, gingesulfonic acid, zingerone, geraniol, neral, monoacyldigalactosylglycerols, gingerglycolipids.

Zingerone is present in a significant amount of about 9.25% in ginger. It is a member of Methoxyphenol family and its related derivatives. They have a basic phenolic ring with a methoxy group attached to benzene ring. Zingerone is known to have potent pharmacological activities. Zingerone is primarily present in dry ginger, but cooking or drying also converts gingerol (another component in ginger) into zingerone by retroaldol reaction. [48]

Use of high profile liquid chromatography has shown that the contents of 6-gingerol, 8-gingerol, and 10-gingerol are usually low in fresh ginger while on drying and roasting the amount of zingerone increases significantly. Its pharmacological properties are varied including antioxidant, anti-inflammatory, anticancer and antimicrobial activity.[49]

Pharmacokinetics of zingerone has revealed that administration of zingerone either orally or intraperitoneally results in oxidation of side chain at all available sites. During catabolismof zingerone, glucuronidation and sulfation occur which leads to excretion of glucuronide compounds and sulphate conjugates in urine within 24 hours of consumption.[40]

The oil of ginger is a mixture of constituents, consisting of monoterpenes (phellandrene, camphene, cineole, citral, and borneol) and sesquiterpenes (zingiberene, zingiberol, zingiberenol, ß-bisabolene, sesquiphellandrene, and others). Aldehydes and alcohols are also present.

The therapeutically useful portions of ginger are the rhizome (root), the active ingredients in ginger are thought to reside in its volatile oils, which comprise approximately 1-3% of its weight. . Active constituents are called gingerols found in ginger's oleo-resin. These compounds have the following properties antipyretic, analgesic, antitussive, cardiac inotropic and sedative1. Dehydration products of gingerols are called shogaol homologues [48], the major active ingredients in ginger oil are the sesquiterpenes: bisapolene, zingiberene, and zingiberol. The concentrations of active ingredients vary with growing conditions. Ginger's active ingredients have a variety of physiologic effects. For example, the gingerols have analgesic, sedative, antipyretic and antibacterial effects in vitro and in animals [49]. The sensory perception of ginger in the mouth and the nose arises from two distinct groups of chemicals; Volatile oils and Nonvolatile oils. The volatile oil components in ginger consist mainly of sesquiterpene hydrocarbons, predominantly zingeberene (35%), curcumene (18%) and farnesene (10%) with lesser amounts of bisabolene, monoterpenoid hydrocarbons, 1, 8-cineole, linalool, borneol, neral, and geraniol and b- sesquiphellandrene. Many of these volatile oil constituents contribute to the distinct aroma and taste of ginger. Non-volatile pungent compounds contain biologically active constituents including the non-volatile pungent principles, such as the gingerols, shogaols paradols and zingerone that produce a ‘‘hot" sensation in the mouth. The increasing attention was paid to the anticancer actions of zerumbone. It wasreported that zerumbone exhibited a strong ability to treat liver cancer, lung carcinogenesis, and leukemia through increasing the apoptosis and inhibiting the invasion. [49]

1.3.5 Pharmacology and Clinical Studies

A- Anti-emetic Activity:

Early animal studies had explained the anti- metic property of fresh ginger, but it was Mowrey and Clayson clinical work which generated a spacious interest in this use of ginger. They compared the effects of 1.88g of dried powdered ginger, 100mg dimenhydrinate (Dramamine) and placebo on the symptoms of motion sickness in 36 healthy subjects who reported very high capability to motion sickness. Motion sickness was induced by placing the blind folded subject in a tilted rotary chair. Ginger was found to be superior to dimenhydrinate and placebo in preventing the gastrointestinal symptoms of motion sickness and the authors supposed a local effort in the gastrointestinal tract for ginger. This was particularly probably since it was given as a powder only 25 minutes before the test. The main anti-emetic compounds in ginger was gingerols and shogaols. [43]

B- Improvement of digestive function:

Early Chinese and Japanese research found that oral and intragastric application of fresh ginger decoction produced a stimulant action on gastric secretion1. German scientists found that chewing 9g of crystallised ginger had a profound effect on saliva production. [43]

Amylase activity was also increased and the saliva was not more watery, although it contained slightly less mucroprotein. Intraduodenal doses of ginger extract increased bile secretion in rats. Total secretion of bile solids was also increased, but not to the same extent as bile flow. 6-gingerol and 10- gingerol were identified as the active components. Fresh ginger also contains a proteolytic enzyme. [60]

Ginger, in conjunction with other pungent Ayurvedic herbs, increased the bioavailability of a number of drugs by promoting their absorption and/or protecting them from being metabolized in their first passage through the liver .Oral doses of 6-

shogaol accelerated intestinal transit in rats. Also an extract of ginger, and isolated 6-shogaol and gingerols, enhanced gastrointestinal motility in mice after oral doses. [45]

C-Antibacterial Activity:

Ginger also shows antimicrobial and other biological activities due gingerol and paradol, shogaols and zingerone. [37]

D-Anti-ulcer Activity:

Ginger and 6- gingerol inhibited experimental gastric ulcers in rats. Fresh ginger decocted in water resulted in symptomatic improvement in 10 patients with peptic ulcers. [43]

E-Antiplatelet Activity:

Srivastava and co-workers found that aqueous extract of ginger inhibited platelet aggregation induced by ADP, epinephrine, collagen and arachidonic acid in vitro. Ginger acted by inhibiting thromboxane synthesis. It also inhibited prostacyclin synthesis in rat aorta. The antiplatelet action of 6-gingerol was also mainly due to the inhibition of thromboxane formation. [43, 45]

F- Anti-inflammatory Activity:

Inflammation is a complex physiological response to infections, injury and damaged tissues that mediates the healing process. Chronic inflammation itself drives patho logical processes leading to diseases such as allergy, atherosclerosis, autoimmune diseases and cancer. Inflammation is therefore, finely tuned to protect from in fections and injury while preventing its excesses from causing chronic diseases. For over half a century, increased understanding of mechanisms and mediators of inflammation have directly contributed to the discovery of large and successful clinical application of numerous anti-inflammatory drugs. However, almost all current pharmaceutical drugs have serious side effects. [47]

Ginger extract inhibited carrageenan-induced paw swelling and was as active as aspirin. Essential oil of ginger inhibited chronic adjuvant arthritis in rats. Ginger and its pungent components are dual inhibitors of arachiodonic acid metabolism. That is, they inhibit both cyclooxygenase (prostaglandin syntheses) and lipoxygenase enzymes of the prostaglandin and leukotriene biosynthetic pathways.

G- Antipyretic Activity:

Ginger extract given orally reduced fever in rats by 38%, while the same dose of aspirin was effective by 44%. The antipyretic activity of 6-shogaol and 6- gingerol has also been observed.

H- Cardiovascular Effects:

Ginger exerted a powerful positive inotropic effect on isolated guinea pigs left atria. Gingerols were identified as the active components.

I-Antioxidant Activity:

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer, cardiovascular, disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcer-active colitis, and atherosclerosis.[45,50]

An antioxidant may be defined as "substances that when present in low concentrations, compared to those of an oxidisable substrate significantly delay or prevent oxidation of that substance". Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more

effective over the long term. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery.[51] For convenience, antioxidants have been traditionally divided into two classes, primary or chain chain-breaking antioxidants and secondary or preventative anti-oxidants. Secondary or preventative antioxidants are compounds that retard the rate of oxidation. This may be achieved in a number of ways including removal of substrate or singlet oxygen quenching. Primary antioxidants, AH, when present in trace amounts, may either delay or inhibit the initiation step by reacting with a lipid radical or inhibit the propagation step by reacting with peroxyl or alkoxyl radicals:

$$L^{\bullet} + AH \rightarrow LH + A^{\bullet}$$

$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet}$$

$$LO^{\bullet} + AH \rightarrow LOH + A^{\bullet}$$

The antioxidant free radical may further interfere with chain propagation reactions by forming peroxy antioxidant compounds:

$$A^{\bullet} + LOO^{\bullet} \rightarrow LOOA$$

$$A^{\bullet} + LO^{\bullet} \rightarrow LOA$$

The activation energy of the above reactions5 increases with increasing A–H and L–H bond dissociation energy. Therefore the efficiency of the antioxidant increases with decreasing A–H bond strength. [47]

Chain-breaking antioxidants may occur naturally or they may be produced synthetically as in the case of BHT, BHA. The synthetic antioxidants are widely used in the food industry and are included in the human diet. The use of naturally occurring antioxidants has been promoted because of concerns regarding the safety of synthetic

antioxidant, with natural alternatives (e.g., plant biophenols) possessing antioxidant activity similar to or even higher than that of synthetic antioxidants. [52]

Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress. This concept is supported by increasing evidence indicating that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this, thus lowering the risk of disease.

Zingiber officinale contains a number of antioxidants such as beta-carotene, ascorbic, acid, terpenoids, alkaloids, and polyphenols such as flavonoids, flavones glycosides, rutin, etc. Easily cultivable, Zingiber officinale with its wide range of antioxidants can be a major source of natural or phytochemical antioxidants. Although various extracts are obtained from ginger, it is the ethanolic extracts that are richest in polyphenol compounds and have a composition that closely resembles that of the rhizomes. Since ginger has been widely speculated to be beneficial for human health because it exerts antioxidant activity. [51]

Antioxidants affect the process of lipid oxidation at different stages due to the differences in their mode of action. Because of the complexity of the oxidation process itself, the diversity of the substrates and the active species involved, the application of different test methods is necessary to evaluation antioxidants. The objectives of the present study are to determine the antioxidant activity, in vivo the ROS,MDA,Catalyase, and in vitro antioxidant activity by barbutricacid. [52]

J-Pharmacokinetics

After injection, 90% of 6-gingerol was bound to serum protein and elimination was mainly via the liver. Oral or intraperitoneal dosage of zingerone resulted in the urinary excretion of metabolites within 32 hours, mainly as glucuronide and/or sulphate conjugates. Appreciable biliary excretion (40% in 12 hours) also occurred. [43]

K- Toxicity and Adverse Reactions:

The mutagenic activity of ginger extracts has been observed in several strains. As a result of component fractionation of ginger juice, it was found that 6- gingerol was a potent mutagen. When mutagenicity of gingerol or shogaol was tested in the presence of zingerone, it was observed that zingerone suppressed the mutagenic activity of bothcompounds. [47]

Ginger extract caused no mortality at doses of up to 2.5g/kg in mice (equivalent to about 75g/kg of fresh rhizome). This low acute toxicity was confirmed in a separate study, which also found that ginger extract at 100mg/kg per day for three months caused no signs of chronic toxicity. Topical application of ginger may cause contact dermatitis in sensitive patients. [43, 50]

M- **Phytochemistry:**

Ginger has been reported to contain usually 1-3% of volatile oil, pungent principles viz, gingerols and shogaols and about 6-8 lipids and others. Ginger oil contains Zingibereneand bisaboline as major constituents along with other sesqui and monoterpenes. Ginger oleoresin contains mainly the pungent principles gingerols and shogaols as well as zingiberone. Shogaols have recently been found to be twice as pungent as gingerols. [50, 51]

1.3.6 Dosage

Ginger can be consumed as a fresh or dried root and is often prepared in teas soft drinks (including ales), and breads. No specific dosing studies have been performed. however, most clinical research has used between 250 mg and 1 g of the powered root in capsular form, taken one to four times daily. For pregnancy-induced nausea and vomiting, most research studies used 250 mg four times daily. [53]

Ginger is usually regarded as safe in small amounts, or approximately 2-4 grams per day[54], High doses (presumably more than 4 grams per day) of ginger may have

uterine stimulating properties, Do not use in pregnancy or lactation, unless prescribed by a health professional, as the possible effects on the developing fetus have not yet been fullyascertainedIn one controlled study in humans, ginger ingested in various forms during pregnancy did not appear to increase the rates of major fetal malformations, In animal experiments, ginger has not shown any teratogenic effect when applied during pregnancy, found that ginger tea applied orally to rats was not materno-toxic, but increased fetal loss, although augmenting growth in the surviving fetuses.[42]

Rubbing the oil of ginger into painful joints and inhaling the fumes in steamed techniques have not been studied.[53]

1.4 Nanoparticle

The term "nanoparticles" is used to describe a particle with size in the range of 1nm-100nm, at least in one of the three possible dimensions. In this size range, the physical, chemical and biological properties of the nanoparticles changes in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk materials. Nanoparticles can be made of materials of diverse chemical nature, the most common being metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon and biomolecules. Nanoparticles exist in several different morphologies such as spheres, cylinders, platelets, tubes etc. Generally the nanoparticles are designed with surface modifications tailored to meet the needs of specific applications they are going to be used for. The enormous diversity of the nanoparticles arising from their wide chemical nature, shape and morphologies, the medium in which the particles are present, the state of dispersion of the particles and most importantly, the numerous possible surface modifications the nanoparticles can be subjected to make this an important active field of science now-a-days.[55]

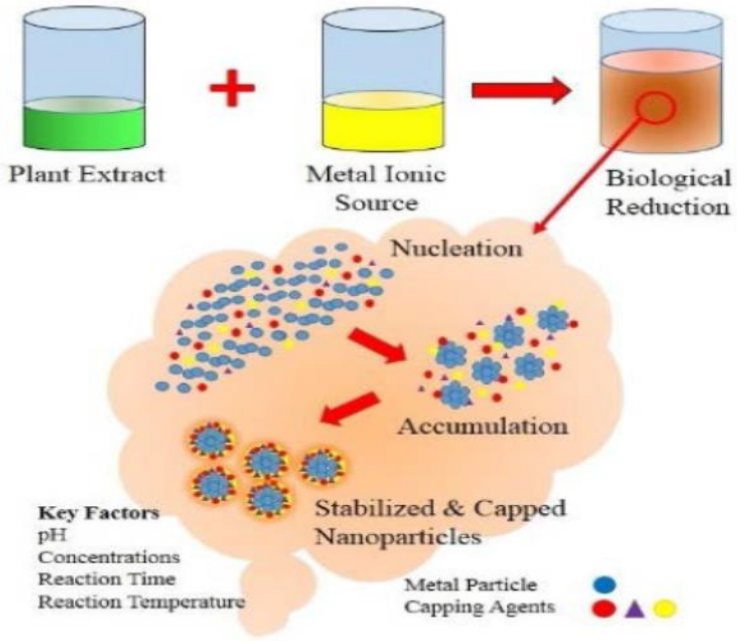
Importantly nanoparticles are highly dependent on their size, shape, and surface structure and processing tends to introduce surface imperfections. These surface imperfections can significantly impact on the overall nanoparticle surface physicochemical properties .In the bottom up approach, nanoparticles are built from atoms, molecules and smaller particles/monomers. In either approach, the resulting nanoparticles are characterized using various techniques to determine properties such as particle size, size distribution, shape, and surface area. This is of particular importance if the properties of nanoparticles need to be homogeneous for a particular application.[56]

In the case of chemical and biological synthesis of nanoparticles, the aqueous metal ion precursors from metal salts are reduced and as a result a colour change occurs in the reaction mixture. This is the first qualitative indication that nanoparticles are being formed. One interesting property of colloidal particles in solution, due to their size and shape, is their ability to be seen when a laser beam passes through the colloidal solution. This effect is known as the Tyndall effect and is a simple and straightforward technique that can be used to detect the presence of nanoparticles in solution. After the reaction, nanoparticles can be separated from the colloid by high speed centrifugation and then examined using advanced nanocharacterization techniques.[56,57]

Some of the spectroscopy and microscopy techniques routinely used include UV-visible spectroscopy (UV-vis), atomic force microscopy (AFM), transmissionelectron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive spectroscopy electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and Raman spectroscopy. Microscopy based techniques such as AFM, SEM and TEM are considered direct methods of obtaining data from images taken of the nanoparticles. In particular, both SEM and TEM have been extensively used to determine size and morphological features of nanoparticles. [56]

1.4.2 Types of Nanoparticles

Nanoparticles can be broadly grouped into two, namely, organic nanoparticles which include carbon nanoparticles (fullerenes) while, some of the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semi- conductor nanoparticles (like titanium oxide and zinc oxide). There is a growing interest in inorganic nanoparticles i.e. of noble metal nanoparticles (Gold and silver) as they provide superior material properties with functional versatility. Due to their size features and advantages over available chemical imaging drug agents and drugs, inorganic particles have been examined as potential tools for medical imaging as well as for treating diseases. Inorganic nonmaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good compatibility, and capability of targeted drug delivery and controlled release of drugs.[55]



Figure(1-10)Biological synthesis of nanoparticles using plant extracts[55]

1.4.3 Silver Nanoparticles

Silver nanoparticles are of interest because of the unique properties (e.g., size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles.[55]

The most popular chemical approaches, including chemical reduction using a variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction, and radiolysis are widely used for the synthesis of silver nanoparticles.[56]

Recently, nanoparticle synthesis is among the most interesting scientific areas of inquiry. And there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry). Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. This chapter presents an overview of silver nanoparticle preparation by physical, chemical, and green synthesis approaches.[57]

In conclusion the production of nanoparticles using biological entities has the potential to deliver new sources of novel materials that are stable, nontoxic, cost effective, environment-friendly, and synthesized using green chemistry approach. This green chemistry approach of using biological entities is in complete contrast with conventional chemical and physical processes that often use toxic materials that have the potential to cause environmental toxicity, cytotoxicity, and carcinogenicity. Whilst biological entities have been extensively used to produce nanoparticles, the use of plants offers a straightforward, clean, non-toxic, and robust procedure that does not need any special culture preparation or isolation techniques that are normally required

for bacteria and fungi based techniques. Plant extracts have the potential to produce nanoparticles with a specific size shape and composition. Plant synthesized nanoparticles have the potential to be widely used in current medical procedures involving nanoparticles such as fluorescent labelling in immunoassays, targeted delivery of therapeutic drugs, tumour destruction via heating (hyperthermia), and as antibacterial agents in bandages.[58]

Aims of Study

1.5 Aims of study

Trying to treatment the patients with thyroid cancer by herb (ginger) and knowledge the effect of thyroid cancer on kidney, liver function and level of glucose in blood by doing this steps:

1- Study the changes in levels of thyroid hormones (T3, T4, and TSH), kidney function (Urea, Creatinine), liver function (ALT, AST) and random blood Glucose RBG in patients with thyroid cancer

2- Study the effect of ginger *in vitro* in papillary thyroid cancer cells line (PTC-1) and explore the antioxidant activity *in vivo* in mice.

3- Prepare the ginger nanoparticles and study the properties of GNPs and its effect on papillary thyroid cancer cells line.

CHAPTER TWO

Subjects and Methods

MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant Material

Fresh Ginger (*Zingiber Officinale*) Root Imported From Chain,Iraq Country Not A Producer Of Ginger .

2.1.2 Cell Line

TPC-1 (Thyroid Papillary Cancer Cell Line) supplied by Pharmacology department/ Medicine college/ Malaya university.

2.1.3 Chemicals And Other Materials

Table (2-1): List of chemicals used.

Material	supplier	purity %
Macconkey Ager Medium	SIGMA-ALDRICH	-
Nutrient Ager Medium	SIGMA-ALDRICH	-
MTT	SIGMA-ALDRICH	-
Sabouraud dextrose	SIGMA-ALDRICH	-
Chloroform	GCC	99.5%
DMSO(Dimethyl sulfate oxide)	RIEDEL-DE HAEN AG SEELZE-HANNOVER	99.5%
Acetone	ROMIL-SPS	99.7%
Ethanol absolute	SIGMA-ALDRICH	99.8%
Methanol	ROMIL-SPS	99.9%

Hexane	ROMIL-SPS	99.9%
Silver chloride AgCl	SIGMA-ALDRICH	99.999%

Table (2-2): The Instruments were use in this study.

Instrument	Source	Model
Atomic Force Microscope(AFM)	Angstrom advanced Inc. ,USA	AA2000
Water Bath Shaker	Alta Alta laboratorium, USA	BS-11
Transmission electron microscopy(TEM)	PHILPS, USA	CM10
Microplate Washer	Biotek, USA	ELX50
Absorbance Microplate Readers	BioTek, USA	ELX800
Scanning electron microscopy(SEM)	FEI, Japan	INSPECT S50
infrared spectroscopy (FTIR)	SHIMADZU, Japan	IRAFFINITY-1
Autoclave	Autoclave LABTECH, Indonesia	LAC-5040S
Hotplate stirrer	LABTECH, Indonesia	LMS-1003
CO2 Incubator	Sanyo, Japan	MCO-17AIC
Digital Balance	Technical Advantages, Australia	OHAUS Analytical Scales
Vacuum Oven	Gallenkamp, UK	OVL 570 010J
spectrophotometer	APEL, Japan	PD-303
flow laboratories	Gelaire, Germeny	TC60

X-Ray Diffraction(XRD)	PANalytical, Japan	X'Pert PRO
Centrifuge	Hermle LaborTechnik GmbH,Germeny	Z 200 A

2.2 Methods

2.2.1 Part One

2.2.1.1 Patients subjects

Fifty female were selected according to age less than 45year and fifty male with thyroid cancer, which were done in Alamal hospital/Baghdad/Iraq. Radiotherapy department during the period from October 2015 to January 2016.

2.2.1.2 Control subjects

For the purpose of comparisons, 50 healthy male and 50 healthy female these age less than 45 without cancer, matched for age, sex and ethnic background (Iraqi) were incorporated into the study.

Control was selected among subjects that apparently healthy in condition of non-diabetic, non-hypertensive and with no family history thyroid cancer or other types of cancer. In addition, they had no history of smoking or alcohol consumption.

2.2.1.3 Characteristic of patients and control

Thyroid cancer patient and control were characterized in condition of age, gender, family history, urea, creatinine, ALT, AST, Glucose, T3, T4 were measured for patients and control.

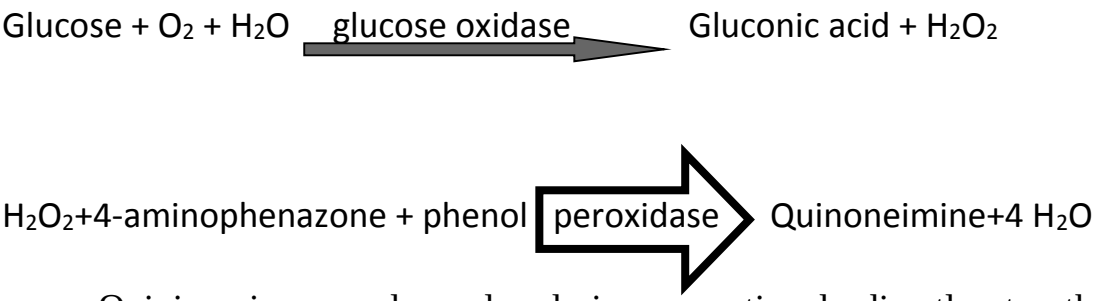
2.2.1.4 Blood Sample

Three milliliters of blood samples have been compiled from each subject by vein puncture using 5ml disposable syringe between (8 am and 11 A.M). Poured the blood samples in activator clotting tubes, then were centrifuged at (3000rpm) 10 min after allowing the blood to clot at R.T. The sera were frozen at (-20 °c) until the assay day.

2.2.1.5 Biochemical Test

2.2.1.5.1 Determination of Serum Glucose

Determination of Serum glucose by the enzymatic oxidation method, which was measured the activity of the enzyme glucose oxidase at 500nm. Kits were supplied HUMAN Gesellschaft für Biochemica und Diagnostica mbH



Quinine is a red –colored is proportional directly to the concentration of glucose in original sample, measurement done at 505 nm by used spectrophotometer supplied by APEL.

2.2.1.5.2 Determination of Serum Urea

Determination of Serum Urea by Kit was supplied HUMAN, Germeny. Urea is hydrolyzed in the existence of water and urease to yield carbon dioxide and ammonia. In a changed Berthelot reaction

hypochlorite and salicylate react with ammonium ions to yield a green dye.

The absorbance increase at 546 or 578nm is change with concentration of urea in the sample. Measurement done at 578 nm by used spectrophotometer supplied by APEL.

2.2.1.5.3 Determination of Serum Creatinine

Determined serum creatinine by Kit was supplied HUMAN kit, Germany. Creatinine forms in alkaline solution colored with organic-red complex with picric acid. The absorbance of this complex is indicator to the concentration of creatinine in the sample.

Creatinine + picric acid → creatinine-picrate complex

Measurement done at 492 nm by used spectrophotometer supplied by APEL.

2.2.1.5.4 Determination of Serum AST

Aspartate aminotransferase (AST) catalyzes amino group transferred from L-aspartate to α-ketoglutarate to yield oxaloacetate and L-glutamate. The reduction of oxaloacetate with simultaneous oxidation of $NADH^{+}$ to NAD catalyzed by Malate dehydrogenase (MDH). The resultant rate of lessening in absorbance at 340 nm is proportional directly to the activity of AST. Added (LDH) lactate dehydrogenase to avoid interference from endogenous pyruvate which is usually existing in serum.[72]

$$\text{L-aspartate} + \alpha\text{-ketoglutatrate} \xrightarrow{\text{AST}} \text{oxaloacetate} + \text{L-glutamate}$$

$$\text{NADH} + \text{Oxaloacetate} + H^+ \xrightarrow{\text{MDH}} \text{L-malate} + NAD^+$$

2.2.1.5.5 Determination of serum ALT

The ALT procedure is based on principles outlined by Wroblewski and LaDue1 and utilizes a modification of the methodology recommended by the (IFCC). The amino group transferred by ALT from alanine to α-oxoglutarate to procedure glutamate and pyruvate. The pyruvate enters a lactate dehydrogenase (LDH) catalyzed reaction with NADH to produce lactate and NAD+. The decrease in absorbance due to the consumption of NADH is measured at 340nm and is proportional to the ALT activity in the sample. [59]

$$\text{L-Alanine} + \alpha\text{-Oxoglutarate} \xrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+$$

2.2.1.6 Hormones

Total triiodothyrionin T3, Total thyroxine T4 and Thyrotropin TSH the Kits supply by AccuBind ELISA Monobind Inc, USA.

2.2.1.6.1 Total triiodothyrionin T3

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration.

The enzyme activity, determined by reaction with a substrate that generates light, in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

2.2.1.6.2 Total thyroxin T4

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration.

The enzyme activity, determined by reaction with a substrate that generates light, in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be

generated from which the antigen concentration of an unknown can be ascertained.

2.2.1.6.3 Thyrotrophin (TSH)

The essential regents required to an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex.

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration.

The enzyme action in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing sevral different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

2.2.2 Part Two: Ginger Examination

2.2.2.1 Preparation plant

Ginger rhizome fresh imported from china .it was washed with clean water ,cut ,and dried in vacuum oven for 48h(2day) in 80C° temperature ,then using milling machine, the plant cutlets were milled to powder. The powder was weighted using Electronic weighting balance.

Weight of the total powder was: 1kg of ginger yield 80.00g dried powder

2.2.2.2 Extraction

2.2.2.2.1 Aqueous Extraction

1.10g of ginger rhizome powder was boiled in 400ml of distilled water till one of fourth of the extract initially taken was left behind after evaporation [37].

2. The solution was filtered by using muslin cloth.

3. Centrifuged the Filter at 5000 rpm for 15 min.

4. Filtered the supernatant using Whatman filter paper No.1 under aseptic condition.

5. Collected the filtrate and stored at 4C° until further use.

2.2.2.2.2 Organic extraction

1. 10g of ginger rhizome powder was mixed with 100 ml organic solvent (viz. Ethanol, methanol, hexane, acetone, chloroform).

2. Placed the mixtures at 25C for 24h under magnetic stirring.

3. Filtered the Solutions throw muslin cloth and then re-filtered through Whatman No.1.At 25C^{o} the filtrate thus obtained was concentrated by completed evaporation of solvent to yield the pure extract.

4. repeated this process of extraction to recover larger quantity of the extracts and they were stored in the refrigerator at -4C˚ for further use.

Recovered the yield as percentage of the quantity of the initial plant material (10g) used.

Yield (%) = ((yield*100)/10g)

2.2.2.3 Preparation concentration

1. Prepared the stock solutions of organic solutions by dissolve organic extracts dried by DMSO (Dimethyl sulfoxide) and Stock solution of aqueous extract prepared by dissolve aqueous extract dried by distil water.

2. Prepared from it various concentration (1000, 500, 250, 125, 62.5) mg\ml diluted with DMSO for organic extract and diluted with water for aqueous extract.

3. Then filter it with micro filter 0.45µm and used or stored at 4C˚ until further use.

2.2.2.4 Biological activity

2.2.2.4.1 Antibacterial activity

2.2.2.4.1.1 Bacterial strains selected for susceptibility Assay

The tow bacteria namely Escherichia coli (gram-negative bacteria) and Staphylococcus aureus (gram-positive bacteria) were screened for present investigation. These bacterial cultures were maintained in nutrient agar slants at 37°C. Each of the microorganisms was reactivated before to sensibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.

2.2.2.4.1.2 Antibacterial assay

Antibacterial activities of all aqueous and organic extracts of ginger rhizome were determined by standard agar well diffusion assay [54].

1. Petri dishes (100 mm) containing 18 ml of red MacConkey ager medium for Escherichia coli and nutrient ager for Staphylococcus aureus seeded with 100µl inoculum of bacterial strain take it from third dilution of bacteria (100µ of stock bacteria in 10 ml normal saline then take 100µl of that in 10ml normal saline in other tube, finally take 100µl of that in 10 ml normal saline, this last dilution will use in culture).

2. Allowed media to solidify and then individual petri dishes were marked for the bacteria inoculated .wells of 6mm diameter were cut in solidified ager media with help of sterilized cup-borer.

3. 50µl of each concentration of extracts were poured in the wells and plates were incubated at 37C˚ for 24h. In organic solvent DMSO used as

negative control. And with aqueous used water as control, the zone diameter of inhibition were measured and recorded.

2.2.2.5 Determination of Antifungal activity

2.2.2.5.1 Culture methods

The test organisms (The fungal strains used were Aspergillus niger, Aspergillus flavus, Candida albicans) were stored on Sabouraud dextrose agar slopes, prepared from Sabouraud Broth base. The fungal strains were obtained from fungal laboratory of AL-Nahrain University in Medical microbiology department.

2.2.2.5.2 Antifungal assay

The antimicrobials present in the Ginger are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The fungicidal effect of the Ginger extracts can be assessed by the inhibition of mycelial growth of the fungus and is observed as a zone of inhibition near the wells. The inoculum density of test fungi were adjusted by using 0.5 McFarland standard tubes then plated onto Sabouraud dextrose agar in three directions by sterile swabs. Wells (7 mm diameter) were punched in the plates using a sterile stainless steel borer. The wells were filled with 1-32 µg/ml containing 0.1mg/ml, 1mg/ml, and 10 mg/ml from Ginger per well respectively (table 1) and incubated at 35°C for 72 hr.[60]

Table (2-3): serial two fold Ginger dilution to determine the MIC using agar well diffusion method

Ginger concentration Mg/ml	**Volume of Ginger Stock solution (µl/ml)**	**Final concentration (µl/ml) When adding 25 ml D.W or DMSO.**
0.1	250	1
1.0	50	2
1.0	100	4
1.0	200	8
10.0	40	16
10.0	80	32

2.2.2.6 Cytotoxicity Assay (MTT assay)

2.2.2.6.1 Cell culture and treatment for ginger extract

Thyroid papillary carcinoma cell line(TPC-1) were cultured in 100 µl of RPMI 1640 (Roswell Park Memorial Institute medium) media containing 10% fetal bovine serum (FBS).TPC-1 cells were incubated overnight at 37°C in 5% CO_2 for cells attachment.

2.2.2.6.2 MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

2.2.2.6.2.1 Kit contents

- Hidex Chamelon plate Reader with a 570nm filter.
- MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide (MW = 414), 10 vials, each containing 5 mg

- Multi-channel pipette (8 to 12 channel)
- Pipette tips (10-100µL)
- phosphate-buffered saline (PBS), dimethylsulfoxide (DMSO) optional.
- CO_2 incubator (5%).

2.2.2.6.2.2 Preparation Instructions

MTT Solution: 5 mg/ml MTT in PBS. Solution must be filter

Sterilized after adding MTT.

2.2.2.6.2.3 Procedure

1. Seeded the cancer cells in 96-well plates at a density of 1×10^4 cells/well in 100 µl RPMI. 24 h

2. After seeding, removed the medium and then incubated the cells for 3 days with RPMI in the absence and the presence of various concentration of ginger extracts.

3. Added Ginger extract solution at various concentrations ranging from (1ooo, 500,250,125, 62.5, 31.25, 15.7, 7.87) µg/ml and incubated.

4. Reagent was added into each well after incubation 20 µl of MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide]. Incubated this plate again for 4 h in CO_2 incubator at 37°C.

5. The resulting MTT–products were determined by measuring the absorbance at 570 nm using a micro plate reader represents the mean of triplicate experiments. The sell viability was determined using the formula:

Viability (%) = (optical density of sample/optical density of control) ×100

IC50 values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line. Non-treated cells were used as control.

2.2.2.7 Determine antioxidant activity of ginger *in vivo* in mice

2.2.2.7.1 Animals and experimental design

Fourteen adult (mixed Gender) mice weighted 24±5g (obtained from animal house of biological department, Science College of women, Baghdad University), they fed stander diet consisting of corn, bean and bread and allowed food and water. After acclimatization for 1 week, the animals were maintained in animal's house of Biotechnology Research Center, Al-Nahrain University. Animals were handled with human care. The mice were randomly divided into four groups each consisting of four animals as in following design: the first group fed basal diet and tap water and kept as control group. The 2nd group the mice were treated orally with ginger at dose body weight (250mg/Kg) for 14 days. The 3rd group the mice treated with ginger at dose body weight (500mg/Kg) for 14 days. The 4th group the mices treated with ginger at dose body weight (750mg/Kg) for 14 days. [61]

2.2.2.7.2 Preparation of blood Samples

At the end of experiment, blood samples were withdrawn from the heart of each mice and each sample was collected into clean tubes. The blood samples were allowed to coagulate and then centrifuged at 3000 rpm for 10 min. The separated serum were kept at -20°C until used for the estimation of serum activity of MDA, SOD, and Catalase enzyme.

2.2.2.7.3 Antioxidants analysis

2.2.2.7.3.1 Measure of malondialdehyde (MDA)

The level of malondialdehyde was determine by modified procedure. The peinceple of the test was based on the reaction of MDA with thiobarburtic acid (TBA); forming MAD-TBA_2 product that absorbs strongly at 532 nm as following reaction.

Molondialdehyde + 2 TBA red complex

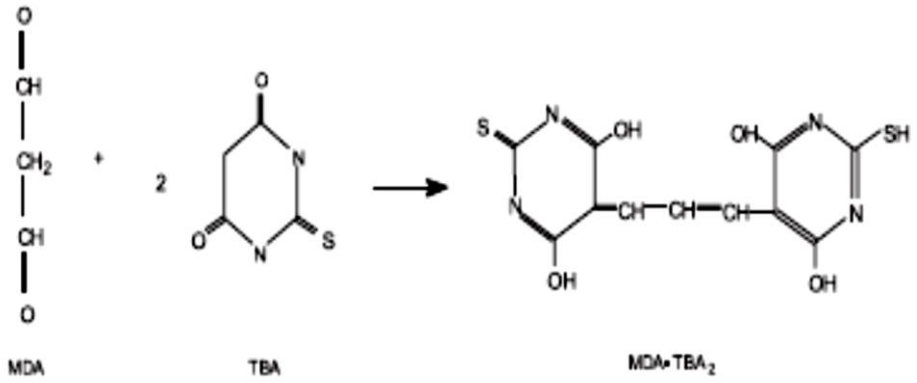


Figure (2-1): The Reaction between MDA and TBA to form the MDA-TBA2 pigment [78]

2.2.2.7.3.2 Quantitative colorimetric determination of SOD activity (BioAssay System Kit)

SUPEROXIDE DISMUTASES (SOD, EC1.15.1.1) are enzymes that catalyze the dismutation of superoxide into O2 and H_2O_2. They are an important antioxidant defense in all cells exposed to O_2. There are three major families of superoxide dismutase: Cu/Zn, Fe/Mn, and the Ni type. Aberrant SOD activities have been linked to diseases such as amyotrophic lateral sclerosis, perinatal lethality, neural disorders and cancer. BioAssay Systems' SOD assay provides a convenient colorimetric means for the quantitative determination of SOD enzyme

activity in biological samples. In the assay, superoxide (O_2^-) is provided by xanthine oxidase (XO) catalyzed reaction. O_2^- reacts with a WST-1 dye to form a colored product. SOD scavenges the O_2^- thus less O_2^- is available for the chromogenic reaction. The color intensity (OD440nm) is used to determine the SOD activity in a sample.

2.2.2.7.3.3 Quantitative colorimetric /Fluoriemetric catalase determination

CATALASE (EC 1.11.1.6), is a ubiquitous antioxidant enzyme that Catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and Oxygen.

$$2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2\,H_2O$$

By preventing excessive H_2O_2 build up, catalase allows important cellular Processes which produce H_2O_2 as a byproduct to occur safely Deficiency in catalase activity has been associated with grey hair and peroxisomal disorder acatalasia. Simple, direct and high-throughput Assays for catalase activity find wide applications. BioAssay Systems' Improved assay directly measures catalase degradation of H_2O_2 using a Redox dye. The change in color intensity at 570nm or fluorescence Intensity (λem/ex = 585/530nm) is directly proportional to the catalase Activity in the sample.

2.2.3 Part Three: Nanoparticle

2.2.3.1 Preparation Ginger Nanoparticle

1. Extracted 1gm of ginger powder with water and filtered.

2. Added 1mM silver nitrate solution to the filtrate slowly under magnetic stirring conditions for even coating of silver and subjected to heating at 12°C for 10 min. used the extract as reducing and stabilizing agent for 1mM of Silver nitrate. This one pot green synthesis was the modified method followed by Vigneshwaran *et al*.[55]

2.2.3.2 Characterization of synthesized GNPs

Through sampling the bioreduction of Ag+ in aqueous solution of ginger extract was monitored. Diluted 0.2 ml reaction media with 2 ml deionized water and the resulting diluents were subsequently measured by UV-Vis spectra. UV-Vis spectroscopy analyses of silver nanoparticles produced were carried out as a function of bioreduction time on Ultraviolet-Visible spectroscopy (shimadzu)

Samples of the aqueous solution of the silver nanoparticles (GNPs) were prepared by dried on room temperature for 2 days. The pellet was subjected to FT-IR analysis by KBR pellet (FT-IR grade) method and the spectrum was recorded using spectral range of 4000~400 cm^{-1} (shimadzu).

The interaction between protein-silver nanoparticles were analyzed by Fourier transform infrared spectroscopy (FTIR) in the diffuse reflectance mode at a resolution of 4cm-1 in the KBr pellets and the spectra were recorded in the wavelength interval of 4000 to 400nm-1. FTIR measurements were carried out to identify the biomolecules

responsible for the reduction of the Ag+ ions and the capping of the bioreduced GNPs synthesized by ginger extract. For comparative study, the ginger filtrate was mixed with KBr powder, pelletized after drying and subjected to measurement.

X-ray diffraction (XRD) measurement of the seaweed reduced GNPs was carried out using powder X-ray diffractometer instrument (X-RAY ROWDER DIFFRACTION 'X'Pert PRO PANalytical) in the angle range of 10°C-80°C at 2θ, scan axis: 2:1 sym.

Thin films of the sample were prepared on a carbon coated copper grid by dropping a minute of the sample on the grid. The film on the SEM grid were allowed to dry under a mercury lamp for 5 min prior to measurement using INSPECT S5 SEM machine.

The presence of elemental Silver was confirmed through EDAX. The EDAX spectrum recorded in the spot mode from one of the densely populated silver nanoparticles region on the surface of the film. The nan crystallites were analyzed using INSPECT S5 SEM machine

Sample for Transmission Electron Microscopic (TEM) analysis were prepared by coating GNPs solutions onto carbon coated copper TEM grids. The film on the TEM grid was allowed to dry prior to measurement using CM10 Philips instrument operated at an accelerating voltage of 100 KV.

2.2.4 Statistical analysis

Performed Statistical analysis by statisticians with the SPSS 15.01 Statistical Package for Social Sciences and also Excel 2003. Data analysis was done using chi- square test for tables with frequencies, while independent sample t-test was used for tables with means and standard Deviation. P value of ≤ 0.05 was used as the level of significance. Used correlation coefficient to find the correlation between studied markers by using Pearson correlation.

CHAPTER THREE

RESULTS and Discussion

Results of part one: clinical part

3.1 results of assay for patient with thyroid carcinoma

One hundred Iraqi thyroid cancer samples were included in this study. The results of clinical analysis for thyroid carcinoma patient when Comparison between groups for age,T3,T4, Creatinine, Urea, ALT, AST and Glucose showed in table(3-1) in which mean T4(66.297 vs7.110) µg/dl for females and (75.552 vs 6.758) µg/dl for males , T3(2.575 vs 0.101) µg/dl for females and (3.188 vs 0.097) µg/dl for males, Urea (14.165 vs 10.94) mmol/L for females and (16.135 vs 12.64) mmol/L for males, Creatinine (3.717 vs 1.162) mmol/L for females and (2.793 vs 1.101) mmol/L for males, ALT (20,080 vs 17.828) U/L for females and (20.529 vs 18.48 U/L) for males , AST (28.485 vs 23.24) U/L for females and (38.009 vs 29.72) U/L for males and Glucose (7.575 vs 5.018) mmol/L for females and (7.979 vs 5.830) mmol/L for males ,showed high significant in two thyroid cancer patients group (G1) and (G2) (P=<0.001) for male and female with all parameters accept age and Creatinine (p=<0.001) for female and p=(0.008) for male when compared with healthy controls groups. T4 mean value for G1 and G2 so higher than controls groups G3 and G4 the increase was 10.7% for females and 8.9% for males as showed in figure (3-2).T3 mean value for patients groups G1,G2 also was higher than healthy control groups G3,G4 the increase was 3.9% for female and 3% for males as showed in figure (3-3) , Urea level was increase with increasing of T3 andT4 , the increasing was 77.2% for female and 78.3% for males as showed in figure (3-4). Creatinine for patients groups G1,G2 also higher than healthy control groups G3,G4 the increasing was 31.2% for females and 39.7% for males as showed in figure (3-5) , Thyroid hormone plays an important role in the growth, development, and physiology of the kidneys. Thyroid dysfunction causes remarkable changes in renal blood flow, glomerular filtration rate, tubular secretory and absorptive capacity, electrolyte pumps, and kidney structure

given the critical role of the kidney in maintaining optimal whole-body homeostasis, kidney dysfunction may be anticipated to impact negatively on other organ systems. ALT mean value for patients group G1, G2 higher than healthy control groups G3, G4 the increasing was 88.9% for females and 90% for males as showed in figure (3-6). AST mean value for patients groups G1, G2 was higher than healthy control groups G3, G4 the increasing was 81.5% for females and 78.1% for males as showed in figure (3-7). The clinical parameters we analyzed to evaluate liver function showed elevated AST and ALT. Other reports demonstrated that hypothyroidism induces hepatic dysfunction The liver has an important role in thyroid hormone metabolism and the level of thyroid hormone is also important to normal hepatic function and bilirubin metabolism, that in excess of thyroid hormone, it over stimulate metabolism and exacerbates the effect of the sympathetic nervous system causing speeding up of various body system and symptoms and increase the enzyme activities and releasing into blood stream in patients .The proliferation is often associated with increased risk of cancer, hepatocyte proliferation induced by T3 accelerates, it has been known that excessive amounts of thyroid hormone decreased the glycogen in the liver . Increased consumption of hepatic enzymes, and enhanced protein and glucose catabolism. These factors could be the cause of liver dysfunction in hyperthyroidism. It is known that some cases of Hashimoto's thyroiditis are accompanied by chronic progressing liver disease[60]. Glucose mean value for patients groups G1, G2 was higher than healthy control groups G3, G4 the increasing was 66.2% for females and 75.9% for males as showed in figure (3-8). Thyroid dysfunctions caused Carbohydrate metabolism disorders the increased degradation of insulin in hyperthyroid subjects was a consistent finding in thyrotoxicosis, thyroid disease prevalence in patients with diabetes is significantly higher than in the general population. In this study observed the increasing for T3, T4 levels for thyroid cancer patients (males and females) caused increasing in kidney function, liver function and Glucose level. The age of females patients <45 Premenopausal so not compared with male those take from various age, in males thyroid cancer patients situation mean age

was 46.8 years that Indicates older people more susceptible to infection with thyroid cancer, thyroid cancer diagnosis in women more than in men, it is believed that the increased morbidity of thyroid nodules with increasing age was associated with changes in the thyroid that accompany aging. These age-related changes include degeneration of thyroid cells associated with fibrosis, inflammatory cell infiltration, thyroid follicle alteration, and the formation of nodules [61] .These age-related changes include degeneration of thyroid cells associated with fibrosis. Type of thyroid cancer observed 18% follicular, 4% medullary and 88% papillary thyroid cancer based one this was selected for cytotoxicity test. Hypertension for all patients was normal. No one of patients was smoking or Exercised. These patients were treated with Radioactive Iodine Therapy (RAI) also known as I-131. Is taken into the body in capsule form. The nuclear receptor HNF-4α is an important regulator of hepatic metabolism it was demonstrated that TSH significantly decreased the expression of nuclear HNF-4α in vivo and in vitro. The nuclear localization of HNF-4α was affected by TSH-mediated phosphorylation Interestingly, HNF-4α has been suggested to play numerous roles in many metabolic pathways, such as glucose homeostasis and lipid metabolism. [62] There is a higher prevalence of thyroid nodules in insulin-resistance patients and that the insulin-like growth factor 1 signaling pathway is involved in the proliferation of thyroid cells, an effect mediated by TSH. Insulin-resistance itself can promote the development of thyroid nodules. [63]TSH levels were not significantly altered across the four renal function groups, although there was a trend toward higher TSH levels outside of the normal reference range in patients with mild, moderate and severe renal dysfunction. That change in thyroid function during severe illness may actually have a protective effect by preventing excessive tissue catabolism. [64]

Table (3-1): comparison between groups for (Age, T4, T3, Creatinine, Urea, ALT, AST and Glucose) as mean (±SD) levels.

Parameters	**Female(G1)** **Patients** **No.**=50	**Female**(G3) **Control** **No.**=50	**P-value**	**Male**(G2) **Patients** **No.**=50	**Male**(G4) **Control** **No.**=50	**P-value**
Age years	33.771 ±9.226	35.45 ±6.092	0.745	46.882 ±7.895	48.76 ±7.09	**0.801**
T4 (µg/dl)	66.297 ±32.314	7.110 ±2.147	<0.001**	75.552 ±20.920	6.758 ±3.012	**<0.001****
T3 (µg/dl)	2.575 ±1.480	0.101 ±0.021	<0.001**	3.188 ±0.415	0.097 0.015	**<0.001****
TSH (µg/dl)	52.214 ±20.305	5.123 ±1.58	<0.001**	45.134 ±10.004	4.998 ±2.657	**<0.001****
Urea(mmol/L)	14.165 ±0.92	10.94 ±2.598	<0.001**	16.135 ±1.611	12.642 ±7.533	**<0.001****
Creatinine(mmol/L)	3.717 ±0.342	1.162 ±0.610	<0.001**	2.793 ±0.362	1.110 ±0.216	**0.008**
ALT (U/L)	20.042 ±2.143	17.828 ±5.711	<0.001**	20.529 ±1.412	18.48 ±9.71	**<0.001****
AST (U/L)	28.485 ±2.559	23.24 ±6.354	<0.001**	38.009 ±1.870	29.72 ±11.31	**<0.001****
Glucose (mmol/L)	7.575	5.018	<0.001**	7.679	5.830	**<0.001****

	±3.22	±2.42		±5.97	±3.27	

**=highly significant.

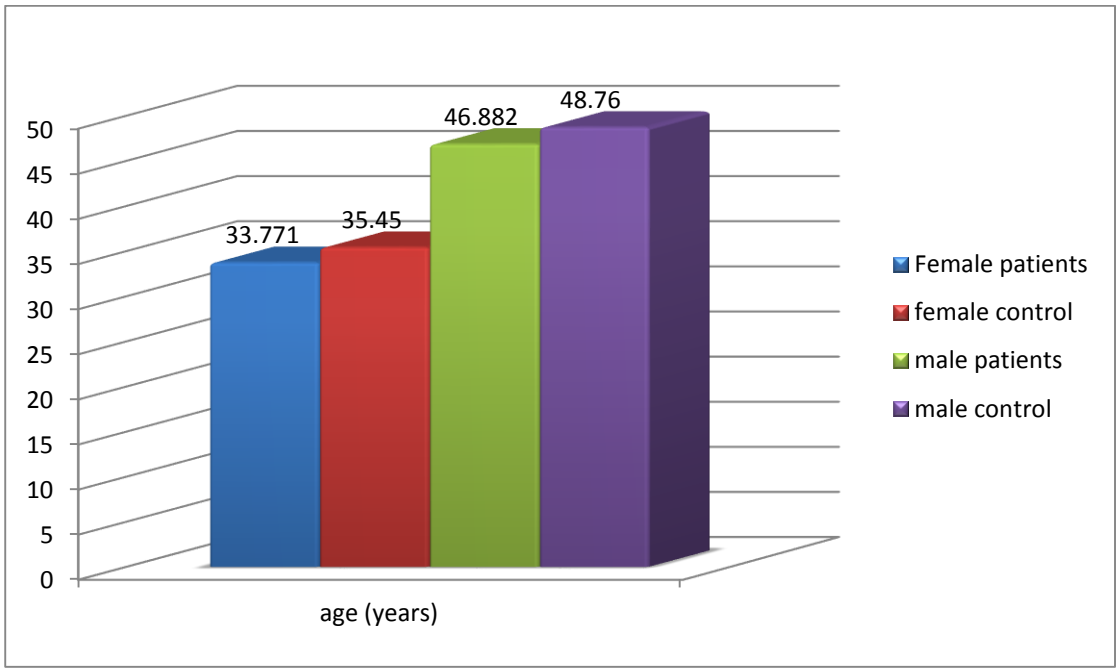


Figure (3-1): The comparison between G1, G2, G3 and G4 for the age.*

* P-Value<0.001

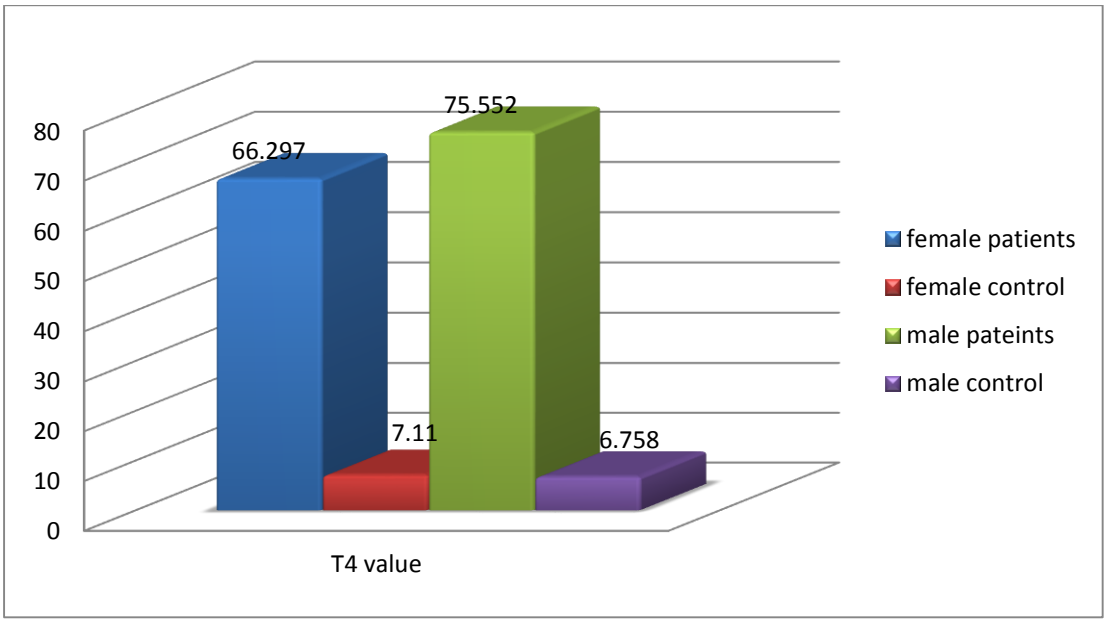


Figure (3-2) comparison between G1, G2, G3 and G4 for T4 values.*

*P-Value<0.001

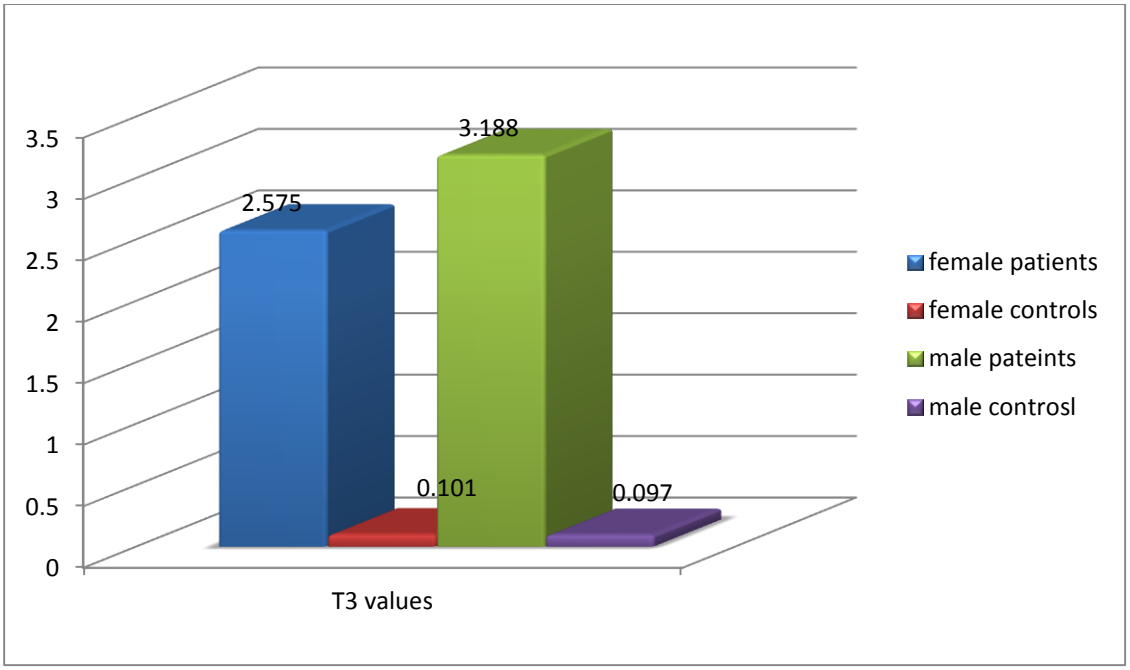


Figure (3-3) comparison between G1, G2, G3 and G4 for T3 values.*

*P-Value<0.001

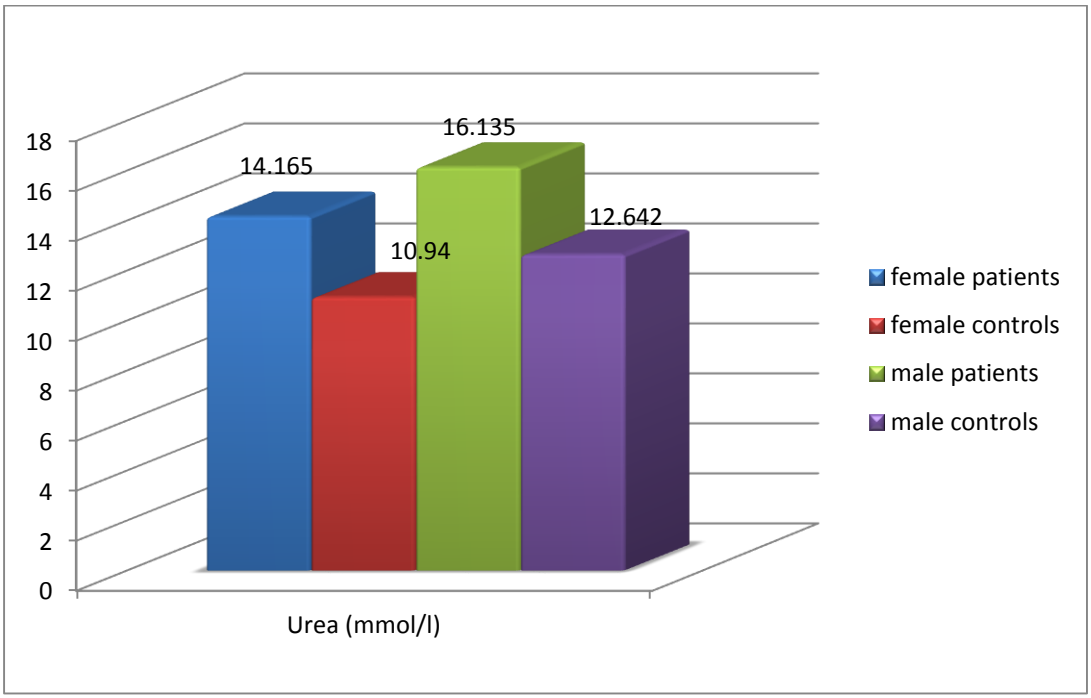


Figure (3-4) comparison between G1, G2, G3 and G4 for urea values (mmol/L).*

*P-Value<0.001.

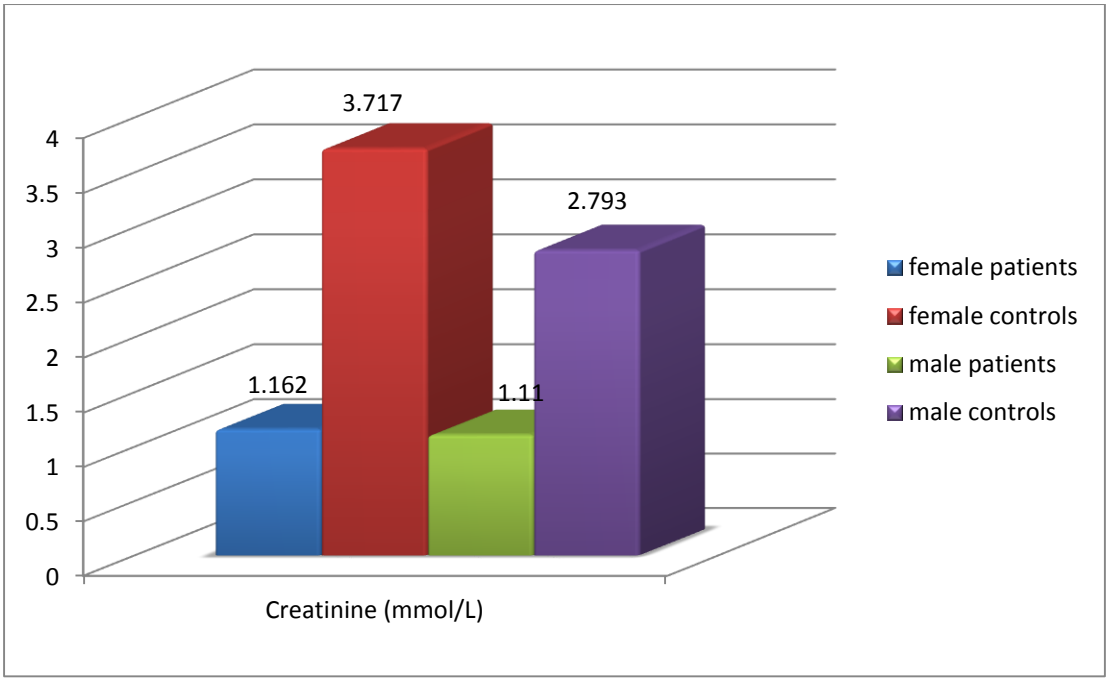


Figure (3-5) comparison between G1, G2, G3 and G4 for Creatinine (mmol/L). **

*P-Value<0.001

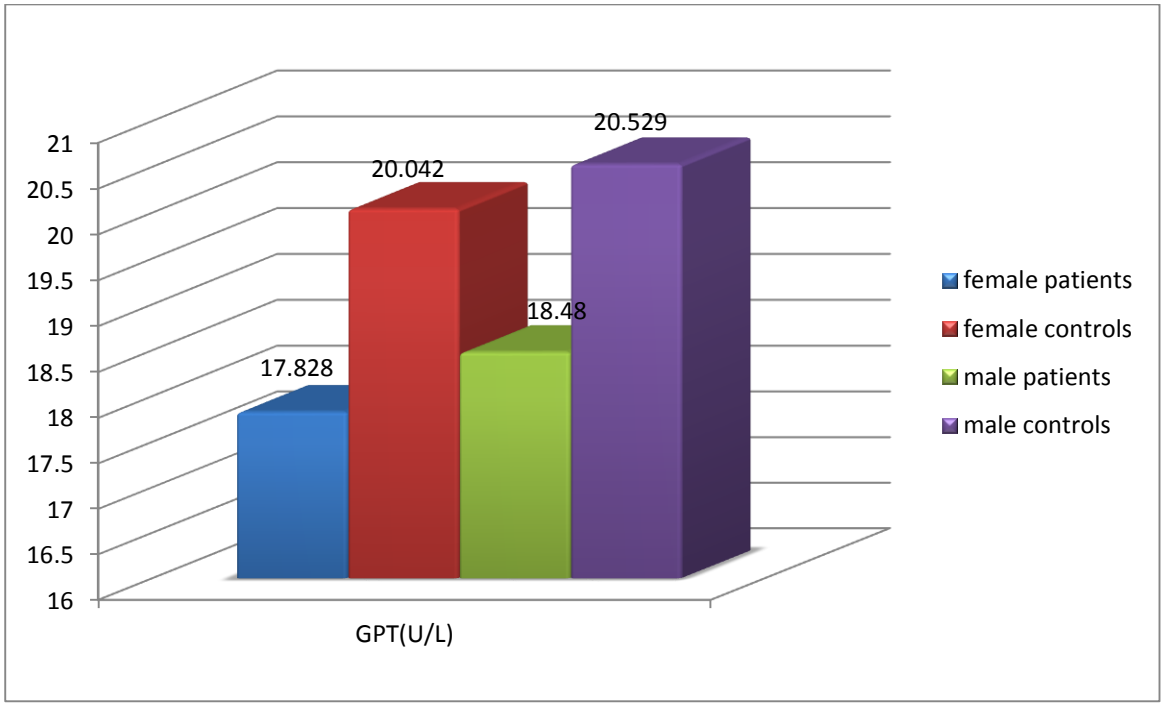


Figure (3-6) comparison between female G1, G2, G3 and G4 for ALT (U/L) values.*

*P-Value<0.001

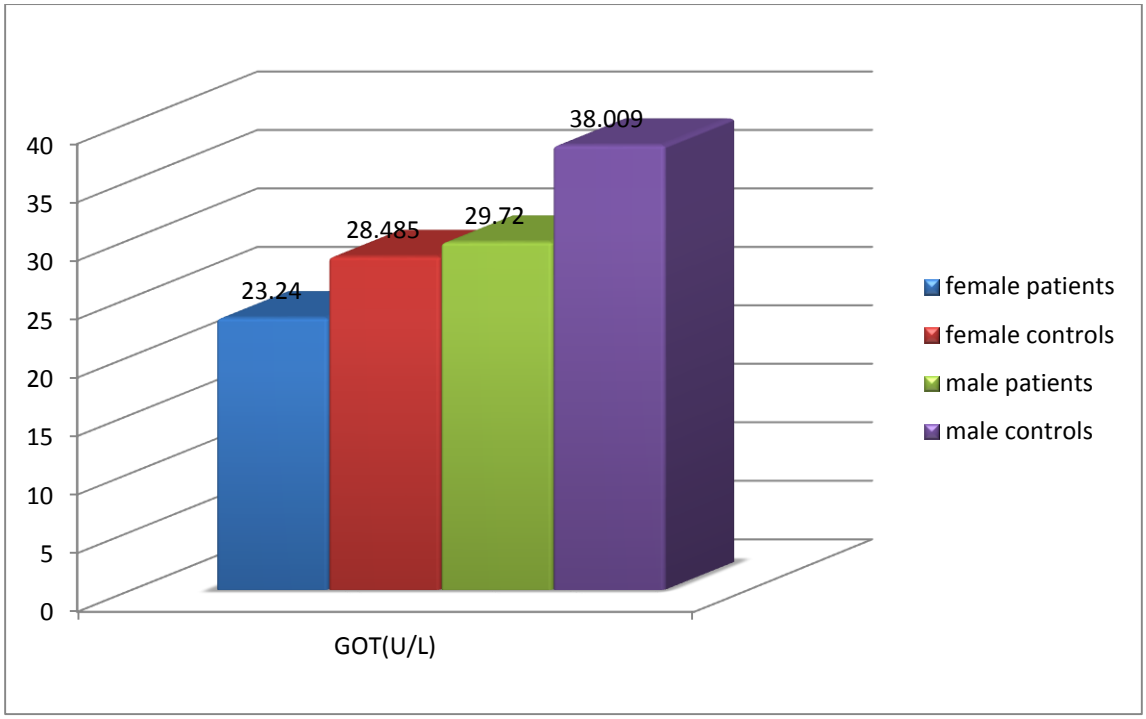


Figure (3-7) comparison between G1, G2, G3 and G4 for AST (U/L) values).*

*P-Value<0.001

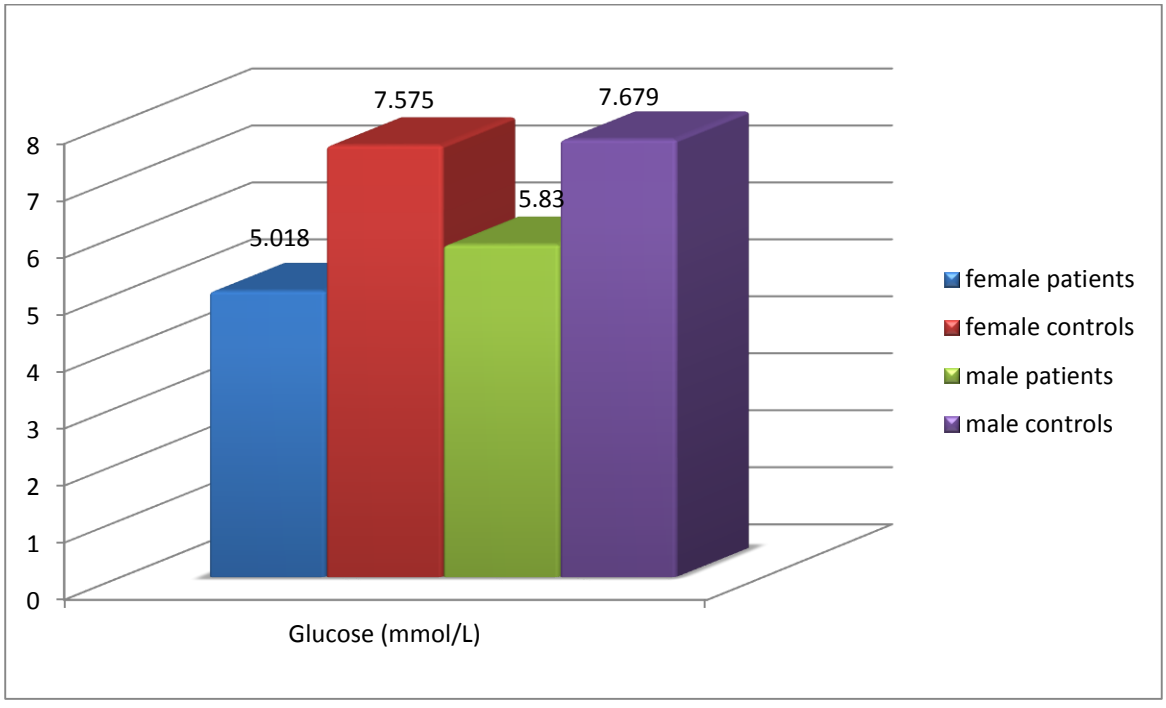


Figure (3-8) comparison between female G1, G1, G3 and G4 for Glucose (mmol/L) values.*

*P-Value<0.001

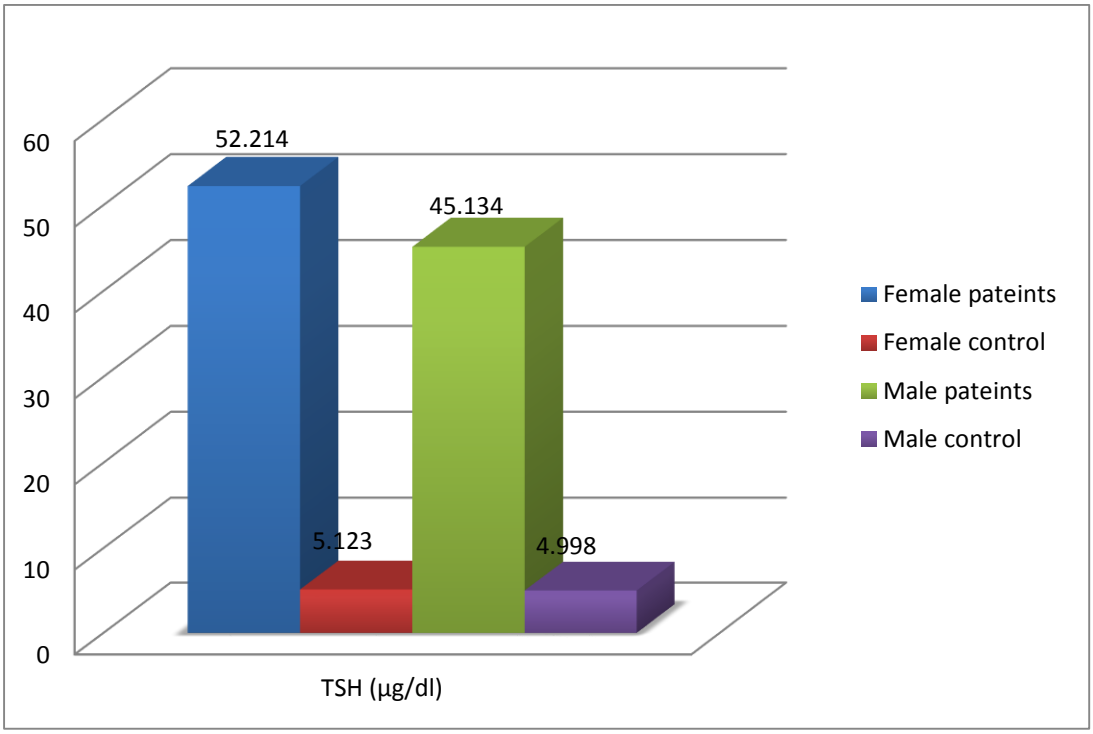


Figure (3-9): comparison between G1, G2, G3 and G4 for TSH values.*

* P-Value<0.001

As shown in the table (3-2), T4 level of the group (G1) showed a significantly positive correlation with Creatinine ,Urea and Glucose s showed in figure (3-10). T4 level of the group (G2) showed a significantly positive correlation with Creatinine, Urea and Glucose s showed in figure (3-10) also. Thyroid hormones have important effects on kidney tubular transport.

Table (3-2) the correlation (r) between T4 with (Creatinine and Urea) for studied groups.

T4 correlation with		
Parameters	Female patients group	Male patients group
Creatinine	0.500	0.505
Urea	0.607	0.504
Glucose	0.411	0.455

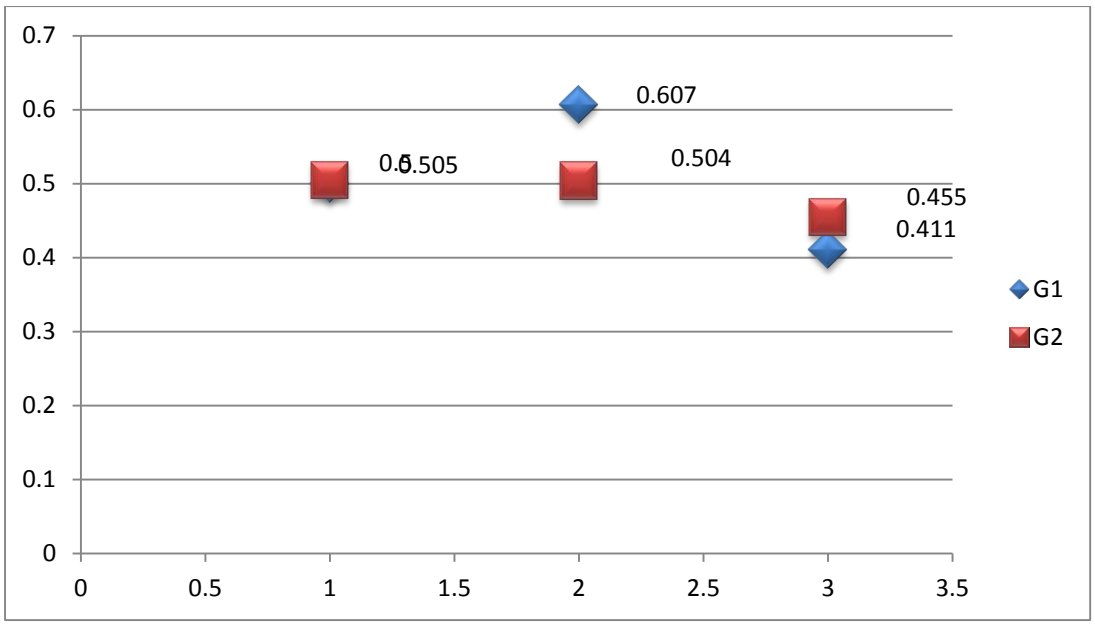


Figure (3-10): correlation between T4 and (Urea, Creatinine, Glucose) for patients groups for G1 (female patients) and G2 (male patients).

As shown in the table (3-3), T4 level of the group (G1) and group (G2) showed a significantly positive correlation with ALT and AST showed in figure (3-11).

Table (3-3) the correlation (r) between T4 with (ALT and AST) for studied groups.

T4 correlation with		
Parameters	Female patients group	Male patients group
ALT	0.429	0.372
AST	0.584	0.481

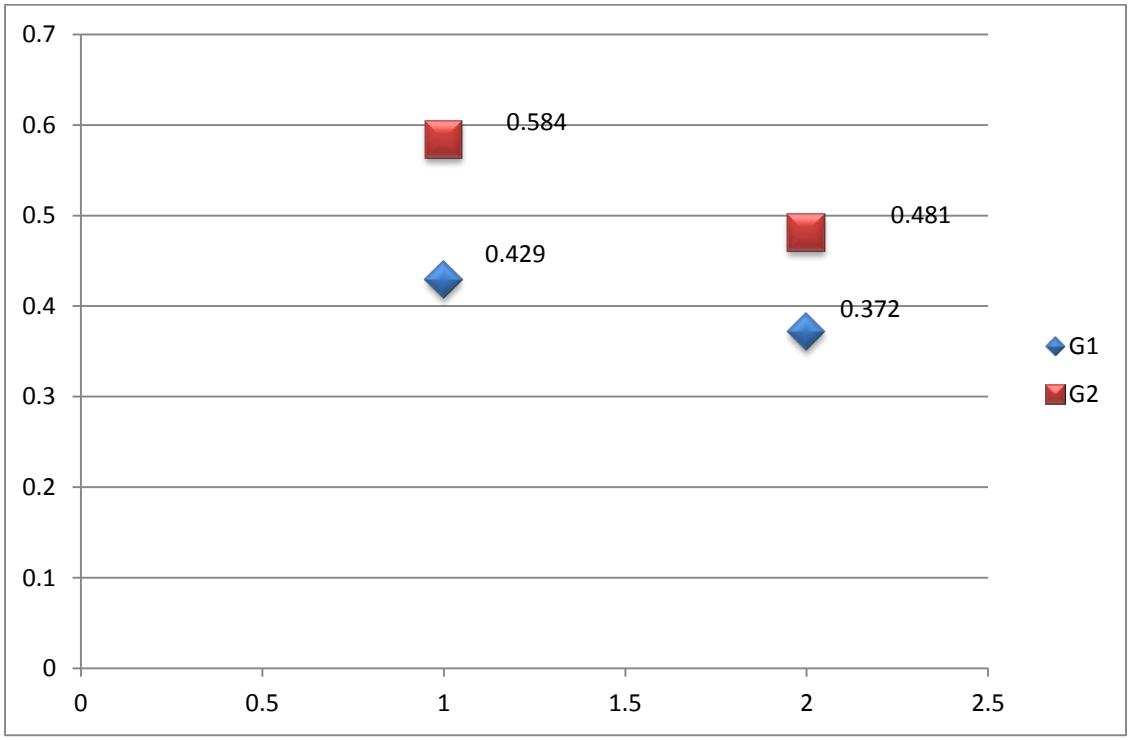


Figure (3-11): correlation between T4 and ALT AND AST for patients groups G1 (female patients) and G2 (male patients).

As shown in the table (3-4), T3 level of the group (G1) and group (G2) showed a significantly positive correlation with Creatinine, Urea and Glucose s showed in figure (3-12).

Table (3-4) the correlation (r) between T3 with (Creatinine and Urea) for studied groups.

T3 correlation with		
Parameters	Female patients group	Male patients group
Creatinine	0.547	0.514
Urea	0.521	0.521
Glucose	0.423	0.580

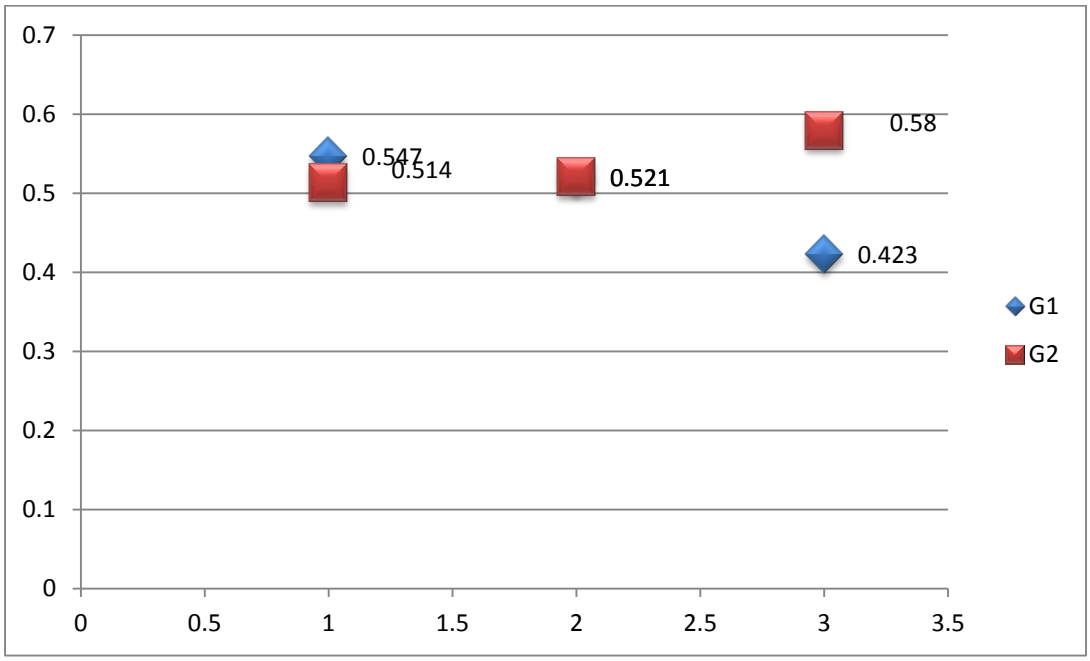


Figure (3-12): the correlation between T3 and (Creatinine, Urea, Glucose) for patients groups for G1 (female patients) and G2 (male patients).

As shown in the table (3-5), T3 level of the group (G1) and group (G2) showed a significantly positive correlation with AST and ALT as showed in figure (3-13).

Table (3-5) the correlation (r) between T3 with (ALT and AST) for studied groups.

T3 correlation with		
Parameters	Female patients group	Male patients group
ALT	0.422	0.474
AST	0.540	0.458

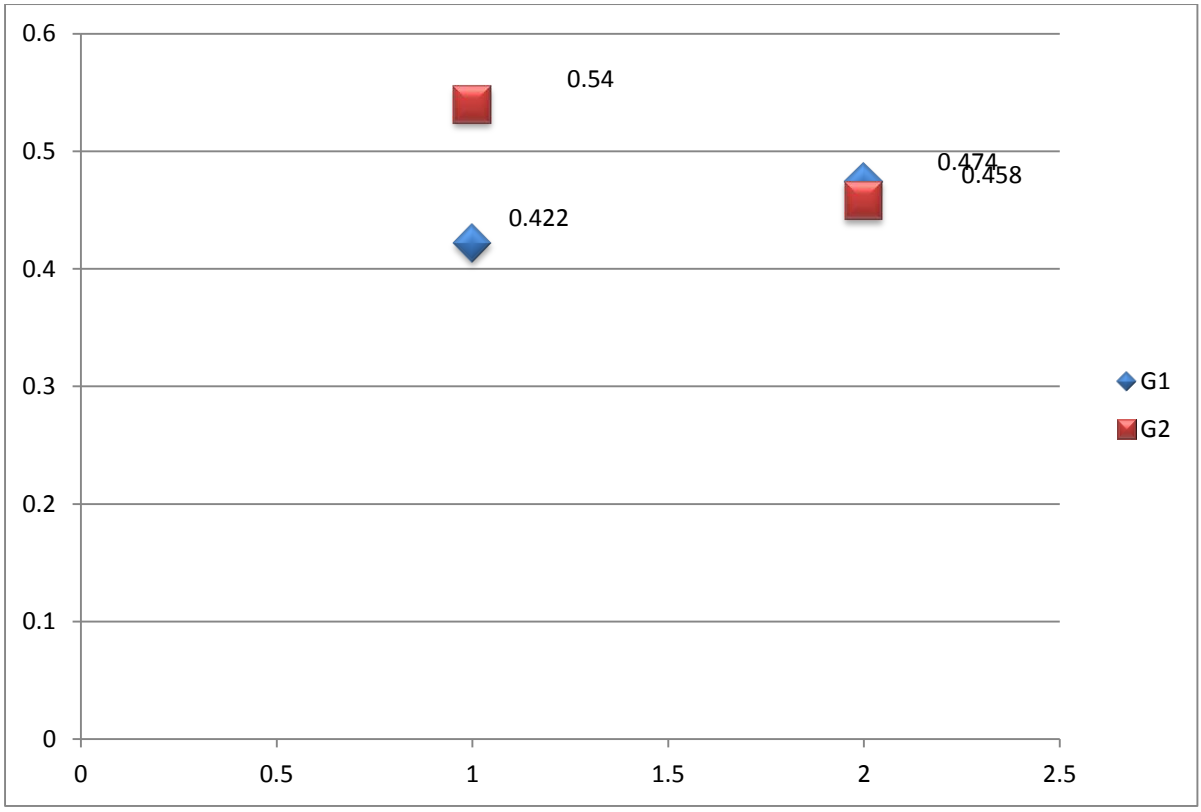


Figure (3-13): The correlation between T3 and (ALT, AST) for patients groups for G1 (female patients) and G2 (male patients).

As shown in the table (3-6), Time of treatment level of the group (G1) and (G2) showed a significantly negative correlation with Creatinine, Urea and Glucose s showed in figure (3-14).

Table (3-6) the correlation (r) between Time of treatment with (Creatinine and Urea) for studied groups.

Time of treatment correlation with		
Parameters	Female patients group	Male patients group
Creatinine	-0.307	-0.376
Urea	-0.433	-0.416
Glucose	-0.445	-0.431

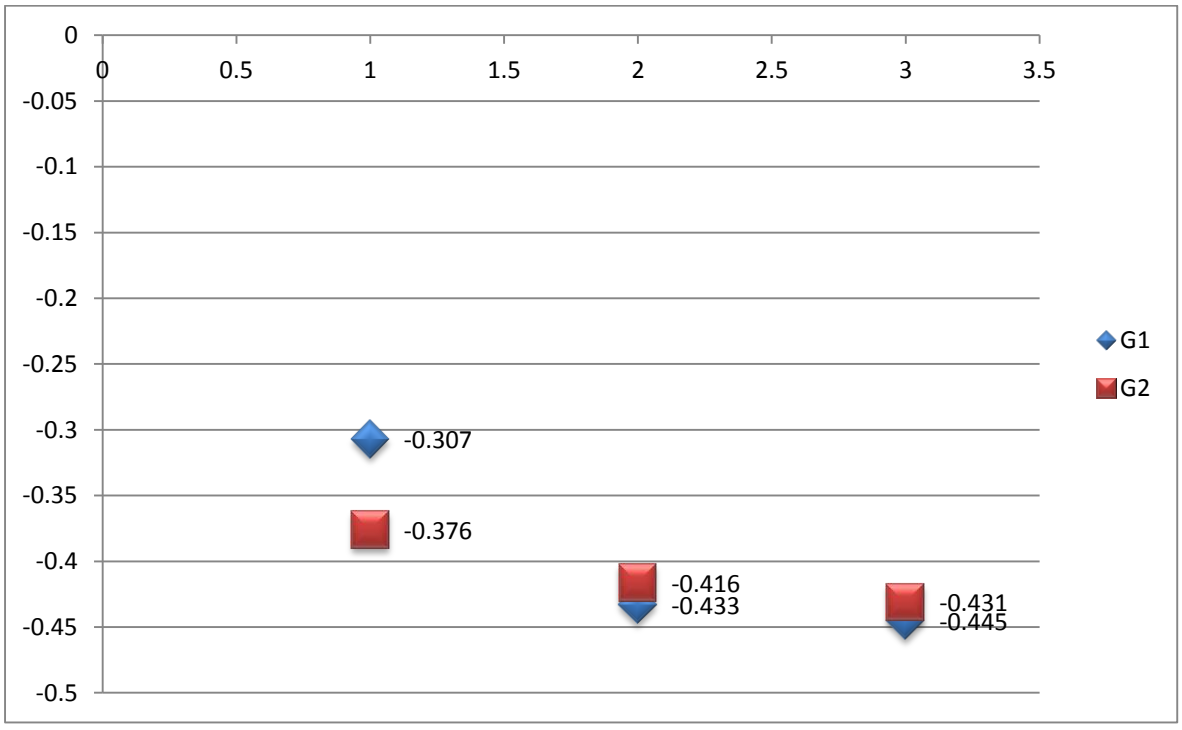


Figure (3-14): The correlation between time of treatment and (Creatinine, Urea, Glucose) for patients groups for G1 (female patients) and G2 (male patients).

As shown in the table (3-7), Time of treatment of the group (G1) and (G2) showed a negative correlation with ALT. Time of treatment for the group (G1) showed a negative correlation with AST and showed significantly positive correlation for (G2) as showed in figure (3-15).

Table (3-7) the correlation (r) between Time of treatment with (ALT and AST) for studied groups.

Time of treatment correlation with		
Parameters	Female patients group	Male patients group
ALT	-0.416	-0.421
AST	-0.640	0.570

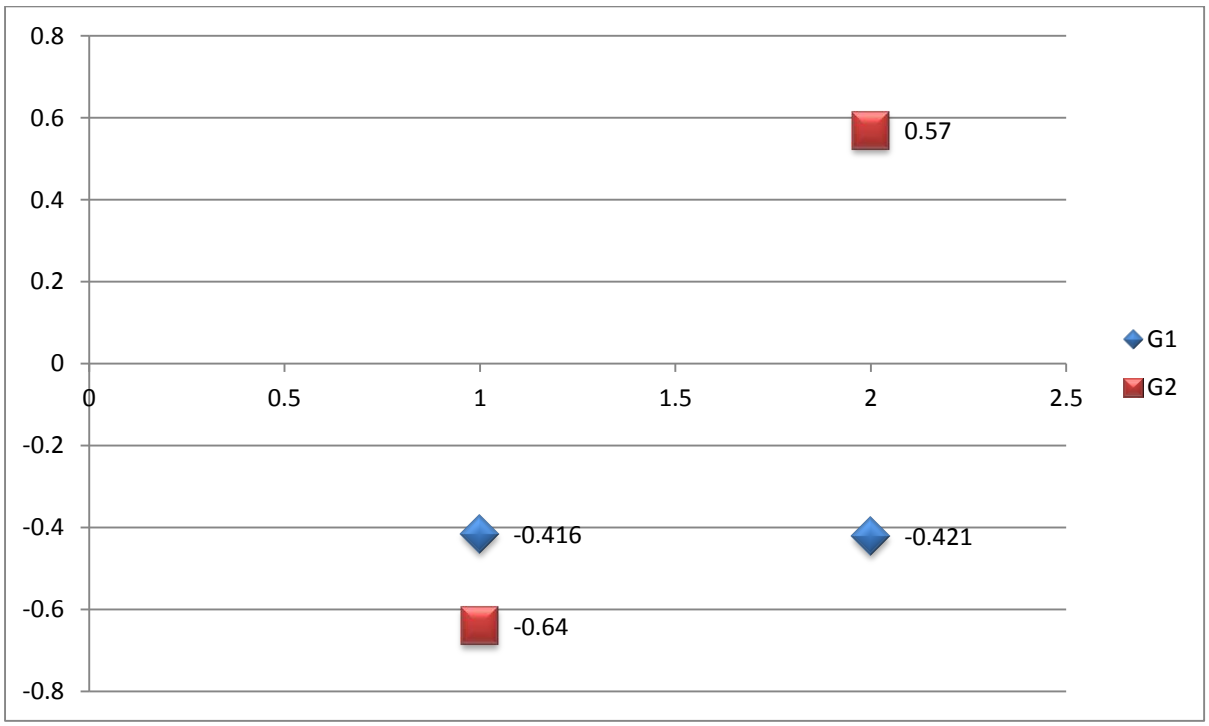


Figure (3-15): The correlation between Time of treatment and (ALT, AST) for patients groups for G1 (female patients) and G2 (male patients).

3.2 Part two

3.2.1 Ginger extraction

Table (3-8) show the quantity of ginger, Volume in (ml) and type of solvent, Weight of pure extract yield and the Color of extracts.

weight of ginger	Volume (ml) and type of solvent	Weight (g) of pure extract yield	Color of extract
10g of ginger powder	100ml of ethanol	0.34g	orange yellow
10g of ginger powder	100ml of methanol	0.41g	orange yellow
10g of ginger powder	100ml of acetone	0.2g	light brown
10g of ginger powder	100ml of hexane	0.15g	yellow
10g of ginger powder	100ml of chloroform	0.4g	light brown
10g of ginger powder	400ml of water	3.2g	brown

Figure (3-16) extracts of ginger before dried.

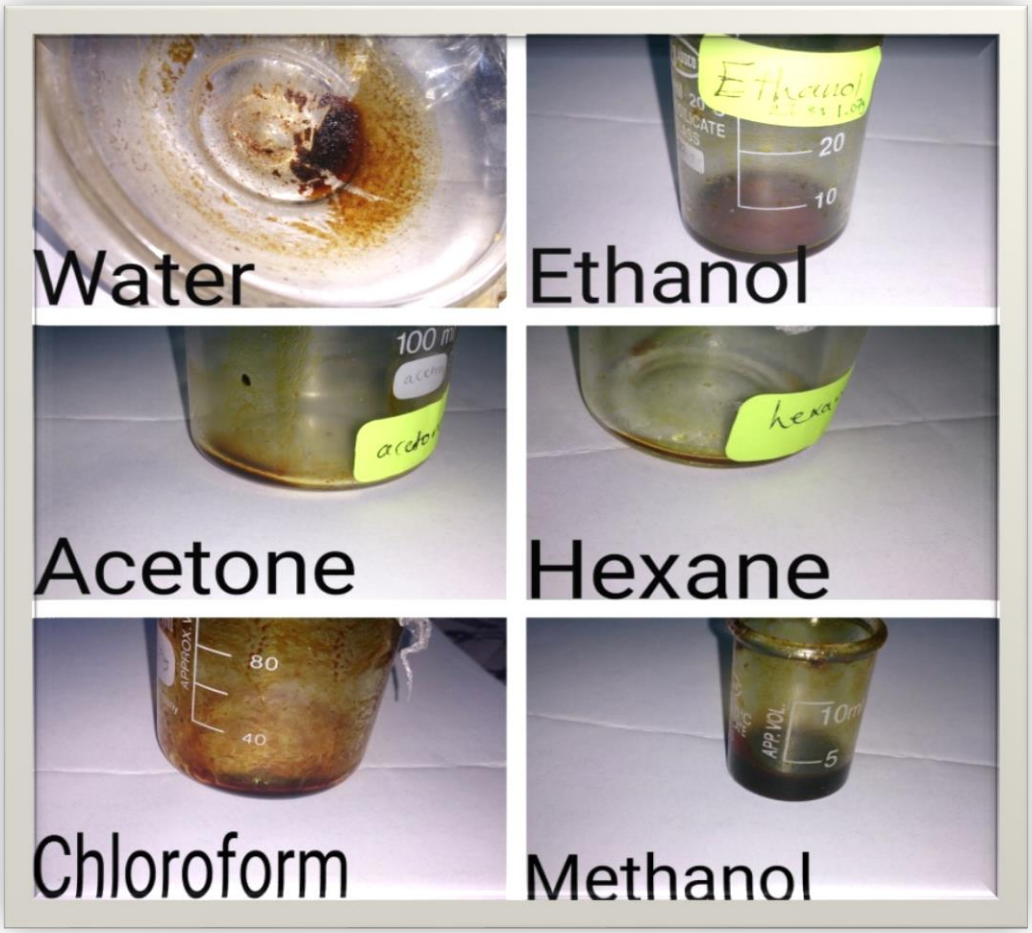


Figure (3-17) extracts of ginger after dried.

The yield of the extracts of zingiber officinale root with respect to solvent are shown in table (3-9).

The percentage yield of aqueous extract of ginger (was highest with 32%). The next was methanol extract with (4.1%). The percentage was (3.4%, 4%, 2%, and 1.5%) for ethanol, chloroform, acetone and hexane extracts, respectively.

Table (3-9): Yield of extracts of ginger with respect to solvents. The percentage yields were calculated against 10g powder of ginger material subjected to each extraction method.

Solvent	Yield(g)	Yield (%)
Ethanol	0.34	3.4
Methanol	0.41	4.1
Hexane	0.15	1.5
Acetone	0.2	2
Chloroform	0.4	4
Water	3.2	32

Ginger, the rhizome of the Zingiber officinale plays an important role in prevention of diseases. But the exact mechanism of action in diseases management is not understood fully.

3.2.2 FT-IR spectrum of ginger extracts

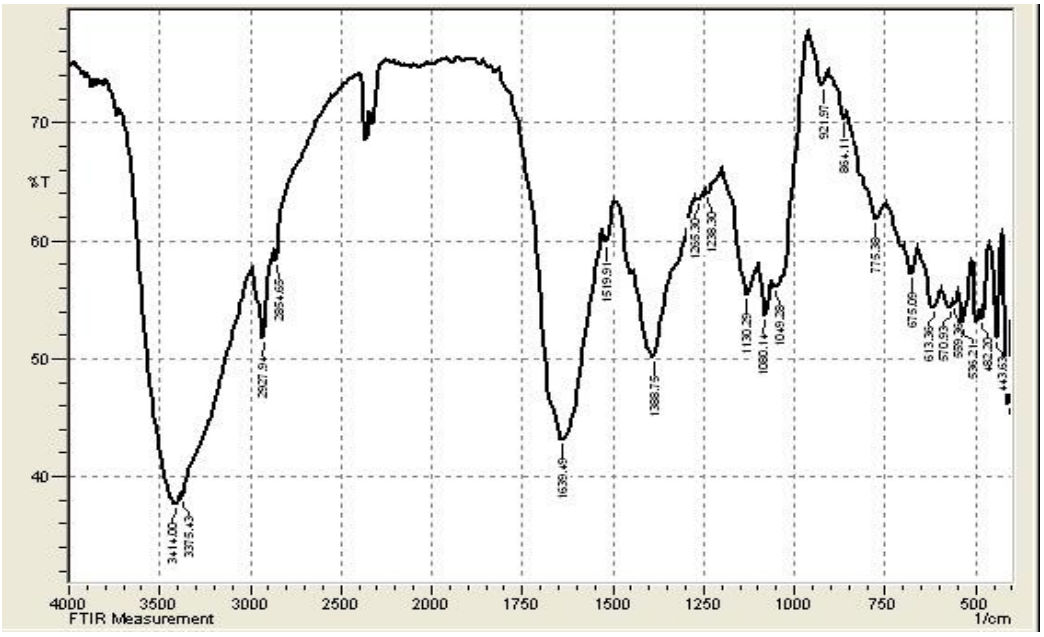


Figure (3-18): FT-IR spectrum of aqueous extract of ginger.

FT-IR of aqueous extract of ginger has the active group that gave ginger antioxidant property , in figure (3-18) appear (C=O) at 1693 ,(O–H) alcoholic group at 3400 , (C–O) group at 1300 , (C–H) group at 2977,2957 two broad's that mean ginger peak at 1130 cm^{-1} to C-O-C stretching aromatic ring. Compounds presented in this extract as gingerol, paradol, shagole and other compounds.

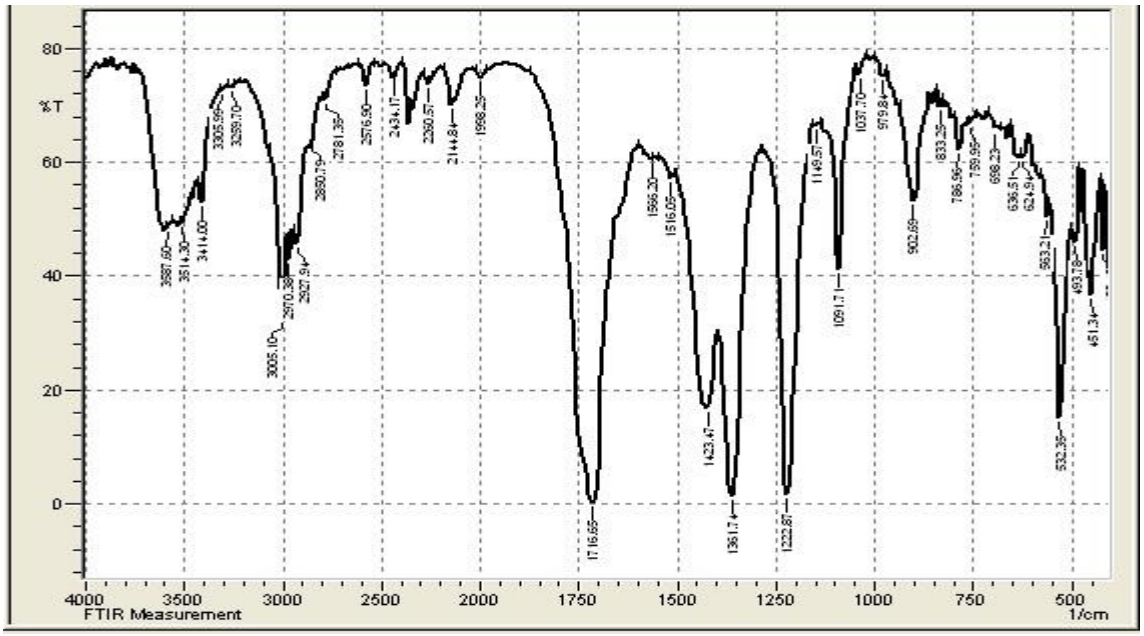


Figure (3-19): FT-IR spectrum of acetone extract of ginger.

FT-IR of acetone extract showed in figure (3-19) , in this chart many active group appear like (C=O) carbonyl group at 1716cm^{-1} ,(O–H) alcoholic or phenolic group at 3587-3541Cm^{-1} ,(C–O) group at 1361 ,1423 , 1222Cm^{-1} and (C–H) at 2970, 2927Cm^{-1} peak at 1149 cm^{-1} to C-O-C stretching aromatic ring. That mean ginger active compound that use as antioxidant Exist in this exetract like a shagole and gengerol and paradol ..ect.

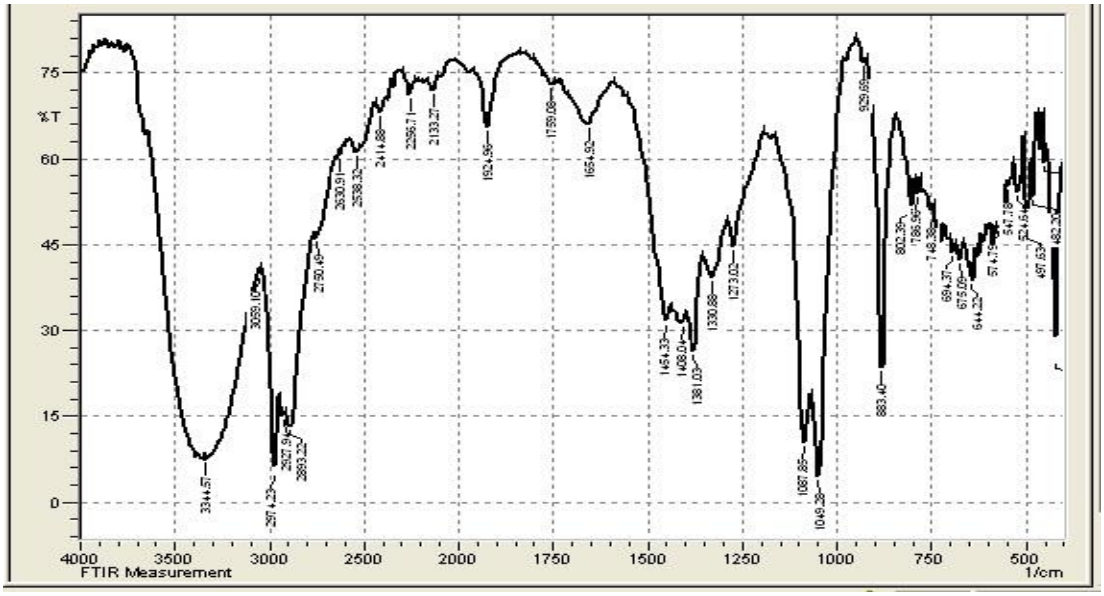


Figure (3-20): FT-IR spectrum of ethanol extract of ginger.

FT-IR spectrum of acetone extract showed in figure (3-20).in that chart many active group appear like (O–H) hydroxyl alcoholic group in 3344Cm^{-1} ,(C–H) with two broad's in 2947 and 2927 Cm^{-1} ,carbonyl group (C=O) in 1759Cm^{-1} and (C–O) group at 1454 Cm^{-1}, peak at 1067 cm^{-1} to C-O-C stretching aromatic ring, that mean exist ginger active compound in this extract like gengerol and shagol and paradol.

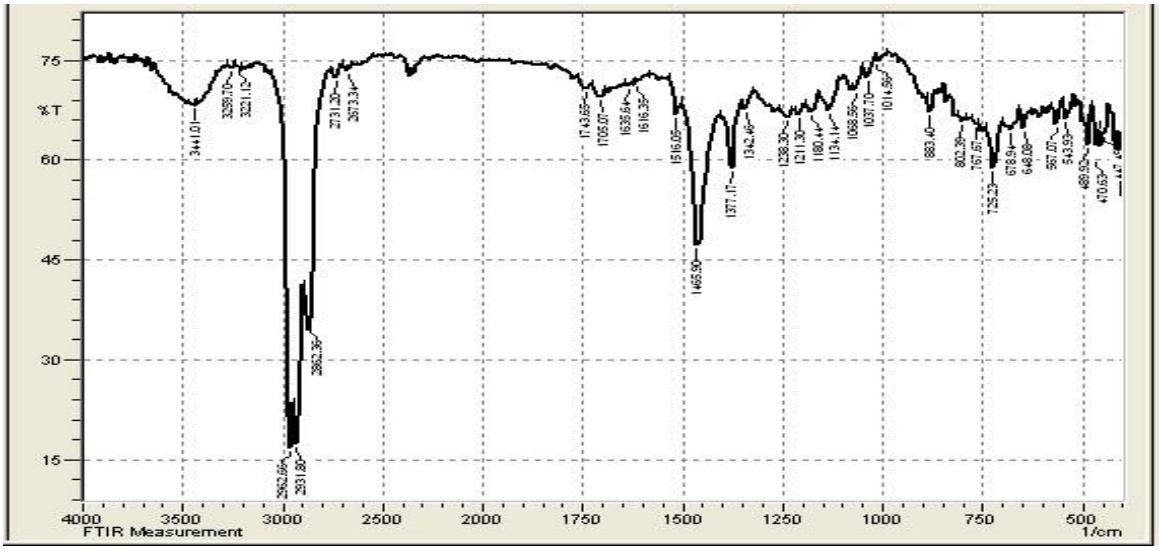


Figure (3-21): FT-IR spectrum of hexane extract of ginger.

FT-IR spectrum of hexane extract of ginger showed in figure (3-21),in that chart many active group like (C=O) carbonyl group in 1743 Cm^{-1} ,(C–H) group in 2 broad's at 2962 and 2931 Cm^{-1}, hydroxyl alcoholic group (O–H) at 3441 Cm^{-1} and (C–O) group at 1465Cm^{-1} , peak at 1143 cm^{-1} to C-O-C stretching aromatic ring ,that mean ginger active compound exist in this extract like gengerol ,shagol and paradol ..ect.

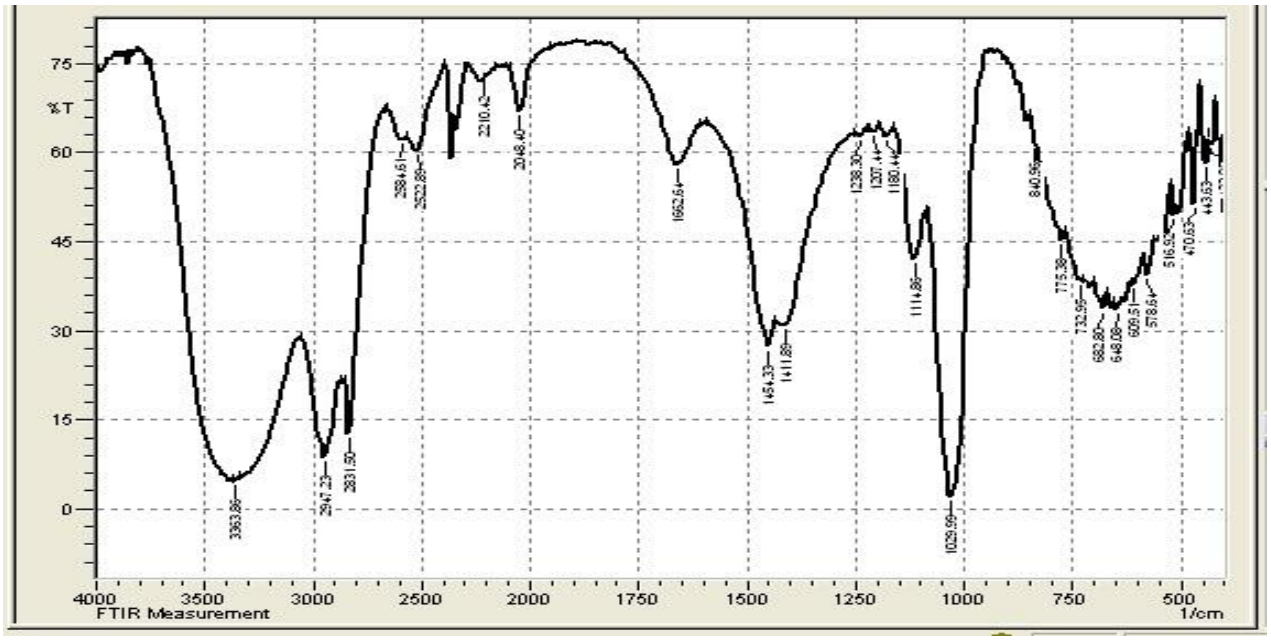


Figure (3-22): FT-IR spectrum of ethanol extract of ginger.

FT-IR spectrum of ethanol ginger extract showed in figure (3-22), in that chart many active group its make extract as antioxidant like a hydroxyl alcoholic group as broad peak (O–H) at 3353Cm^{-1}, (C–H) group as two peaks 2947and 2831 Cm^{-1}, carbonyl group (C=O) at 1662Cm^{-1} and (C–O) group at 1454Cm^{-1}, peak at 1114 cm^{-1} to C-O-C stretching aromatic ring that mean ginger active compounds exist in this extract like a gingerol, a shagol and a paradol..ect.

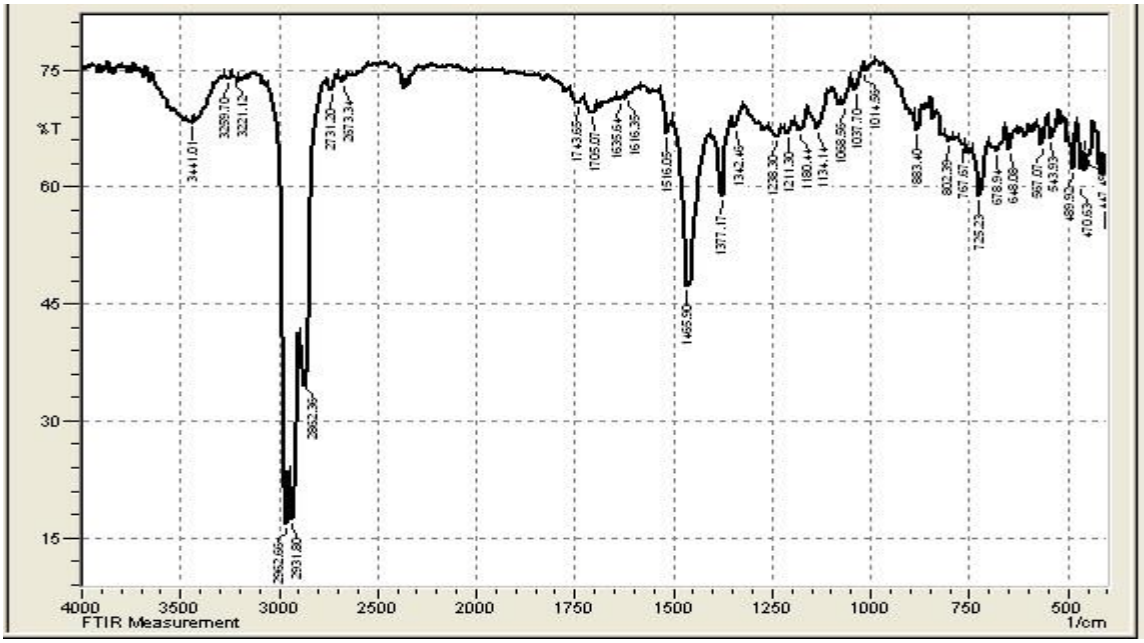


Figure (3-23): FT-IR spectrum of chloroformic extract of ginger.

FT-IR spectrum of chloroformic extract showed in figure (3-23). the active group of that extract make ginger antioxidant, in that chart many active group appear like carbonyl (C=O) at 1743Cm^{-1} ,(O–H) hydroxyl alcoholic group as broad peak at 3441Cm^{-1} , (C–O) group at 1465Cm^{1} , (C–H) group at two peaks 2977,2957Cm^{-1} ,peak at 1134 cm^{-1} to C-O-C stretching aromatic ring. That mean ginger active compound exist in this extract like a gingerol , a paradol and a shagol ..ect.

Ginger has been identified as an herbal medicinal product with pharmacological effect. Ginger suppresses prostaglandin synthesis through inhibition of cyclooxygenase- 1 and cyclooxygenase- 2. [33]

3.2.3 Antibacterial activity of ginger for organic and water extract

Ginger powder of organic extracts dissolve in DMSO but aqueous extract dissolving in water and forming stock solution. Not all organic extract exhibited inhibition on the test microorganism, just ethanol, methanol and hexane extract exhibited inhibition, another acetone and chloroform could not inhibit, aqueous extract could not exhibited inhibition anymore. Because ethanol and methanol and hexane are an organic solvent and will dissolve organic compounds better, hence liberate active components required for antimicrobial activity. The sensitivity of different organisms with the concentrate-on use well 6mm are shown in table (3-10).

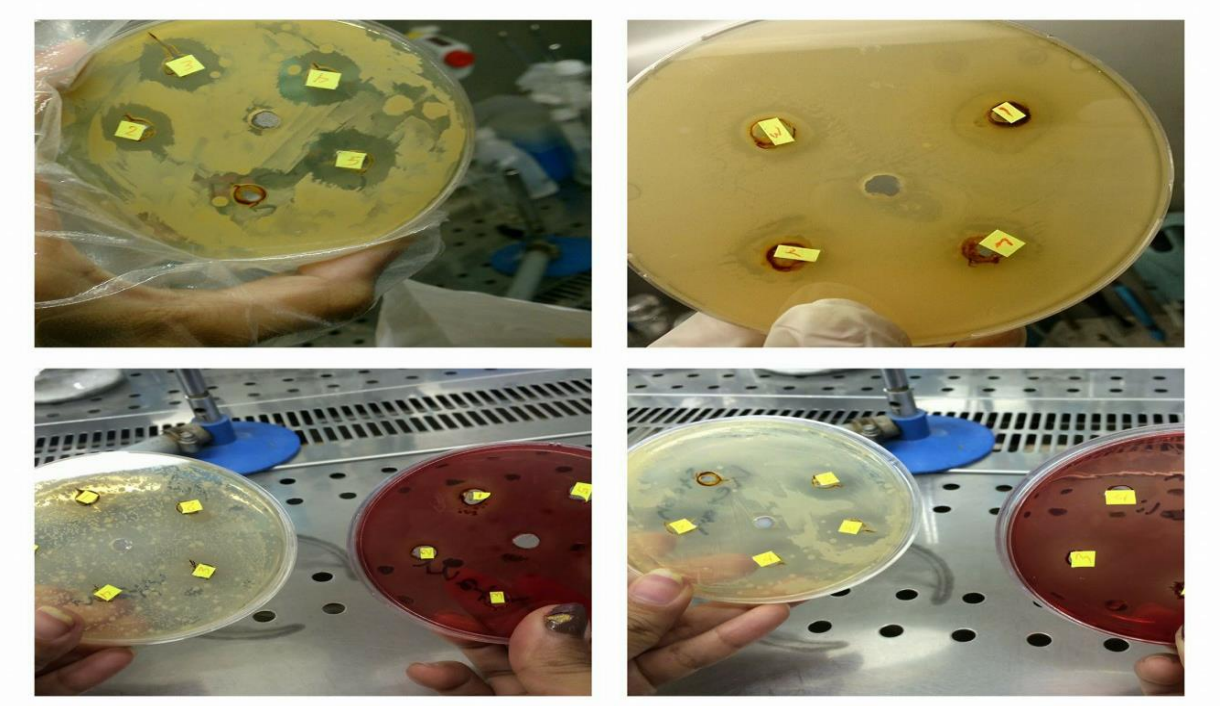


Figure (3-24): Antibacterial potential of crude extracts of rhizome of Zingiber officinale.

Table (3-10): antimicrobial activity of the various extracts of ginger.

Key: ++ =inhibition > 8.00mm diameter,-= no inhibition

extract	**Escherichia coli**	**Staphylococcus aureus**
Ethanol	+	+
Methanol	+	+
Hexane	+	+
Acetone	-	-
Chloroform	-	-
water	-	-

It was clear from this work that the solvent of extraction and method affected the degree of antimicrobial activity. From the test, it was observed that ethanol, hexane and methanol extracts of ginger give the widest diameter zone of inhibition (16.00, 20.00, 15.00) mm using the concentration 1000mg\ml. no detectable growth of bacteria was found around the wells containing 1000mg\ml of these organic extracts of ginger.

Table (3-11): diameter of inhibition, Figure above are zones of clearing in millimeter of the original working solution.

Extract	Escherichia coli	Staphylococcus aureus
Ethanol	16.00 mm	14.00 mm
Methanol	15.00 mm	13.00 mm
Hexane	15.00 mm	20.00 mm
Acetone	0.00 mm	0.00 mm
Chloroform	0.00 mm	0.00 mm
Water	0.00 mm	0.00 mm

Mean zone of inhibition that ethanol, hexane and methanol extracts showed inhibition of varying diameter with the different test organisms. The mean zone (x-) of inhibition of ethanol, hexane and methanol extracts were (16.00,15.00,15.00)mm on E. coli, respectively.

The highest mean zone (x-) of inhibition ethanol, hexane and methanol extracts on Staphylococcus aureus were (15.00, 20.00.13.00) mm, respectively, shown in table (3-12)

Table (3-12): mean zone diameter of inhibition (mm) of extracts with respect to various concentration in mg\ml. The mean zone was deduced from the summation of various zones of inhibition.

extract	*Escherichia coli*						*Staphylococcus aureus*					
Conc.	1000	500	250	125	62.5	x-	1000	500	250	125	62.5	x-
Ethanol	16.00	15.00	13.00	12.00	11.00	13.4	15.00	13.00	12.00	11.00	10.00	12.2
Methanol	15.00	14.00	14.00	13.00	11.00	13.4	13.00	12.00	11.00	11.00	10.00	11.4
Hexane	15.00	11.00	8.00	0.00	0.00	6.8	20.00	18.00	17.00	15.00	14.00	16.8
Acetone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloroform	11.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.oo	0.00	0.00	0.00
Water	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

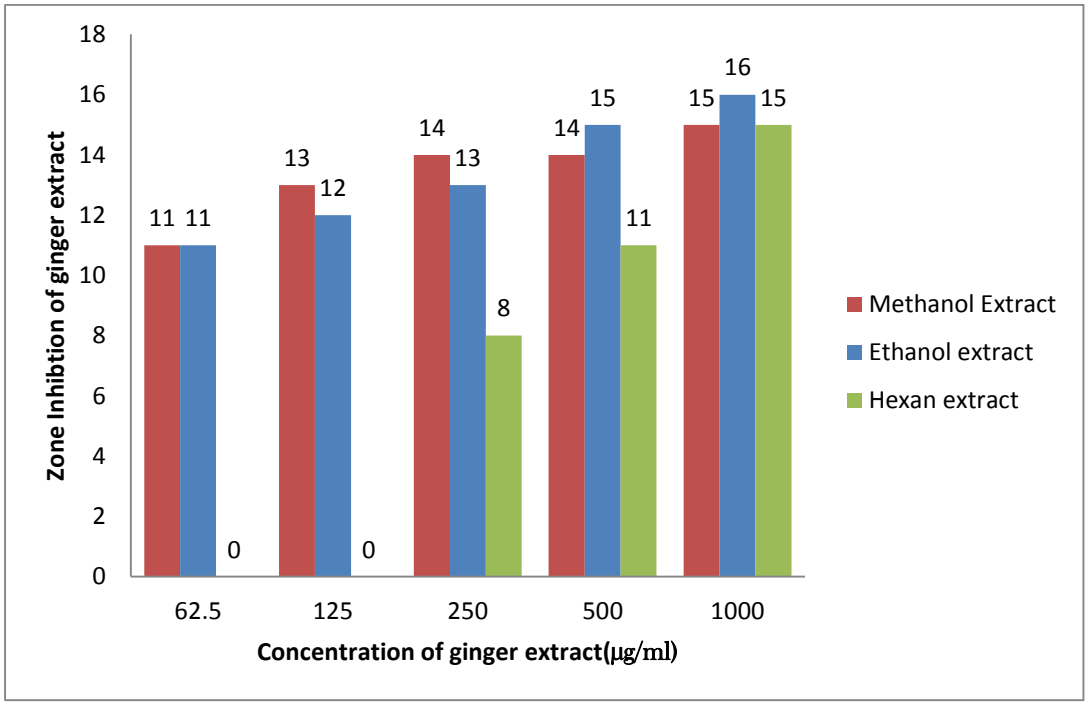


Figure (2-25): Antibacterial activity for whole extracts of ginger with E.coli.

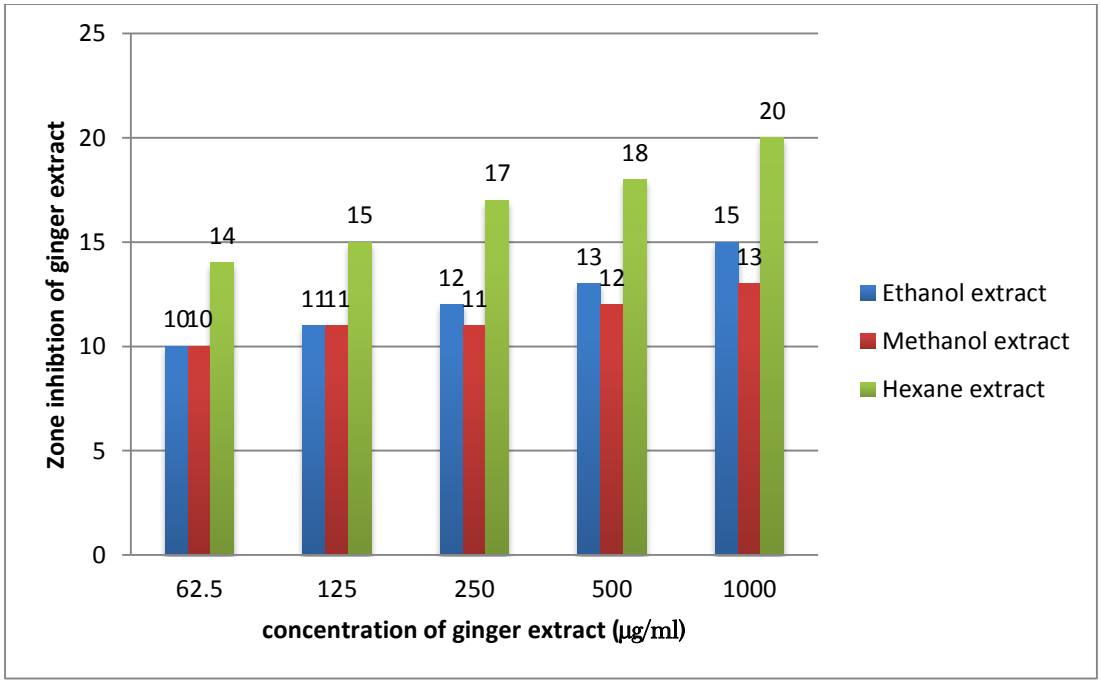


Figure (2-26): Antibacterial activity for whole extracts of ginger with S.aureus.

The solvent of extraction and method affected the degree of antibacterial activity. During the introductory testing, it was observed that ethanol, methanol and

hexane extracts gave the widest diameter zone of inhibition (16.00,15.00,15.00) mm with E.coli and (15.00,14.00,20.00)mm with Staphylococcus aureus when using concentration 1000 mg\ml, but weak inhibition or there is no inhibition with acetone , chloroform and aqueous extracts with same concentration.

The antibacterial for those extracts dependent on the concentration of the extract for example 1000 mg\ml of ethanol extract inhibition e. coli with 16 mm and inhibition Staphylococcus aureus 15 mm , while 125 mg\ml of same extract inhibition e. coli 12 mm and inhibition Staphylococcus aureus 11 mm diameter inhibition zone.

From these results, it become appropriate to detect the minimum inhibitory concentration (MIC) of different extracts. Ethanol extract had the overall widest diameter zone of inhibition e. coli (10.00) mm with 1000 mg\ml and hexane extract had the overall widest inhibition Staphylococcus aureus (20.00) mm with 1000 mg\ml concentration.

Table (3-13): Minimum inhibition concentration (mg/ml) of organic and aqueous extracts (MIC).

Extract	**E. coli**	**S. aureus**
Conc.	1000 500 250 125 62.5 MIC	1000 500 250 125 62.5 MIC
Ethanol	62.5 mg/ml - 11.00 mm	62.5 mg/ml - 10 mm
Methanol	62.5 mg/ml- 11.00 mm	62.5 mg/ml - 10 mm
Hexane	250 mg/ml- 8.00 mm	62.5 mg/ml - 10 mm
Acetone	NI	NI
Chloroform	1000 mg/ml -11 mm	NI
aqueous	NI	NI

Table (3-14): Antimicrobial sensitivity of different extracts on the test microorganism concentration (mg\ml). Key: +++≥ 16 mm diameter, ++≥ 10 mm diameter, + ≥ 8 mm diameter,* = weak inhibition, - = no inhibition.

Extract	**E. coli**	**S. aureus**
Conc.	1000 500 250 125 62.5 MIC	1000 500 250 125 62.5 MIC
Ethanol	+++ ++ ++ ++ ++	++ ++ ++ ++ ++
Methanol	++ ++ ++ ++ ++	++ ++ ++ ++ ++
Hexane	++ ++ + - -	+++ +++ ++ ++ ++
Acetone	- - - - -	- - - - -
Chloroform	* - - - -	- - - - -
Aqueous	- - - - -	- - - - -

The MIC result of different extracts were gained from extrapolation of zone diameter of inhibition of the concentration. For example at the dilution of 62.5 mg\ml for ethanol extract , the MIC was observed for e. coli and Staphylococcus aureus, which gave 12.00 mm and 11.00 diameter zone of inhibition respectively.

There was no MIC detected for the aqueous and acetone extracts because there was no inhibition with highest concentration of 1000 mg\ml as shown in tables 5.

In conclusion, *Zingiber officinale* Roscoe (ginger root) produced marked inhibitory effect on S. *aureus* and E. *coli* with ethanol, methanol and hexane extracts, while aqueous and acetone extracts not inhibitory to the test organisms and chloroform extract had weak inhibition with S. aureus and had no inhibition with E.

coli bacteria. The results indicated that the plant have growth inhibitory effect in vitro against pathogenic bacteria.

3.2.4 Antifungal activity

In this study antifungal activity show no significant zone inhibition recorded on all ginger extract (ethanol, methanol, and acetone, hexane, chloroform, aqueous).

3.2.5 Antioxidant activity of ginger in mice (*in vivo*)

Table (3-15) antioxidant activity of ginger in mice. **-significant

NO. OF Groups	MDA(µM)	SOD(U/ml)	Catalase(U/ml)
Group1(control)	1.82±0.05	7.85±0.35	6.15±0.77
Group2(250mg/Kg)	1.32±0.29	8.85±0.07	7.75±0.21
Group3(500mg/Kg)	1.11±0.04	10.55±0.21	9.4±0.07
Group4(750mg/Kg)	0.94±0.03**	12.55±0.35**	10.25±0.91**

Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation; it can also be generated during prostaglandin biosynthesis in cells MDA is one of the most known secondary products of lipid peroxidation, and it can be used as a marker of cell membrane injury, MDA is far the most popular indicator of oxidative damage to cells and tissues. MDA reacts with amino groups on proteins and other biomolecules to form a variety of adducts Increased levels of lipid peroxidation products, by measurement of MDA, have been associated with various conditions and pathological states of diseases. The results of serum MDA level was showed in table (3-15) figure (3-28) in which observed when dose increase MDA was decreasing , that mean dinger extract decreasing MDA level Therefore increasing antioxidant level.

There is very close association between oxidative stress, inflammation and tumer promotion. in the intracellular system of every aerobic organism, a balance between oxidant and antioxidant is vital for physiological process. Reactive oxygen spcies ROS such as superoxid and hydrogen peroxid are initially produced as a normal host –defense meachnicm. However due to their high reactivity. ROS are prone to cause damage to normal tissue and are theraby potentially toxic, mutagenic, carcenogenic, to epithelium and connective tissue. Antioxidant serve to protact cell and organisms from the lethel effect of excessive ROS formation by eleminating the unpaired electron or action as free radical scavenger.[36]

Superoxide dismutase (SOD) s an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. The results of serum Sod level was showed in table (3-15) figure (3-29) in which observed when dose increase SOD was increasing also that mean dinger extract increasing sod level Therefore increasing antioxidant level.

Hydrogen peroxide is highly important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell by reacting with Fe^{2+} and possibly Cu^{2+} ions .This assay shows the ability of ginger to inhibit hydrogen peroxide in the reaction mixture. From the results, it appeared that activities of the plant extract were nearly the same with the reference compounds. This could be due to the presence of phenolic compounds that donate electron to H2O2 and thus neutralizing it to water.

Catalase is another antioxidant enzyme widely distributed in the animal tissues. It decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals .The reduction of activity of this enzyme may lead to deleterious effects as a result of superoxide and hydrogen peroxide assimilation. In the present study, the percentage stmulation of catalase was shown to increase after the

administration of the aqueous extract in a dose related pattern. The results of serum catalase level was showed in table (3-15) figure (3-27) in which observed when dose increase catalse was increasing also that mean dinger extract increasing catalase level and antioxidant level.

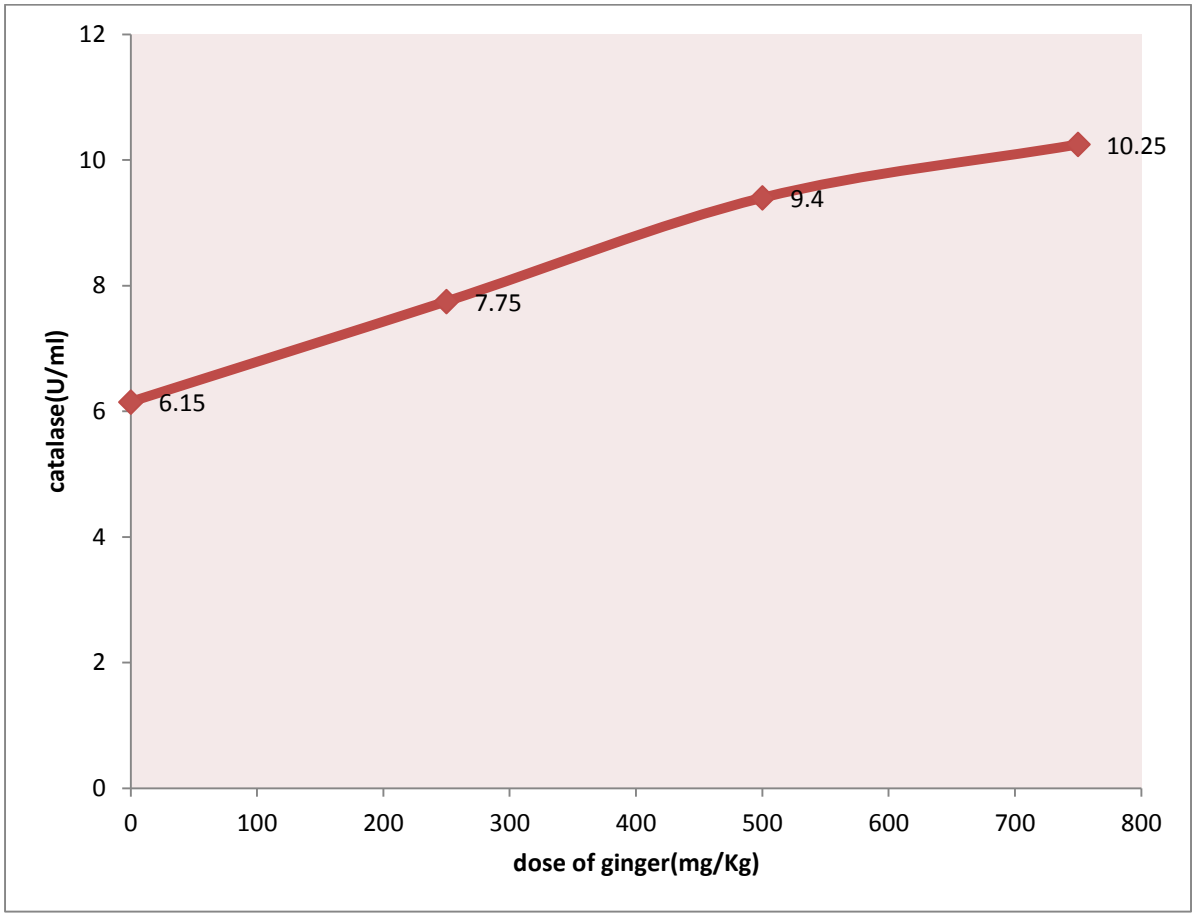


Figure (3-27) : fluctuation of catalase in different dose of ginger

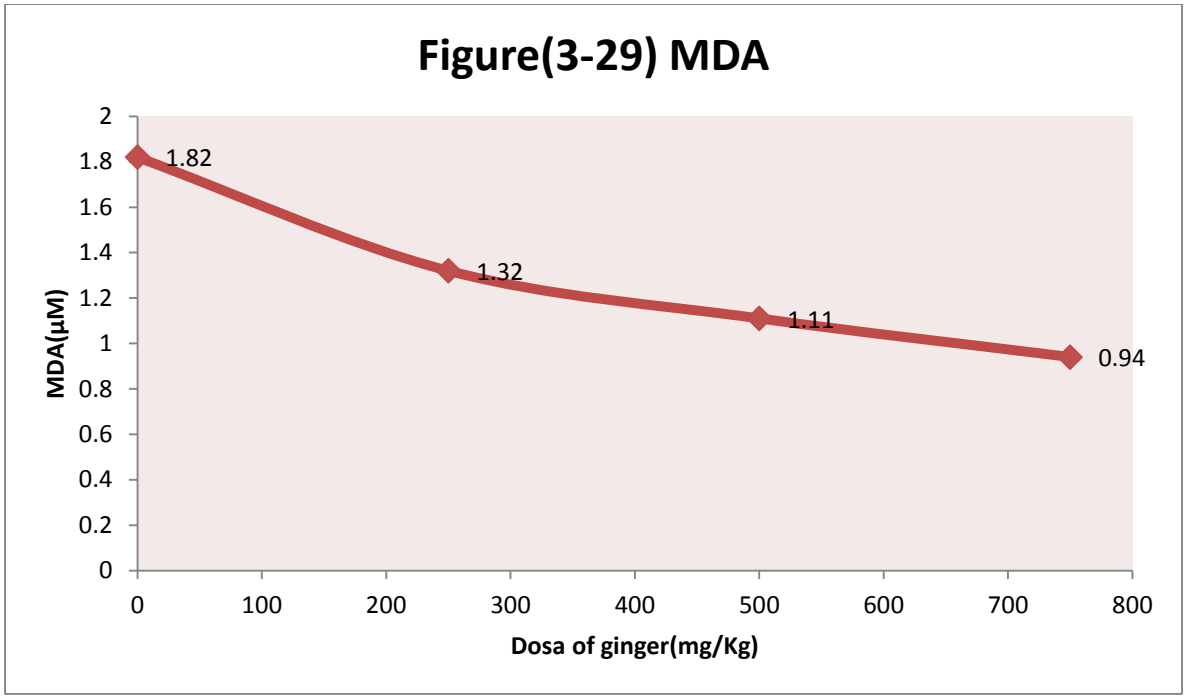


Figure (3-28): fluctuation of MDA in different dose of ginger.

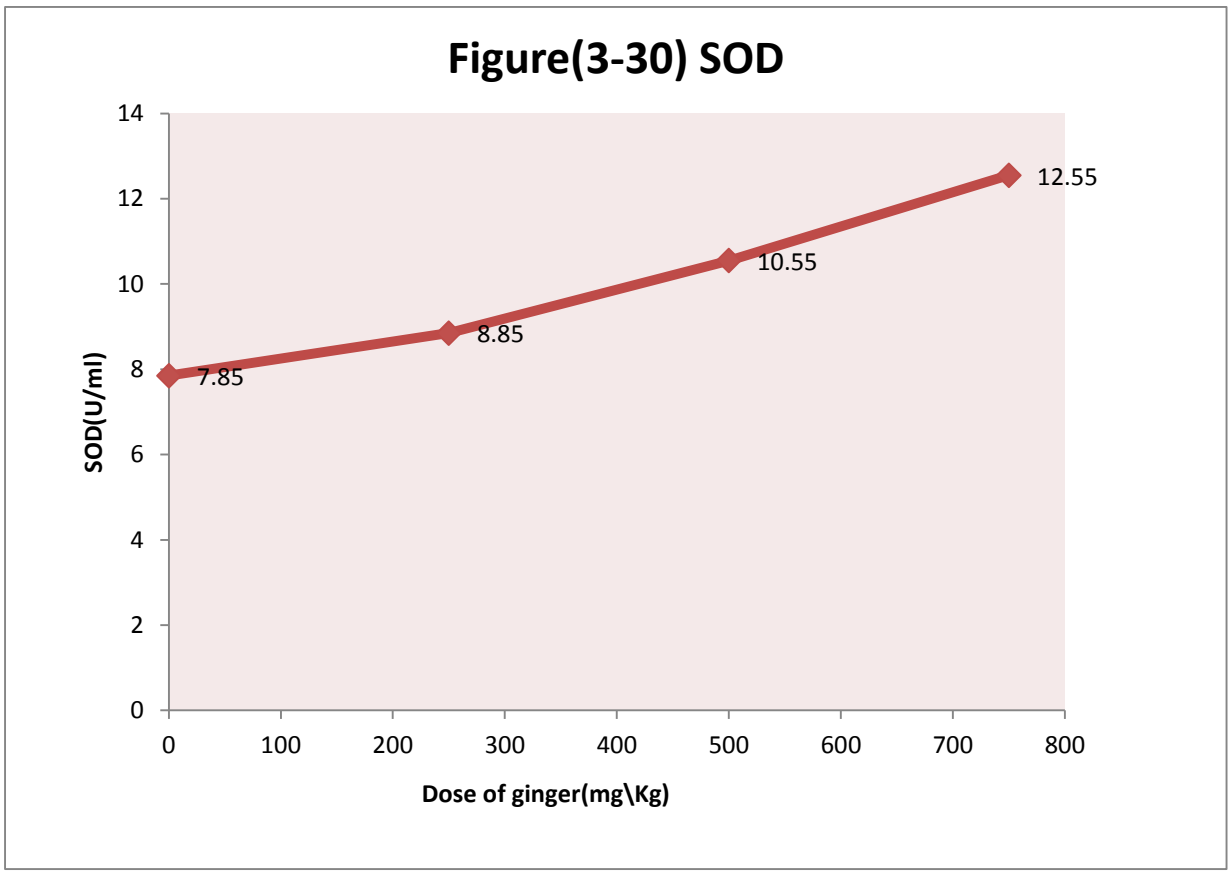


Figure (3-29): fluctuation of SOD in different dose of ginger.

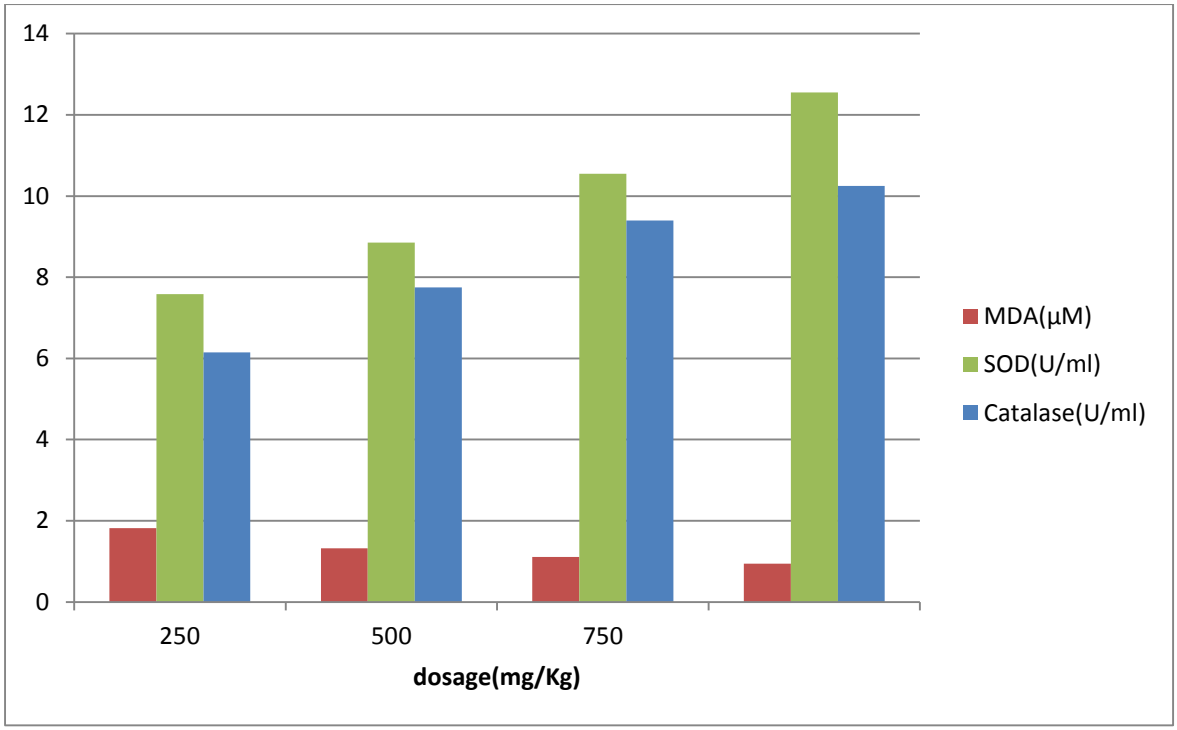


Figure (3-30): Data for antioxidant activity test *in vivo*

This study showed that ginger aqueous extracts have good free radical scavenging ability and can be used as a radical inhibitor or scavenger, acting possibly as a primary antioxidant. Kikuzaki reported that methanol extracts may include phenolic and hydrox-phenolic compounds with acid, alcohol, sugar or glycoside [65]. Part of the antioxidativeactivity may be due to these components or flavonoids. In addition, antioxidative activities observed in ginger varieties could be the synergistic effect of more than two compounds that may be present in plant. [66]

3.2.6 Cytotoxicity of ginger ethanol extract

Table (3-16): Results of the MTT assay test on TPC-1 cell lines after treatment with ginger ethanol extract .**IC50=2969µg/ml**

S.NO	Concentration (µg/ml)	Dilutions	Cell viability (in percentage)	
			TPC-1 cell lines	
			sample	STD
1	1000	neat	44.94±5.9	82.45±7.47
2	500	1:1	62.81±4.83	86.36±6.857
3	250	1:3	86.04±3.28	95.13±2.32
4	125	1:8	94.33±3.54	94.81±3.38
5	62.5	1:16	95.56±3.79	92.88±6.23
6	31.25	1:32	96.51±2.26	96.04±3.56
7	15.7	1:64	95.35±3.42	97.43±1.76
8	7.85	1:128	99.33±1.97	97.16±1.36

The cytotoxic effect of the ethanol ginger extract was determined with Thyroid Papillary Carcinoma cell line (TPC-1). Analyses were done using MTT method. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cytotoxicity assay is based on the ability of a mitochondrial dehydrogenase enzymes from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form dark purple formazan crystals which are largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. The number of surviving cells is

directly proportional to the level of the formazan created. Results shown in table (3-16) and figure (3-31) and (3-32), indicated that 1000µg/ml was the most significant cytotoxic concentration.

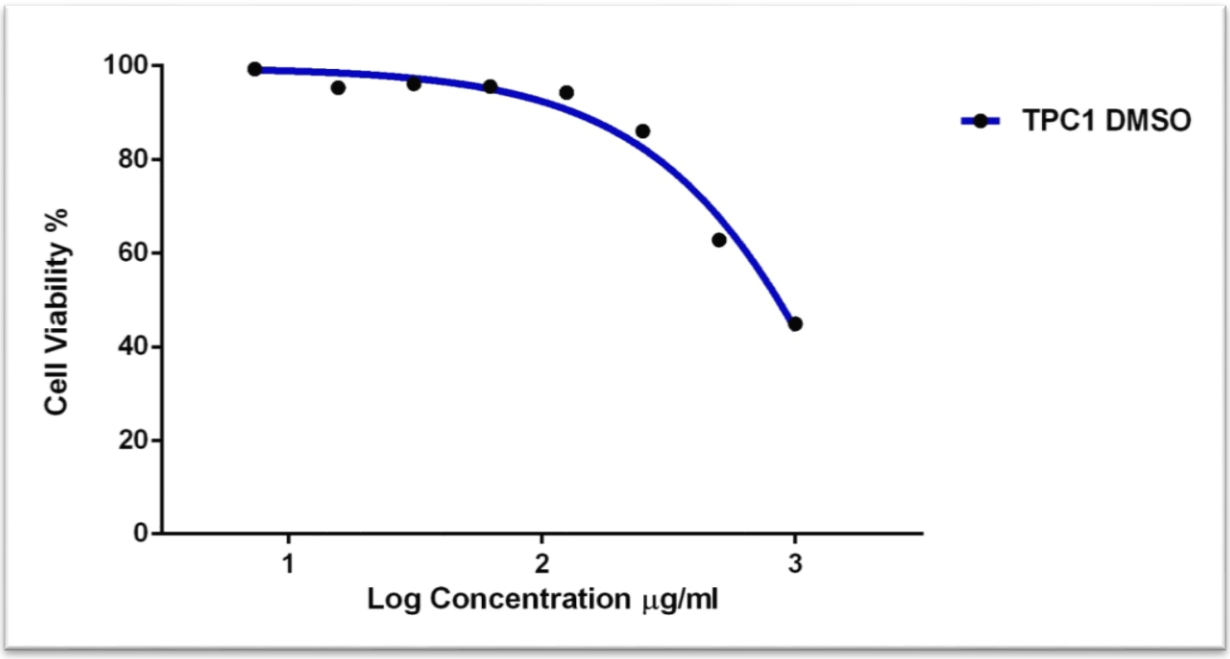


Figure (2-31) Cytotoxicity of ethanol ginger extract with TPC-1 cell line.

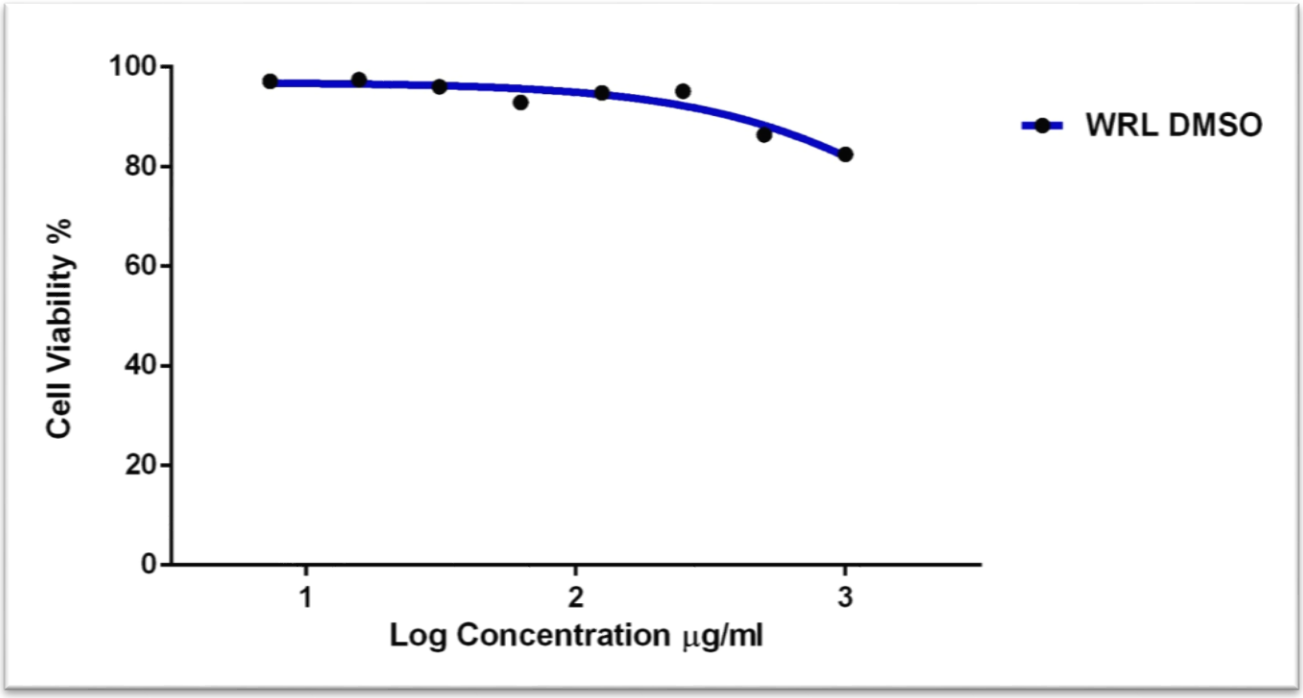


Figure (3-32): cytotoxicity of ethanol ginger extract with normal cell line.

Based on the several *in vivo* and *in vitro* studies, many mechanisms of anticancer action may be involved. These include cell cycle arrest, carcinogen inactivation, antiproliferation, inhibition of angiogenesis, induction of apoptosis and differentiation, antioxidation and reversal of multidrug resistance or a combination of these mechanisms .Flavonoids are among the best candidates for mediating the protective effect of diets rich in fruits and vegetables with respect to colorectal cancer. To gain additional information about their effects on cancer cells and their mechanisms of action, a series of related flavonoids was added to cultures of cancer cells. All flavonoid compounds increased growth inhibition and cell loss at concentrations of 1 to 100 mM

thyroid cancer is the most common endocrine malignancy, with an estimated 44,670 new cases diagnosed in the United States in 2010. Its prevalence continues to rise; in 2008 it became the sixth most diagnosed cancer in women For reasons that are unclear, thyroid cancer is 2-3 fold more common in females than males. Although the peak incidence of thyroid cancer diagnosis is 45 to 49 years in women and 65 to 69 years in men, it does affect young people.

Thyroid cancer accounts for approximately 10% of malignancies diagnosed in persons aged 15 to 29 years. Thyroid carcinoma can arise from either follicular or non-follicular thyroid cells. Follicular cancers include papillary thyroid cancer (PTC, 80%), follicular thyroid cancer (FTC, up to 11%), Hürthle cell cancer (HCC, 3%) and anaplastic thyroid cancer (ATC, 2%). PTC and FTC, which together account for the vast majority of cancers, are commonly referred to together as differentiated thyroid cancer (DTC). HCC, a subtype of FTC, is often classified on its own because it has a distinct histological appearance and is often less responsive to standard therapy.

3.3 Part Tree: Nanoparticles

Zingiber officinale Rhizome (ginger root) contain a number of biodynamic compounds of therapeutic value. These compounds provide valuable ideas for the development of new drugs against cancer, microbial infections and inflammations. Many of the secondary metabolites biosynthesized by the Rhizome plants are well known for their cytotoxic property.

3.3.1Antibacterial activity

In table (3-17) it was observed that nanoparticle of ginger give the widest diameter zone of inhibition 27mm Compared to other ginger extracts. Nanoparticles was synthase from aqueous extract which when experience antibacterial activity no detectable growth around wells, so with nanoparticle give antimicrobial activity.

Mean zone of inhibition that showed inhibition of varying diameter with the different test organisms. The mean zone (x-) of inhibition was (20.8) mm on E. coli. The mean zone (x-) of inhibition on Staphylococcus aureus was (16.6) mm, shown in table (3-9).

It was observed nanoparticles gave the widest diameter zone of inhibition 27mm with E.coli when used concentration 500mg/ml, and gave diameter zone inhibition 21 mm with S.aureus. The minimum inhibitory concentration (MIC) of nanoparticles with E.coli and S.aureus was 31.25mg/ml.

Table (3-17): antimicrobial activity, mean zone inhibition diameter, minimum inhibition concentration and antimicrobial sensitivity of synthesized AgNPs of ginger.

Conc.	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml	x-	activity	MIC	sensitivity
E.coli	27mm	23mm	21mm	17mm	16mm	20.8	+	75mg/ml	+,+,+,+,+
S.aureus	21mm	19mm	15mm	15mm	13mm	16.6	+	75mg/ml	+,+,+,+,+

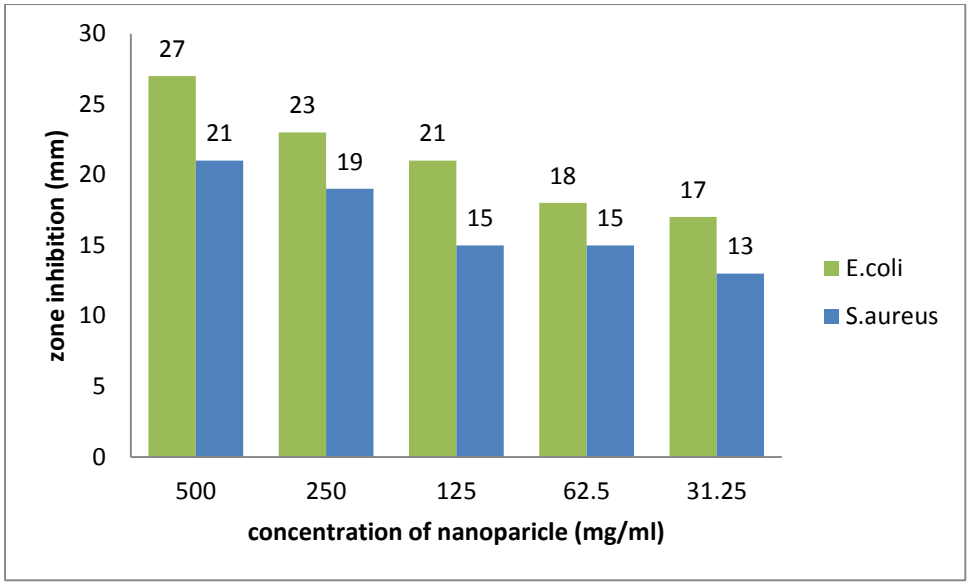


Figure (3-33): antibacterial activity of nanoparticles.

3.3.2 Cytotoxicity of ginger nanoparticle with PTC-1 cell line

It is thought that ginger act as anticancer, due to various constituents such as vallinoids viz. [6]-gingerol and [6]-paradol, shogaols, zingerone, and galanals A and B and constituents show a therapeutics role in diseases control via modulation of various biological antioxidant effect of ginger stress. The free radical production is balanced by the antioxidative defense system of our body. Any alterations between reactive oxygen species (ROS) generation and its neutralization by antioxidant defense [67, 68] cause oxidative stress. Ginger is a source of a large number of antioxidants and also plays an important role in the reduction of the lipid oxidation and inhibits the pathogenesis of diseases. The essential oil and oleoresin of Zingiber officinale exhibited significant antioxidant and antimicrobial activities. 6 Dehydroshogaol 6-shogaol and 1-dehydro-6-gingerdione has shown potent inhibitors of nitric oxide (NO) synthesis in activated macrophages.

The favor of ginger as antioxidant showed that 6-shogaol has potent antioxidant properties which can be attributed to the presence of unsaturated ketone moiety. That phenolic substances possess strong anti-inflammatory and antioxidative properties and

considerable anticarcinogenic and antimutagenic activities and showed role as in scavenging of H_2O_2 which donate electrons to H_2O_2 ,thus neutralizing it to water. Ginger and its constituents show a vital effect in the control of tumour development through up regulation of tumour suppressor gene, induction of apoptosis and inactivation of VEGF pathway. Angiogenic factor such as VEGF play a significant role in the development and progression of tumour. Therefore, Inhibition of VEGF is an important step in the prevention of tumour development/management. Earlier in vestigation has shown that, 6-gingerol has role in the suppression of the transformation, hyperproliferation, and inflammatory processes that involve in various steps of carcinogenesis, angiogenesis and metastasis. [69]

As shown in Table (3-18) a concentration of 500 μg/ml most of extract exhibited strong anticancer activity , exhibit cell viability 32.54% , In this concentration, normal cell treated with ginger extract showed 70.18% in same concentration. The plates were observed under an inverted microscope to detect morphological changes. The result showed that PTC-1 cells proliferation were significantly inhibited by GNPs with an IC50 value of 557.1 μg/ml of the concentration. Thus the synthesized nanoparticles were found to be potently cytotoxic agent against PTC-1.

It is well known that phenolic and flavonoid compounds act as hydrogen donors in that reaction mixture and therefore, the formation of hydroperoxides were decreased. The free radical scavenging of phenolic compounds was mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers.

Table (3-18): result of cytotoxicity of ginger with PTC-1 cell line with various concentration of synthesized GNPs.

S.NO	Concentration (µg/ml)	Dilutions	Cell viability (in percentage)	
			TPC-1 cell lines	
			sample	STD
1	1000	neat	32.54±7.82	70.18±2.16
2	500	1:1	46.06±5.23	78.07±2.72
3	250	1:3	68.16±8.54	89.40±2.73
4	125	1:8	70.42±8.77	93.88±2.40
5	62.5	1:16	83.79±7.45	94.90±3.13
6	31.25	1:32	86.29±6.17	96.31±2.05
7	15.7	1:64	90.00±2.31	97.71±2.680
8	7.85	1:128	92.16±233	98.38±3.03

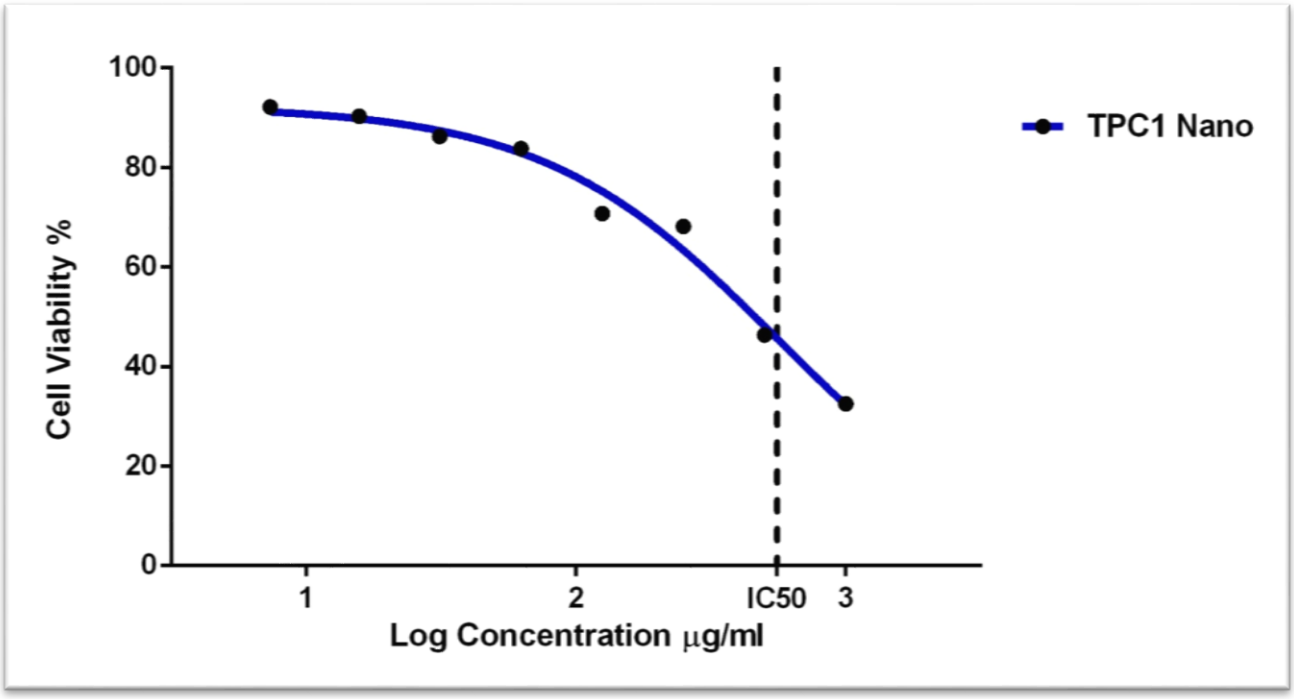


Figure (3-34): cytotoxicity of synthesized GNP with TPC-1 cell line.

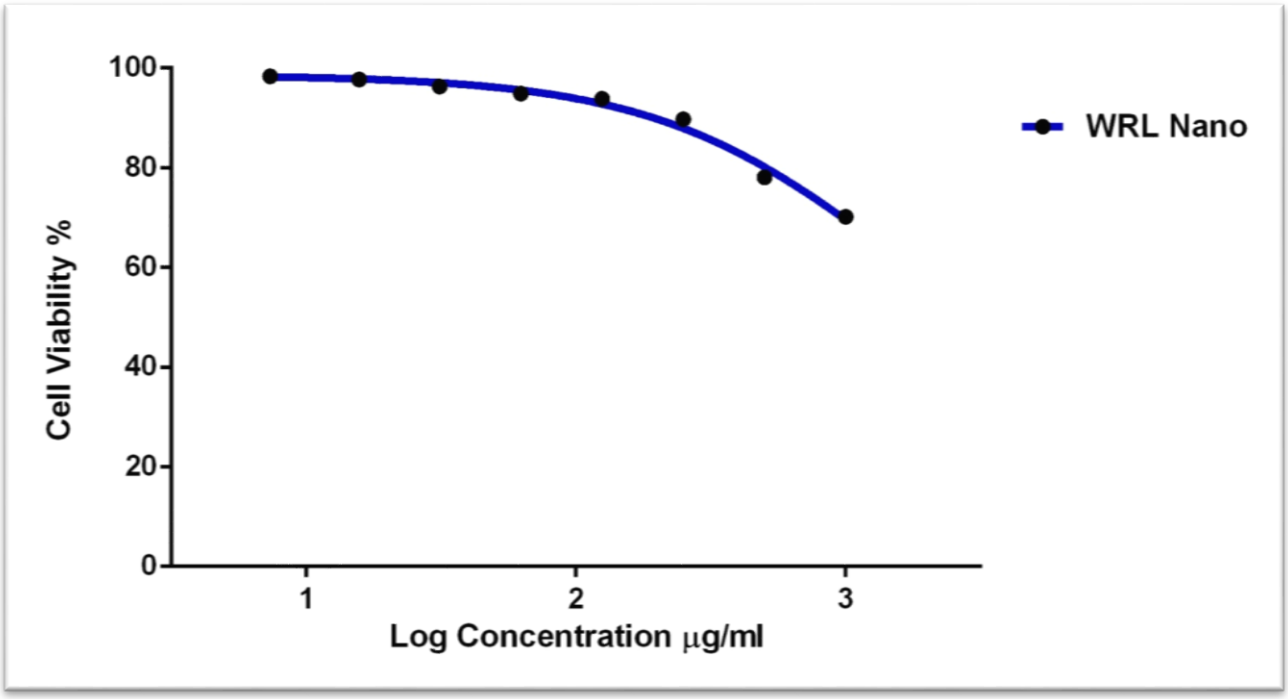


Figure (3-35): cytotoxicity of synthesized GNP with normal cell line.

By Compared cytotoxic activity of ethanol extract with cytotoxic activity of synthesized GNPs, the results in figure (3-36) were showed that GNPs have cytotoxic activity more than twice time the ethanol ginger extract. It's good to remind concentration prepared with water while ethanol ginger extract prepared with DMSO, maybe DMSO protect cell line because DMSO used in kept cell line but DMSO used cause not found another solvent dissolve organic ginger extracts.

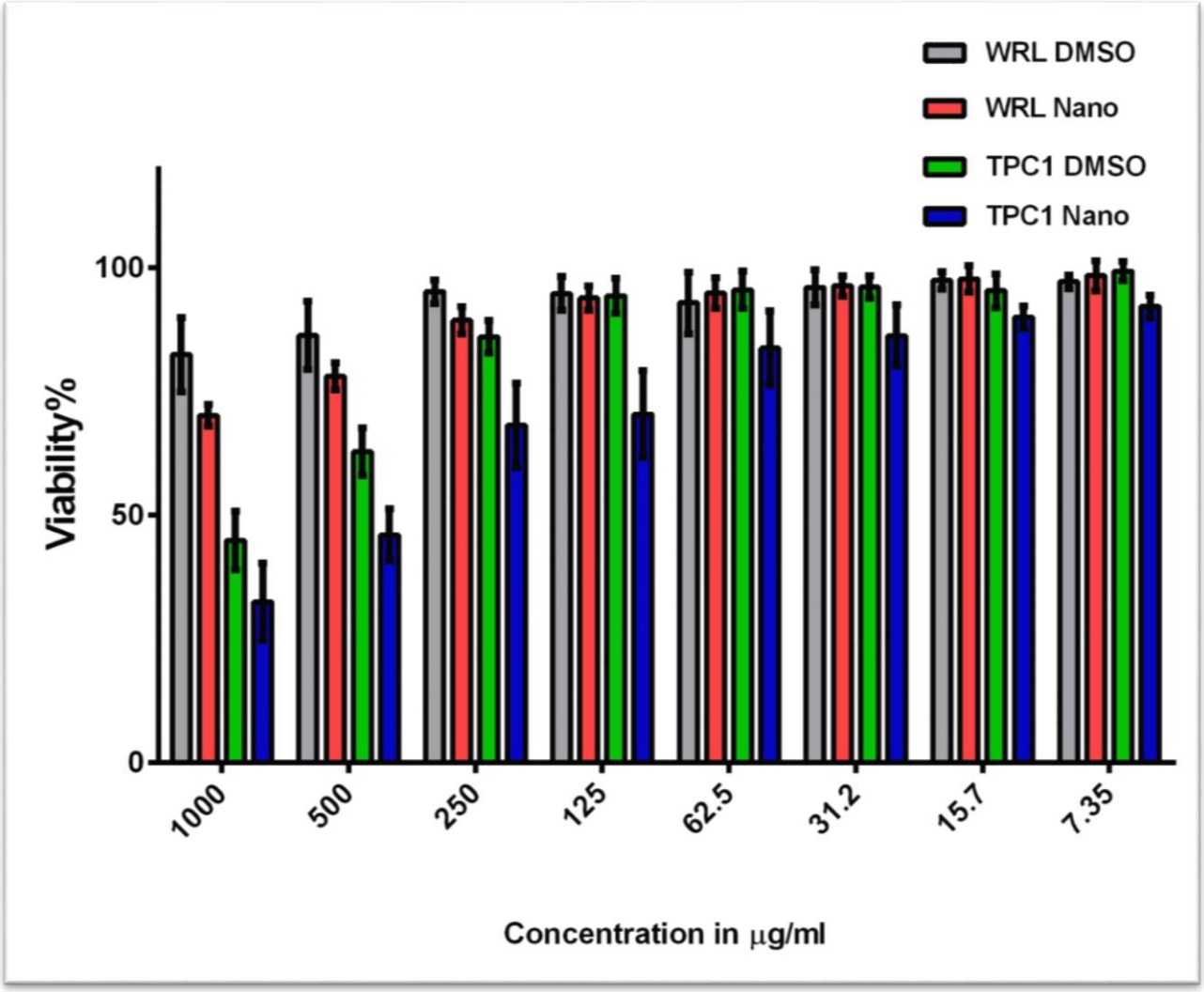


Figure (3-36): cytotoxic activity for ethanol ginger extract and synthesized GNPs on normal cell line and PTC-1 cell line.

3.3.4 UV visible spectra

The present study was conducted to screen the Zingiber officinale Rhizome for the synthesis of GNPs and its cytotoxic potential against cancer cell lines. Reduction of silver ions into GNPs during exposure of the ginger extracts could be followed by color change when kept in dark place for 2 day. The reaction mixture turning brown after the addition of silver nitrate solution is a clear indication of the formation of silver nanoparticles. At the same time control without silver ions was also run along with the experimental flask.

UV-Vis absorption spectra are known to be quite sensitive to the formation of GNPs. Thus the presence of GNPs was characterized by using a UV-Vis spectrum. A single broad peak was observed at 434 nm Figure (3-37), which corresponds to plasmon excitation of the GNPs. A control without the addition of the silver nitrate solution was also recorded. Several investigators have observed absorption maxima of colloidal silver solution between 410 to 440 nm, which is assigned to surface plasmon of various metal nanoparticles.

Table (3-19): UV-visible spectra of synthesized GNPs against the aqueous extract of ginger.

Wavelength (λ)	Absorbance of water extract of ginger	Absorbance of ginger nanoparticle
330	0.94	0.97
350	0.919	1.07
400	0.399	0.498
450	0.247	0.331
500	0.151	0.232

550	0.095	0.161
600	0.064	0.124
650	0.053	0.097
700	0.048	0.091
750	0.041	0.084
800	0.036	0.076
850	0.033	0.061
900	0.031	0.053

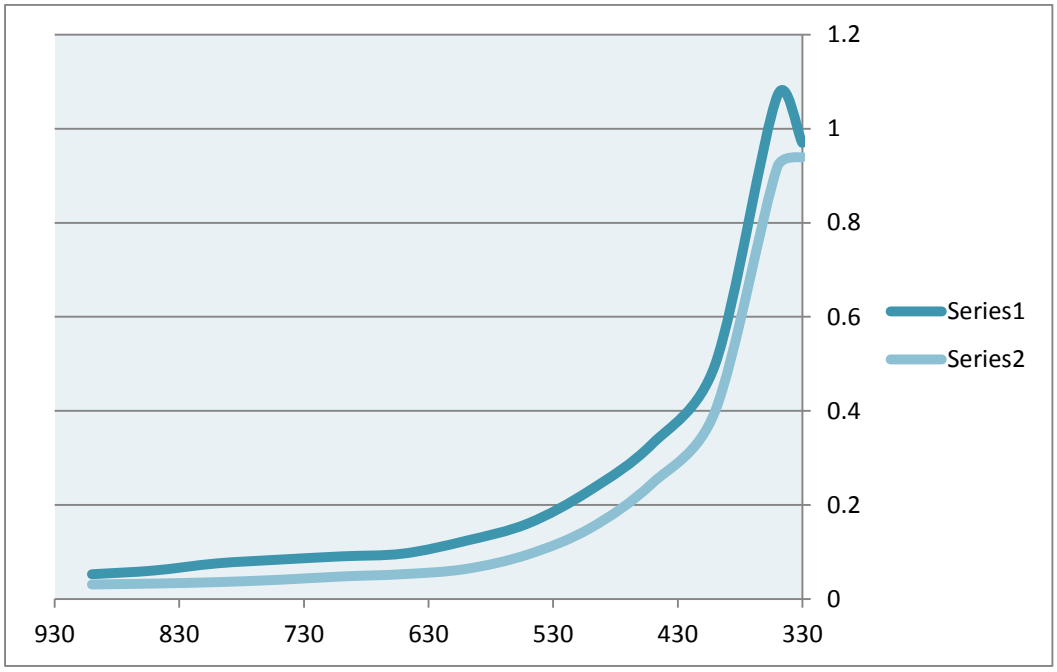


Figure (3-37) UV visible spectra of synthesized AgNPs against the aqueous extract of ginger.

3.3.5 FT-IR spectrum of synthesized GNPs

FT-IR has become an important tool in understanding the involvement of functional groups in relation between metal particles and biomolecules which is used to search the chemical composition of the surface of the silver nanoparticles and identify the biomolecules for capping and efficient stabilization of the metal nanoparticles. There were many functional groups present which may have been responsible for the bio-reduction of Ag^{+} ions.

FTIR measurements were carried out to identify biomolecules responsible for the stabilization of the newly synthesized GNPs. The FT-IR spectrum of the ginger extract in Figure (3-38) showed the presence of $AgNO_3$ with transmission peaks at (93433.29, 3410.15, 2962.66 & 1639.49) cm^{-1} respectively. The broad and intense peak at 3433 cm-1 corresponds to OH stretching vibrations of phenol/carboxylic group present in extract. This evidence suggested the release of protein molecules that probably had a role in the formation and stabilization of GNPs in aqueous solution. A peak observed at 2922 and 2886 cm-1 is due to C-H stretching of alkanes. The peak at 1384 cm-1 assigned to nitro N-O bending and peak at 1109 cm-1 to C-O-C stretching aromatic ring. It showed peak in the range of 628 cm-1 relating to the alkyl halides band especially the C-Cl bond. Therefore, it may be inferred that these biomoleculesare responsible for capping and efficient stabilization of synthesized nanoparticles.[70]

Figure (3-38): FT-IR spectrum of synthesized GNPs.

3.3.6 Scanning Electron Microscope (SEM)

A Scanning electron microscope (SEM) micrographs of AgNPs obtained showed that they are spherical shaped, it is showed in figure (3-39) .

Energy Dispersive Analysis of X-ray (EDAX) gives qualitative as well as quantitative status of elements that may be involved in the formation of GNPs. Figure 5 shows the peak in silver region at 3KeV which is typical for the absorption of metallic silver nanocrystalline due to surface plasmon resonance as showed in figure (3-40).

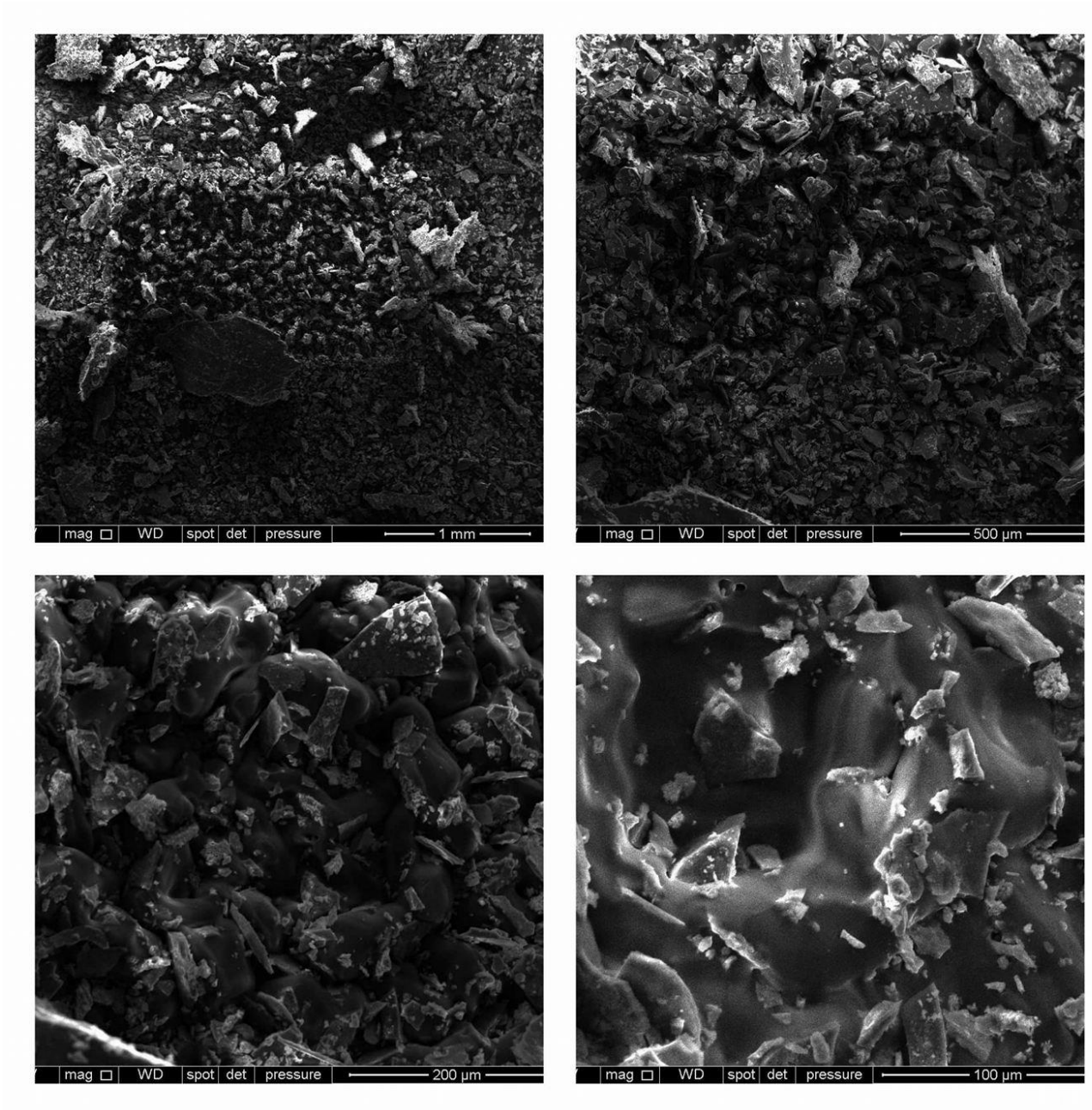


Figure (3-39): SEM images of synthesized GNPs.

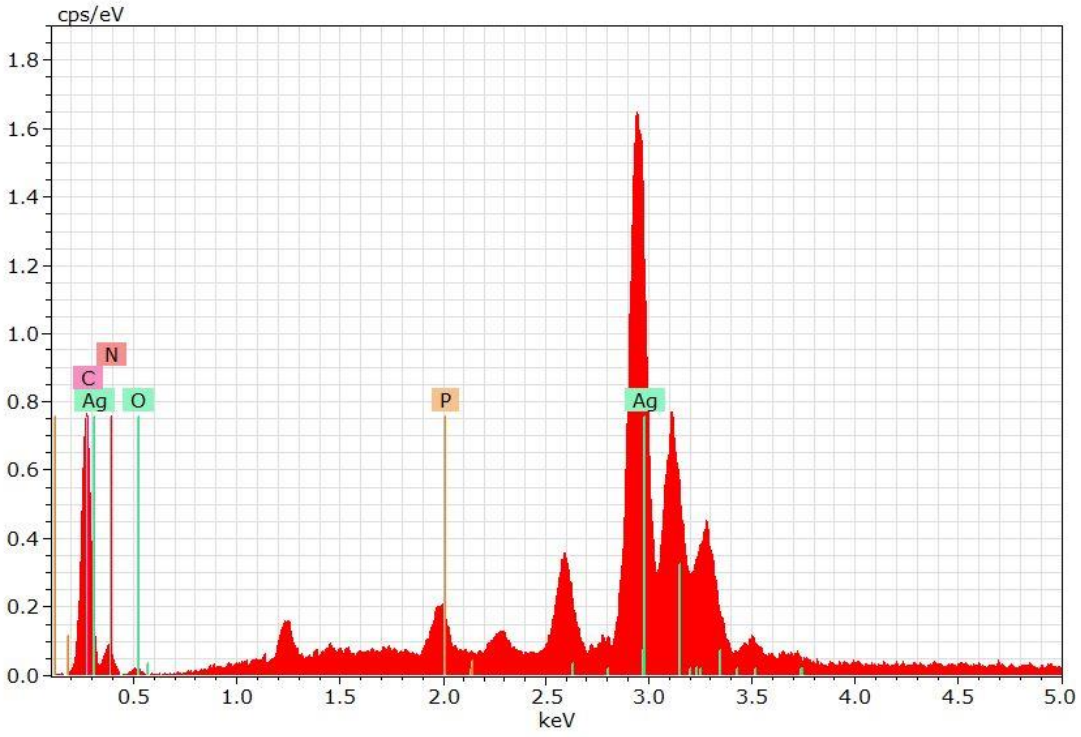


Figure (3-40): EDAX profiles of GNPs along with the elemental composition of Ag.

3.3.7 Transmission Electron Microscopy (TEM)

A Transmission electron microscopy (TEM) micrograph recorded from the silver nanoparticles deposited on carbon coated copper TEM grid was shown in Figure (3-41). This micrograph shows spherical GNPs with low density dispersion and are in the range of 22-30nm in size.

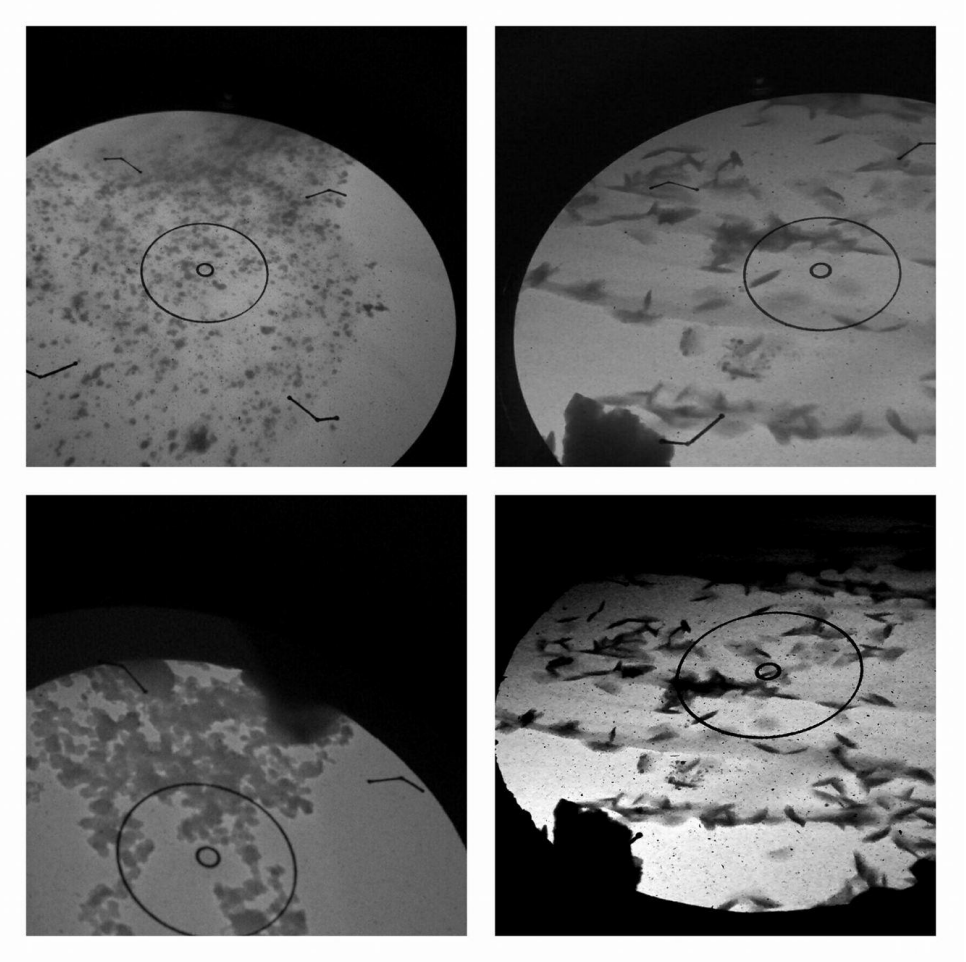


Figure (3-41): TEM images of synthesized GNPs.

3.3.8 Atomic-force microscopy (AFM)

Nanoparticle analysis is an important challenge in present nanoscale metrology. Their proper characterization is therefore very important. The size of a nanoparticle can be determined easily from the AFM image by measuring the nanoparticle image height [71]. Nanoparticle analysis can be performed using scanning probe microscopy methods (AFM), AFM has several advantages over SEM/TEM for characterizing nanoparticles. Images from an AFM represent data in three dimensions, so that it is possible to measure the height of the nanoparticles quantitatively. With an SEM/TEM, the images measured are only two-dimensional, The AFM scans more slowly than an SEM [68]. From this analysis detect size of nanoparticles easily before tested GNPs on cell line or antibacterial. As shown in figure (3-42-a) 3D image of synthesized GNPs and a darker color mean very small particle and lighter color mean particle more larger up to 78.29nm size, in b one dimensional image as showed in figure (3-42-b), the darker color mean particle too small and a lighter color mean larger particle. Size of Ag nanoparticle synthesized ranging from 1.00nm to 78.29 nm but the larger proportional less than 60nm. [72]

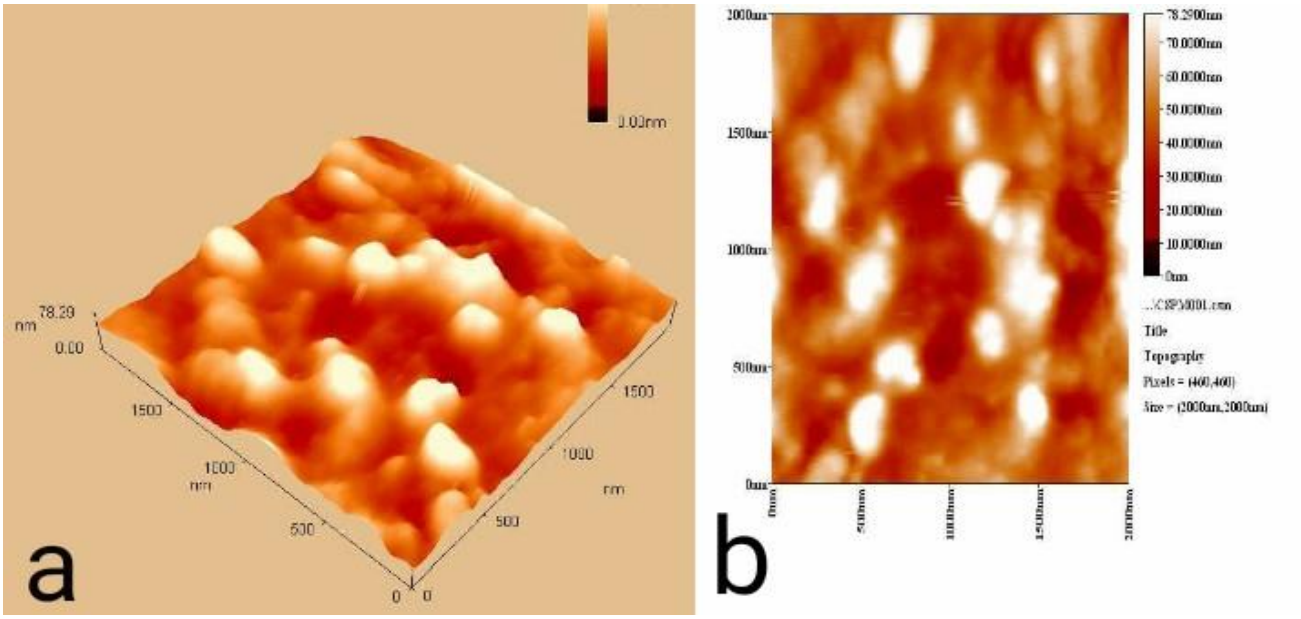


Figure (3-42): AFM images of synthesized GNPs .

3.3.9 X-Ray Diffraction Analysis

X-ray diffraction analysis the dry powders of the silver nanoparticles were used for XRD analysis. The diffracted intensities were recorded from 20◦ to 80◦ at 2 theta angles. The diffraction pattern in Figure (3-43) corresponds to pure silver metal powder The XRD pattern indicates that the nanoparticles had a spherical structure. No peaks of the XRD pattern of Ag_2O and other substances appear in Figure (3-43), and it can be stated that the obtained silver nanoparticles had a high purity. The observed peak broadening and noise were probably related to the effect of nanosized particles and the presence of various crystalline biological macromolecules in the plant extracts. The obtained results illustrate that silver ions had indeed been reduced to Ag0 by ginger plant extract under reaction conditions. [73]

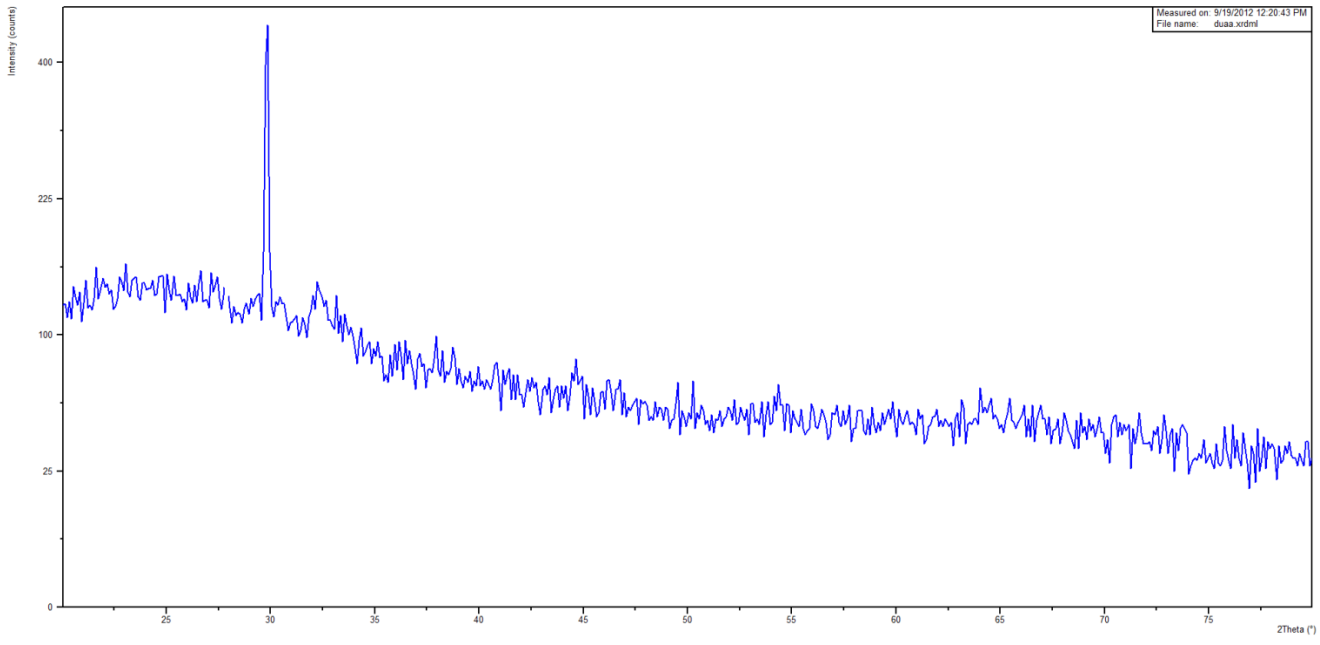


Figure (3-43): XRD pattern of synthesized GNPs.

CHAPTER FOUR

CONCLUSIONS

Conclusions

Based on the findings of present study, it is possible to reach the following most important conclusions:

1. The results showed that thyroid cancers patients had high T3,T4 and light rise in kidney function (Urea,Creatinine) ,liver function(AST, ALT) and glucose levels , and reveal a positive and strong correlation between T3,T4 and(Urea, Creatinine, AST, ALT, Glucose) .the papillary thyroid carcinoma was popular more than another type of thyroid cancer(follicular and medullary) in our study.
2. In this study ginger was extracted with water and with five organic solvent (ethanol, methanol, acetone, hexane, chloroform), then it has been tested for biological activity (antibacterial activity and antifungal activity) and FT-IR spectrum for all extracts. Hexane extract was most powerful biological activity.
3. The results showed significant antioxidant activity (MDA, Catalase, SOD) for aqueous extract of ginger *in vivo* in mice.
4. Preparation of Ag nanoparticles from aqueous ginger extract and check nanoparticle size by TEM, AFM, SEM and XRD and was 25-30nm and that silver ions had indeed been reduced to Ag0 by ginger plant extract under reaction conditions.
5. Ethanolic ginger extract and synthesized GNPs had cytotoxic effect against papillary thyroid carcinoma PTC-1 determined *in vitro* by MTT assay. Cytotoxic activity of GNPs was more than ethanolic ginger extract.

CHAPTER FIVE

RECOMMENDATIONS

Recommendation

1. According to the results separation ginger root extracts with HPLC to it Components and test biological activity for each component separately.

2. Trying to dissolution organic ginger extract with other solvent than DMSO because this solvent was not working with cell line and decreasing cytotoxic of extract.

3. Examine cytotoxic of ginger extract in vivo in mice infect with cancer then measure effect of the extract on the organism, and use another extract than aqueous extract because activity of aqueous extract less than ethanolic, methanolic and hexanic extracts, or use separated ginger extracts compounds by HPLC or GNPs synthesize from separated compounds of ginger extracts.

4. preparation Ag nanoparticles with other ginger extract because organic ginger extract have more activity than aqueous extract, or preparation GNPs with separated compounds by HPLC, it will be take better biological activity results.

Chapter six

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APPENDIX

The assay procedure of parameters

1. Serum Glucose

1- Test tubes were filled in triplicate with 10 µL distilled water (blank) glucose standard or serum sample.

2- Added 1 mL of a reagent solution containing peroxidase, glucose oxidase, phosphate buffer (pH 7.5), 4-Aminoantipyrine, phenol, mutarotase and sodium azide. Tubes were then vortexed and incubated at 37° C for 30 minutes. During the incubation period, glucose in the blanks, standards and samples reacted with glucose oxidase, releasing hydrogen peroxide. The formed Hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-Aaminoantipyrine to a red-violet quinoneimine dye as indicator.

3- Read at 500 nm by the spectrophotometer. The intensity of the color in the samples in comparison to the intensity of the color in the standards provides an indication of glucose concentration.

2. Serum Urea

1- Test tubes were filled in triplicate with 10 µL distilled water (blank) urea standard of serum sample.

2- Added 1 mL of a reagent 1 solution containing phosphate buffer (pH=7.0), sodium salicylate, sodium nitroprusside and EDTA mixed and incubated for 3min at 37C˚.

3- Added 1mL of reagent2 solution containing phosphate buffer (pH<13) and hypochrite mixed and incubated for 5min in 37C°.During the incubation ammonium ions react with salicylate and hyochlororite to form a green dye.

4- Read at 550 nm by the spectrophotometer. The intensity of the color in the samples in comparison to the intensity of the color in the standards provides an indication of Urea concentration.

3. Serum Creatinine

1- Test tubes were filled in triplicate with 100 µL creatinine standard or serum sample.

2- Added 1 mL of a working reagent mixed and started the stopwatch.

3- After 30 sec read the absorbance A1 at 492.

5- Exactly after 2 min read absorbance A2 .A2-A1 =ΔA, the different in intensity ΔA of the color sample was comparison to the different ΔA in intensity of the color slandered provides an indication of creatinine concentration.

4. Serum GOT

1- 1. added 0.1ml sample to 0.5ml of reagent one(buffer), mixed and incubated for 30 min at 37C°

2- 20min at 20-25C°

3- Added 5ml of reagent3 (NaOH) for 5 min, during the incubation Aspartate react with oxoglutarate to form Oxaloacetic and glutamate.

4- Yield a colored hydrazine that was read at 546nm by the spectrophotometer. Absorbance compared with table come with kit and found concentration.

5. serum GPT

1- Added 0.1ml sample to 0.5ml of reagent one (buffer), mixed and incubated for 30 min at 37C˚.
2- Added 0.5ml of reagent 2(2,4-DNP) mixed and incubated for 20min at 20-25C˚.
3- Added 5ml of reagent3 (NaOH) for 5 min, during the incubation Alanine react with oxoglutarate to form Pyruvate and glutamate.
4- Yield a colored hydrazine that was read at 546nm by the spectrophotometer. Absorbance compared with table come with kit and found concentration.

6. Total triiodothyrionin T3

1- Formatted the micro plate wells for each serum reference, control and patient specimen to be assayed in duplicate.
2- Pipetted 25µl of the appropriate serum reference, control or specimen into the assigned well.
3- Added 100µl of working reagent A, T3 enzyme reagent to all wells.
4- Swirl the micro plate 20-30 second to mixed and covered.
5- Incubated 60 minutes at room temperature.
6- Discarded the contents of the micro plate by decantation or aspiration.
7- (3times) Added 350µl of wash buffer.

8- Added 100µl of working substrate solution into wells.

9- Incubated at room temperature for 15 min.

10- Added 50µl stop solution to each well and gently mix for15-20min.

11- Read the absorbance in each wells at 450 nm.

7. Total thyroxine T4

1- Formatted the micro plate wells for each serum reference, control and patient specimen to be assayed in duplicate.

2- Pipetted 25µl of the appropriate serum reference, control or specimen into the assigned well.

3- Added 100µl of working reagent A, T4 enzyme reagent to all wells.

4- Swirl the micro plate 20-30 second to mixed and covered.

5- Incubated 60 minutes at room temperature.

6- Discarded the contents of the micro plate by decantation or aspiration.

7- (3times) Added 350µl of wash buffer.

8- Added 100µl of working substrate solution into wells.

9- Incubated at room temperature for 15 min.

10- Added 50µl stop solution to each well and gently mix for15-20min.

11- Read the absorbance in each wells at 450 nm.

8. Thyrotropin (TSH)

1- . Formatted the micro plate wells for each serum reference, control and patient specimen to be assayed in duplicate.
2- Pipetted 25µl of the appropriate serum reference, control or specimen into the assigned well.
3- Added 100µl of working reagent A, TSH enzyme reagent to all wells.
4- Swirl the micro plate 20-30 second to mixed and covered.
5- Incubated 60 minutes at room temperature.
6- Discarded the contents of the micro plate by decantation or aspiration.
7- (3times) Added 350µl of wash buffer.
8- Added 100µl of working substrate solution into wells.
9- Incubated at room temperature for 15 min.
10- Added 50µl stop solution to each well and gently mix for15-20min.
11- Read the absorbance in each wells at 450 nm.

9. serum MDA for mice

Preparation of reagents

1. TCA reagent (70%): seventy drams of trichloroacetic acid (TCA) was taken and dissolved in a final volume of 100ml of DW.
2. TCA reagent (17.5%): Five milliliters of 70% TCA was taken and the volume was completed to 20ml with DW.

3. TBA reagent (0.6%): 0.06g of TBA was dissolved in a final volume of 10ml of DW using a water bath for complete dissolving of TBA.

Procedure

1. one hundred µl of serum sample was poured in a test tube and 1 ml of 17.5% TCA was addad.
2. One milliliter of 0.6% TBA was added.
3. the tubes were mixed well by vortex, incubated in boiling water bath for15minutes, and then allowed to cool.
4. One milliliter of 70% TCA was added.
5. The mixture was left to stand at room temperature for 20 minutes.
6. The tubes were centrifuged at 2000xg for 15 minutes, and the supernatant was taken out for measuring sepctrophptometrically at 532nm.

10. SOD activity for mice

1. serume diluted 1:5 .
2. Mixed 160µL Assay buffer, 5 µL xanthine and 5µL wst-1.treansfered 160µL working reagentto each sample and tapped tube to mixed.

For each sample, dilute the xo enzyme 1:20 in diluent. Quickly was added 20µL diluted XO enzyme to each samle. Tube was tapped to mix.

3. Immediately read OD440nm (OD420-460nm) (OD0). Sample incubated for room temperature (25C˚) in the dark. Read OD440nm again.

〖ΔOD〗_60=〖OD〗_60-〖OD〗_0

ΔΔOD=〖ΔOD〗_std8-ΔOD

11. serum catalase for mice

1. transfered 10μL sample into tube. In addition for each assay run, was prepared one sample blank that contains only 10μL assay buffer.

400μL assay buffer was added to positive control tube and mixed well. Transfer 10μL of reconstituted positive control (catalase).

2. Enzyme reaction: mixed 5μL 3%H2O2 and 914μL dH2O (Final 4.8Mm).

Enough 50μM H2O2 prepared Substrate for sample, positive control and sample blank by mixed, for each tube 1μL of the 4.8mM H2O2 with 95 μL assay buffer.

90μL of 50μM substrate added to these tube to initiate the catalase reaction. Tubes was tapped quick to mix. Tubes incubated 30min at room temperature during the incubation time.

3. Detactin reagent was prepared enough by mixed for each reaction tube (sample, control and standard tube), 102μL assay buffer, and 1μL dye reagent and 1μL HRP enzyme. At the end of incubation,

100µL detection reagent was added into each tube. Tubes was tapped to mixed well. Incubated for 1o min.

4. Samples optical density was read at 570nm .

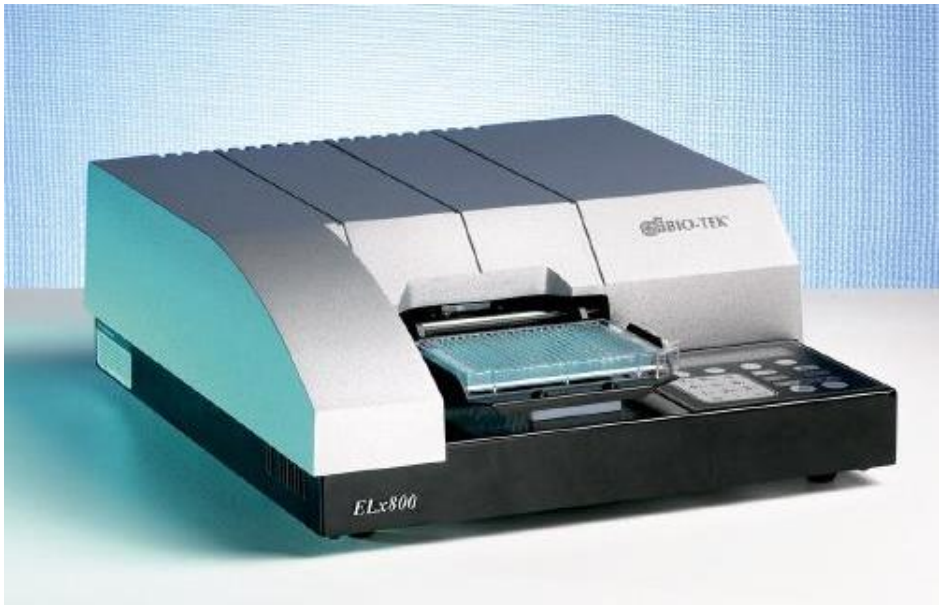
Catalase (U/L) = $R_{Sample\ Blank} - R_{Sample}$/ Slope (µM-1) x 30 min × n

R sample and R sample blank are optical density. Slop determine from standard curve.30min is the catalase reaction time. n is the sample dilution factor.

Devices that used to measure the parameters

1. Washer device from biotek ELx50 Washer, USA

2. Reader device ELX800 Absorbance Microplate Readers, USA



3. Centrifuge device from Hermle LaborTechnik GmbH,Germeny

4. Spectrophotometer device from APEL

الملخص

المقدمة:

الغدة الدرقية هي غدة صماء، وتتكون من فصين تقع في الجزء الأمامي من الرقبة، وتحت تفاحة آدم. الغدة الدرقية تفرز هرمونات الغدة الدرقية التي تؤثر على معدل الأيض، تخليق البروتين، ولها مجموعة واسعة من التأثيرات الأخرى. يتم تخليق هرمونات الغدة الدرقية T3 و T4 من اليود والتيروسين. تنتج الغدة الدرقية أيضا الكالسيتونين، الذي يلعب دورا في توازن الكالسيوم.

الزنجبيل يمتلك العديد من الخصائص الطبية ومن خصائصه الطبية انه يستخدم كمضاد للاكسدة ومضاد للبكتريا ومضاد للسرطنة و مضاد للالتهابات بالأضافه الى أن له تأثيرا على مرض السكرو المفاصل و الزهايمر.

. في الآونة الأخيرة دراسة الجسيمات النانوية اصبحت من المجالات العلمية الأكثر إثارة للاهتمام. وهناك اهتمام متزايد لإنتاج الجسيمات النانوية باستخدام طرق صديقة للبيئة (الكيمياء الخضراء)، الفضة النانوية ذات أهمية لأنها ذات خصائص فريدة من نوعها.

اهداف الرسالة:

١- لدراسة التغير في مستويات هرمونات T3,T4,TSHو وظائف الكلية ووظائف الكبد و السكر في المرضى المصابين بسرطان الغدة الدرقية.
٢- هذه الدراسة صممت لقياس تأثير السمية الخلوية للمستخلص الايثانولي للزنجبيل و الجسيمات النانوية المحضرة على خط خلايا سرطان الغدة الدرقية.
٣- دراسة فعالية الزنجبيل كمضاد للاكسدة على الفئران ودراسة الفعالية البايلوجية للمستخلص المائي والمستخلصات الخمسة العضوية.
٤- . تحضير الجسيمات النانوية و دراسة خصائصها بعدة تقنيات.

المرضى و طرق العمل:

هذه الرسالة تضمنت خمسين مريضا ذكرا وخمسين مريضة انثى (يشرط فيها ان تكون تحت سن اليأس)أضافة الى خمسين رجل و خمسين أمرأة يتمتعون بصحة جيدة كمجاميع قياسية للمقارنة.هذه الدراسة اجريت اثناء الفترة من تشرين الثاني ٢٠١٥ الى كانون الثاني ٢٠١٦.

الجزء الاول :هذه الدراسة صممت لتحري التغير الحاصل للمتغيرات التاليه لمرضى سرطان الغدة الدرقية:

١. المتغيرات الكيمياوية الحيوية: وتشمل مستويات وظائف الكلية و وظائف الكبد و مستوى السكر في الدم و قيست بطريقة التلون الانزيمي.
٢. الهرمونات: هرمونات الغدة الدرقية thyroid hormones Total (triiodothyrionin T3, Total thyroxine T4, Thyrotropin (TSH و قيست بواسطة ELISA.

الجزء الثاني: هذه الدراسه صممت لتحري تأثيرات مستخلص الزنجبيل من خلال دراسة:

١. استخلاص الزنجبيل بخمسة مستخلصات عضويه اضافه الى مستخلص بالماء، و حللت بجهازمطيافية الاشعة التحت الحمراء FT-IR.
٢. تحديد الفعالية البايلوجيه لجميع مستخلصات الزنجبيل.
٣. تحديد السمية الخلوية على خط خلايا سرطان الغدة الدرقية لمستخلص الايثانول.
٤. تحديد فعاليه الزنجبيل كمضاد للاكسدة داخل اجسام الفئران.

الجزء الثالث: في هذه الدراسة حضرت الجسيمات النانوية من المستخلص المائي للزنجبيل و تمت دراسة خصائصها من خلال:

١. الجسيمات النانوية المحضرة من مستخلص الزنجبيل المائي تم قياسها بجهاز المطيافية المرئية و الفوق البنفسجية ومقارنتها بالمستخلص المائي للزنجبيل.
٢. الجسيمات النانوية حللت بعدة تقنيات ،تقنية المطيافية الاشعه تحت الحمراءFT-IR، وتقنية مجهر القوة الذرية SEM، وتقنية المجهر الالكتووني النافذ TEM، وتقنية مجهر الطاقة الذرية AFM، وتقنية حيود الاشعة السينية XRD.
٣. قياس الفعالية البايلوجية للجسيمات النانوية .
٤. قياس السمية الخلوية للجسيمات الناموية على خط خلايا سرطان الغدة الدرقية.

النتائج:

الجزء الاول:

اظهرت النتائج وجود اختلاف معنوي ايجابي ($P<0.001$) في الهرمونات , T3,T4,TSHو وظائف الكبد (AST,ALT) و وظائف الكلية (Urea, Creatinine) و مستوى السكر في كل مجاميع المرضى مقارنة بما يقابلها من مجاميع قياسية.

اظهرت نتائج هرمون T3 ارتباطا ايجابيا مع وظائف الكبد (AST,ALT) و وظائف الكلية (Urea, Creatinine) و مستوى السكر في الدم.

اظهرت نتائج هرمون T4 ارتباطا ايجابيا مع وظائف الكبد (AST,ALT) و وظائف الكلية (Urea, Creatinine) و مستوى السكر في الدم.

اظهرت نتائج فترة المعالجة ارتباطا سلبيا مع وظائف الكبد (AST,ALT) و وظائف الكلية (Urea, Creatinine) و مستوى السكر في الدم في جميع المجاميع عدا مجموعة المرضى الذكور كان ارتباطا ايجابيا.

الجزء الثاني:

أظهرت النتائج قياس مستخلصات الزنجبيل بجهاز مطيافية الاشعة تحت الحمراء وجود مجاميع فعالة و تشير الى وجود المركبات الفعالة في مستخلصات الزنجبيل.

نتائج الفعالية البايلوجية لمستخلص الزنجبيل اظهرت وجود منطقة تثبيط عاليه بالنسبة لمستخلص الايثانول و الميثانول و الهكسان في البكتيريا الكروية العنقودية الذهبية و البكتريا الإشريكية القولونية، بينما لا توجد فعالية بكتيرية لمستخلص الكلورفورم و الاسيتون و المستخلص المائي.

اظهرت نتائج الفعالية الفطرية بأنه لا يوجد منطقة تثبيط لمستخلصات الزنجبيل.

اظهرت نتائج السمية الخلوية لمستخلص الايثانول بأنه كانت له سمية خلوية مع تراكيز عالية و قورنت بالخلايا الطبيعية.

أظهرت نتائج قياس فعاليه مضادات الاكسدة لمستخلص الزنجبيل على الفئران ان مضادات الاكسدة في سيرم الفئران يزيد بزيادة تركيز جرع الزنجبيل التي تم تطعيمها.

الجزء الثالث:

أظهرت النتائج ان حجم الجسيمات النانوية المصنعة كان من٢٥-٣٠ nm بتقنية المجهر الالكتروني النافذ و ١-٧٥ nm بتقنية مجهر الطاقة الذرية، و اظهرت النتائج في جهاز مطايفية الاشعة التحت حمراء وجود مجاميع فعالة من ما يعني بأن المركبات الفعالة للزنجبيل بقيت بعد تحضير جسيمات النانو، اما تقنية مجهر القوة الذرية فقد وضح لنا شكل الجسيمات اضافة الى كمية الفضة الحرة في جسيمات النانو و أظهرت منتوج عالي. أظهرت نتائج تقنية حيود الاشعه السينية وجود أيونات الفضة لAg^0 بواسطة مستخلص نبات الزنجبيل في ظل ظروف التفاعل.

أظهرت النتائج الفعالية البايلوجية للزنجبيل ظهر منطقة تثبيط كبيرة و أكبر من جميع مستخلصات الزنجبيل الأخرى. و أظهرت نتائج الفعالية السمية لجسيمات النانو المصنعة على خط خلايا سرطان الغدة الدرقية وجود فعالية سمية كبيرة مع تراكيز اقل من تراكيز مستخلص الايثانول .

الإستنتاجات:

١. كانت هناك زيادة ملحوظة في مستويا T3,T4,TSH و في مستويات وظائف الكبد (AST,ALT) و وظائف الكلية (Urea, Creatinine) في مرضى سرطان الغدة الدرقية، نوع سرطان الغدة الحليمي هو من اكثر الانواع شيوعا في دراستنا.
٢. في هذه الدراسة أَستخلص الزنجبيل مع الماء و مع خمسة مذيبات عضوية (إيثانول، ميثانول، كلورفورم، استون، هكسان)و تم قياس الفعالية البايلوجية للمستخلصات وقياسهم بتقنية مطيافية الاشعة تحت الحمراء، مستخلص الايثانول و مستخلص الهكسان كانو من افضل المستخلصات فعالية.
٣. أظهرت نتائج قياس فعالية مضاد الاكسدة في الزنجبيل في سيرم دم الفئران وجود فعالية للزنجبيل كمضاد اكسدة .
٤. حضرت الجسيمات النانوية من المستخلص المائي للزنجبيل و تم قياس حجمها بعدة تقنيات منها ال TEM المجهر الالكتروني النافذ و مجهر الطاقة الذرية AFM و كان حجمها ٢٥-٣٠ نانومتر وبتقنية حيود الاشعة السينية استنتج انه تم اختزال ايون الفضة الى Ag^0 في ظروف التفاعل.

٥. قياس الفعالية السمية الخلوية لمستخلص الايثانول و للجسيمات النانوية المصنعة على خط خلايا الغدة الدرقية الحليمي، وكانت الفعالية السمية للجسيمات النانوية المصنعة اعلى منها لمستخلص الايثانول.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم الكيمياء

دراسة التأثيرات النانوية والسمية الخلوية و فعالية مضادات الاكسدة لمستخلص الزنجبيل في مرضى سرطان الغدة الدرقية

رسالة
النهرين جامعة/ العلوم كلية الى مقدمة
كجزء من المتطلبات نيل درجة الماجستير في علوم الكيمياء

من قبل

دعاء ياسين رشيد

بكلوريوس ٢٠١٠

بأشراف

(الاستاذ المساعد)

د.آمال إسماعيل إبراهيم

محرم ١٤٣٨ ٢٠١٦تشرين الأول