

Republic of Iraq
Ministry of Higher Education and
Scientific Research
University of Babylon
College of Sciences for Women
Biology Department



**Histological and Physiological Study of Gold
Nanoparticles Effect on Some Reproductive
Organs in Male Wister Rats
(*Rattus norvegicus*)**

A thesis

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By

Nadia Kamil Mohammad AL-mashta

B.Sc. /Biology/University of Kufa/College of Science (2001)

Supervised by

Asst.Prof.Dr.

Manar Mohammad Hasan

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بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

۱۰. وَلَوْ لَا فَضْلُ اللّٰهِ عَلَیْكَ وَرَحْمَتُهُ لَهَمَّتْ طَائِفَةٌ
مِّنْهُمْ أَنْ يُضِلُّوكَ وَمَا يُضِلُّونَ إِلَّا أَنْفُسَهُمْ وَمَا
يَضُرُّونَكَ مِنْ شَيْءٍ وَأَنْزَلَ اللّٰهُ عَلَیْكَ الْكِتَابَ
وَالْحِكْمَةَ وَعَلَّمَكَ مَا لَمْ تَكُن تَعْلَمُ وَكَانَ فَضْلُ اللّٰهِ

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صدق الله العلي العظيم

CERTIFICATE

I certify that this thesis, entitled (Histological Study of the Effect of **Gold** nanoparticle on Some Reproductive Organs in Male **Rats**) was prepared by (**Nadia kamil muhammed Al-mashta**) under my supervision at the College of Science for women, University of Babylon as a partial requirements for the degree of Master of Science in Biology.

Signature

Assist. Prof. Dr. Manar Mohammad Hasan

College of Science for women/ University of Babylon

Date: / / 2022

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

Signature

Assist. Prof. Dr. Hadi Mizal Khudair

Head of Biology Department College of Science for Women/ University of Babylon

Date: / / 2022

DEDICATIONS

I dedicate my effort and my thesis in seeking Knowledge to my father, may God have mercy on him ,who gave his life for us, peace be upon you from the earth to the highest paradise.

To my dear mother who was flooding me with her prayers which I feel is reason for to support me in life.

To my dear husband Ali who gave me the opportunity to complete my study and created the appropriate condition for me.

To the light of my eyes ,my dear son Hussain . To my heart beats, my dear daughter Zainab.

To my brothers and my sister .

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Summary:

This study showed the current concept of toxicological effect of gold nano particles(Au NPs) on the male reproductive system of male rats (*Rattus nirvegicus*).It investigated the histological and physiological, the study also included tissue changes for each components of testes ,epididymis ,prostate and seminal vesicle, measurement standards such as hormonal (Testosterone hormone(T), Luteinizing hormone (LH) ,Follicle stimulating hormone(FSH) and Estradiol hormone(E)) as well as effect gold nanoparticles on total antioxidant(T.A.O.). It also includes the result of a study Histomorphometric for testis, measure the diameter of seminiferous tubules , lumen of seminiferous tubules and thickness of germinal epithelium ,as well as weight changes for body and organs.

This studied had been conducted at the animal house of the Faculty of Science/ University of Babylon according to the where this study has been conducted in the laboratories of the tissues of the Faculty of Sciences, University of Babylon, laboratories of AL-Fadhel Institution and Hilla general teaching hospital. The study has included 42 male white rats sexually mature , their ages ranged between 10-12week , and their weights ranged 200-230mg.The study had been conducted during the period from November 2021 to April 2022.The rats had been distributed randomly into 6 groups each group had 7 rats in each group as follows:

-Group I : Control animals divided into two sub groups each group containing 7 rats injected intraperitoneally with normal physiological saline solution for 30 and 60 days .

-Group II: include 7 rats injected intraperitoneal with 40 µg/Kg body weight of Au Nps diluted in distilled water(D. W.) for 30 days

-Group III include 7 rats injected intraperitoneal with 80 µg / Kg body weight of Au Nps dissolved in diluted water(D. W.) for 30 days .

-Group IV: include 7 rats injected intraperitoneal with 40 µg/kg body weight of Au NPs diluted in distilled water(D. W.) for 60 days.

-Group V: include 7rats injected intraperitoneal with 80 µg /kg body weight of Au NPs dissolved in diluted water(D. W.) for .60 days.

-Note :

The injection was every 15 days, per month twice the full duration of the injection 4 times within 60 days. Injected gold particles sized 5-20nm.

After completing the injection the rats had been sacrificed after 30 and 60 days as the injection of gold nanoparticles causes a histological and physiological effects, but less than the rest of the toxicity of metals and this characteristic of gold nanoparticles as a chemically inert material, but this does not prevent there to be toxic when treated on the long time .It produced decrease significantly difference($p \leq 0.05$)($p \leq 0.01$) in both total antioxidants, and hormone level Testosterone hormone(T), Luteinizing hormone(LH), Follicle Stimulating hormone(FSH). But according to the duration of exposure and histological changes were disparate after injection for 60 days. Causing degeneration and exfoliation of germ and leydig cells and penetration of the blood testicular barriers causing high estradiol cause of hyperaromatase syndrome.

As for the weight changes there is an increasing in body weight as for the members, the weight changes varied as weight of the testicles, epididymis and seminal vesicles decreased and increased prostate weight causing benign prostatic hyperplasia compared to control group.This study concluded that the gold nano particles had the effects of less on the male

reproductive system depending on concentration and exposure period, so the harmful effects remain dependent on the concentration of 80 μ g/kg for 60 day of the duration of exposure.

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List of Abbreviation

ABP	Androgen –binding protein
Au NPs	Gold nanoparticles
BTB	Blood testis- barrier
CAT	Catalase
D.W.	Distiled water
DHEA	Dehydroepiandrosterone hormone
DHT	Dihydrotestosterone
DPX.	Distrine Plasticizer Xylene
E	Estradiol
EDCS	Endocrine disruption chemicals
FSH	Follicle stimulating hormone
GnRH	Gonadotropin –releasing hormone
GPX	Glutathione Peroxidase
GSH	Glutathione
H&E	Hematoxylin and Eosin
IL	Interleukin
L.S.D.	Least Significant deference
LH	Luteinizing Hormone
MDA	Malondiadehyde
Nm	Nanometer
NP	Nanoparticles
OD	Optical density
ROS	Reactive Oxygen Species
SOD	Super oxide dismutase
Spss	Statistic Package for Social Sciences
T	Testosterone
T.A.O	Total anti- oxidant
TLRs	Toll –Like Receptors
TNF	Tumor necrosis factor(cytokine)

Chapter one

Introduction

1. Introduction:

Nanoparticles becomes more common in commercially available products, Nano particles is defined according to ASTM (American Society for testing) as standard from (1-100 nm) length range in two or three dimensions " ASTM international , 2006". Therefor the nanotechnology can be defined as a branch of biotechnology engineering (Beer *et al.*, 2012) The uses of nanoparticle (NPs) and Nano delivery system in the medical field such us diagnosis and drug delivery this application become more important in medicine (Habas *et al.*, 2021). Applications and large production of nanoparticles, NPs lead to environment impact of releasing such materials (Khan *et al.*., 2019)and then cause harm to human health (Massarasky *et al.* ., 2014) . Nps enter into the cells easily via free penetration interact with cellular components as (proteins , lipid , genomic DNA) and receptor - mediated endocytosis , when released into the environment (Shalviri *et al.* ., 2012) . The male reproductive system has more liable to different tension from other organs system (Ban *et al.* ., 2018).

Nps enter body of human by skin adsorption, breathing, ingestion and via given intravenously when used for medicine (Chen *et al.*, 2010) . One of the most important nano materials is gold nanoparticles (Au NPs) which have been widely used in drug delivery (Singh *et al.*, 2017) , cancer targeted and photo thermal therapy PTT (Chen *et al.*, 2015) and imaging diagnosis (Wilson ,2008) .They were easily synthesized , chemically inert , combine ligand and high stability. Gold has higher bioactivity in biomedical fields but the increasing use of Au Nps has raised concerns regarding in body fate and its harmful effects in human (Liu *et al.*, 2020). Au NPs have toxicity effects on important members such as lung, kidney, liver (Vines and Faunce , 2009). But they have more effect on the male reproductive

system as it weakens testicular function including decreased sperm quality and fertility, changes in testicular histology and alteration in testosterone levels (Velikorodnaya *et al.*, 2015).

More efficiently spherical Au Nps than gold nano rods (Chithrani *et al.*, 2006). Small Au Nps (4 and 13 nm) have longer residence time and more widespread organs distribution than large gold nanoparticles (100 nm). (Cho *et al.*, 2010). Oxidative Stress is one of the reasons for the deterioration of human male reproductive system . (Wright *et al.*, 2014; Guz *et al.*, 2013). NPS increase in production reactive oxygen species lead to inflammation and cancer (Hsin *et al.*, 2008). Few studies have been reported on the potential adverse effects of AuNps on male reproductive health despite concerns (Wiwanitkit *et al.*, 2009). Au NPs can penetrate the blood testes barrier (BTB) there is limited evidence that declare enter the testis in animal models (Li *et al.*., 2013). The average concentration of Au element in normal people of whole blood is about 0.05 ng /g (Brune *et al.*., 1966) ,and is about 0.23 ng/g in platelets (Kaspersk *et al.*, 1979). The reported number of NPs studies induce on reproductive system cytotoxic effects of male on man fertility (Pinho *et al.*, 2020). There are several investigations that have linked reproductive cancer and the use of NPs in targeted therapy (Barkalina *et al.*, 2014). Gold NPs have been used (Au Nps) for the treatment of prostate cancer have been used widely (Wang *et al.*, 2008; Kim *et al.*, 2010). Development and function of reproductive organ , Physiological structure of germ cell and fertility NPs may have negative effects on it, in humans and animals (Brohi *et al.*, 2017).

1-2 Aims of the Study: -

Based on the above literature, little research about histological effect of gold nanoparticles and their association with reproductive system in male rats. and little or no study related to their association of this subject (the histological and physiological effect) has yet been undertaken in our country. Therefore, the current study has been conducted to evaluate the histological and physiological effect of male rats .

The current study aims to:

- 1- Determine the effect of gold nanoparticles on reproductive organs (Testes, Epididymis, Prostate, Seminal vesicles) and study histological morphometric .
- 2- Study the histological changes
- 3- Evaluate the effect of gold nanoparticle on (T, LH, FSH, E) hormone levels.
- 4- Determine the total antioxidant.
- 5- Explain the effects of Au NPs on body and organs weight.

Chapter Two
Literature
Review

2-Literature Review

2.1 Nanotechnology:

The word " Nano " which means "dwarf " is derived from the Greek word , therefore a nanometer (nm) is equivalent to one part of billion as example a cold virus measures it is about 100 nm (Hulkoti and Taranth , 2014). The use of nanoparticles has developed quickly in different fields , because to their possessing developed properties that depend on size , shape and ranges should be from (1-100) nanometers (Rao and Gan ,2015).NPs have different properties from material which they were formed. Nanotechnology is the science that deals with nano materials (Madkour , 2019). In recent years interest has increased in the production of metallic nano materials due of their uses in different fields industrial , biomedical , agricultural , and environmental fields (Singh *et al.*, 2017) .

2.1-1 Gold Nano Particles (Au NPs):

Au NPs are highly used in bio nanotechnology because of their unique properties and multiple surface functionalities (Yeh *et al.*, 2012) . Bio conjugates of Au NPs design of biomaterials for the investigation of biological system, as the nane biological assemblies with "antibodies" (Mukherjee *et al.*, 2007),"oligonucleotides" (Zhang *et al.*, 2010a ; Guo *et al.*, 2010 ; Park *et al.*, 2010), " Protein "(Calzolari *et al.* 2010) ,because their use in bio nanotechnology easy in Au NPs functionalization . Au NPs have the Physicochemical properties such as redox behavior , conductivity and surface plasma resonance (Uehara , 2010). And high surface area of Au NPs serves as therapeutic agents , e.g. (drugs), (Brown *et al.*, 2010) . And (targeting agents) , (Khan *et al.*, 2019) .

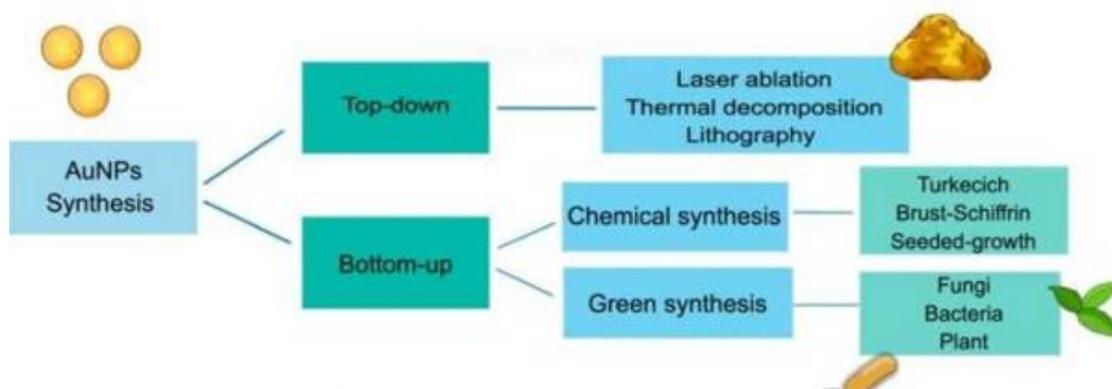
2.1-1.1 Gold nanoparticles Synthesis:

Gold nanoparticles Synthesis by using two Pathways:

1-top - down

2- down - top

The first strategy is the breaking down gold for generating nanomaterials with different dimensions that includes methods like sputtering, laser ablation, thermal decomposition, bulk metal grinding and lithography. Second Strategy is the down – top, the atoms are arranged to forming nanomolecular. This Strategy uses chemical and green methods (Zhoa *et al.*, 2013 and Kumari *et al.*, 2019). As shown in the figure(2-1) (Soto *et al.*, 2021).



Figure(2-1)Representation of Gold Nanoparticles of Different Synthesis (Soto *et al.*, 2021).

2.1-1.1.1 Chemical Synthesis of Gold Nanoparticles :

Au Nps were created by a developed synthetic method in 1951 by treating(HAuCl_4) in boiling water with Citric acid (Turkevich *et al.*, 1951). Citric acid acts as Stabilizing and reducing agent, this method is improved by Frens to control particle size, by changing the gold to citrate ratio. (Frens, 1973). This protocol is used to prepare dilute solution of

spherical stable from Au NPS with different diameters of 10-20 larger 100 nm . During functionalization of these citrate stabilized for Au Nps can undergo irreversible including using surfactant (tween 20) conquer this problem to prevent aggregation (Aslan and Prerez , 2002) .In 1994 Brust and Schrieffin made a progress through in Au Nps synthesis, by creating organic soluble alkanethiol- stabilized Au NPs by using sodium borohydride (Na BH₄) as the reducing agents and tetra-octylammonium bromid as the phase transfer reagent (TOAB) (Brust *et al.*, 1994). Thiol - gold interaction have higher stability because the synergic effect and Van der waals attraction (Hostetle *et al.* , 1998).

2.1-1.1.2 Green Synthesis:

Researchers have developed new methods to synthesize safe gold nanoparticles based on biological system like bacteria , plants , yeasts and fungi better from traditional chemicals method that generation toxic chemicals and solvents (Teimouri *et al.*, 2018 ; Kumari *et al.*, 2019and Majoumouo *et al.*, 2020) .

2.1-1.1.2.1 Microorganism Based Au Nps Synthesis :

Au NPs are synthesised by using of microorganism because low cost , non - toxic products and production on a large scale, include viruses , bacteria , yeast , and mycetes It can produce Au NPs in two shapes :

Intracellular synthesis contain transport ions into the cell wall and then enzymes present convert into nontoxic nanoparticles from the toxic metal as reductive components. Other shape of extracellular Synthesis includes reductive enzymes such as hydrogenase , nitrate (Menon *et al.*, 2017; Teimouri *et al.*, 2018). The extracellular production is very important in bio

imaging , sensor technology , optoelectronics (Rana *et al.*, 2020 ; Rajasekar *et al.* , 2020) .

2.1-1.1.2.2 Plant , Fruit , Waste Extracts Based Au Nps Synthesis:

Synthesis of Au Nps plant is done through two methods, the first is intracellular Synthesis which Puts plant in medium fullled with metal ions. This ion transport from the medium to plant from 4-6 nm is size(Noruzi , 2015) .The second method of synthesis is by using plant part such as flowers, stems, leaves, roots. This route is more promising than microorganisms because extracts are not needed when making the complex process , cheaper , safer and easily obtainable . Less toxic nanoparticles are based on shapes ,size ,temperature , extract Concentration and PH (Islam *et al.*, 2019) . This type of synthesis is used as an anti -fungal because these particles presented by antioxidant . Plant extract have large amount of antioxidant compounds able of reducing metal salts such as phenolic compounds , ascorbic acid and other vitamins (Kumar *et al.*, 2020 ; Botteon *et al.*, 2021),and flavonoids polysaccharides. These components not only serve as reducing agents but also as stabilizers and prevent agglomeration , involves the extraction of juice or pulp mixed with metal salt (Xin *et al.*, 2016; Soto , 2019).

2.1-1.1.3 Physical Synthesis :

Producing stable gold nanoparticle colloids by laser fragmentation in liquids used femto - second laser radiation to gold target (plate) to produce nanoparticle c olloids in aqueous β -cyclodextrin solution (Sylvester *et al.*, 2004) . Laser ablation is suitable for further applications for produce pure colloids (Tsuji *et al.*, 2002).

2.1-1.2 Properties of gold nanoparticles :

Size and shape are useful attributes related to optoelectronic properties of spherical Au Nps (Hu *et al.*, 2006; Sau and Rogach, 2011). Low toxicity and excellent bio compatibility happen large surface to volume ratio (Khlebtsov *and* Dykman ,2011). These qualities make Au NPs important in bio nanotechnology, ability to fluorescence and surface plasmon resonance from important physical properties , exhibit a range of color brown , red , orange and Purple of spherical Au Nps .The core size increases from (1-100 nm)in aqueous solution (Jain *et al.*, 2006) by incident photons that cause the resonant excitation by the collective oscillation of the conduction electrons, and this absorption band is called a " surface plasmon band" (Mie,1908) .This phenomenon is influenced not only by surface ligand but also by temperature , shape , size , solvent and core charge, sensitive to proximity of other nanoparticles (Toderase *et al.*, 2008). Even in the presence of 1 nm Au Nps observed fluorescence resonance energy transfer phenomenon, (FRET) in the photo induced electron transfer (PET)Process (Bigioni *et al.*, 2000;Mohamed *et al.*,2000; Dulkeith *et al.*,2005).

2.1-1.3 Application of Au NPs Bio– nanotechnology :

Antibodies or oligonucleotides readily conjugated with Au Nps and this bonding makes them important in detection and diagnosis in vitro and in vivo (Fang *et al.*, 2009 ; Huo *et al.*, 2011). Au NPs that combine between selectivity and affinity of aptamers therefor conjugate with aptamer for detect Cancer (Medley *et al.*, 2008 ; Zang *et al.*, 2010a). Conjugates of Au NPs and flurophore (lock and key) to provide high sensitivity sensing of bio detection method contain from quaternary ammonium and functionalized Au NPS the initial sensor system with poly paraphenylene ethynylene(PPE) as a fluorescence transduction element (Bunz and Rotello, 2010 ; Bajaj *et*

al., 2009). PPE can disrupt by cation Au NPs . This Strategy is a rapid and accurate assay, it is used differentiate normal and cancerous and metastatic cell (Bajaj *et al.* , 2009) and is replaced the polymer transducer by GFP (green fluorescent protein) to provide more sensitivity in mammalian Cancer.

2.1-1.4 Therapeutic:

Several research groups are used to transport of therapeutic agents to cell by functionalized Au NPs to investigate the interaction with cell membrane (Arvizo *et al.*, 2010; Verma and Stellacci 2010 ;Cho *et al.*, 2011), important process in bio medical. Demonstrated that Can regulate cell membrane Penetration by surface ligand arrangement (Verma *et al.*, 2008 ; Chou *et al.* , 2011) .There are passive or active targeting mechanisms for delivered Au NPs therapeutics into cell . Passive targeting based on the retention or enhanced Permeability . Au NPs ordered arrangement and amphiphilic molecules penetrate the cell membrane . But the Au Nps coated and random arrangement trapped vesicula bodies (Perrault *et al.* , 2009; Kennedy *et al.*, 2011).

Active - targeting provide specificity and selectivity based on surface functional ligand designed for target analysis (Li *et al.*, 2011). Using of Au Nps is developed for therapeutic methods such as photo thermal therapy (Huang *et al.*,2010 ;Van de *et al.*, 2011) ,for controlling protein expression in cell used intracellular gene regulation agents such as Au Nps - oligonucleotide (Rosi *et al.* , 2006), or Conjugates RNA – Au NPs luciferase expression (Giljohann *et al.* , 2007) . Au NPs are used for loading of drugs by covalent interactions or non-covalent conjugation that use hydrophobic or hydrophilic pockets monolayer to drug encapsulation with Au Nps (Cheng *et al.*, 2008 ; Kim *et al.*, 2009). Drug release mechanism

is done through the non-covalent conjugates with Au NPs and passive accumulation lead to penetrate deeply into tumor, and release their drugs quickly within hours, Au NPs conjugated to drugs covalently by glutathione (GSH) displacement or through split linkers can be released (Hong *et al.*, 2006; Nakanishi *et al.*, 2009). Cationic ligand (Cationic Surface) and fluorescent genetic ligands of Au NPs facilitate penetration of the cell membranes and the payload release through intracellularly by GSH (Podsiadlo *et al.*, 2008). In tumor model, GSH investigate the movement of Au NPs that carry doxorubicin or fluorescein molecules (Kim *et al.*, 2010). Anionic Au NPs deliver drug deep into tissue but cationic Au NPs are more active in delivering payloads to the majority of tumor cells. Using Au NPs mixed thiol monolayer as coating inhibits bacterial growth (Bresee *et al.*, 2011).

2.1-1.5 The Use of Gold Nanoparticles as Therapeutic Agent :

Gold nanoparticles were used not only in cell photothermal lysis and diagnosis but also in therapy in 1997. Colloidal gold is used in therapy for rheumatoid arthritis patients (Abraham and Himmel, 1997). There are positive results when using colloidal gold in rats upon intra-articular introduction in a patient with rheumatoid arthritis (Tsai *et al.*, 2007), due to the binding between gold nanoparticles and vascular endothelial growth factor, increasing anti-angiogenic activity. Therefore, it reduces macrophage infiltration and inflammation in (solid tumors) that use a colloidal gold vector to carry TNF in mice (Paciotti *et al.*, 2004; 2006). AuNP conjugates with TNF when intravenous injection rapidly accumulates in tumor cells in mice (Tumor Necrosis factor TNF). The colloidal gold-TNF vector has higher efficacy and lower toxicity in tumor therapy. Au NPs inhibit activity of heparin, binding glycoproteins, growth factor of cardiac endothelium, vascular permeability factors and fibroblasts when Au NPs interact with them and these agents mediate angiogenesis. The Process of

formation of new blood vessels in tumor tissues , Au NP prevent angiogenesis that is considered as one of the main tumor growth factors (Mukherjee *et al.*, 2005) . AuNP properties can make promises for cancer therapy, and enhance the apoptosis of the leukemia cells (lymphocytes) (Mukherjee *et al.*, 2007).

2.1-1.6 Gold Nanoparticles as Drug Carriers :

The medical use of gold nanoparticles is for targeted drug delivery (Duncan *et al.*, 2010 ; Pissuwan *et al.*, 2011).The most popular objects of target delivery antitumor agents and antibiotics conjugated with Au NPs (Paciotti *et al.*, 2006) such as methotrexate (Chen *et al.*, 2007).The conjugation with Au NPs via the use of simple physical absorption or use of alkanethiol linkers .

There are several therapeutic agents both (hydrophilic and hydrophobic) and auxiliary agents when a gold nanoparticle is loaded it. Such as dyes for photodynamic therapy, target molecules (Kim *et al.*, 2009) , example : antibiotic and antibacterial agents can be delivered by gold nanoparticles such as: vancomycin and colloidal gold against intestinal pathogenic Strains (Gu *et al.*, 2003) , ciprofloxacin and colloidal gold show antibacterial against E. coli (Rosemary *et al.*, 2006). The anti- leukemia drug conjugates with gold, (5 - fluorouracil + Colloidal gold) show antibacterial and antifungal against *Micrococcus luteus* , *Staphylococcus aureus* , *Pseudomonas* , E. Coli , *Aspergillus* (Selvaraj and Alagar,2007) .It is noted that all of the listed drugs with gold nano particles are stable , but there are some antibiotics when they are mixed with colloidal gold, they are not stable complexes such as Ampicillin and Streptomycin . (Saha *et al.*, 2007; Grace & Pandian , 2007) .

Nevertheless, their activity when mixed with gold from using alone by 12-4% when insulin conjugates with gold give to models that suffer of diabetes mellitus a decrease in blood sugar levels comparable with effect of giving them under the skin (Joshi *et al.*, 2006). Gold nanoparticles are used as an efficient agent in gene therapy for therapeutic purposes delivering the genetic material into cell nucleus and cytoplasm (Patel *et al.*, 2010). The desired effect due the expression of inserted gene or gene suppression of the damaged or over expressed gene (Zilber *et al.*, 1929).

2.1-1.7 Immunologic Properties of gold Nanoparticles:

Researchers show interest colloidal metals in the immunological , Zilber succeeded to obtain agglutinating sera from colloidal gold (Zilber *et al.* , 1929). By number of studies inter a rigorous antigen with colloidal gold lead to production antibodies and introduction haptens adsorbed on colloidal Particles also production antibodies (Dykman ,2020). The effect of colloidal gold on immune reactions (non-specific) is noted when introduced intravenously after 2 hour 5 mL of colloidal gold into rabbits increases the Leukocytes content in 1 mL of blood from (9900 to 19800), decreasing monocytes from (5200 to 4900), and increasing in Neutrophil (Polymorphonuclear) from (4700 to 14900) , but not been observed when introduction other colloidal metals. It is known that the antibody synthesis need agents that have developed structure (immunogenicity) such as proteins , polysaccharides, polymers (synthetic) contrary biologically active compounds as vitamins, antibiotics, hormones ... etc was low molecular weight therefore they cause a lower immune response known (haptens) are chemically bound to high molecular - weight (carriers) such as proteins carrier to produce specific antisera (Kovalev *et al.*, 1985) . In 1986 study by Japanese researchers was a successful attempt that used colloidal gold particles as a carrier to glutamic acid at producing antibodies (Shiosaka *et*

al., 1986). It noticed that when produce specific antisera contain accompany antibodies to antigenic structures who used as Carrier (kovalev *et al.* , 1985).These methods were developed and applied to produce antibodies to the following rigorous antigens and haptens (Dykman *et al.*, 2010) . In 1993 it suggested that when production of antibodies hapten be bound to the carrier protein before conjugated with colloid gold, it obtained high specificity antibody (Pow & Crook,1993) .

In 1996 colloidal gold was used in vaccine of the antiviral as carriers of antigen of the tick - borne encephalitis virus (Demenev *et al.*, 1996). Despite the vaccine did not contain adjuvants but the experimental vaccine that used colloidal gold had better protective properties.

2.1-1.8 Bio-distribution and Toxicity of Gold Nano Particles:

More from the acute question concerning Toxicity and bio-distribution of Au NPs took place during the Past years (Bioisselier & Astruc , 2009). The main target for the accumulation Au NP of the organs the reticular endothelial system , need 3-4 months to be excreted from the liver and Spleen (Dykman & Khlebtsov , 2011) .Common mechanism of Au NPs is oxidative stress induced toxicity which activates NF - KB signaling of pro-inflammatory genes as TNF and IL - 1 , IL - 6 , IL - 8 result in apoptosis and DNA damage (Khanna *et al.*, 2015) .

2.1-1.9 Toxicity Effect of Au Nps on Male Reproductive System:

The male reproductive system in human subject for exogenous material causes for its deterioration (Guz *et al.*, 2013). Gold nanoparticles induced ROS can cause damage in sperm cells and impaired fertility may result in congenital defects in offspring (Van Gelder *et al.*, 2010). AuNPs (

10-30 nm) repeated intraperitoneal injection result increased number of abnormal spermatozoa and Au NPs penetrate the BTB, and accumulation in testicular tissue (Nazar *et al.*, 2016). Au NPs may impair testicular function (Velikorodnaya *et al.* , 2015). Potential health and environmental risk due Au Nps present with the widespread use in biomedical fields necessary known their fate in vivo and toxicology effects especially on male reproductive system (Liu *et al.*, 2021). After incubation of human sperm with Au Nps the internalization inside the nucleus of human sperm compared to other cells ,the humane sperm nucleus is relatively complex , relating not only to physicochemical properties of Au NPs but also to the state of sperm membrane (Acrosome reaction) ,and may alter as soon as particles reach the sperm fluidity and composition (Moretti *et al.*, 2013).

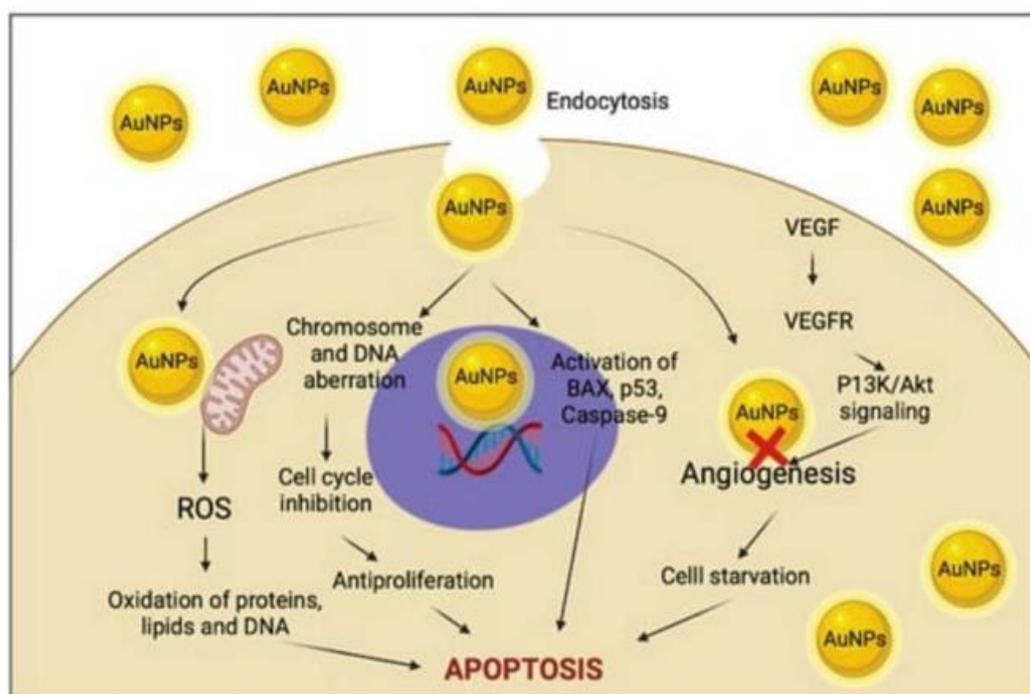
2.2-1.10 Genotoxic Effect of Au NPs :

AuNPs interact directly or indirectly with DNA that cause DNA break , oxidative DNA , Point mutation (Asharani *et al.*, 2009 ; Foldjerg *et al.*, 2011) , in direct interaction by time and concentration dependent result of DNA damage and cytotoxicity against TM3 cell (leydig cell).

2.1-1.11 Ros production:

Au NPs not induced apoptosis in TM3 but enhanced ROS production - the intercellular lead to DNA damage and apoptosis.as shown in the figure(2-2) (Soto *et al.*,2021). Therefore the production of ROS is the main cause of toxicity , but unstable ROS molecular do not travel from their site (Wells *et al.*, 2005) .Which induced inflammation , leydig cell responsible on production and secretion hormone testosterone , Au NPs treatment is reduced from production the TM3 cell of testosterone and significant reduced plasma testosterone level because repeated dose of Au Nps of intraperitoneal injection (Behnammorshedi *et al.*, 2015).Au NPs treatment

induced testosterone production inhibition are analyzed both TM3 and testicular tissue lead to reduce the expression of 17 α - hydroxylase , which inhibit the conversion to Pregnenolone to dehydroepiandrosterone (DHEA) and the end reduce the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) it should regulated by the hypothalamic pituitary gland (Zuber *et al.*, 1986 ; Behnammorshedi *et al.*, 2015).BTB blood testicular barrier that located between seminiferous tubules and blood vessels form tight junction in the animal testis between sertoli cell prevent cytotoxic agents inter seminiferous (Sharma *et al.*, 2018), but NPs exposure expanding the size of BTB gap induce inflammatory and promote cytokine secretion (Park *et al.*, 2010). Testosterone hormone can support integrity of BTB (Meng *et al.*, 2005) . Se Nps (Selenium nanoparticle) with antioxidant prevent lipid peroxidation and protect reproductive system . Therefore the potential negative impact of Au NPs on male reproductive function should be carefully in their biomedical application.

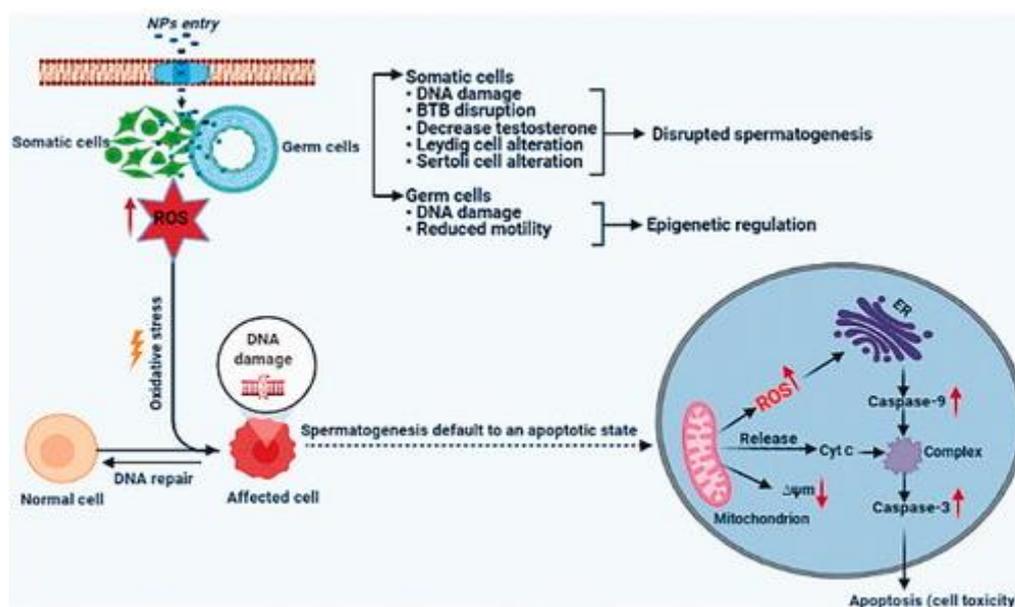


Figure(2-2)representation mechanism of gold nanoparticles(Soto *et al.*,2021) explain ROS and apoptosis s formation .

2.1-2 Mechanisms of Nanoparticles Implicated in Somatic Cell Toxicity and Male Germ Cell Toxicity:

Nano Particles enter the reproductive system by different methods of drug delivery. when penetration is used in many medicine applications it may cause induce DNA damage and negative effects on sperm motility and morphology (Taylor *et al.*,2012), dermatological therapy and skin care (Niska *et al.*, 2018). When exposure to 7 or 28 day it declines HL and FSH hormone with histological abnormalities , and reduces motility and number sperm in the testicles of male rat (Olugbodi *et al.*,2020) .Enter by absorbed in gastrointestinal (Hansson , 2012) oral administration reduce number of sperm in male rats (Sleiman *et al.*, 2013). Entering the human in respiratory tract (lung) induce inflammation , necrosis generation of ROS is resulted(Del Vento *et al.*, 2019).The important potential adverse health effect of NPs to produce ROS , molecules and free radicals are the main pathway for toxicity (Wells *et al.*, 2005), and there is an imbalance between oxidants and antioxidant .The Particles traveling end up in the reproductive system throughout the body and enter the circulation systemic causes inflammation (Erdely *et al.*, 2011). Further inflammation by receptors (TLRS) contributes to the inflammatory reaction brought on by exposure to NPs (Koga *et al.* , 2014) .Recent researches the toxicity of NPS caused inflammatory response and generation of ROS in male reproductive system . Induced more toxicity for NPs by inhibition of antioxidant (defense systems) and increased ROS generation . These effects depend on concentration increasing and duration of exposure inducing DNA damage with apoptosis of germ cell (yan *et al.*, 2016) .Nps pass through the gonads ‘ outer membranes stimulate the damage antioxidant result generation of free radicals and disrupting metabolism of cells (Perez - Labrada *et al.*,

2019).Whereas,AuNPs negatively affect somatic cells and germ cells simultaneously as shown in following figre(2-3) (Habas *et al.*,2021).



Figure(2-3) Schematic of Mechanism and Potential Biological Effects of NPs on the Reproductive System of Male, the Cytotoxic Effect of NPs on Supporting and Germ Cell(Habas *et al.*,2021)

2.1-3 NPS induced DNA damage:

NPs cause oxidative damage to DNA on the 2-deoxyribose and on DNA bases, DNA back bone result single strand break ,and oxidized DNA bases (Carrière *et al.*, 2017) . Inducing genotoxic effects when used NPS average size 40-70 nm on human sperm (Gopalan *et al.*, 2009) . DNA damage is in spermatocytes because increased ROS production that result from NPs are a dose and time dependent (liu *et al.*, 2016) .

2.1-4 NPs Induced Oxidative Stress:

The male reproductive system in human susceptible has many to exogenous material (Knez ,2013). Oxidative stress effects reducing the thickness of spermatogenic epithelium and effect after reach to target on the quantity and quality of Sperm (Bai *et al.*, 2010).

The most vital causes of male infertility and deterioration is the reproductive system because oxidative stress (Hussien *et al.*, 2016). Oxidative stress is mitochondrial apoptosis by NPs comprises mitochondrial respiration and depletion of antioxidant enzymes (Manke *et al.*, 2013). Daily oral exposure decreases mitochondrial function and damage the plasma membrane and acrosome of sperm (Mathias *et al.*, 2015). NPs induce toxicity causing oxidative stress , damage DNA and apoptosis of cells (Khanna *et al.*, 2015) .Industrial Au cause the potential spermatotoxicity as well as epididymitis and male sterility (Manin *et al.*, 2007) .NPs induce cell apoptosis and necrosis because they induce damaged mitochondrial and membrane function (Braydich - Stolle *et al.*, 2005). ROS formation leads to nuclear DNA damage and results apoptosis and necrosis in sertoli and leydig cell. Testicular tissue induce apoptosis and autophagy because exposure Nps by cleaved caspas 8, cleaved caspas 3and increases in gene and protein (Shen *et al.*, 2019).

2.1-5 NPS disrupted hormones :

In Studies there are endocrine disruption chemicals (EDCs) effect on essential hormones of the male reproductive system , and cause a number of damaging such as testicular cancer , poor semen quality , prostate diseases , and high number and low number abnormal Sperm (Knez, 2013) .The number leydig cell and sperm parameter indices have interruption in function of sex hormones (Baki *et al.*, 2014). NPS in adult male rat show

significant differences in LH , FSH , and testosterone h. (Negahdary *et al.*, 2015). When exposure of NPS can cause alteration or modulation in the cell , in the cell processes involving signaling ,NPs act as EDCS, cause hormonal disorder , effect on fertility and EDCS disrupt the bodies (Dagar and Bagchi , 2020) .Au NPS decrease testosterone production in leydig cell when accumulate in testes by decrease the expression 17 - α hydroxylase, and NPs can diminish an influencing sperm quality (Liu *et al.* , 2020).

2.2 Anatomy of Male Rat Reproductive System :

The reproductive System in male rats Consists of a pair of gonads , the testes which are mixed glands ,and these gland are characterized by their two main functions . The first one is the production of sperm from seminiferous tubules in this case the testicle is an exocrine gland, and the second function includes the production of steroid hormones from the interstitial cells of the testicle , which are Known leydig cells , and are located between the tubules that make the sperm and these cells under the influence of hormonal control coming from the pituitary gland .The secretes androgen which is essential and important in males is called the testosterone hormone in which case the testicle is an endocrine gland (Osman and Ploen , 1986). Each testicle consists of a head extremity that connects to the head of the epididymis and a Tail extremity it matches to the tail of the epididymis , and each testicle includes an epididymis border. This edge is weakly connected with the body of the epididymis consisting of the epididymis sinus and free border . It is located in the opposite side of the epididymis border and it is usually convex . The testes contain two surface , the medial nearly concave surface , and lateral convex surface . There are differences in the size , weights and shapes of the testicles depending on the type of animal .The testes of rodents are large and disproportionate to the size of the body (التميمي , ٢٠٠٣) . Either the male sheep , goats and pigs are be relatively large , but be a small testes in

dogs , cats and camel compared with animal size . The male reproductive system consists of two testes and genital channels transfer of sperm (efferent ductules which includes vas deferens , epididymis , ejaculatory duct and Penis , either the additional gland extension of the male reproductive are prostate , seminal vesicles and cowpers glands(Wilson,1979)

2.2-1 Testes:

It is an oval - shaped gland ,Mixed glandular installation with external and internal secretion. The left testes is larger and more effective because to the increased amount of blood in it. and also located at a level below the right testicle and during embryonic development the testicle grows in the abdominal cavity and then descends before or after birth in a little while in scrotal sac . It is a dermal cyst that produces external dent of skin and shaped like an external pocket of the body wall which is divided into two parts by a superficial connective tissue called (superficial Raphe) (الحسني والهيبي, ١٩٩٠). The scrotum consists of several muscle layer smooth shrinks in the cold and flatters in the heat , the function of the scrotum is to preserve the testicles at a temperature lower than body temperature one to one and a half degrees lower than body temperature (34-35) C°. The reason for this is that the process of sperm production in the testicles cannot stay for long time at body temperature and the low temperature of the scrotum is maintained by the special muscles that extend between the body and the scrotum sac . When the temperature go down these muscles contract and bring the testicles closer to the body (Berne *et al.*, 1998) , the testicle is surrounded by a serous membrane on all of its anterior and lateral surfaces except for the posterior surface called Tunica Vaginalis. This tunica consists of inner visceral layer and outer partietal layer, under this tunic the testicle is surrounded by a sheath or capsule of dense connective tissue that includes Some smooth muscle fibers called the tunica albuginea , which thickens the

posterior surface of the testicles to form an inner layer of loose connective tissue rich in blood vessels from vascular testicle cover, the structure that lies under the tunica albuginea is called Tunica Vasculosa and from the thickened posterior part of the tunica albuginea, fibrous septa extend inside the testicle dividing to (250-300) cubby or pyramidal lounge, which are called testicular lobules, it contains each lobule of (2-5) many of tubules twisted to each other abundantly are called seminiferous tubules (الحسيني, ٢٠٠٤). It produces sperm and the process of producing sperm is called spermatogenesis (Hickman *et al.*., 1993).

2.2-1.1 Histological Structure of the testicle :

The testes Consists of two main parts : -

1. Seminiferous tubules.
2. Interstitial tissue .

first : seminiferous tubules :

The Seminal ducts, are located between the lobes, the number of which reaches 840 tubule the length 30-70 cm and diameter 0.2 mm and each tubule loses torsion at the apex of the lobule and becomes a straight tubule these tubules are scattered in the primary connective tissue or the stromal which is rich in blood vessels and nerves. The spermatic tubule is either mouthful with an adjacent tubule or with blocked end. the end of each of collecting tubes opens into testis Rete which is located close to the head of the epididymis (Athur *et al.*, 1996). The seminal tubule is lined with seminiferous epithelium and is called Germinal epithelium which is a stratified epithelial tissue made up of cubic or columnar epithelial cell and this tissue rests on a thin basal plate covered from the outside by a sheath of fibrous tissue called the determinant tissue. It contains many connective tissue cells, fibers and some smooth muscle fibers. It is believed that the

contraction of smooth muscle cell can lead to a change in the diameter of convoluted tubules , which helps in the movement of sperm along the convoluted tubule . (Kalthoff , 1996) .The convoluted tubules occupy approximately 75 % of volume of the testes and these tubules are lined internally by a layered epithelium consisting of two types of cells(Dekretser , 2002) :

- Sertoli Cells
- Spermatogenic cell

Sertoli Cell :

It is called by the supporting cells which are large cells , long , pyramidal to columnar, irregular in shape and they are found along the spermatic tubule between the generative sex cell .

Its broad base rests on the basement membrane perpendicularly and its funnel edge is open at the lumen of seminiferous tubule (Pineda & Dooly , 2003) . The cell includes an oval nucleus that is few distances above the base of the cell and has one or more large and clear nucleoli . It is located towards the base of the nucleus and the cytoplasm contains a smooth and rough endoplasmic reticulum and mitochondrion and many fat droplets , the Golgi apparatus , and glycogen , as well as the cytoplasm of sertoli cells in human includes crystalloids function unknown . The lateral rims of the cell also include tubules and intermediate filaments , which are believed to contribute to the movement of cells from the basal region to the adjacent region for the lumen of the Seminiferous tubule , and its side walls are united by tight links. Its function is to prevent the passages of large particles from interstitial space into the seminiferous tubules , Sertoli cells perform multiple functions as they contribute to the regulation of Spermatogenesis and its development and continuity by preparing the formed Sperm with the necessary nutrients,

support and Protection , and devouring the diseased decomposing sperms. It also allows the passage of Some materials necessary for the formation of Sperm through the formation of a special compound that regulates the secretion of testosterone and the return of Some harmful substances that hinder the development of sperm . Thus, preparing the blood - testicular barrier, which prevents the formation of antibodies to new Sperm cells (Johnston *et al.*, 2004) .

Sertoli cells also secrete the androgen - binding protein (ABP) under the control of testosterone and FSH which participates in the regulation of spermatogenesis by transporting and concentrating male androgens around the germ cells and the concentration of testosterone in the spermatic tubule . (Chaudhary *et al.*, 2004).As shown in the following figure

(2-4)(Fraser *et al.*,2021).

Spermatogonia :

It is an epithelial layer that compose the biggest part of the seminal epithelial tissue and the cells are arranged in multiple rows (4-8) from cells lining the seminiferous tubule . They are arranged in the form central layers of different ages , starting from the basal region of the tube to its lumen , and when they multiply , grow and specialize they rush towards the cavity and turn into sperm to separate from the epithelial tissue and become free in the cavity (white , 1976) .The types of cells that create sperm from the basement membrane of the tubule to the inside include the following(Hussien *et al.*,1997) :

1. Spermatogonia .
2. primary spermatocytes.
- 6- Secondary Spermatocytes.
- 7- Spermatides.

8- Immature Spermatozoa.

9- Mature Spermatozoa

Interstitial tissue :

It is a tissue that located with-in the lobules and between the seminiferous tubules . It consists of loose connective tissue rich in blood and lymphatic vessels, nerves , and endocrine cells called leydig cells .They are large , round or polygonal cells that are found either singly or in groups in the interstitial tissue between the seminiferous tubules so they are also Called interstitial cells (Payne *et al.*, 1996). Its nucleus is oval or spherical containing pigment granules and a single nucleolus the cells contain of two nuclei is a common case while the cytoplasm contains the smooth endoplasmic reticulum , which manufactures hormones (Hooker , 1970) .

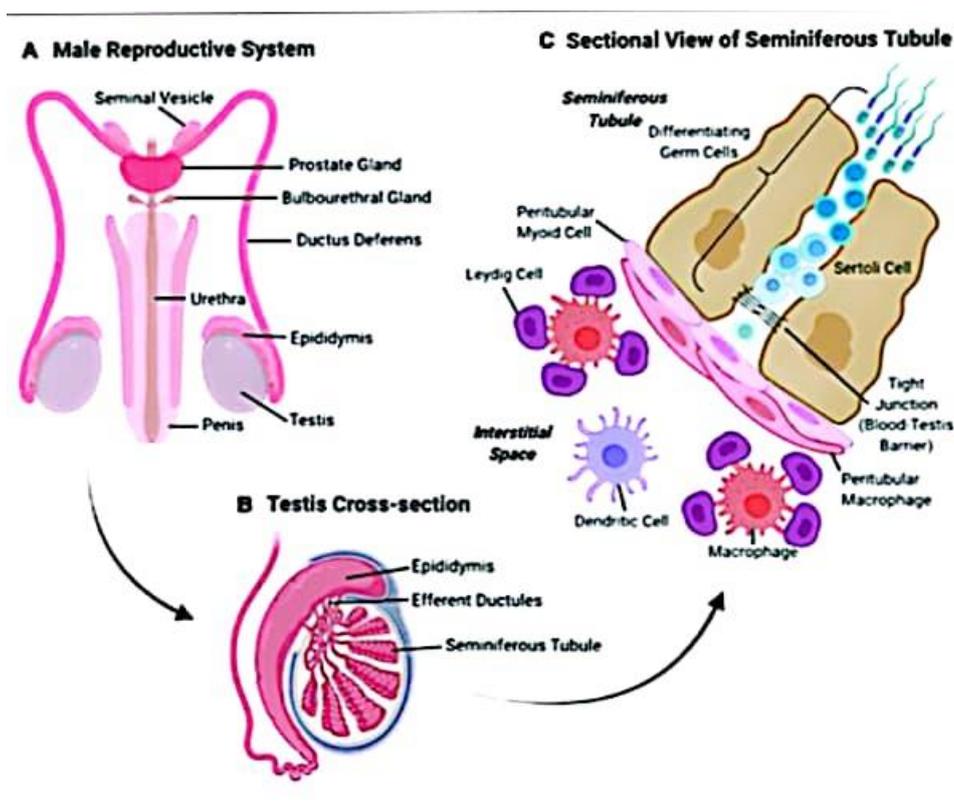
2.2-2 Epididymis:

Cummins *et al.*, (1986) defined the epididymis as , it is long often torsional tube approximately 7M and lind with columnar cells and a ciliated pseudo – layer resting on a basement membrane , consists of a loose connective tissue rich in blood vessels and smooth muscle fibers , and the epididymis is located with the testicle within the scrotal cavity.

It is attached to edge of the testicle from behind by a connective tissue composed of narrow efferent ducts through which sperms move from the testicle to the Vas deferens . The epididymis consists of three regions , the first of which is the head of the epididymis caput and represents its enlarged front end close to the testicle , the head of epididymis is connected to testicle by efferent ducts . Their number ranges from 13-23 coiled helical cannulas surrounded by connective tissue and the length of each cannula is 6-8 cm has a diameter 0.05 mm . These ducts are irregularly undulating separated ,

and have a smooth regular surface , they are lined with simple epithelium consisting of columnar ciliated cells and some cuboidal non- ciliated secretory cells which rest on the basement membrane and frequently exchange groups of long columnar cells with other groups of short cells due to the different heights of the epithelial tissue cells . The lining of the long cells contain the cytoplasm which has lipid droplets and pigment granules these cells contain cilia whose function is to transport sperm through the canals .For the short cells the cytoplasm contains lysosomes and micro villi located on its free surface , they are also absorbing cells its function is because it absorbs a large proportion of the secreted fluid in the Seminiferous tubules and their lining becomes columnar . It is only at the end of the efferent canals and its lumen is flat , the cannulas twist on each other to form the head of the epididymis then they unite with each other to form a large canal called the epididymis ducts which extends from the head of the epididymis to the body of the epididymis which is a narrow middle region that ends in the tail of the epididymis called Cauda it is located at the bottom and connects with the vas deferens and occurs in the head and body region the process of sperm maturation. As for the tail of the epididymis , it is the main region for Sperms to acquire the ability to move and fertilize (كاظم, ٢٠٠٦) .

also indicated(العزب , ٢٠٠٨) that storing and preserving live sperm is one of the most important functions of the epididymis as it contains some nutrients necessary for its activity. In addition, the development and maturation of sperms and their transfer takes place inside the epididymis , as the sperms gain the ability to move when they pass through the epididymis canal.



Figure(2-4)(A)Overview of Human Male Reproductive System. (B)Schematic Diagram of the Testes is Showing the Epididymis and Seminiferous Tubes of cross-section.(C)Sectional View of Cells Forming the Seminiferous Tubules and Surrounding Interstitium(Fraser *et al.*,2021).

2.2-3 Prostate :

The prostate is a small walnut – like, gland located below the urinary bladder surrounding the posterior upper urethra. The part of urethra passing through it. Consists four lobes anterior , dorsal, ventral and lateral lobes (Latayia Aaron *et al.*2016) ,surrounded by a network of veins called prostatic cluster (Villers ,1994).Prostate consists of two parts the first glandular located inside the other is fibrous muscular tissue ,the prostate is made up of three glandular regions the central zone surrounding the ejaculatory duct ,the transitional zone surrounding the urethra ,peripheral zone surrounds both of them(Mc Neal,2008).It participates in formation of semen containing

nutrients that give sperm the ability to motility and fertilize. hyperplasia prostate occurs in the transitional zone while cancer is happening in peripheral zone, prostate contains three unique types of epithelial cell basal, luminal, and endocrine cells(Hudson *et al.*,2001).

2.2-4 Seminal vesicle:

Seminal vesicles are a pair of glands sited behind the bladder, lateral to the vas deferens in front of your rectum., and in linking with the base of the prostate, Function of Seminal vesicles release up 60% from the fluid set up in semen(Tjionas *et al.*, 2015).The histological structure of the seminal vesicles is lining with columnar epithelium and smooth muscle that from mucosal folds, composed three layer of pseudostratified columnar epithelial include goblet cells and lamina propria, layer of muscular outer longitudinal inner circular from smooth muscle and finally layer adventitial loose connective tissue(Zang *et al.*,2018).

The vesicles may also be called seminal glands or vesicular glands. The seminal vesicles are accessory glands of the male reproductive system. They are a pair of contorted or twisted tubes, which are located between the bladder and the rectum(Mikuz ,2019). The vesicle secretion Alkaline fluid that survival of spermatozoa, Fructose too facilitate survival of sperm, Prostaglandins have role suppressing the immune response of semen in female(Druart and Graaf ,2018).One of substances contained in vesicular secretion is the clotting factors that maintain semen in the female reproductive tracts (wang ,2018).

2.3- Seminal Fluid:

The prostate and seminal vesicles are responsible for producing most of the semen. The latter is defined as the fluid that work to prepare the food needed for the sperm's metabolism and movement while it forms %60 from the volume of semen , which is secreted from the seminal vesicle and the ratio is 13- 33% the semen has a milky appearance from the prostate gland. The secretion contains citric acid , calcium , acid phosphate and proteolytic enzyme the latter is responsible for dissolving semen and also secretes cholesterol whose function is to protect the sperm from environmental shocks as for the lowest percentage of secreted fluids it is from the epididymis and cowper gland at a rate of 5 % (عبد الطيف والبازي, ٢٠٠٥). One of the most important qualitative measures of semen characteristics is the movement of sperms , as it is a good indicator that positively correlates with the percentage of normal live sperms, and negatively with the percentage of dead and deformed sperms , and this movement is evidence of the vitality of the sperms and their ability to penetrate the barriers that they encounter and intercept in the reproductive system for the purpose of reaching the fertilization site (Saake , 1982). Sperm can live in the ducts for several weeks , but after ejaculation their maximum life span is 72 hours . If it is frozen at (100-) degrees celsius ,it will remain for one year , one of the important characteristics of good quality semen , which gives a higher percentage of pregnancy , is the one in which the high percentage of live sperms with forward movement .

2.4 Spermatogenesis :

This process is conducted in seminiferous tubules , which leads to mature sperm production spermatozoa , and begin during the duration of puberty and continues throughout male life and include the process of spermatogenesis involves a complex series of specialized cell divisions during which entire number of chromosomes is reduced Diploid ($2n$) across of animal type to half Haploid ($1n$) .If the sex cells develop and go through various stages transformation then leads to the production of sperm (Hickman *et al* . , 1993) .This process includes two stages , the first stage is the stage of mitotic and meiosis divisions and the doubling of the number of cells while the second stage is

spermiogenesis (المختار والراوي, ٢٠٠٠). The process of spermatogenesis begins with the division of germ cells to form spermatozoa.

- A. Type A spermatogonia : Most of which are divided to form intermediate , Spermatogonia. and most of them are divided to form
- B. Type B spermatogonia. that undergo last mitosis to form primary Spermatocytes , they contain the full number of chromosomes and then these cells move away from the basement membrane and increase in size and prepare to enter the stage of meiosis the mediastinum of the chromosomes which form secondary spermatocytes and then spermatids which undergoes a series of morphological changes to transform them into sperm a process that called (cellular specialization) of the sperm spermiogenesis (Ganong , 2010). The process of spermatogenesis pass through a series of stages and this causes the different arrangement of the spermatogonia, Primary and secondary spermatocytes , spermatids and spermatozoa from across section in the seminiferous tubules to another for this reason absorbed a series of interconnected cells arranged in a

certain way along the seminiferous tubule , and the sequential changes in all stages along the length of the seminiferous tubules between two types of cells are called cycle of the seminiferous epithelium . As for the total changes in the cells that start from the activation of the germ cells or sperm progenitors until the release of the sperm into the lumen of the seminal tubule they are called spermatogenic cycle which takes 30 days in rats (Beamer *et al.* , 1983 ; Guyton & Hall , 2006) .

2.5 Mature Sperm:

A mature human sperm consists of a head, a middle piece and tail (الحسيني والهيتي, ١٩٩٠). The head consists of the nucleus and the vertical cap containing the terminal body at its front edge and the bulk of the head occupies the nucleus, which in turn contains the genetic material DNA . There is a belief that the function of the terminal body is to form a substance of an enzyme nature called sperm lysins in mammals. This enzyme is called (hyaluronidase) as these enzymes degrade the egg membranes in the region where the sperm meets the egg to facilitate the passage of the sperm to the surface of the egg as for the middle piece separated from the head . By narrow neck , it contains an axis of longitudinal tubules forming what is called a (axial filament complex) surrounded by nine thick fibers and surrounded by a sheath of mitochondria . It is believed that the midsection controls the movement of the tail (المختار والراوي, ٢٠٠٠).

2.6 Hormones Control the process of Spermatogenesis:

The process of activating the male reproductive system is involved in three endocrine glands , which are the hypothalamus which secretes a hormone gonadotropin - releasing hormone (GnRH), pituitary gland and testis, which secretes testosterone and inhibin hormone . The overlap among the functioning of these glands is called the hypothalamic - pituitary - testis

axis who is in habited before Puberty (Kuiri - Hanninen *ét al.*, 2014) . The hypothalamus is activated upon reaching puberty and secretes a hormone GnRH which is less than ten minutes old and then destroyed by enzymes found in the cells of the pituitary gland then it travels through the hypothalamic - pituitay – portal system by blood vessels it is affects the cells of the anterior part of the pituitary ,which in turn respond to secretion of stimulating hormones FSH , LH (Popa *et al.*, 2008 ; Clasadonte and Prevot , 2018) .FSH travels in the blood to the seminiferous tubules and binds to receptors on sertoli cells urging them to secrete androgen - binding protein to male hormones ABP which binds testosterone leading to an increase in its concentration on the surfaces of sertoli cells . Thus contributing to the maturation and differentiation of sperm is one of the basic and most important functions of the testicle (Allan *et al .*, 2010 ; Lindgren *et al.*, 2012). As for the LH hormone , it travels in the blood to the testicles to affect the leydig cells , which are located between the seminiferous tubules to build and secrete the Testosterone which is important in the formation, growth and development of sperm. That's a working mechanism axis hypothalamic - pituitary- testis axis regulated by negative feedback as shown in figure(2-5)(Tornoe *et al.*, 2007) ,the increase in testosterone inhibits the formation of the (GnRH) hormone.

FSH is inhibited by a hormone Inhibin which is secreted by sertoli cells inside the seminiferous tubules , and when the number of sperm is increased, this hormone is transmitted through blood to affect the pituitary and inhibits the secretion of FSH by the negative feedback process . (Mclachlan *et al.*, 2002 ; Mcneilly *et al. ,* 2003). It should be noted that sexual stimuli send nerve signals through the sacral parasympathetic nerves (the second , third and fourth vertebrae of the spinal cord) which affect the smooth muscles in the arteries (pudendal) the internal nutrients of the penis

lead to the secretion of nitrogen oxide, which causes relaxation in the muscles of the arteries and their widening and increase the flow of blood from them towards the cavernous bodies where they became engorged with blood , which lead to an erection (العبد الله, ٢٠١٢).

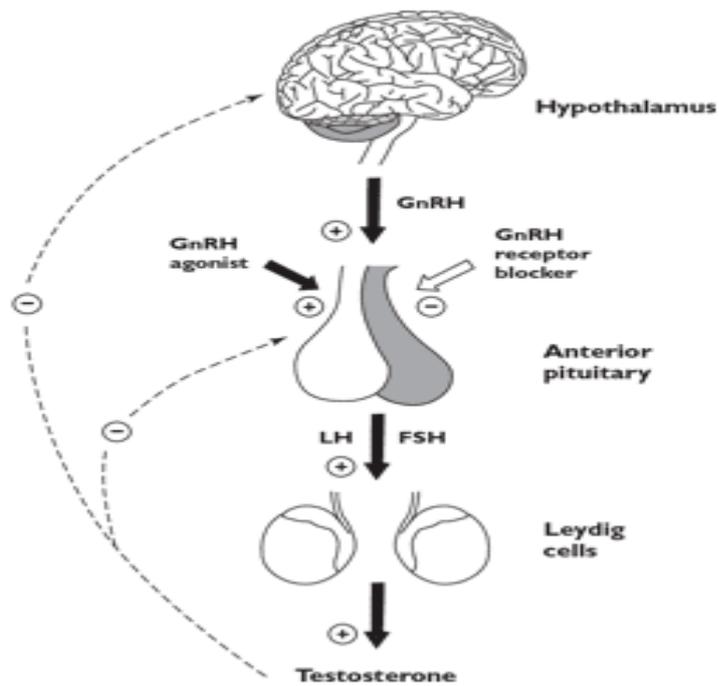


Figure (2-5) showing the mechanism action of the of hypothalamic-pituitary-testicular axis(Tornoe *et al.*,2007)

Chapter Three

Materials and Methods

3- Material and Methods

3.1 Materials :

Chemical materials, kits, tools, and equipment used in present study with their suppliers are listed in tables(3.1 and 3.2).

Table (3-1) List of chemicals, reagents and their suppliers.

Chemical material, Kits	Supplier
Chloroform	SDFCL ,India
D.P.X.	SDFCL ,India
Distilled water	AL-joud company, Iraq
Eosin	Fulka, Germany
Ethanol	BDH,UK
Formaldehyde	Sigma ,USA
Gold nanoparticles (Au NPs)	VCN Materials , Iran
Hematoxyline	Fulka, Germany
Kit measurement of Total antioxidant	Elabscience , Biotechnology China
Kits to measurement of Hormone T,FSH, LH,E	Elabscience, Biotechnology China
Methanol	LAB-SCAN, Ireland
Normal physiological salin 0.9%	SDI, Iraq
Paraffin wax	Merek, Germany
Xylene	Alfasan, Woerden. Hlland

Table (3-2) The an equipment and tools used in study with supplier

Equipment, Tools	Supplier
Centrifuge	Hermlse, Germany
Disposable Insulin Syringes	Shan chuan ,China
Disposable Syringes	Shan chuan ,China
Dissecting Set	Pakistan
Electronic Balance	Germany
Elisa device	Organ Teknika,Beelchum
Eppendrof tube	China
Gel Tube	Asco, Jordon
Hot plate	Gallen-Kamp ,UK
Incubator	Memmert Germany
Light Microscope	Olympus,Japan
Micro pipette	Germany
Rotary Microtome	Duran Germany
Sensitive Scale	Memmert Germany
Slides	Shenzhen BDJK Technology Industry ,China
Slides Cover	Shenzhen BDJK Technology Industry ,China
Vortex	China
Water path	Histoline,Italy

3.2 Methods

3.2-1 Animals :

In this study adult white male laboratory rats were used (*Rattus norvegicus*) and aged 10-12 week, with 200- 230g weight. The number was 42 rats that were raised in the animal house of the College of Science University of Babylon. The animals remained in animal house for acclimatization Then divided to five groups each group was placed in the floor Covered cage with sawdust as Plastic Cages covered with metal lid its dimensions (20 x 20 x 40) cm, taking care of cleanliness of cages, irrigation bottles, and shelter room from appropriate laboratory conditions, such as the appropriate temperature range 20-24C°, and light 12 hours dark 12 hours . All of the procedures were carried out with minimal stress . The animals were provided with water and standard diet.

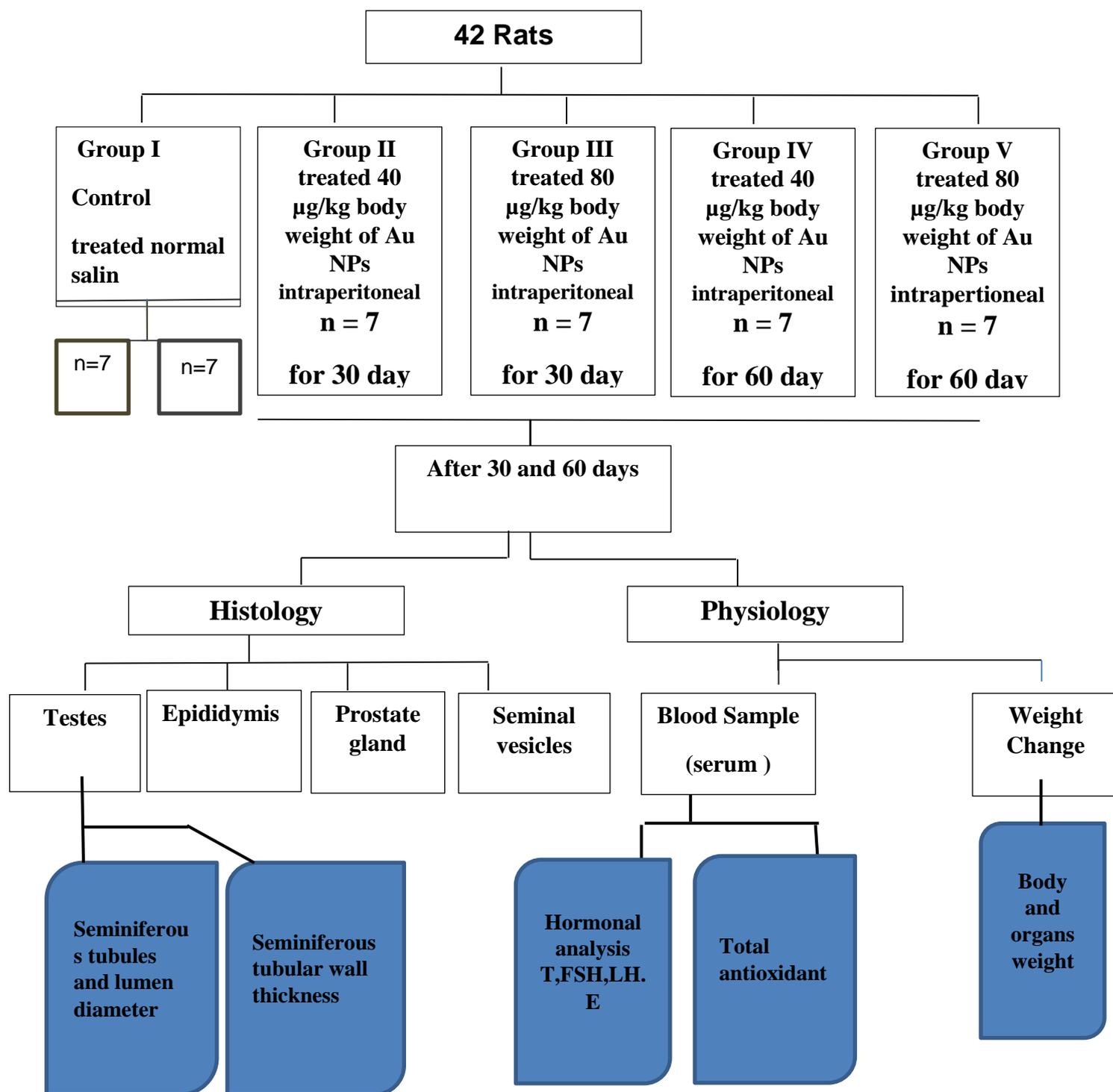
3.3 Experimental Groups:

Five groupings were created from the animals

- 1- Groups 1 : Control animals divide into two subgroups, contains 7 rats injected intraperitoneally with normal physiological saline for 30 and 60 days.
- 2- Group II: includes 7 rats injected intraperitoneal with 40 µg/Kg body weight of Au Nps diluted in(D. W.) for 30 days
- 3- Group III includes 7 rats injected intraperitoneal with 80 µg / Kg body weight of Au Nps diluted in(D. W.) for 30 days .
- 4- Group IV: includes 7 rats injected intraperitoneal with 40 µg/kg body weight of Au NPs diluted in (D. W.) for 60 days.
- 5- Group V: includes 7rats injected intraperitoneal with 80 µg /kg body weight of Au NPs diluted in(D. W.) for .60 days

After 30 days half animals were sacrificed after anesthetized with chloroform of injection with Au gold nano particles solution intraperitoneal. Continue dosing for another 60 day to study the accumulation in reproductive system for the rest of the animals. Figure (3-1)

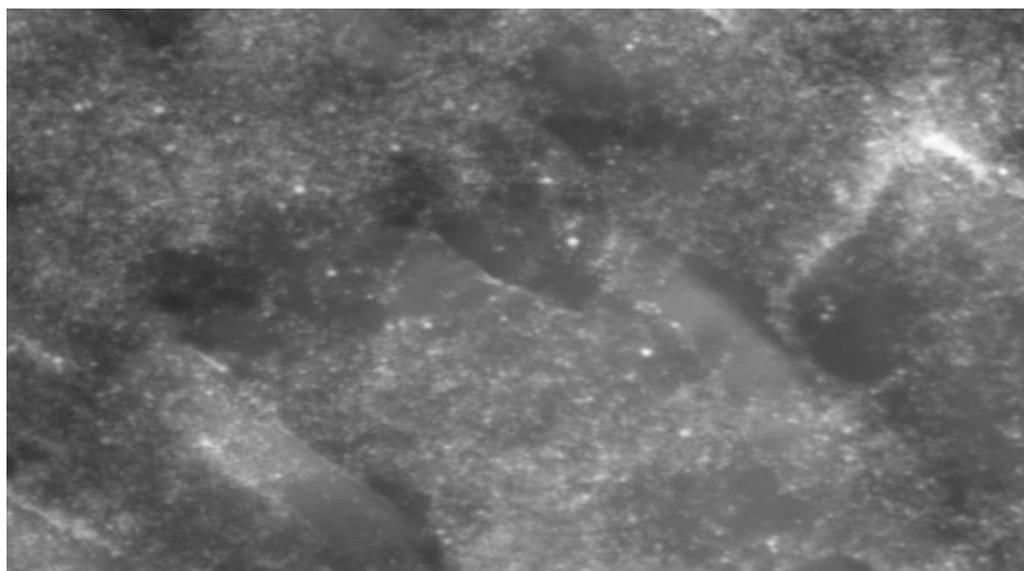
3.3-1 Experimental Design :



3.4 Gold Nano Particles (Au Nps) Solution Preparation :

Au NPs that used in the current study were obtained from (Iran), Au NPs have characteristic

- Appearance : Color Red Solution
- weight Concentration : 100 ppm
- additives: Au
- morphology: Spherical
- Size range : 5-20 nm
- Product Number : VCN 4021 W
- Iran Address: Science and Technology Incubator persian gulf. university intersection mahini street.
- Malaysia Address: B-12-10, Scott Garden, Klang Lama, Kuala Lumpur, Malaysia . it was examined with a Scanning electron Microscope, figure (3-2) Shows gold nanoparticles prepared dose (40- 80) $\mu\text{g}/\text{kg}$ by dilute it in D.W. (weight of Au NPs was determined based on the weight of the animal).



Figure(3-2)Show Gold Nanoparticles by (scanning Electromicroscope)

3.5 Blood Collection:

The animals were anesthetized using a cotton swab containing the anesthetic substance chloroform in a large box containing the rat by breathing, blood sample were obtained from animals by using 5ml syringe from the heart by stabbing the heart to get the biggest amount of blood. then using gel tube was placed in centrifuge (3000 RPM for 5 min to separate the serum and kept them in eppendorf tubes and them stored in refrigerator at a temperature (-20°) to complete the chemical testes a measuring hormone of T, LH, FSH,E and (T.A.O.).

3.6 Collection of Organs:

The ventral cavity of the animal is opened by a scalpel and scissors after anesthesia, then organs (testis, epididymis, prostate and seminal vesicle) were left out and weighed and placed in petri dish contain normal saline for washing then dried by filter paper and saved in formalin at a concentration of 10% .

3.7 Measuring the Weight Changes:

Using electronic balance, the weights of animals were measured before the start of experiment, then average for weight were taken after end the experiment to know changes that obtained from the beginning the experiment to end the experiment of each group and the rest, organs which removed also their weight was taken.

3.8 Measurement of hormone levels:

Serum hormone concentrations has been measured by using a device ELISA reader (Enzyme linked Immune Sorbent Assay), which is a special Kit made by China ,Elabscience company . Hormones level of serum were

measured and the absorbance was read at a wavelength of 450 nm. The hormonal tests were carried out in physiology laboratories of the university of Babylon .For assessment of T ,FSH ,LH,E level.

3.9 ELISA Working Principle:

(ELISA)

(Enzyme linked Immuno Sorbent assay)

- There are two type of reactions:
 - 1- Immunological Reaction (Antibody (Ab) - Antigen (Ag))
 - 2- Chemical Reaction (Enzyme (E))

Three main Steps:

- 1- Specimen (Anglyte).
- 2- Conjugate (enzyme. lobled Ab or (enzyme lobled Ag)
- 3- Substrate (chromogen)

These steps include Sub- steps, except for the last step which contains a stopping solution instead of wash, Solution :

- a- Incubation.
- b- washing

- Type of ELISA

Non-Competitive (conjugate take from animals)

- 1- Direct
2. Indirect
3. Sandwish

Competitive (conjugate take from human)

3.9-1 Estimation testosterone hormone:

Concentration of the hormone was measured according to the way (Tietz , 1995).

- The appropriate number of wells was provided above its own Stand, which is equipped with the hormone.
- An amount of 20 μ L of each of the serum and standard substance was taken and then placed in the wells Prepared for it.
- Added 50 μ L from Testosterone -HRP (Horse rich Peroxidase) Reagent to each well.
- Added 50 μ L from Rat anti testosterone Reagent rat extract for each well mixed the contents of the well thoroughly for 20-30 Seconds. Then the plate was a incubated at temperature of 37C° for 60 min
- The Mixture was poured from wells then washed by wash buffer 5 times, in interrupted form .
- Added 100 μ L from (Tetramethyl benzidine) TMB (Substrate Solution) for each well..
- Wells were placed in a dark, covered incubator for 20 minutes between 18 and 28 degrees Celsius..
- For each well then added 50 mL of the stop solution (HCL) 1N and gently mix for 15-20 seconds.
- Use an ELISA reader, the absorbance reading for each well was calculated at a wavelength of 450 nm.

3.9-2 Measuring the Level of Follicle- Stimulating Hormone (FSH) and Intercellular- Stimulating Hormone (LH) :

Concentration of the hormones was measured according to the way(Kosasa,1981;Simoni *et al.*, 1997)

3.9-2.1 Principle:

The sandwich ELISA concept is employed by this ELISA kit. Samples (or standards) are put to the micro ELISA plat wells on the kit's included micro ELISA plate, which has been pre-coated with an antibody specific to rat FSH, LH Next, a biotinylated detection antibody that is specific for rat FSH is applied gradually to each microplate well, followed by the removal of the incubated free components. When the substrate solution is added to each well, the only wells that become blue are those that contain rat FSH, biotinylated detection antibody, and Avidin-HRP conjugate. The enzyme-substrate process can be interrupted by including stop solution, which causes the color to become yellow. A wavelength of +/- 2 nm 450 is used to spectrophotometrically quantify the optical density (OD). Rat FSH concentration and OD value are inversely related. One may figure out the concentration of Rat FSH in the samples by comparing the OD of the samples to the standard curve.

3.9-2.2 Reagent :

Note. Before use, bring all reagents to room temperature (between 18 and 25 °C). Standard working solution, Biotinylated detection Ab working solution, and wash buffer -Working solution for concentrated HBR conjugate

1. Determine the correct number of wells above its own stand for the diluted standard, add a blank and a sample, and then add 100µl of each dilution of the standard the plate was then sealed with the kit's included

- sealer. kit .At 37C°,incubate for 90 min. Note: To avoid foaming ,solution should only be added to the well at the bottom of the microplate.
2. Do not wash, just solution from each well should be decanted. Insert 100 ml of away. After one hour at 37°C, cover with a fresh incubate.
 3. After adding 350 µl of Wash Buffrr to each well, decant the solution from each well. After soaking for one minute, decant or Take the solution out of each well, and then wipe it dry with new absorbent paper. 3 times total should be shaved. NOT: This step and others involving washing may be completed with a micro plate washer. As soon as the wash phase is finished, put the tested strips to use. Keep wells from drying out.
 4. Fill each well with 100 µlof the HRP conjugate working solution. Apply fresh sealer to the plate. for 30 minutes at 37 °C.
 5. Pour the solution from each well, then carry out steps 3 and 4 five more times.
 6. Pour 90 l of the substrate reagent into each well. Over the plate, smear some new sealant. Incubate for approximately 15 minutes at 37 °C. while shielding the plate from light. Reaction times can be lowered or prolonged depending on the actual color shift, but not beyond 30 minutes. Pre-heat the Micro plate Reader for the OD measurement for around 15 minutes.
 7. Fill each well with 50 µl of stop solution. It should be noted that the stop solution should be added similar to how the substrate solution did
 8. Calculate the optical density (OD value) of each well using a microplate reader set to 450 nm.

3.9- 3 Determination of Estrogen Hormone:

The kit for estimating estrogen manufactured by Elabscience company was used and the test was conducted according to the instructions of the producing company as follows ,Concentration of the hormone was measured according to the way (Tietz ,1995). :

1. Put The wells of the plate were first filled with 50 ml of the standard or sample, followed by the addition of 50 ml of the biotinylate detection Ab as a working solution, and finally, after the plate had been incubated for 45 minutes at 37°C.
2. The plate was washed and aspirated three times.
3. Pour 100 ml of the After applying the HRP conjugate working solution to the plate, it was incubated for 30 minutes at 37 °C. The plate was washed three times.
4. Add 90 ml of the substrate reagent, and then incubate the plate for 15 minutes at 37 °C.
5. Fill each well of the plate with 50ml of the stop solution.
6. Immediately at 450nm, the optical density was read, and the results were computed (according to the Elabscience firm).

3.10 Bio chemical Parameters:

3.10-1 Total Antioxidant Capacity Assay

3.10-1.1 Principle:

According to the test's guiding principle (Apak *et al.*, 2005)



Maximum at 450 nm: Cu + (2,9-dimethyl-1,10-phenanthroline complex)

3.10-1.2 Reagents:

1. Chloride of copper (II), one CuCl₂. 2H₂O, weighing 0.4262g, was dissolved in water, and 250 mL of water was added to create a solution with a concentration of 10⁻² M.
2. Dilution Buffer: Ammonium acetate (NH₄ Ac) buffer with a pH value of 7.0 was made by dissolving 19.27g of NH₄ Ac in water and completing 250 milliliters are the total volume.

3. Trolox the Standard Solution of Sample antioxidant were Prepared at 1.0×10^{-3} M Trolox
4. Stop Solution : Neocuproine (Nc) { 2,9 - dimethyl - 1.10 Phenanthroline } Solution at a Concentration of 7.5×10^{-3} M was prepared by dissolving 0.039 g Nc in 96% Et OH, the Volume was Completed to 25ml with Ethanol.

3.10-1.3 procedure:

Copper Solution, dilution buffer, and stop solution 30 minutes should pass for the assay to reach room temperature before use. In the dilution buffer, dilute both the standards and the sample 1:40 (for example, 15 mL serum + 585 ml buffer).

1. Fill each well with 50 μ l of diluted samples or Standards.
2. For a reference measurement, read the plate at 450 nm.
3. Add 1mL of the Cu Solution to each well, then let it sit at room temperature for 30 minutes.
4. Add 1mL to the Stop Solution.
5. Next time 450 nm reading of the plate Test tubes were vortexed a spectrophotometer was used to measure the absorbance at 450 nm after being prepared and incubated for 30 minutes at 37°C.

Calculation :

$$\text{Total antioxidant levels} = \frac{A_{\text{test}}}{A_{\text{STD}}} * \text{Cons of STD (mmol/L)}$$

3.11 Histological study:

After the animal was Sacrificed the organs were removed and washed with normal saline and then transferred for preservation in 10% formalin solution and after 48 hours it was extracted from formalin and washed with ranning water for two hours after which a series of steps were performed on it depending on method described in (Suvarna *et al.*, 2013). This method called (fixation) for link proteins and inactivate degradation enzyme which preserves cell and tissue .

3.11-1 Dehydration :

The tissue transferred through Series of concentrated ethyl alcohol (70% , 80% , 90%, 100%,100%) to remove all water, for an (1 hour) at each concentration.

3.11-2Clearing :

The samples placed in Xylene for removing alcohol for a period (2 minute).

3.11-3Infiltration :

The tissue is then placed in melted paraffin until completely infiltrated with substance Paraffin wax (57-60c^o) melting point the process repeated 2-3 time for a period (1hours) inside an electric oven at a temperature of 60 in order to keep the wax melted the number of times of wax change depends on the type of sample so that it decreases whenever the sample is soft and increases whenever the sample is solid . Wax gives it a strong support to prepare it for microtome cutting and help to keep tissue along time with any harm.

3.11-4 Embedding :

The paraffin - infiltrated tissue is placed in melted paraffin and allowed to harden in room temperature, inside iron molds or special capsules in which the models were buried.

3.11-5 Sectioning :

Used for Cut the models (Rotary Microtome) is fixed and cutes to a thickness of 5 micrometers then the strips of the Sections placed in a water bath (45- 50 c°) for (1-2 min) and then were carried on glass slides and put the slides on hot plate to dry left in room temperature 37C" for (1 hour).

3.11-6 Staining and Mounting :

The dyeing was done using two dyes Haematoxylin - Eosin stain, placed the slides are placed in xylene to clean slides from wax residue for (5 minutes) and then passed on series from descending ethyl alcohol (100%, 100% , 90%, 80%, 70%) and for two minutes in each concentration then stained of the histological Sections.

A-Table (3-3) A-shows hematoxylin stain p reparation

Material	Quantity
Haematoxylin Powder	2.5gm
AIK (So4)2. 12H20 ² or NH4Al (S04)2. 12H2o	50gm
Absolute ethanol	25mL
D.W	500 mL
Oxide of mercuric red	1.25gm
Acetic acid in ice	20ml

Hematoxylin preparation according to (suvarna *et al.*, 2013) dissolved the hematoxylin powder by absolute alcohol, then it is added to the AlK (S04)₂-12 H₂O in warm distilled water, and Add red mercury oxide to the mixture after bringing it to a boil.. cool the mixture by placing it in a beaker of cold water and then add glacial Acetic acid but filter the mixture before use it.

B-Table(3-4)B-shows eosin stain preparation

Material	Quantity
Eosin Powder	1 gm
Glacial Acetic acid	1 mL
Alcohol 70% Ethel	99mL

Dissolved the Eosin Powder by Ethel alcohol and add Glacial Acetic acid but filter the mixture before use it according (suvarna *et al.*, 2013). Transfer the slide to containers containing the dyes, where they are colored with hematoxylin dye for one minute then it was washed with distilled water for two minutes then dipped in alcohol two or three times to remove the excess dye, then it was colored with eosin and then transferred to an ascending chain of ethyl alcohol (70%, 80%, 90%, 100%, 100 %) for two minutes except for the last concentration put in it for 5 minutes then clearing with Xylene for 10 minutes and then we Perform mounting process by using (D.P.X.) Distrine plasticizer xylene to fixed cover on slide and left the slide to dry on hot plate for 8 hours to be ready for examination.

3.12 Histological mophometry :

3.12-1 Calculation of the Average Diameter of the Seminiferous Tubules, the Thickness of Germ Layer in the Testis and the Lumen Diameters:

Diameter of the seminiferous tubule was measure by using an optical microscope with an objective lens 40X on average, the diameter of 10 seminiferous tubules of regular, circular shape in each section was calculated, then the general average was calculated to extract the average seminiferous tubules' diameter, as well as the thickness of the germ layer measured by measuring the thickness from the basement membrane to the space of seminiferous tubule with 10 readings for each animal and then extracting the general average (Akderek *et al.*, 2015).

3.13 Photo Micro Graph :

The tissue Sections were taken after preparation and the glass slides were examined and changes in the studied tissue sections were determined using the light microscope type olympus equipped with digital camera of Carl Zeiss type, the pictures was taken and checked at the appropriate locations of them, then measurement radicals and cell by objective lens on magnification 40X,10X.

3.14 Statistical Analyses :

The data was analysed using SPSS(version 23,SPSS Inc .Chicago, Illinois,USA). Descriptive statistics(mean ,standard deviation),and differences were compared by one-way ANOVA ,by using Duncan's test .As well as ,it was carried out using student's-t test, followed by chi-square. A statistically significant result was one with a($p < 0.05$). The relationship between studied parameters was determined by person's correlation coefficient (r).Analyses of statistical according (Duncan ,1955)

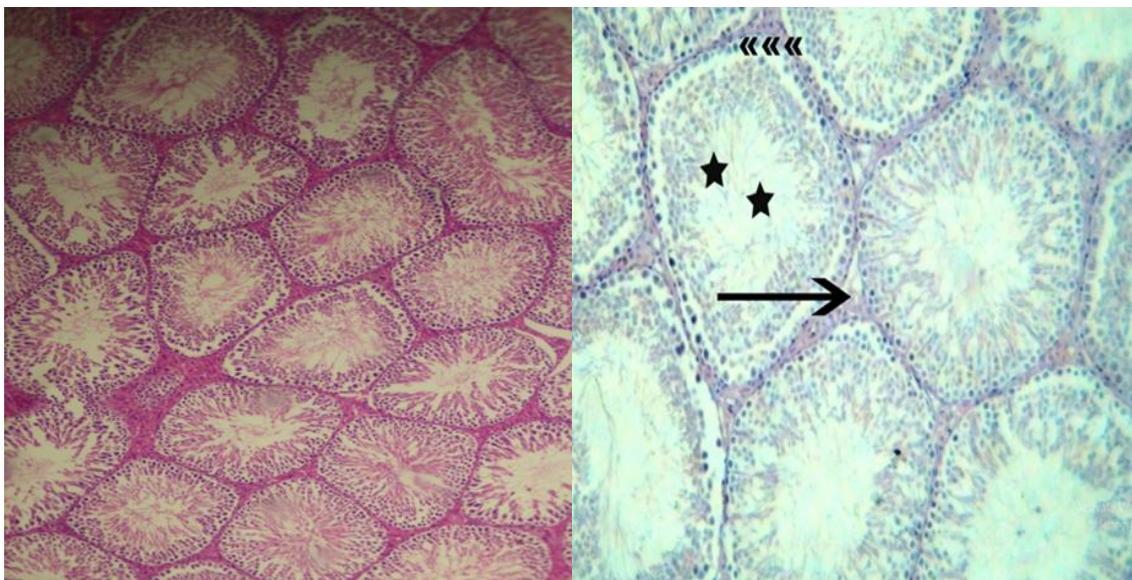
Chapter Four

Results and Discussion

4.Result and Discussion:

4.1 Histological study:

4.1-1 The Effect of Au NPs on Testes:



Figure(4-1):cross section of control rat testis show seminiferous tubules ,seminiferous tubules lumen(star) leydig cell (arrow) sertoli cell(head arrow) (H&E,10X, 40X).

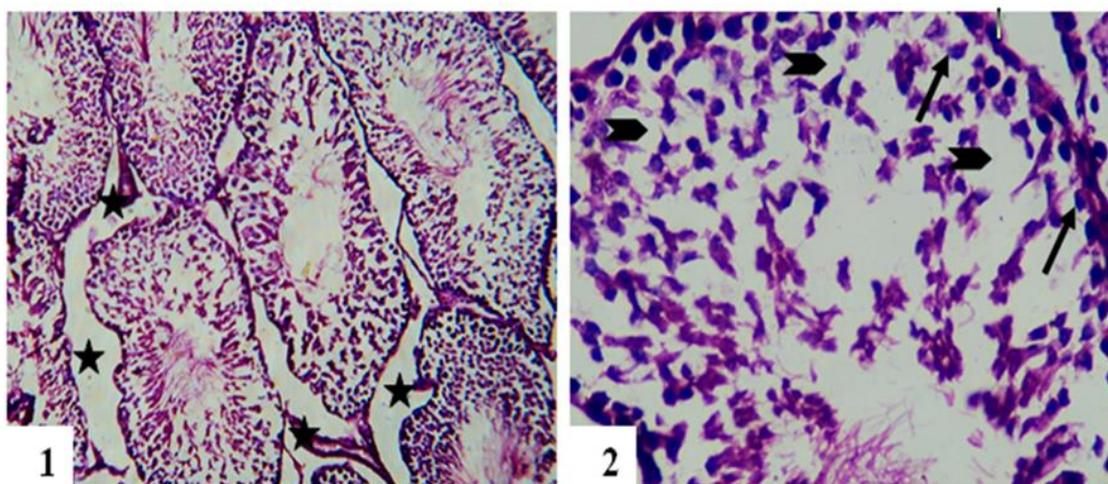


Figure (4-2) :cross section of the testis represent 40µg/kg for30 day (1)showed abnormal widening of interstitial spaces with degeneration of interstitialcells(stars)(10X).

(2)germinal epithelium degeneration(arrow),and disorganization and vacuolization in the spermatogenic series(arrow head)(H&E,100X).

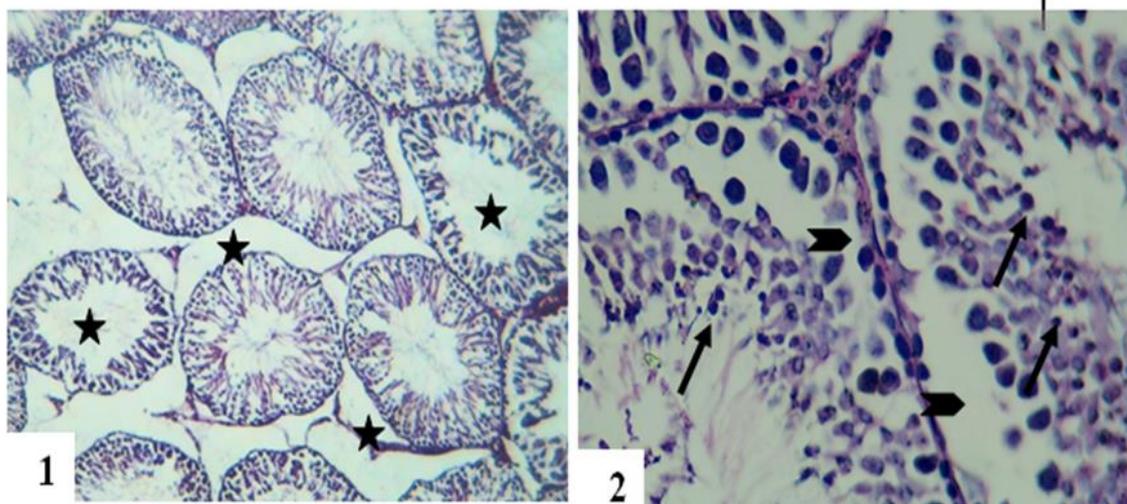
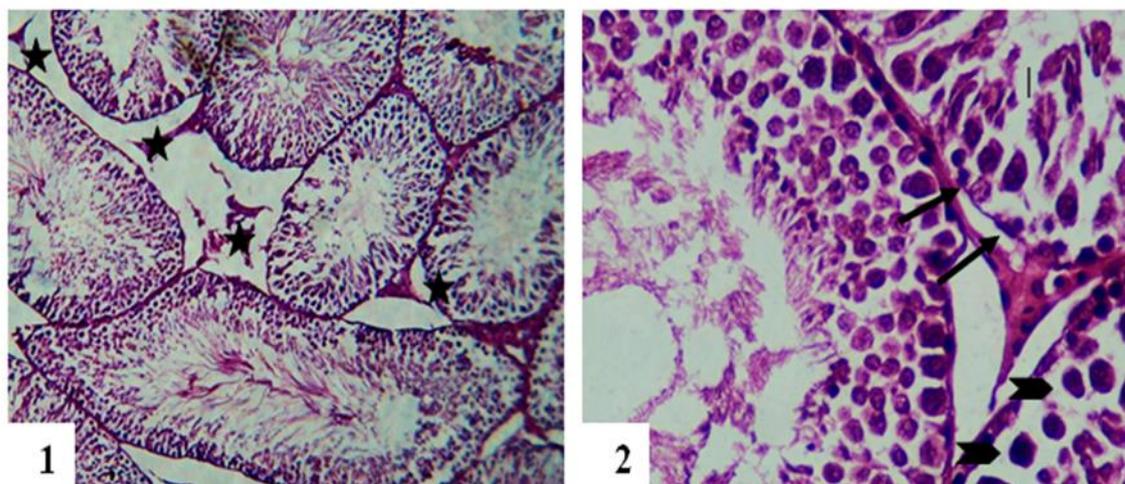


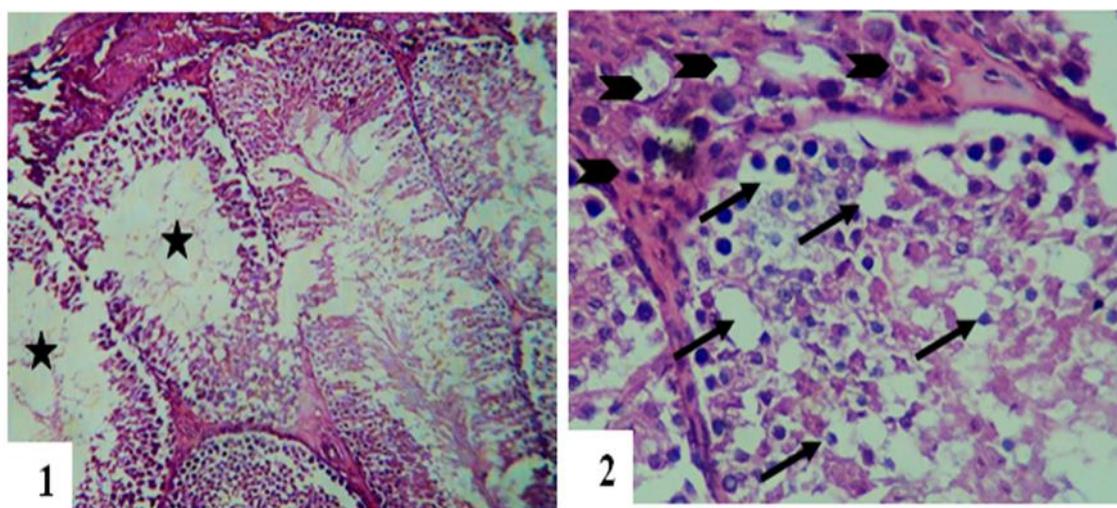
Figure (4-3): Cross section of the rat testis represent $80\mu\text{g}/\text{kg}$ for 30 day ,(1) showed some seminiferous tubules are dilated and increased interstitial space (star) (10X). (2) detachment of germ cell from basal lamina (arrow heads), degeneration and pyknosis of nuclei of some cells (arrows) (H&E, 100X).

The histological study showed the effect of injection for a period of 30 days for doses 40, $80\mu\text{g}/\text{kg}$ when compared to the control group. An abnormal widening of the interstitial spaces was noted with cell degeneration, disorganization of the spermatogenetic chain, emptying and enlargement of some nuclei of the cells of the spermatogenic layer. This agrees with Nazar *et al.*, (2016) by intraperitoneal injection at dose of $40\mu\text{g}/\text{kg}$ and $200\mu\text{g}/\text{kg}$ for 7-35 days to know the acute and chronic effect of Au NPs, as he showed that small particles penetrate the blood testes barrier which is considered as a filter composed of Sertoli cell junction aimed at protecting the process of meiosis as he showed that small particles Au NPs penetrate this barrier (Moretti *et al.*, 2013) causing toxic effect such as creating disturbances in the function of enzymes by the effect of these particles on the genetic structure. That sperm chromatin and DNA are very sensitive to external and internal influences during mitotic changes unlike the chromatin of somatic cell which contains histones while sperm contains protamine, the current study also agrees with (Lasagna-reeves *et al.*, 2010). Since the period of sperm

formation in mice is about 31 days, and to ensure the effect of gold, It is made for 5.



Figure(4-4)Cross section of the rat testis 40μ/kg for 60 day (1) showed increased interstitial space with reduced interstitial cells(star)(10X).(2) detachment of germ cells from basal lamina(arrows) and decrease in the number of spermatogenic cells (arrow heads)(H&E ,40X).



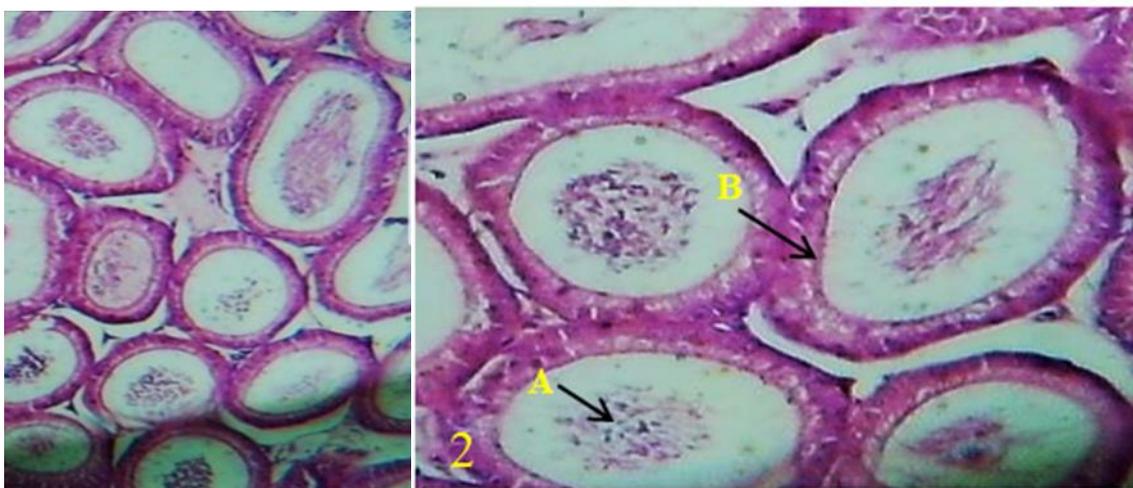
Figure(4-5)Cross section of the rat testis 80μg/kg for 60 day,(1)showed these seminiferous tubules are dilated and hypospermia (star)(10X).(2)degeneration is characterized by disorganized arrangement of germ cells in seminiferous tubules, vacuolation , and the presence of necrotic germ cells(arrows); shrinkage artefact and poor cellular morphology and vacuolation of interstitial cells (arrow heads). (H&E,40X).

The result of dosing for 60 days showed a clear histological effect, after chronic exposure to gold nanoparticles increased interstitial space ,detachment of germ cell from basal lamina ,necrosis, cellular weakness this

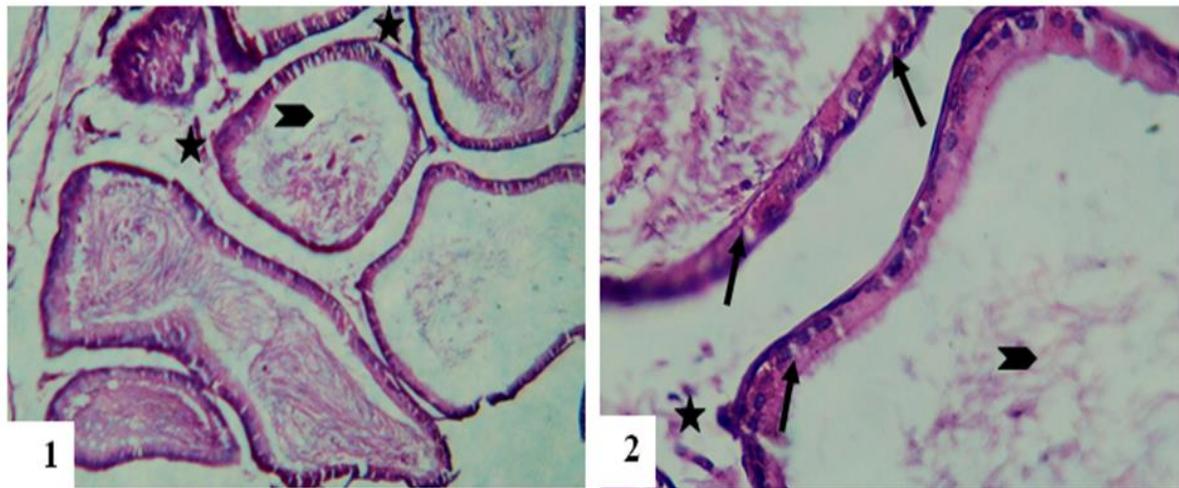
agree with Thakur *et al.*,(2014)who used Ag NPs 20 μ g /kg for 90 days 5 - 20nm;as well Gupta *et al.*, (2018) who used Au NPs 20 μ g /kg also for 90 days ,If we compare it between the two metal to the same concentration we notice that the silver is more toxic than gold, where it was observed and depleted the largest germ cells than gold where the degenerative changes were moderate as the least toxic mineral with penetration BTB but with- out great damage (Morishita *et al.*,2012) .

However toxicity remains dependent on time and exposure. Cytotoxicity mechanism of Au NPs is their relationship with the composition of types of oxygen interactive free ROS which lead to oxidative stress(Pawar and Kaul ,2012). The process of sperm formation is very coordinated with all affected by external and internal conditions and there is consistent with our results current(Xia *et al.*,2019)and can cause gold particles cellular changes during the formation of sperm, which affects the function of cells.

4.1-2 Effect of Au NPs on Epididymis:-

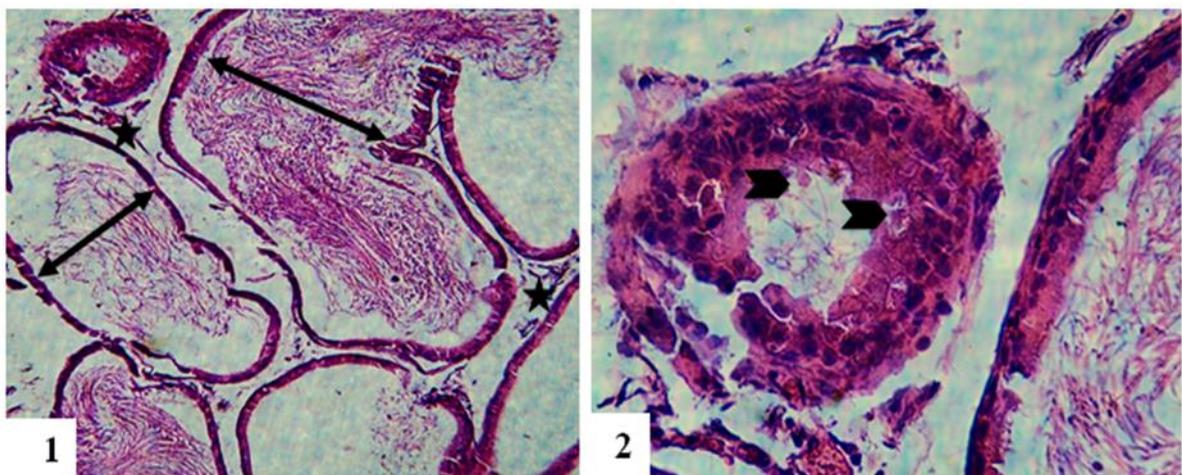


Figure(4-6)Cross section of rat epididymis ,showed normal structure of the epididymis(10X),(2)(arrow B)pseudostratified columnar epithelial cells with stereocilia (arrow A)spermatozoa in lumen(H&E stain,40X).

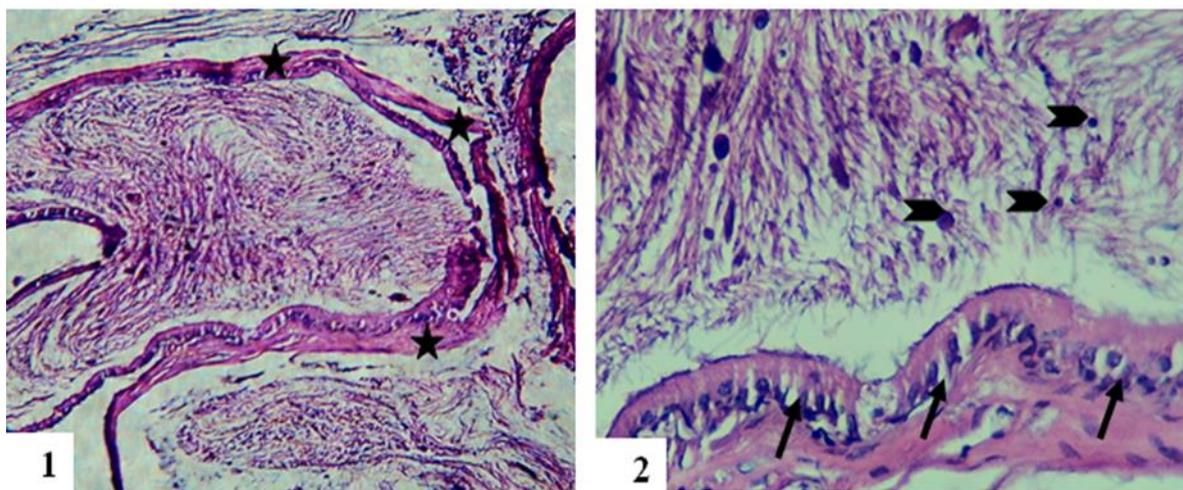


Figure(4-7)Cross section of rat epididymis represent 40µg/kg for30 day (1) showed dilation of the epididymal ducts(10X).

(2) reduce of muscular layer and interstitial tissue between the epididymis canal(stars),vacuolization of epithelial cells(arrows)and oligospermia(arrow head)(H&E,40X).

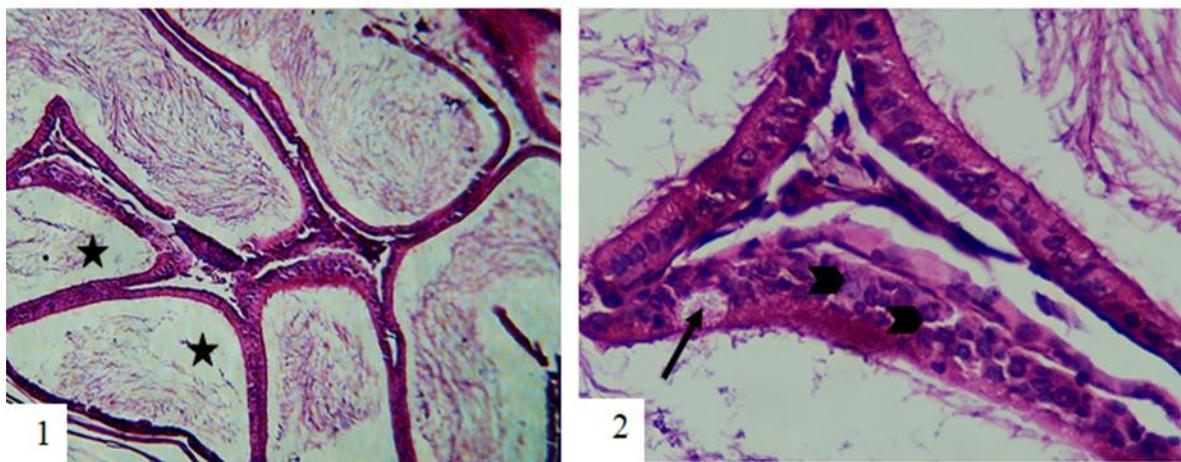


Figure(4-8).Cross section of the rat epididymis represent 80µg/kg for 30 day(1)showed reduce of muscular layer and interstitial tissue between the epididymis canal(stars)and dilation of the epididymal ducts(arrows double head)(10X).(2) massive degenerative change and disruption of architecture with necrotic cellular(arrow head)(H&E,40X).



Figure(4-9).Cross section of the rat epididymis (1) 40 μ g/kg for 60 day showed dilated with irregular ducts in the epididymis of rat and increase of interstitial fibromuscular (stars)(10X).

(2) vacuolization of epithelial cells(arrows),and exfoliated germ cell (arrows head)(H&E,40X)

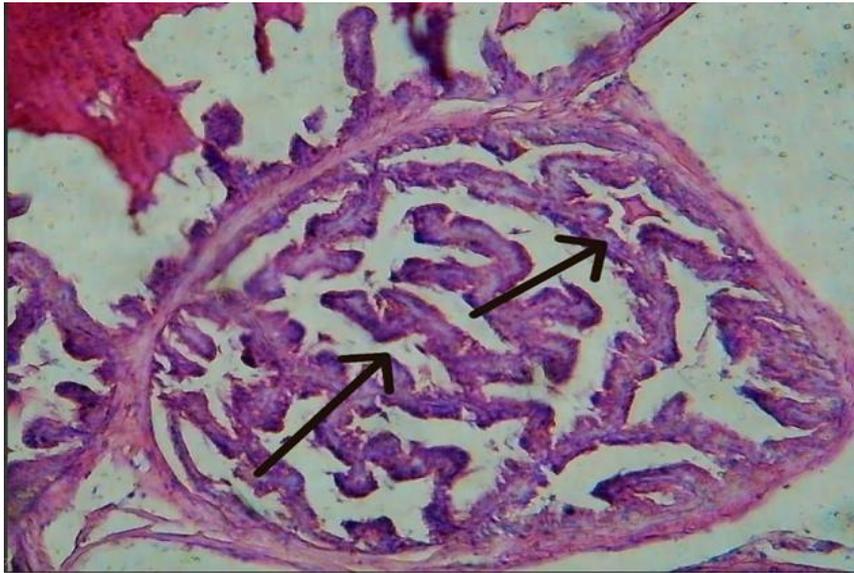


Figure(4-10)Cross section of the rat epididymis 80 μ g/kg for 60day ,(1)showed the hypospermia (star)(10X). (2)denuded epididymal showed hyperplasia (arrows head) and hyperplasia of clear cells(arrow)(H&E, 40X).

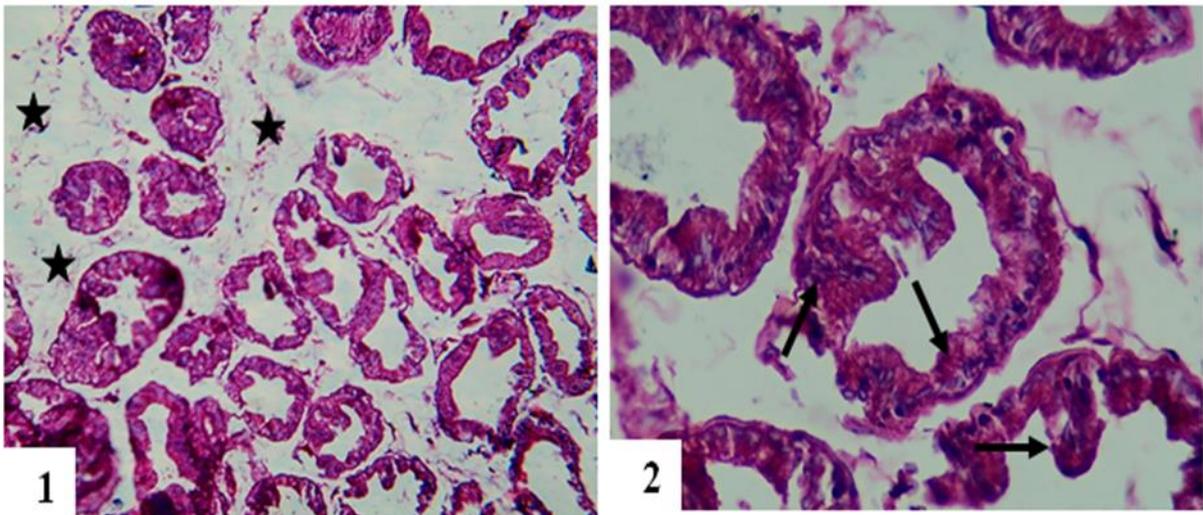
The results showed histological effect on the epididymis of the doses 40 μ g /kg ,80 μ g/kg according to the duration of exposure, as it was shown after intraperitoneal injection for 30 days .The expansion appears in the ducts of epididymis and reduce of muscular layer and vacuolization of epithelial cells and disorder in structure with cell necrosis and degenerative changes due to in malondialdehyde increases (MDA) in the epididymis and

testes ,while decreased antioxidant GSH,SOD , where gold particles cause epididymitis and sperm reduction causing reproductive toxicity and generation of ROS and as a result of oxidative stress causing reduced reproduction this agree with (Manin *et al.*,2007) .

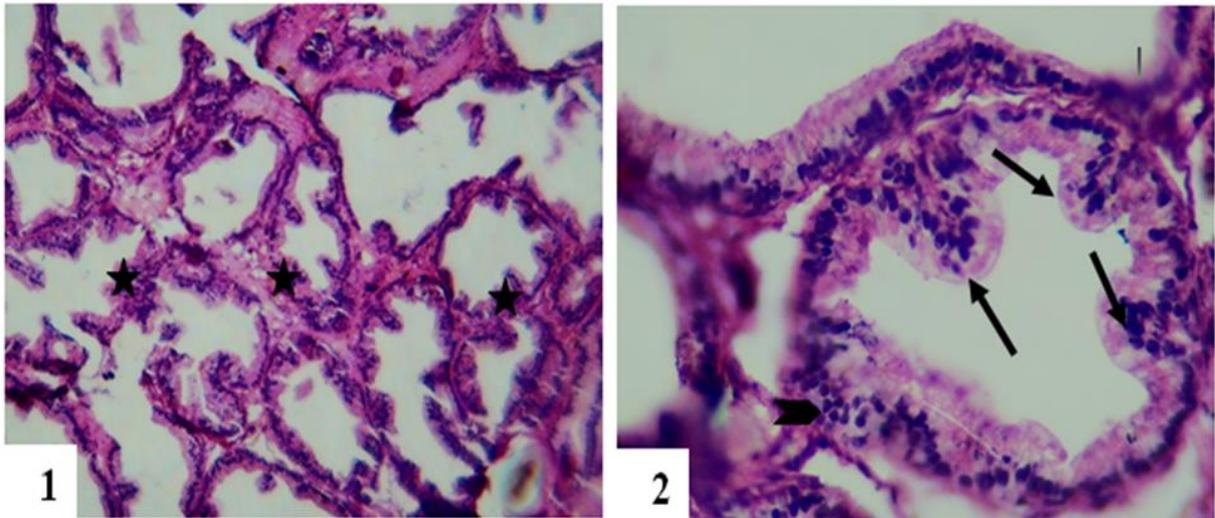
Recently, attention has been directed to the reproductive toxicity of nano materials as they can pass through the blood-testicular barrier and the epithelial barrier that protects the reproductive tissues and lead to damage to the testicles and epididymis causing dysfunction in the cells of leydig and sertoli cells as a whole and negatively affecting the quality, quantity and morphology of sperm and disrupting the level of hormones altered sexual behaviour and oxidative stress and this corresponds with(Wang *et al.*, 2018) .As for the injection for 60 days it was noticed the lack of sperm benign enlargement in the epididymis(hyperplasia), dilated with irregular ducts and increase of interstitial fibro muscular, vacuolization of epithelial cell and exfoliated germ cell. There is very important that the potential impacts of toxic substances on the epididymis and understood as a member who happens where the differentiation of sperm after testicular of storage and maturation of sperm where the show changes epididymals textile occur after deprivation of androgen toxic indirect death epithelial apoptosis lead to a decline in epithelial cells was this is a clear dosage 30 days ,but as for the direct toxicity of the epididymis epithelial it show necrotic and exfoliated the main cells often causing the height of the hyperplasia it appears common in certain areas in the epididymis in head and body and direct toxicity often result disappearance of clear cells in the epididymis tail this agree with(De Grava &Klinefelter , 2015) .

4.1-3 Effect of Au NPs on prostate:

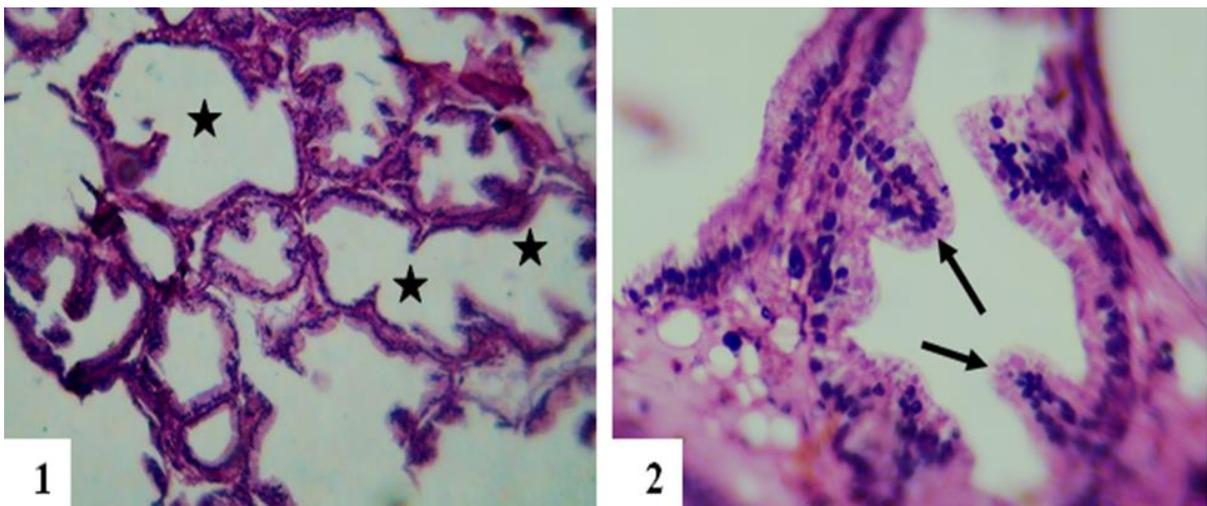
Figure(4-11):cross section of control rat prostate gland showing glandular epithelium cell (acinus epithelial)(arrow) aligned in one layer with epithelial folds(H&E,40X).



Figure(4-12)Cross section of the rat prostate represent 40 μ g/kg for30 day ,(1)showed stromal connective tissue was increased between the secretory alveoli (star)(10X). (2)Arrows indicate multiple areas of hyperplasia (H&E,40X).



Figure(4-13)Cross section of the rat prostate represent 80µg/kg for 30day,(1) showed increase in alveoli folding is considerable(star) (10X) (2)atypical hyperplasia (arrow) and showing focus crowded with epithelial cells(arrow head)(H&E,40X).



Figure(4-14)Cross section of the rat prostate 40µg/kg for 60 day,(1)showed the acinar dilation (star)(10X) (2)atypical hyperplasia(arrow)(H&E,40X)

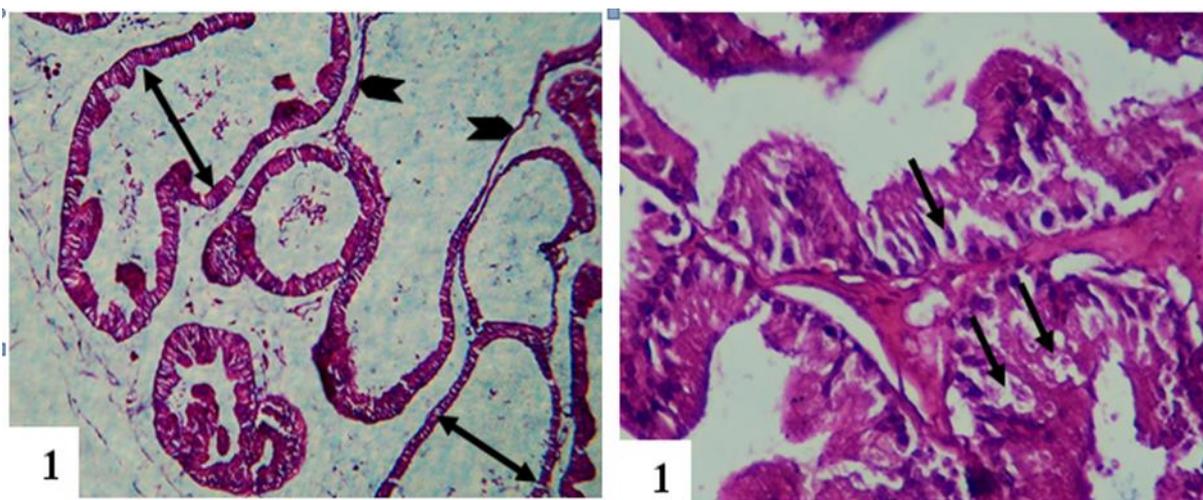


Figure (4-15) cross section of the rat prostate $80\mu\text{g}/\text{kg}$ for 60 day(1) showed the expanding of secretory alveoli (arrow double head) and thin-walled considerable(arrow heads)(10X) .(2)epithelial degeneration is characterized by granular to foamy cytoplasm alteration of enlarged acinar epithelial cells that from a single lining layer (arrow)(H&E, 40X)

The results showed a clear tissue effect in the prostate section that were treated $40\mu\text{g}/\text{kg}$, $80\mu\text{g}/\text{kg}$ for 30 days, and compared it with the control group that appears normal section a columnar epithelium aligned in one layer with epithelial fold in acinus . The section of the prostate shows an increased in alveoli folding ,increased connective tissue(stromal) between the secretory alveoli and atypical hyperplasia . It is known that the effects Au NPs depend on the dose given, duration of exposure and the size of surface area of gold particles and this corresponds with(Al-tarad *et al.*,2019)where morphological abnormalities were found with more spread in the glandular epithelium area and abundant folds with simultaneous administration of Au NPs led to exacerbation and development of BPH and promotion of the inflammatory process. It was found that when the level of the transformed growth factor TGF- β 1 causes the proliferation of the appearance cells, and this corresponds with(Kyprianou *et al.*,1996;Descazeaudet *et al.*,2011).

Also, increased expression of vascular endothelial growth factor(VEGF), an angiogenesis process was also observed in BPH ,as it is the

main driver of its formation increased VEGF expression is implicated in the formation of BPH(Al-tarad *et al.*,2017) . Research has shown that excessive TGF leads to fibrosis, which contributes to the formation of tumours while it is considered as cytokine that has a major role in limiting cellular proliferation and thus leads to apoptosis, but it may act as a traitorous friend by increasing cellular transformation and low apoptosis this agree with(Lee & Peehl, 2004;Zang *et al.*,2006).

The elevated expression of the interleukins IL-17, IL-6 and IL-8 the two major pathways in stromal epithelial growth in BPH, stimulate an inflammatory response that can exacerbate BPH development this agree with (Briganti *et al.*, 2009; De Nunzio *et al.*, 2016;Gandaglia *et al.*, 2013) . But, when dosed for 60 days, it leads to the expanding of secretory vesicles and their thin walls, accompanied by cell degeneration due to the decrease in testosterone which appeared in the current results resulting from oxidative stress in the production of ROS in ledyig cells accompanied by decrease in DHT which is one of the by-products of testosterone, where 10% of testosterone is converted to DHT Dihydrotestosterone by enzyme Alpha-reductase , which it produced in the testes, prostate and ovaries and plays a role in the development of prostate this agree with(Page *et al.*,2006).

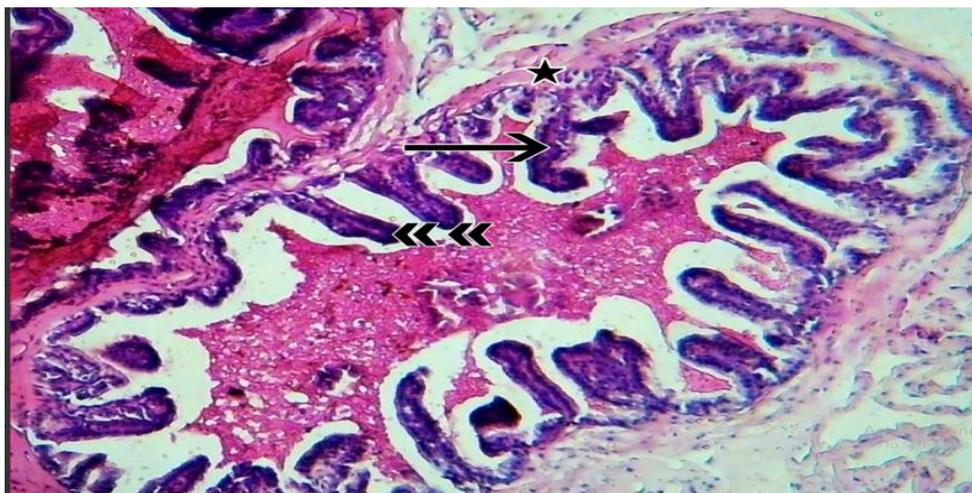
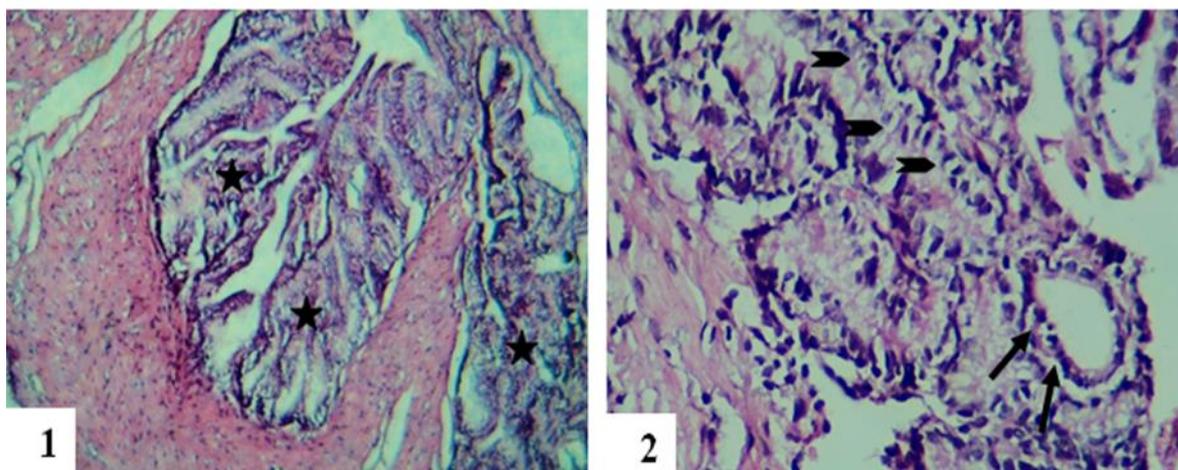
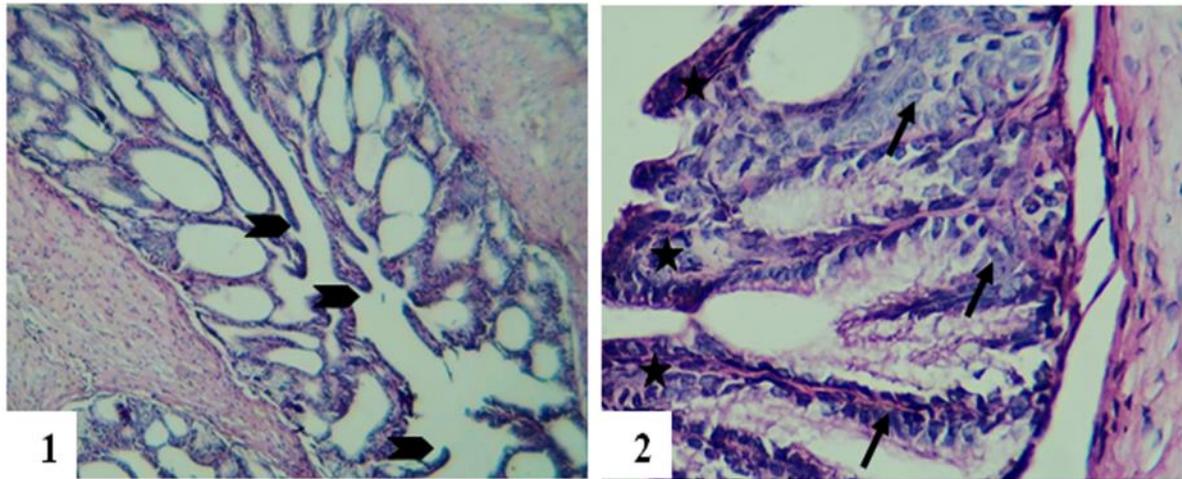
4.1-4 Effect of Au NPs on Seminal Vesicle:

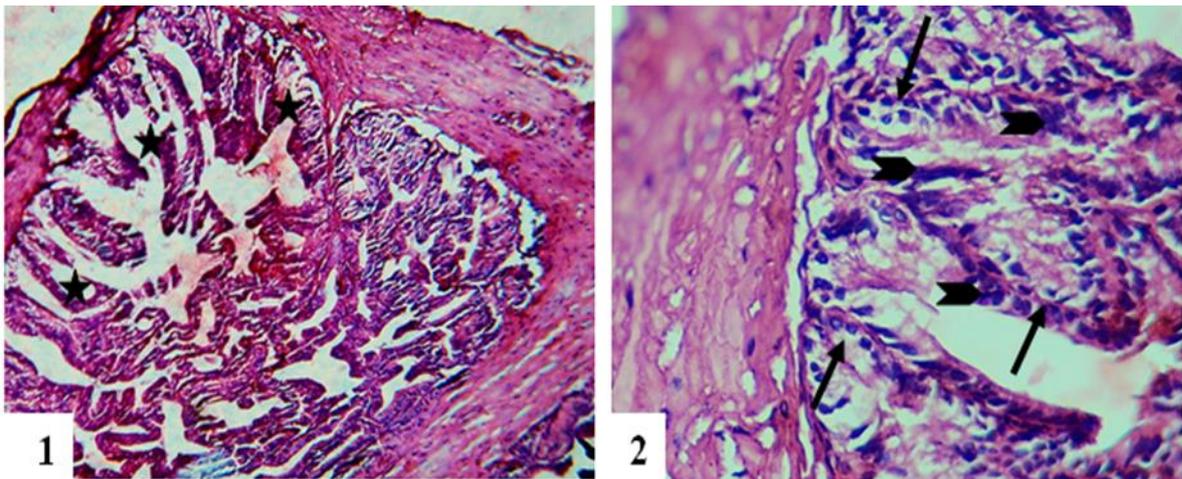
Figure (4-16):Cross section of control rat seminal vesicle with normal tissue show Glandular epithelial (arrow) primary fold in the mucosa(arrow head) lamina propria (star) (40X).



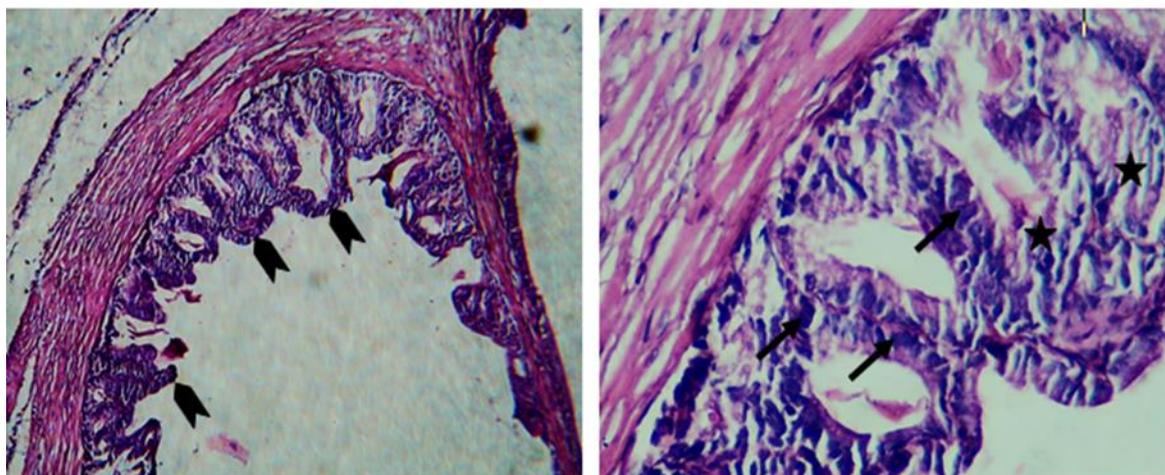
Figure(4-17)Cross section of the rat seminal vesicle 40µg/kg for30 day (1) showed the shrinking of the epithelial cells(stars),(10X).(2) some acini, lined by simple cuboidal (arrow)and(arrow head) point epithelial necrosis but the larger cells with degenerative atypia and vacuolation observed in the cytoplasm(H&E,40X).



Figure(4-18).Cross section of the rat seminal vesicle 80 μ g/kg for 30 day,(1)showed bleb-like apical projections(arrow head)(10X).(2)epithelial hypeplasia,note papillary growth(stars) and epithelial necrosis but the larger cells with degenerative atypia occasional nuclei are hypertrophied (arrow) (H&E. 40X).



Figure(4-19)Cross section of the rat seminal vesicle 40 μ g/kg for 60 day,(1)showed the shrinking of the epithelial cells(star)(10X). (2)hyperplasia of seminal vesicle epithelium with degenerative atypia and vacuolation (arrow) and arrow heads indicate typical hyperchromatic nuclei(H&E,40X).



Figure(4-20)Cross section of the rat seminal vesicle 80 μ g/kgfor 60 day,(1)showed the enlargement and shorting acini (arrow head)(10x).(2) tightly clustered glands with plump, hyperchromatic nuclei(arrow) and sub-epithelial stromal degeneration(star), (H&E,40X).

The result show the effect of Au NPs on seminal vesicle where epithelial cell shrinkage with epithelial necrosis and cytoplasm vacuolation with atypia degeneration at a concentration of 40 μ g /kg ,80 μ g /kg for 30 days comparative with the control group it was observed that normal structures of muscle tissue surrounding the folds lined with columnar epithelial tissue or columnar pseudo stratified . When the dosing for 60 days, tissue sections showed shrinkage of the epithelial cells with atypical degeneration hyperplasia and degeneration of the sub-epithelial stromal this agree with (Taha,2017) using Cu NPs at concentration of 100ppm. It is found that NPs easily pass through the testicular and brain barriers.

The seminal vesicle gland is important in male animals as it is one of the accessory glands that secretes about 60 % of seminal plasma rich in proteins, complex carbohydrates and fructose (Noorafshan&Karbalay-Doust,2012) . The seminal vesicle is highly dependent on androgenic hormones, including testosterone to maintain its structure and function ,which is very sensitive to blood levels of androgens this agree with(Nishino *et al.*,2004). The researchers confirmed the role of testosterone inhibiting the

action of substances that affect the activity of the seminal vesicle agree with (Sastry & Gupta, 2011). The results showed a decrease in seminal vesicle weight hypertrophy, necrosis and atrophy, the reason is due to the decrease in the secretory activity of the Leydig cells of this hormone (Testosterone). This result is in agreement with (Behnmorshedi *et al.*, 2015) which observed a significant decrease in the level of T hormone when the rat was treated with 100mg of Au NPs.

4.2 physiological study:

4.2-1 Effect Au NPS on body weight:

The results of table(4-1) showed that there was no change in the body weight of the male rats that had been dosed with 40µg/kg and significant differences ($p \leq 0.05$) when dosing with 80µg/kg Au NPs for 30 and 60 days and there was an increase in prostate weight, but it did not reach the significant level compared to male rats in the control, with lower weight of the testis, epididymis and seminal vesicles. The result of this study showed that low concentration of gold nanoparticles (Au NPs) did not cause a significant change in the body weight even after its collapse *in vivo*. Whereas, high concentrations of gold nanoparticles cause a slight increase in body weight. And as shown in the figure(4-21).

The histogram show the comparison of body weight for two periods of 30 and 60 days, compare with the control :

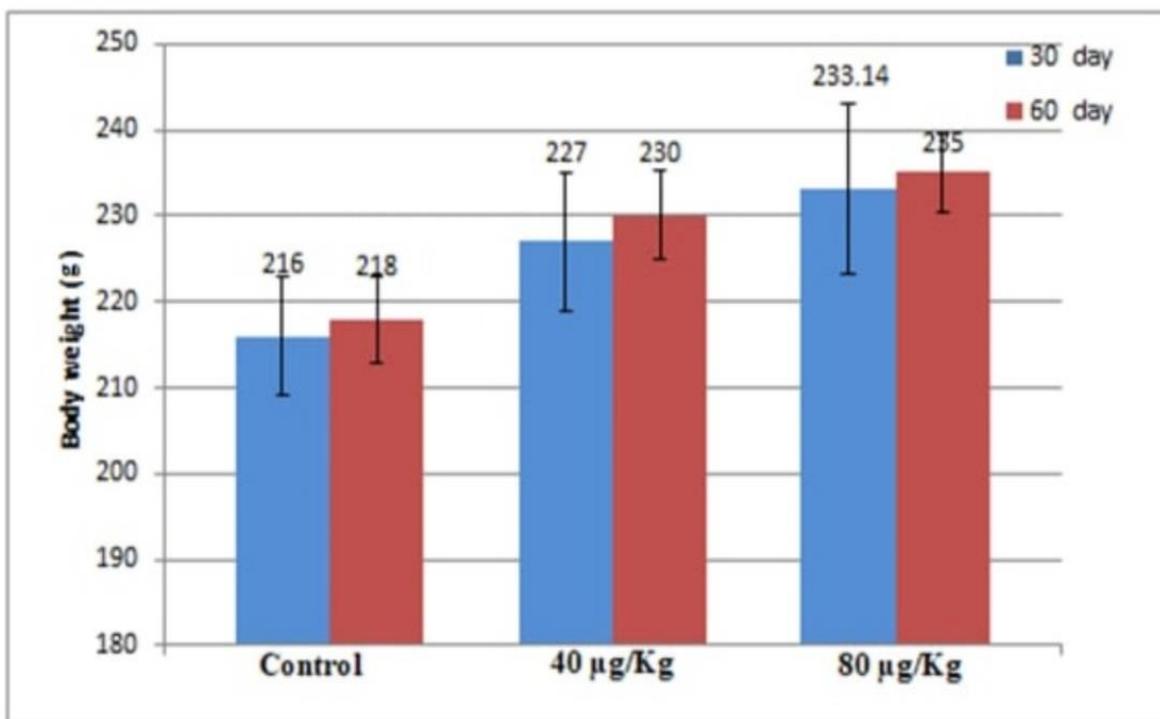


Figure (4-21) demonstrates body weight depending on the dose and duration of exposure.

Table(4-1) Showing the Effect of Au NPs at Concentration of 40µg/kg , 80µg /kg for 30 and 60 day on body weight and the weight of reproductive organs of male rats :

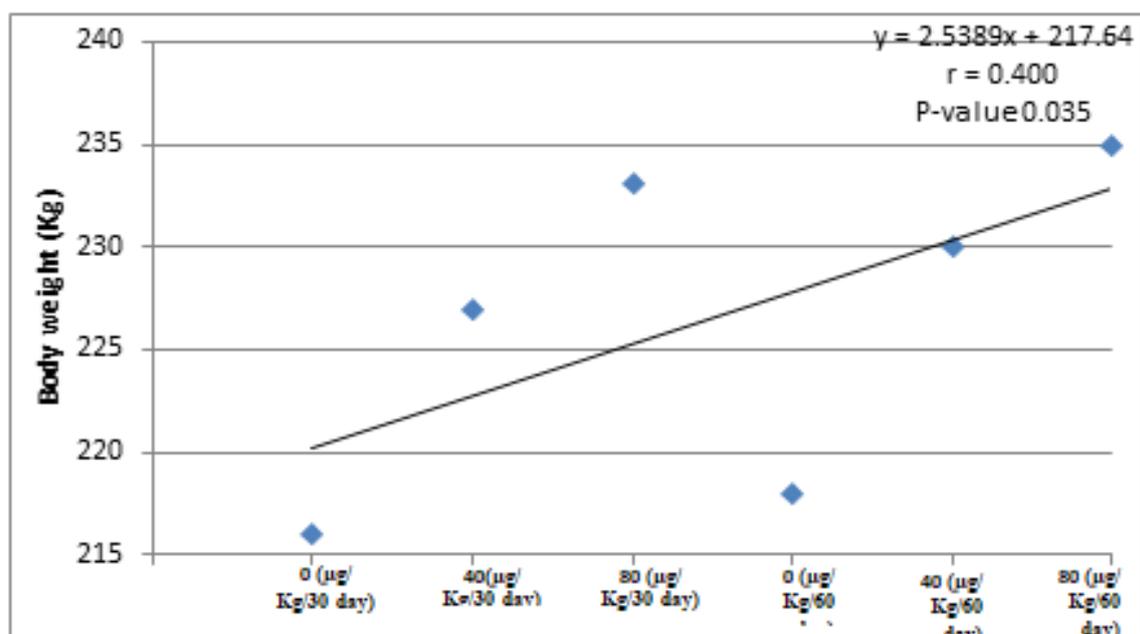
Parameters	Period (day)	Dose(µg/Kg)		
		0 (control)	40	80
		Mean±S.D		
body Weight	30	216.00±7.4 a	227.00±7.3 b	233.14±8.0 b
	60	218.00±5.8 a	230.00±10.5b	235.00±4.5 c
p-value		0.548		0.603
Testis weight	30	1.27±0.03 b	1.24±0.05b	1.18±0.02 a
	60	1.37±0.06 b	1.26±0.02a	1.28±0.04 a
p-value		0.424		0.004 **
Prostate	30	0.26±0.02 a	0.29±0.04a	0.34±0.03 b
	60	0.28±0.03 a	0.32±0.02a	0.40±0.05 b
p-value		0.122		0.030 *
Seminal	30	0.69±0.05 a	0.69±0.12 a	0.62±0.10 a
	60	0.71±0.13 b	0.61±0.12 a	0.58±0.07 a
p-value		0.606		0.383
Epididymis	30	0.65±0.03 b	0.61±0.02 b	0.45±0.09 a
	60	0.67±0.02 c	0.48±0.01 b	0.39±0.02 a
p-value		0.007 **		0.080

Au NPs-gold nanoparticles ($p \leq 0.05$)($p \leq 0.01$)compared to control ;results presented as p-value.

* indicate the significant $p < 0.05$

** indicate the significant $p < 0.01$

Different letters indicate the significant ($p < 0.05$) presented as mean \pm S.D.



Figure(4-22)effect Au NPs on body weight, apposite relationship between body weight and gold nanoparticles according time and dose.

Even at a high concentration slight change may occur in the organs of animals, this may be due to a exposure period that is the exposure to a longer period causes more toxicity ,these result agreement with(Yahyaei *et al.*,2019).The method of administration also affects body weight. The result of this current study correspond with Zhang *et al.*,(2010) which indicated that oral administration of gold nanoparticles caused a significant decrease in body and spleen weight among the three methods of administration it also showed that the oral and intraperitoneal pathways were more toxic and that intravenous injection was less toxic. Thus, It has been suggested that gold nanoparticles are that targeted by vein, may be suitable for enhancing radiotherapy and diagnostic procedures . Gold nanoparticles are known as one of the biocompatible agents due to their large area and smaller size, but It must be considered that particles size ,dose, shape , method of entry, immune response, surface chemistry and metabolism are all important factors for determining toxicity(Zang *et al.*,2010). When peak luminescence in particle size, shape,dosing and exposure duration is taken into consideration, there

was a typical minor difference in the mass of every organ studied in male rats (Abdelhalim, 2013).

In the administered dosage starts to go through the blood and builds up in organs depending on the size from 5-20nm, as 5nm has a greater distribution in tissues compared to large nanoparticles they can also cross the blood-brain barrier and this agree Sonavane *et al.*, (2008), and disagreement with (Nkansah, 2013; Khan *et al.*, 2019) who demonstrated that nervous system drugs failed to cross the blood brain barrier. But in the current study of gold particles through histological anatomy of the reproductive organs, it was proven that nanoparticles cause uneven bio-distribution and toxic features, and this is consistent with Yang *et al.*, (2017). Gold at the nanoscale is toxic, but is safe in bulk shapes (Schrand *et al.*, 2010; Jaclyn *et al.*, 2011). Accumulated Au NPs preserved abdominal adipose tissue mass without altering the daily calorie intake and body weight of the rats.

The toxicity of nano materials has also been reported, as it indicates the effect of gold particles they are nanoparticles of large sizes without causing any harm to the body weight, sizes has injured the brain and liver. It was noted that the nanoparticles in the cytoplasmic areas caused damage (Stefan *et al.*, 2013).

Au NPs cause oxidative stress and reduce antioxidant enzymes such as glutathione and peroxidase activity in the brain and testicles of mice, where 5mg/kg causes blood sugar and an increase in LDL cholesterol in mice which causes weight gain because complications of hyperlipidemia (metabolic syndrome) and increased concentrations cause inflammation (Aravinthan, 2016).

4.2-2 Effect Gold Nanoparticles (Au NPs) on Some Reproductive Organs Weight:

4.2-2.1 Effect on Testis Weight:

The results of the current study elucidate that despite chronic exposure to Au NPs, no effect were observed on testicular weight from atrophy in treated animals just a slight decrease in testes weight for 30 day . Gold nanoparticles have many therapeutic potential due to their ability to cross the blood testicle barrier, and this has been proven in previous studies , where our results reported a 60 day chronic exposure to peritoneal injection to particles ranging in size from 5-20nm in male rat .

It showed that there was a significant decrease ($P \leq 0.01$) compared to the injection for 30 day at a concentration $80 \mu\text{g} / \text{mg}$, this demonstrating capability to cross the blood -testis barrier(BTB), histological examination indicates that there is slight toxicity to the process of spermatogenesis, this agreement with result of Gupta *et al.*,(2018) who used gold nanoparticles with the same diameters at a concentration of $20 \mu\text{g}/\text{kg}$ for 90 days, The research proved by scanning electron microscope that Au NPs is present in various cells of the testes such as sperm cells, sertoli ,leydig cell which corresponds(Sopjani *et al.*, 2008 ; Thakur *et al.*, 2014), research shown that its distribution is much higher after exposure for 90 days compared to exposure for a week, where studies have proven that prolonged exposure causes genitourinary toxicity through degeneration and exfoliation of the tissues of the epithelium the germ cells based on basement membrane and decrease of germ cells and the gold reduces the movement of this sperm corresponds to Comier *et al.*,(2006) with a slight weight effect.

4.2-2.2 Effect on Epididymis weight:

The results of the current study showed a significant decrease ($p \leq 0.01$) in weight of the epididymis at doses $40 \mu\text{g}/\text{kg}$, $80 \mu\text{g}/\text{kg}$ for 30 day, 60day depending on the duration of exposure the reason for decrease in weight is probably due to decrease in the hormone of testosterone as an important element in construction and growth of the reproductive organs the basic structure of body's muscle growth, due to exposure to Au NPs. Performance and the decrease in size of the epididymis depends on the dose and concentrations of hormones T, LH, FSH as any hormone imbalance causes induction of oxidative stress in the testicle and epididymis, and this explanation agreement with a study Maitra and Mitra (2008). Possible mechanisms of weight loss include oxidative stress, apoptosis, ROS stimulation, inflammation that cause pronounced histological change and disruption of hormone levels (Wang *et al.*, 2018). Any dysfunction of the reproductive organs negatively affects the quality of sperm and this is consistent with Manin *et al.*, (2007) which showed that gold bars cause epididymistis which is important because inflammation reduces sperm motility.

4.2-2.3 Effect on prostate weight:

Through it study, it was found that there was a significant increase ($p \leq 0.05$) in the weight of the prostate as it was found that nano-gold as well as nano-silver had harmful effects on the prostate. It is worth mentioning that silver particles were more toxic than gold particles through research, where it has been linked to its well-known work mechanism and this corresponds to (K alynovskyi *et al.*, 2016). They showed that both have toxic effects on prostate.

During the research, it was found that the weight of the prostate at the dose was $40 \mu\text{g}/\text{kg}$, $80 \mu\text{g}/\text{kg}$ there was a slight increase in weight when injected for 30 day when compared with the control, but with the continuation

of injection for 60 day it was found that the gold particles reflected negatively on prostate and possibility of benign prostate hyperplasia which consistent with AL-Trad *et al.*,(2019). This study showed that high and low testosterone induces benign prostate hyperplasia(BPH) which causes prostate enlargement after forty for men , and the simultaneous administration of 50nm Au NPs with testosterone lead to an increase in weight of the prostate.

4.2-2.4 Effect on Seminal Vesicle Weight :

The experiments showed slight decrease in weight as shown in the tables due to the decrease in testosterone, and this interpretation is consistent with Sastry and Gupta(2011) as a result of treatment with Au NPs causing degeneration of glandular tissues depending on the dose and duration of exposure. Morgan *et al.*,(2015)showed that treatment with nano materials it causes tissue breakdown, thus, a defect occurs in the secretion of the gland that have key role in the intensification of sperm to improve fertility and maintain the survival of epididymal sperm and prevent the immune response to sperm in the uterus(Bedford ,2015).

4.2-3 A- Effect of Au NPs at a Concentration of 40µg/kg ,80µg/kg on Some Reproductive Hormone Levels such as Testosterone, LH,FSH and Estradiol for 30 days:

The results showed a slight significant decrease($p < 0.05$)($p < 0.01$) at the 40µg/kg ,80µg/kg compared to the control group, that different doses of gold particles have an effect on testosterone secretion in the testes. The aim of the study is to know the toxicity resulting from nano-gold according to different doses and duration of exposure. As shown in the table(4-2).

This hormonal disorder resulting from the treatment is due to the negative effect on leydig cells (interstitial cells) , whereby the activity of mitochondria decreases and this consistent with Carlson *et al.*,(2008), and Yan *et al.*,(2016) who were reported similar observation ,due to the high

free radicals reactive oxygen species ROS which works on oxidation of molecules (proteins) and cyclooxygenase .

Table (4-2) Showing Effect of Gold Nanoparticles on some Levels of Reproductive Hormones and total antioxidants for 30 and 60 days.

Parameters	Period (day)	Dose ($\mu\text{g/Kg}$)		
		0 (control)	40	80
Testosterone	30	5.11 \pm 0.24 c	4.74 \pm 0.5 b	3.78 \pm 0.4a
	60	5.64 \pm 0.6 c	3.96 \pm 0.1 b	3.17 \pm 0.7a
p-value			0.003**	0.043*
FSH	30	1.75 \pm 0.16 a	1.79 \pm 0.3a	1.42 \pm 0.4a
	60	1.88 \pm 0.40 c	1.52 \pm 0.3b	1.19 \pm 0.3a
p-value			0.191	0.240
LH	30	2.00 \pm 0.25b	1.73 \pm 0.6ab	1.32 \pm 0.4a
	60	2.05 \pm 0.6b	1.37 \pm 0.2a	1.09 \pm 0.1a
p-value			0.152	0.212
Estradiol	30	52.11 \pm 5.7a	61.59 \pm 3.2b	103.99 \pm 12.2c
	60	53.73 \pm 4.9a	118.63 \pm 12.1b	119.39 \pm 26.3b
p-value			0.024*	0.186
TAO	30	1121. \pm 22.10b	1110.99 \pm 65.4b	1012.71 \pm 73.0a
	60	1126.76 \pm 58.4c	975.67 \pm 8.7b	852.74 \pm 85.4a
p-value			0.007**	0.003**

Au NPs-gold nanoparticles ($p \leq 0.05$) ($p \leq 0.01$) compared to control ; result presented as p-value. Different letters indicate the significant difference ($p < 0.05$) presented as mean \pm S.D.

* indicate the significant $p < 0.05$,

** indicate the significant $p < 0.01$

The decrease in antioxidant enzyme causes the onset of oxidative stress on polyunsaturated n fatty acids, which make up a high percentage of tissue cells as a result of being affected by reactive oxygen species, causes degeneration of leydig cells and low testosterone hormone, responsible for perpetuating the work and functions of the reproductive system of the testes, epididymis and gonads (Sikka *et al.*, 1995; Sharma & Agarwal, 1996).

In another study, Omar & Kamar (2021) showed that a concentration 40µg/kg of Au NPs able to cause apoptosis in secretory and hypothalamic cells. the expression of Bax and Caspase3, an intrinsic route for apoptosis, is significantly increased as a result of the accumulation of Au-NPs., thus reducing the hormone FSH, LH. the result of the current study also show that there is a significant increase in the hormone estradiol as a result of injections with different doses and at spaced intervals .The hormone estradiol has a substantial impact on the male reproductive system and sexual function. with the aromatase enzyme that converts testosterone into estradiol in the presence of estrogen receptors in the brain, penis, and testicles .

Estradiol creates the highest level in the brain associated with sexual arousal and aromatase this agree with Savic *et al* (2005) . It has been shown that there is a significant difference between low testosterone and high Estradiol, causing infertility and weak sexual desire due to the accumulated doses of gold particles and a hormonal disorder occure in the pituitary gland or hypothalamus as a result of oxidative stress and formation of ROS this corresponds to(Mancini *et al.*, 2005) ,where there is an indispensable hormonal interaction highly regulated by estrogen in male.

4.2-3 B- Effect of AuNPs at a Concentration of 40µg /kg, 80µg/kg on Some Reproductive Hormones Levels such as Testosterone ,LH,FSH and Estradiol for 60 Day:

The result showed a significant differences($p \leq 0.05$)($p \leq 0.01$) in sex hormone according to duration of exposure to gold nanoparticles, which is the most common mechanism behind the reproductive toxicity of NPs, this corresponds with Morgan *et al.*,(2017) which showed that TiO₂ nanoparticles have a toxic effect on the rat's male reproductive system depending on the exposure time ,and this agree with(Li *et al.*,2009). Au NPs may cross the blood-testicular barrier and accumulate in the genitals

damaging sertoli, leydig and germ cells causing dysfunction disruption of the levels of secreted hormones such as a result of inflammation and oxidative stress and apoptosis (Wang *et al.* , 2018; Hussein *et al.* ,2016) .

Another hypothesis is that the hormonal imbalance due to the gold particles reduces the gene expression of the protein (star) and prevents the transfer of cholesterol in the mitochondria to inner membrane and thus prevents the conversion of cholesterol to pregnenolone and reduce the level of T hormone , where LH binds to its receptors on leydig cells to produce pregnenolone by gene expression of the protein(star) is an acute regulatory protein steroidal origin and this is consistent with Waterman and Keeney (1992). Also, in agreement with Liu *et al.* ,(2020) who suggested that gold particles reduce T hormone production in leydig cells by inhibiting the gene expression of 17 α -hydroxylase after repeated administration, an enzyme important in androgen synthesis. Higher doses of Au NPs cause LH and FSH readings have significantly decreased as a result of pituitary gland disturbance due to increased oxidative of stress and an increase in reactive oxygen species and this is consistent with (Mclahan *et al.*,2002) and the reduction of antioxidant that lead to oxidation of lipids in cell membranes including the brain. Where Knol,(1991) showed that oxidative stress of all kinds leads to activation of the hypothalamic-pituitary-adrenocortical axis which leads to inhibition hypothalamus –pituitary –testis axis and hormone secretion CRH corticotropin releasing hormone inhibits GnRH hormone and latter inhibits and reduces the hormone FSH,LH from the pituitary gland then decrease in level of testosterone which effect on process of spermatogenesis .

Also, a significant increase($P \leq 0.01$) is noticed in estradiol hormone during the 60 day dosing, considering that all cells that participate in the production of sperm contain aromatase and estradiol receptors because of the important role of estradiol in formation of sperm, as the source of estrogen in adult testes in leydig cell and immature sertoli cell . As a result of

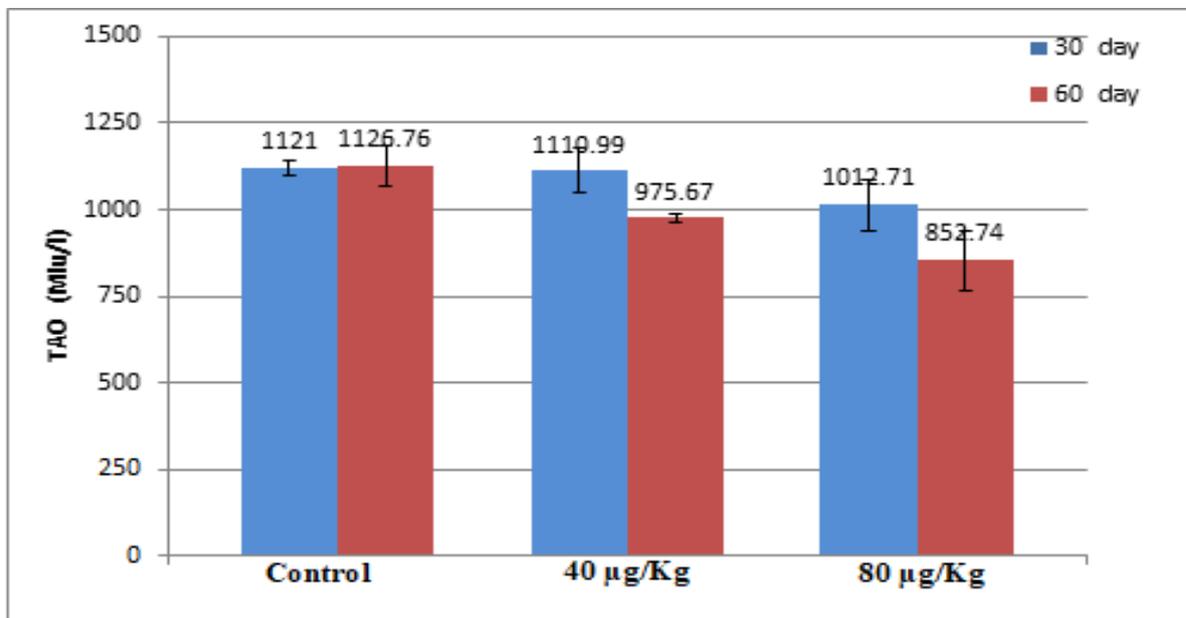
repeated administration of Au NPs, it is increased in estradiol as a result of hormonal imbalances, which led to the possibility of developing hyperaromatase syndrome as a result of genetic mutations, affecting the gene encoding the aromatase enzyme (Yauk *et al.*, 2008), or cerebral hypogonadism may occur as a result of hypothalamic or gonadotropic disorders as a result of nano toxicity, it is a pathological condition in which the body does not produce enough testosterone and an increase in the production of estradiol which are necessary for gonad growth and sperm production, making them compatible with (Dadhich *et al.*, 2017; Rastrelli *et al.*, 2018). There is evidence to suggest that estrogen affects the gonadotropins and then inhibits LH on the Leydig cells thus affecting T-hormone and sperm reduction. This is consistent with (Cigorraga *et al.*, 1980; Atanssova *et al.*, 1999), and Newsholme and Leech, (2010) who show that estradiol appears to be involved in regulating Gonadotropin.

Another assumption is that Au NPs have an effect not only on the reproductive system in terms of hormone production, but also on binding of hormones to their receptors thus affecting the biology of hormone concentration, as nano materials adopt protein adsorption on the surface of the Au NPs. This agrees with (Lynch *et al.*, 2007; Lynch and Dawson, 2008), it means the concentration of the receptor has a necessary role in the effect on the gold particles on hormones associated with the receptor.

Because the mechanism of endocrine hormones does not work directly, but rather combines with receptors which are large protein substances located on the cell membrane, cell cytoplasm, or the nucleus which contains most of the receptors for steroid hormones (Dahlman-wright *et al.*, 2006).

4.2-4 The Effect of Au NPs on Total Antioxidant (T.A.O.):

The histogram show the comparison of total antioxidant for two periods of 30 and 60 days, compare with the control :



Figure(4-23) demonstrates total antioxidants depending dosing and duration of exposure .

The result of the current study showed a significant decrease ($p \leq 0.01$) in level of T.A.O. in serum of male rat that received different doses of Au NPs for 30 days and 60 days for the same dose and comparison with the control group reason for the decrease is the sequence of events of apoptosis signals that occur after a state of cellular free radical generation, oxidative stress, and stimulation of reactive oxygen species generation that inhibit and deplete antioxidants, these result are in agreement with (Esworthy *et al.*, 1997 ;Langer *et al.*, 1996), that exposure to gold particles caused the production of ROS and a decrease in glutathione GSH, which is the best defense against potential toxicity of H₂O₂, as the metabolism of H₂O₂ is mainly by glutathione peroxidase. Higher concentration and exposure time appear to cause more NPs toxicity by increasing the generation of ROS, resulting in DNA damage, cell cycle arrest, inhibition on antioxidant

defensive system, apoptosis of male germ cells (Yan *et al.*, 2016). Metal nanoparticles metabolism, i.e. releases metal ions inside cells leads to cytotoxicity (Derfus *et al.*, 2004; Fukw *et al.*, 2012).

According to a research mice with a diet deficient in antioxidants had low levels of LH, FSH, T and semen, and deficiency in antioxidant enzyme, an increase in DNA damage indicates the role of antioxidants in oxidative stress as a primary mechanism for sperm DNA damage, as well as hormonal disruption in the testicles (Appasamy *et al.*, 2007).

These results agreed with (Richthoff *et al.*, 2002; Saleh *et al.*, 2003). The bulk of GSH is found in the cytosol and ranges from 2-10mM, and there is a small percentage in the mitochondria of total cellular glutathione (%15±10) in the mitochondria (Circu and Aw, 2008). GSH depletion is observed in mitochondria within 48 hours, after which the production of H₂O₂ increases, GSH is depleted intracellularly by Au NPs that have a strong bond with GSH (Au-S), where it was proved that exposure to Au NPs leads to depletion of enzyme glutathione, which is produced by the conjugation thiol that occur in the reaction between Au NPs and GSH (Chen and Chang, 2004). A study showed that excessive high levels of free radicals lead to excessive production of MDA, where its level is a sign of oxidative stress with a decrease in the level SOD, GPX and CAT after repeated doses of treatment with Au NPs. In the same study there was no significant change in oxidative stress as indicated by a significant decrease in MDA and a significant increase in SOD, GPX, and CAT after administration of a single dose of Au NPs after 15 day (Mehanna *et al.*, 2022; Ayala *et al.*, 2014), and compatible with Mangalampalli *et al.*, (2017) when using Mg NPs. This is inconsistent with (Hassan *et al.*, 2020; Orabi *et al.*, 2019). The antioxidant enzymes CAT, GPX, and SOD that protect the body from ROS-induced intracellular cellular damage (Ighodaro and Akinloye 2018).

4.3 Measurements of the Average Diameters of the Seminiferous Tubules, their Lumen Diameters and the Average Thickness of the Epithelial Germ Layer Measured in Micro Meters for Male Rats Treated with Au NPs for 30 and 60 days:

It is evident from the current study of the testicular tissue of the control group treated with physiological saline, as it was noted that the seminiferous tubules are full of sperm and the layer of germ epithelial cells is regular and the cells that form them are distributed naturally from the first layers based on the basement membrane represented by a layer of spermatogenic cell. The results of the current study of the morphological and histological measurements are shown in the tables and pictures of the testicles of a group of rats that were dosed Au NPs 40 μ g/kg ,80 μ g/kg for 30 days showed a slight decrease in the average diameters of the seminiferous tubules and a slight elevation in lumen of the seminiferous tubules and in thickness of the germ layer, measured in micro meters. As shown in the table(4-3)(4-4).

Table(4-3) show the measurements of the seminiferous tubule diameters ,the lumen of the seminiferous tubule, and the thickness of the germinal layer for 30 days :

Parameter Group 0	Seminiferous tubules diameter μ M	Seminiferous tubules Lumen diameter μ M	Thickness Epithelial germinal layer μ M
	Mean \pm S.E		
Control	288.88\pm5.24a	114.00\pm3.22a	102\pm1.78a
40μg/kg of AuNPs for 30 day	281.75\pm20.11b	115.27\pm4.21a	102.28\pm3.47a
80 μg/kg of AuNPs for 30 day	276.23\pm5.11b	117.18\pm5.71a	104.00\pm4.23a

Table(4-4) show the measurements of the seminiferous tubule diameters ,the lumen of the seminiferous tubule, and the thickness of the germinal layer for 60 days :

Parameter Group	Seminiferous tubules diameter μM	Seminiferous tubules Lumen diameter μM	Thickness Epithelial germinal layer μM
	Mean \pm S.E		
Control	287.88\pm6.45a	113.00\pm1.24a	102.73\pm3.55a
40$\mu\text{g}/\text{kg}$ of AuNPs for 60 day	273.70\pm4.11b	118.96\pm2.44a	101.58\pm4.51a
80 $\mu\text{g}/\text{kg}$ of AuNPs for 60 day	189.71\pm3.52c	135.40\pm5.06b	95.90\pm3.78b

Both table(4-3)(4-4)different Letters refer to significant difference at($P\leq 0.05$).

The disorder in the structure of the testis and the process of generating sperm is characterized by morphometric and infrastructural change where there is an abnormal widening of interstitial spaces with the onset of degeneration and enlargement of some cell nuclei in the germinal epithelium and disorganization and vacuolization of the spermatogenic series this agree with (AL mansour *et al.* 2017; Souza *et al.*, 2021) which confirmed a current study aimed at systematic and histomorpho metric to know toxic effects of NMs has progress since 2012 demonstrating its capability to pass across the blood-testes barrier and biological accumulation on the male of reproductive system including testes, epididymis, prostate, seminal vesicle.

But Repeated injection of the same dose for 60 days showed seminal tubular dilation of seminal tubules ,lack of sperm, degeneration germ cell deterioration and necrosis , shrinkage and poor cellular morphology, and vacuolaization of leydig cells appeared during the histological section when compared with the control. Because to damage caused by oxidative stress

caused by ROS(Hubbs *et al.*,2011)the reason is weak and oligospermia it is due to low testosterone due to a defect in leydig cells.

4.4 Correlation Coefficient between Parameters the Result of Injecction with Gold Nanoparticles(Au NPs) :

Table(4-5)Pearson Correlation Coefficient.

Parameters		Weight Body	Testis weight	prostate	seminal	epididymis	testosterone	FSH	LH	Estradiol	TAO
Concentration of treatment	r	.400*	-.574**	.617**	-.353	-.582**	-.606**	-.401*	-	.399*	-.513**
	Sig.	.035	.001	.000	.065	.001	.001	.034	.533**	.035	.005
Weight Body	r	1	-.366	.403*	-.183	-.105	-.428*	.102	-.226	.367	-.098
	Sig.		.055	.033	.351	.593	.023	.604	.248	.055	.619
Testis weight	r		1	.502**	-.331	-.452*	-.379*	-.271	-	.224	-.540**
	Sig.			.006	.085	.016	.047	.163	.579**	.251	.003
prostate	r			1	-.237	-.373	-.690**	-.393*	-.384*	.223	-.600**
	Sig.				.224	.051	.000	.039	.044	.255	.001
seminal	r				1	.171	.195	-.093	.305	-.138	.252
	Sig.					.383	.320	.636	.114	.485	.197
epididymis	r					1	.588**	.625**	.504**	-.422*	.626**
	Sig.						.001	.000	.006	.025	.000
testosterone	r						1	.283	.434*	-.398*	.512**
	Sig.							.144	.021	.036	.005
FSH	r							1	.489**	-.180	.566**
	Sig.								.008	.360	.002
LH	r								1	-.241	.380*
	Sig.									.216	.046
Estradiol	r									1	-.331
	Sig.										.085

* Correlation is significant at the($P \leq 0.05$) level (2-tailed)

** Correlation is significant at the($P \leq 0.01$) level(2-tailed)

(-)Negative is an inverse relationship

(+)Positive is an positive relationship

4.4-1 Correlation between Organs Weight and Sexual Hormones with Duration of Exposure to Specific Concentrations 40 μ g/kg, 80 μ g/kg:

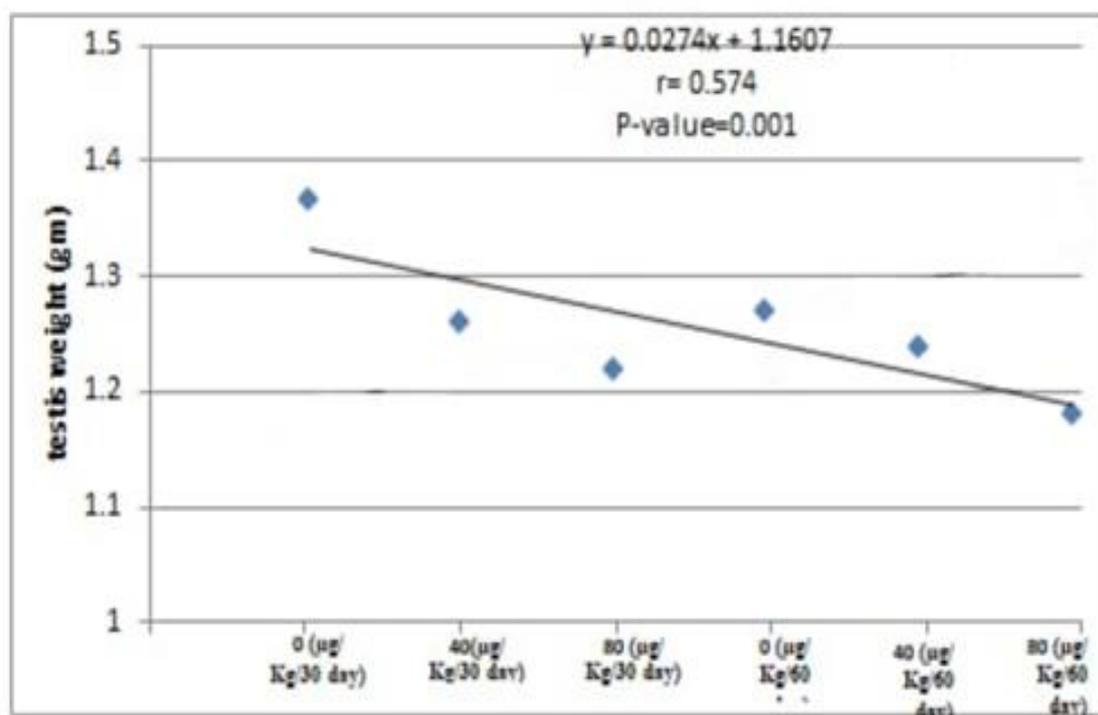
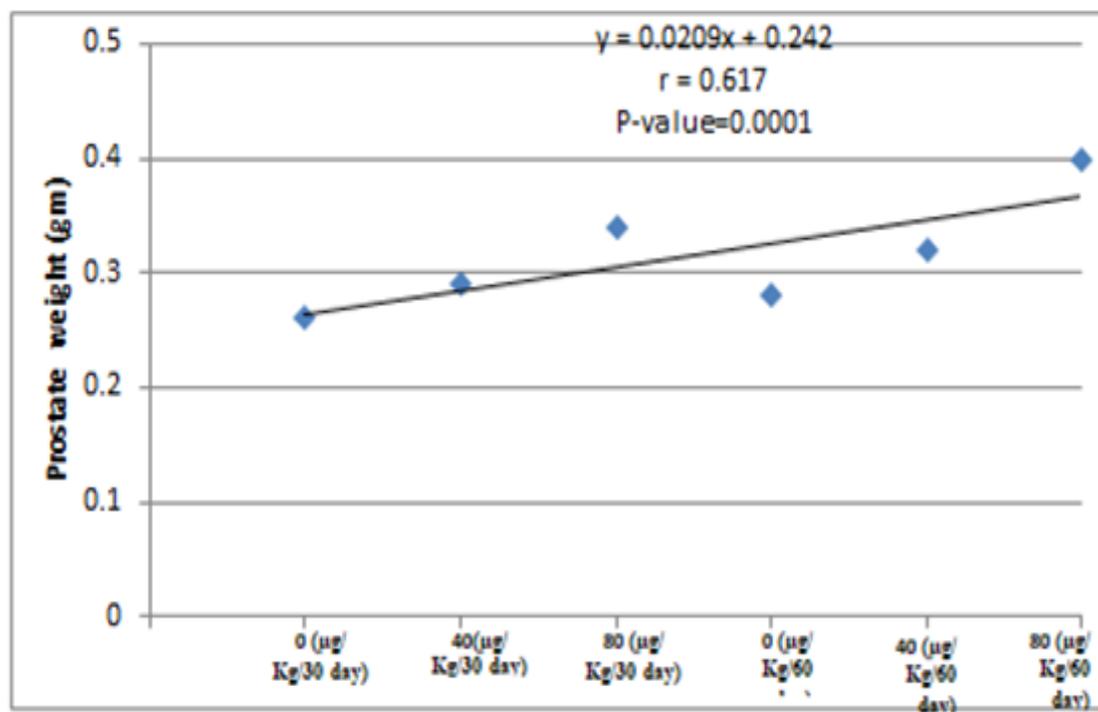
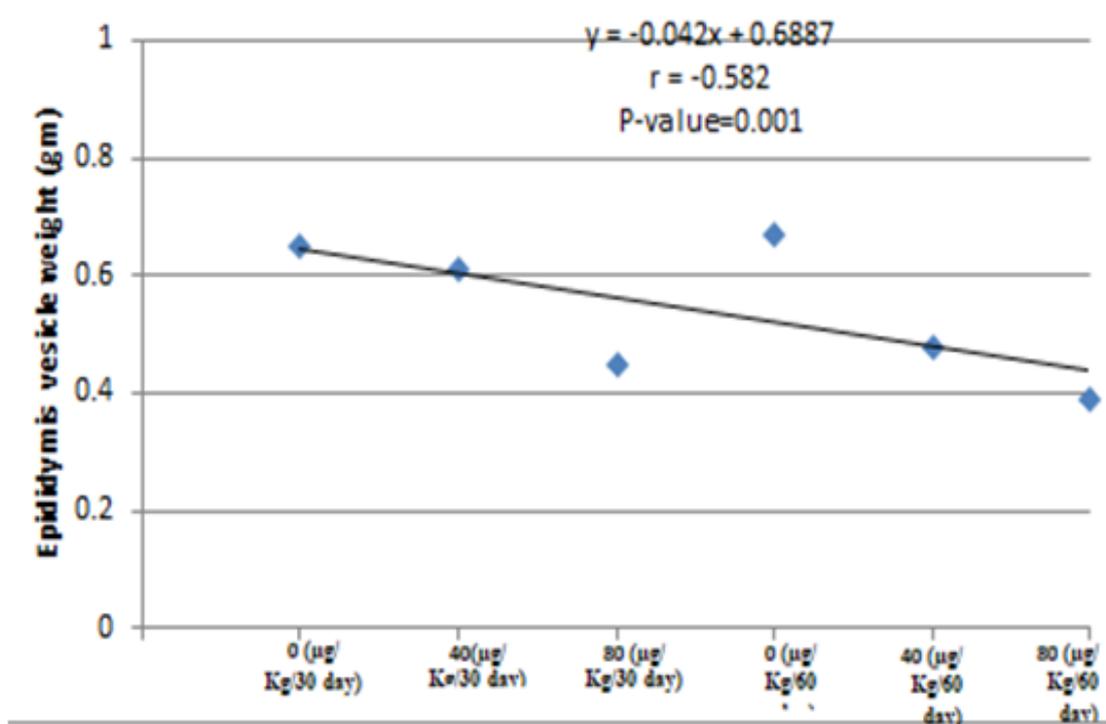


Figure (4-24) Effect of Au NPs on Testis Weight



Figure(4-25) Effect of Au NPs on Prostate Weight.



Figure(4-26)Effect Au NPs on Epididymis Weight.

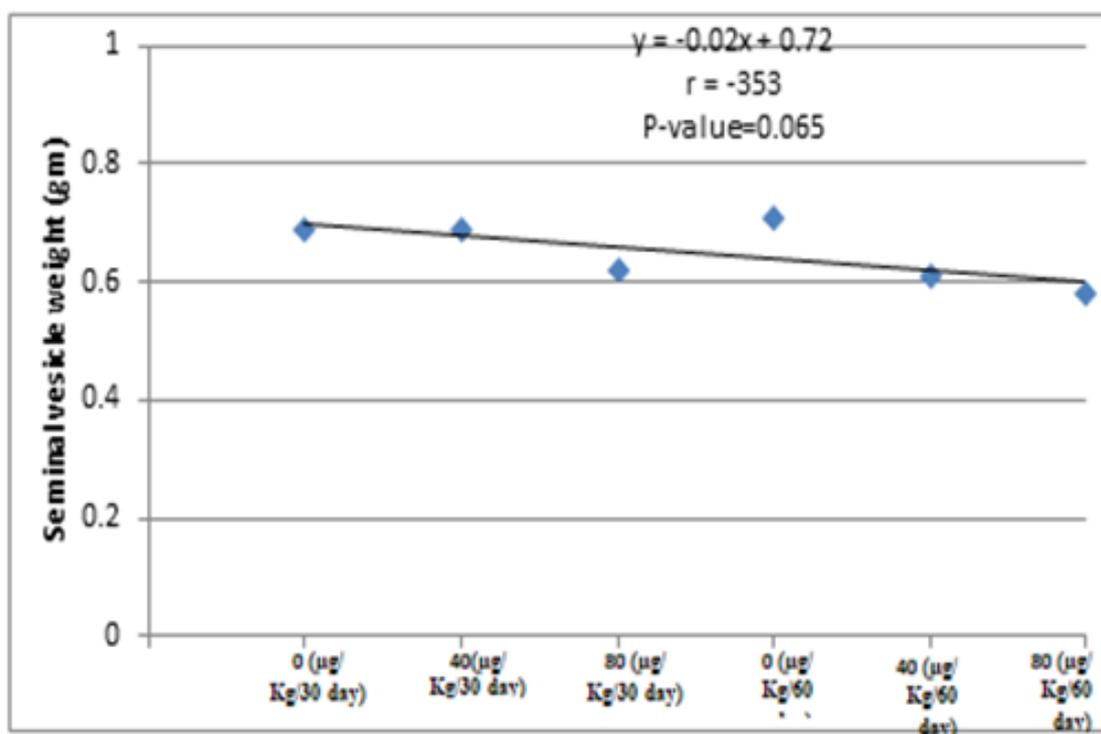
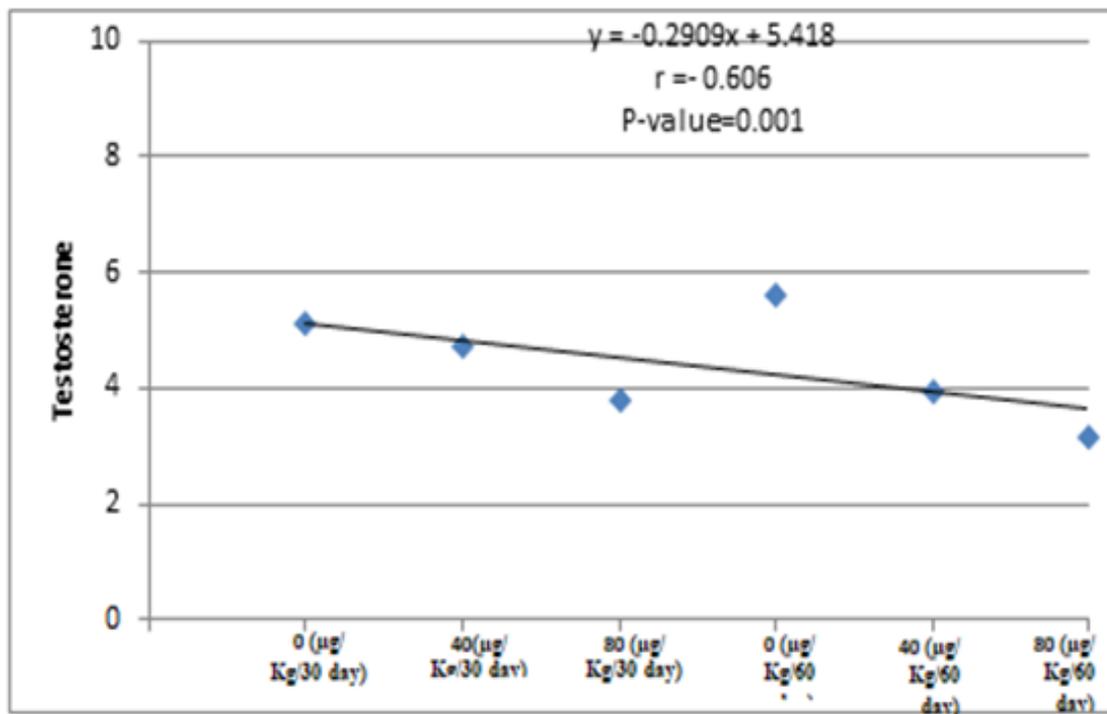


Figure (4-27)Effect Au NPs on Seminal Vesicle Weight



Figure(4- 28)Effect of Au NPs on Testosterone Hormone

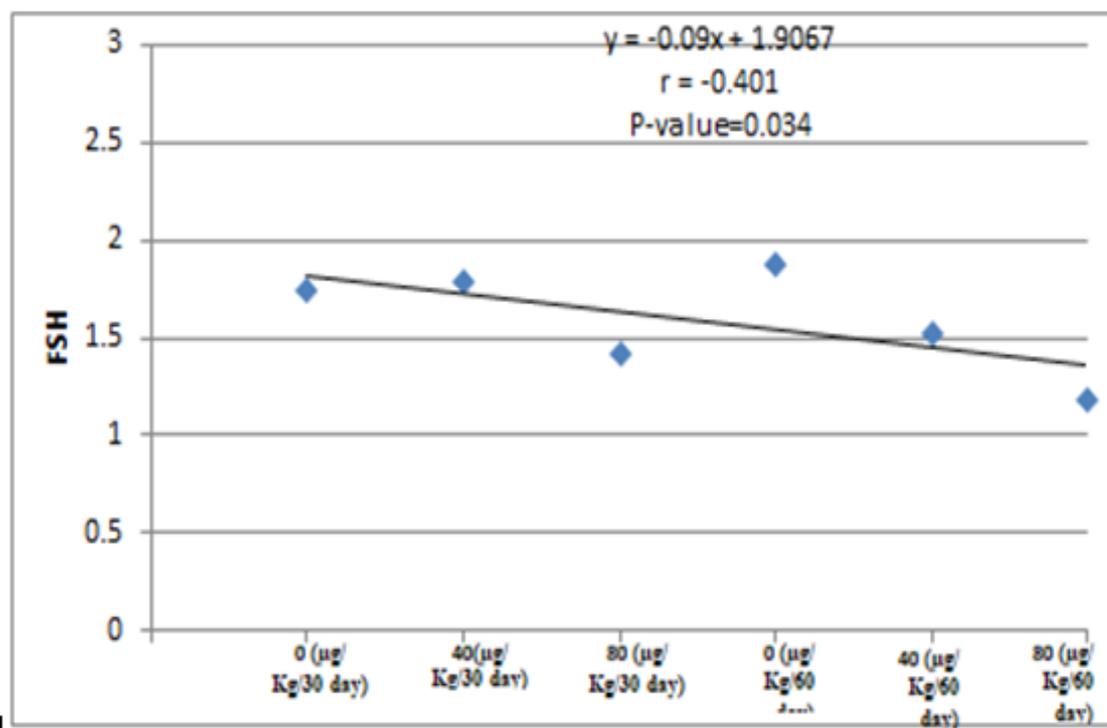


Figure (4-29) The Effect of Au NPs on FSH Hormone

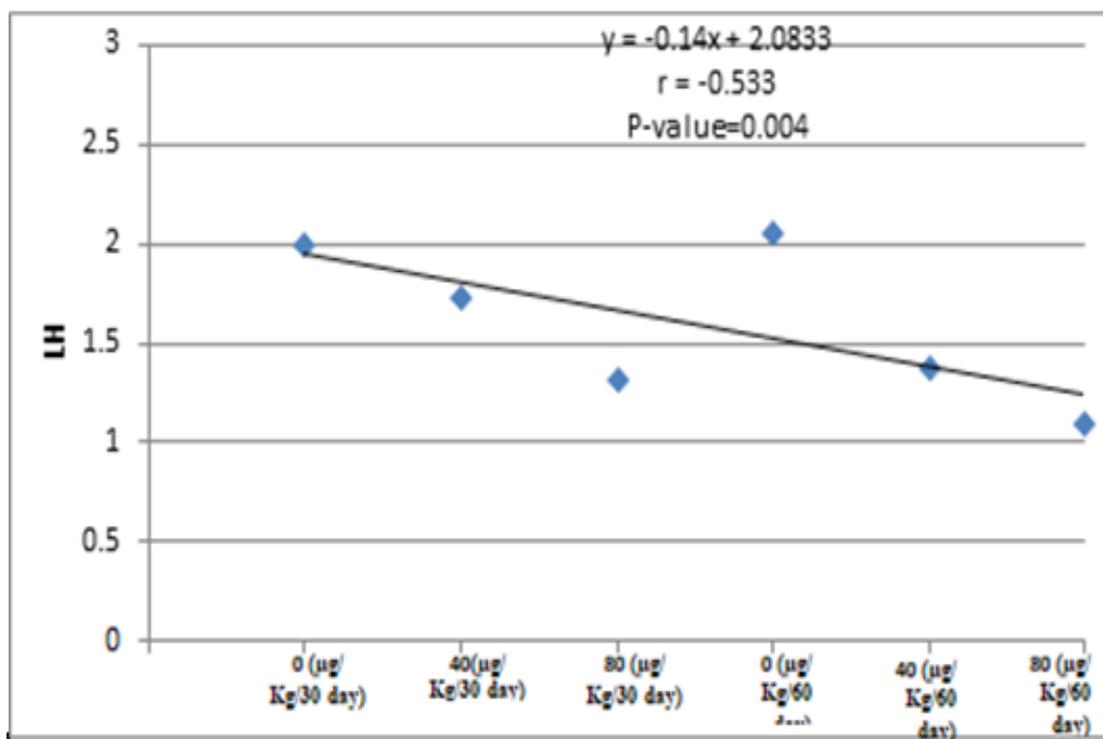
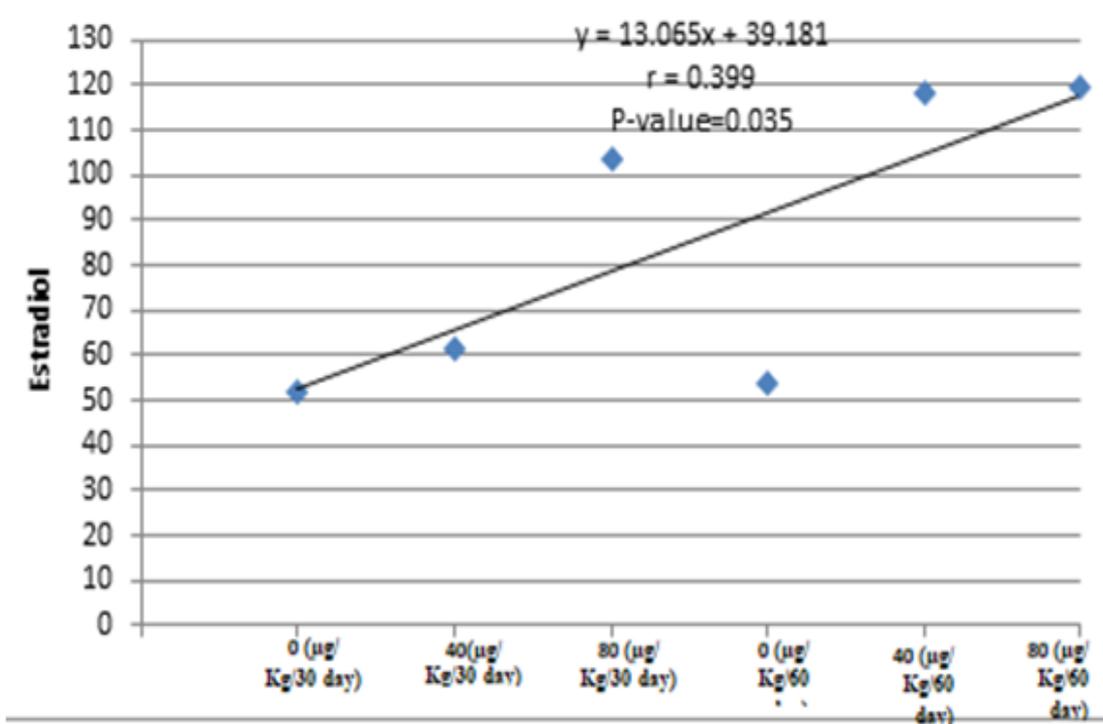


Figure (4-30)The Effect of Au NPs on LH hormone



Figure(4-31)Effect Au NPs on Estradiol hormone.

Conclusions and Recommendations

Conclusions and Recommendations

Conclusions:

This study reached the following conclusion

1. Gold nanoparticles have less toxic effects, gold is a chemically inert substance that activates when it becomes nanoparticles, and the toxic effects depend on the concentration and duration of exposure and method of administration.
2. Au NPs causes dysfunction in male rats due to oxidative stress and ROS formation as a result of the ability of the particles to penetrate the blood-testis barrier and affect the leydig and sertoli cells according to duration of exposure.
3. Gold nanoparticles reduce hormones(T,LH, FSH.)causes of a defect in the hypothalamic-pituitary gonad axis and increased Estradiol hormone causing hyper aromatase syndrome.
4. Gold nanoparticles increase body weight due to complications of hyperlipidemia(metabolic syndrome) and reduce some of the weight of male reproductive organs.
5. Gold nanoparticles at low concentration of 40µg/kg have slight tissue and hormonal effects compared to the control , but harmful effect appear after exposure for the longest period at concentrations of 80µg/kg for 60 days.
6. Gold nanoparticles have ability to penetrate the brain barrier and cause hormonal disorder ,when accumulating for 60 days.
7. The effect of some organs such as the prostate and epididymis as a result of indirect toxicity, which is androgen deficiency.
8. The total antioxidant capacity decrease through 30 and 60 days.

Conclusions and Recommendations

Recommendations

Based on the result of this study, the following recommendations were included for future work:

1. Study of genetic expression for aromatase gene that were inhibited as a result of treatment Au NPs according conclusion of previous studies.
2. Repeating the experiment on female animals and studying the effect of gold nanoparticles on the uterus, ovaries and some hormones of the female reproductive system.
3. Study immunohistochemical with specific histology kits.

References

References

المصادر العربية

- العبده الله , شتيوي (٢٠١٢). علم وظائف الاعضاء. الطبعة الاولى. دار الميسرة . للنشر والتوزيع والطباعة. عمان ٦٠-٧٠
- التميمي, وداد عبد جواد . (٢٠٠٣) . دراسة نسيجية للحوادث الدورية للخلايا المنشئة للنفث في النبيبات المنوية لخنزير غينيا .رسالة ماجستير .كلية التربية . جامعة القادسية.
- الحسني , ضياء حسن والهيبي , صادق محمد امين . (١٩٩٠) . فسلجة الحيوان . كلية الطب البيطري . جامعة بغداد.
- الحسيني , اسماعيل . (٢٠٠٤) . موسوعة الطب الباطني. الطبعة الاولى . دار اسامة للنشر والتوزيع . الاردن . ص٣٠٥ .
- عبد اللطيف , سعد حمد والبازي , وفاق الجبوري.(٢٠٠٥) . النظام الهرموني في اللبائن . مطبعة وزارة التعليم العالي والبحث العلمي . ص ١٨٠
- المختار, كواكب عبد القادر والراوي, عبد الحكيم احمد . (٢٠٠٠) . علم النسيج .مديرية دار الكتب للطباعة والنشر , بغداد.
- كاظم . شيماء عبد الهادي . (٢٠٠٦) . تأثير كلوريد الزنبيق في معالم النفث والتركيب النسيجي لخصى وبرابخ الفئران البيض .رسالة ماجستير . جامعة بابل . كلية العلوم . ص ٥ .
- العزب , محمود عبد السلام.(٢٠٠٨) . رعاية الاغنام والماعز . مجلة البيطرية العربية, مدينة مبارك للأبحاث والتطبيقات التكنولوجية. جامعة بنها - مصر . ص٢-١٦ .

References

References:

- Abdelhalim, M. and Moussa, S. (2013).** The gold nanoparticle size and exposure duration effect on the liver and kidney function of rats: in vivo. *Saudi J Biol Sci* 20:177–181.
- Abraham, G.E. and Himmel, P.B. (1997).** Management of Rheumatoid Arthritis: Rationale for the Use of Colloidal Metallic Gold. *Journal of Nutritional & Environmental Medicine*, 7: 295-305.
- .Agasti, S. S.; Chompoosor, A.; You, C. C.; Ghosh, P.; Kim, C. K. & Rotello, V. M. (2009).** Photoregulated release of caged anticancer drugs from gold nanoparticles. *Journal of the American Chemical Society*, 131(16):5728-5729.
- Akderek, H.; Yurut Caloglu, V.; Tastekin, E.; Caloglu, M.; Turkkan, G.; Mericliler, M. and Mehmet, B.K. (2015).** Acute histopathological responses of testicular tissues after different fractionated abdominal irradiation in rats. *Postgraduate medicine*, 127(1): 73-77.
- Allan, C. M.; Kalak, R.; Dunstan, C. R.; McTavish, K. J.; Zhou, H.; Handelsman, D. J. and Seibel, M. J. (2010).** Follicle-stimulating hormone increases bone mass in female mice. *Proceedings of National Academy of Sciences*, 107(52):22629-22634..
- Almansour, M.; Jarrar, Q.; Battah, A. & Jarrar, B. (2017).** Histomorphometric Alterations Induced in the Testicular Tissues by Variable Sizes of Silver Nanoparticles. *The Journal of reproductive medicine*, 62(5-6):317–323
- Al-Trad, B.; Aljabali, A.; Al Zoubi, M.; Shehab, M. & Omari, S. (2019).** Effect of gold nanoparticles treatment on the testosterone-induced benign prostatic hyperplasia in rats. *International journal of nanomedicine*, 14: 3145–3154.

References

- Al-Trad, B.; Al-Zoubi, M.; Qar, J.; Al-Batayneh, K.; Hussien, E.; Muhaidat, R. ... & Al Omari, G. (2017).** Inhibitory effect of thymoquinone on testosterone-induced benign prostatic hyperplasia in Wistar rats. *Phytotherapy Research*, 31(12): 1910-1915.
- Apak ,K.; Güçlü, M.; Özyürek, S. E.; Karademir, M.; Altun (2005).**Total antioxidant capacity assay of human serum using Refrence 89 copper(II)-neocuproine as chromogenic oxidant: *The CUPRAC method Free Radical Res*, 39:949- 10.
- Aravinthan, A.; Kamala-Kannan, S.; Govarathanan, M. & Kim, J. H. (2016).** Accumulation of biosynthesized gold nanoparticles and its impact on various organs of Sprague Dawley rats: a systematic study. *Toxicology research*, 5(6):1530–1538.
- Arthur, G.H.; Noakes, D.E.,; Pearson, H. And parkin son, T.J. (1996).** Veterinary reproduction and obstetrics. 7th ed. Chapter 29.Sanders, W.B. Co. Ltd. Printed in U.K.: 551-571.
- Arvizo, R. R.; Miranda, O. R.; Thompson, M. A.; Pabelick, C. M.; Bhattacharya, R.; Robertson, J. D.; Rotello, V. M. Prakash, Y. S. & Mukherjee, P. (2010).** Effect of nanoparticle surface charge at the plasma membrane and beyond. *Nano letters*, 10(7):2543–2548.
- Asharani, P.V.; Low, K.M.G.; Hande, M.P. Valiyaveettil, S.(2009).** Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *Acs Nano*.(3),pp.279- 90.penetrate into spermatozoa depending on plasma membrane status. *J Biomed Nanotechnol*. 11:1597.
- Aslan, K. and Pérez-Luna, V. H. (2002).** Surface modification of colloidal gold by chemisorption of alkanethiols in the presence of a nonionic surfactant. *Langmuir*, 18(16):6059-6065.
- ASTM (2006).** Standard test method for oxidative induction time of polyolefin geosynthetics by high pressure differential scanning

References

- calorimetry. American Society for Testing and Materials D5885, West Conshohocken, PA.
- Atanassova, N.; McKinnell, C.; Walker, M.; Turner, K. J.; Fisher, J. S.; Morley, M.; Millar, M. R.; Groome, N. P. & Sharpe, R. M. (1999).** Permanent effects of neonatal estrogen exposure in rats on reproductive hormone levels, Sertoli cell number, and the efficiency of spermatogenesis in adulthood. *Endocrinology*, 140(11):5364-5373.
- Ayala, A.; Muñoz, M. F. & Argüelles, S. (2014).** Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative medicine and cellular longevity*, 2014.
- Bai, Y.; Zhang, Y.; Zhang, J.; Mu, Q.; Zhang, W.; Butch, E.R.; Snyder, S.E.; Yan, B.(2010).** Repeated administrations of carbon nanotubes in male mice cause reversible testis damage without affecting fertility. *Nat Nanotechnol*, 5(9):683-689.
- Bajaj, A.; Miranda, O. R.; Kim, I. B.; Phillips, R. L.; Jerry, D. J.; Bunz, U. H. & Rotello, V. M. (2009).** Detection and differentiation of normal, cancerous, and metastatic cells using nanoparticle-polymer sensor arrays. *Proceedings of the National Academy of Sciences of the United States of America*, 106(27): 10912–10916.
- Baki, M.E.; Miresmaili, S.M.; Pouretezari, M.; Amraii, E.; Yousefi, V.; Spenani, H.R.; Talebi, A.R.; Anvari, M.; Fazilati, M.; Fallah, A.A.(2014).** Effects of silver nano-particles on sperm parameters, number of Leydig cells and sex hormones in rats. *Iran J Reprod Med*. 12(2):139- 30.
- Ban, Z.; Zhou, Q.; Sun, A.; Mu, L.; Hu X. (2018).** Screening priority factors determining and predicting the reproductive toxicity of various nanoparticles. *Environ Sci Technol*. 52(17): 9666–9676.

References

- Barkalina, N.; Charalambous, C.; Jones, C; Coward, K.(2014).**
.Nanotechnology in reproductive medicine: emerging applications of nanomaterials. *Nanomedicine*. 10(5):921-938
- Beamer, W. G., Wilson, M. C. and Leiter , E. H. (1983).** Endocrinology In : The mouse in Biomedical Research .By: Foster H. L., Small J. D. and Fox J. G. American college of Laboratory . New York:218 - 224.
- Bedford, J.M.(2015).**The functions—or not—of seminal plasma? *Biol Reprod*;92:18.
- Beer, C.; Foldbjerg, R.; Hayashi, Y.; Sutherland, D.S. and Autrup, H. (2012).** Toxicity of silver nanoparticles - nanoparticle or silver ion?. *Toxicol . Let . J.*; 208(3): 286-92.
- Behnammorshedi, M.; Nazem, H. and Moghadam, M. S. (2015).** The effect of gold nanoparticle on luteinizing hormone, follicle stimulating hormone, testosterone and testis Iin male rat. *Biomedl Res.*, 26 (2): 348-352.
- Berne, R.M.; Levy, M. N.; Koeppen, B.M and Stanton, B.A. (1998).**physiology. 4th edit. Baltimore. materials, 19(5):711
- Bigioni, T. P.; Whetten, R. L. & Dag, Ö. (2000).** Near-infrared luminescence from small gold nanocrystals. *The Journal of Physical Chemistry B*, 104(30):6983-6986.
- Boisselier, E.; Astruc, D.(2009).** // *Chem. Soc. Rev.*V. (38): 1759–1782.
- Botteon, C.E.; Silva, L.B.; Ccana-Ccapatinta, G.; Silva, T.; Ambrosio, S.; Veneziani, R.; Bastos, J.; Marcato,. P.(2021).***Sci. Rep.*11: 1–16.
- Braydich-Stolle, L.; Hussain, S.; Schlager, J.J.; Hofmann, M.C.(2005).**In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. *Toxicol Sci*. 88(2):412–419.

References

- Bresee, J.; Maier, K. E.; Boncella, A. E. Melander, C. & Feldheim, D. L. (2011).** Growth inhibition of *Staphylococcus aureus* by mixed monolayer gold nanoparticles. *Small*, 7(14):2027-2031.
- Briganti, A.; Capitanio, U.; Suardi, N.; Gallina, A.; Salonia, A.; Bianchi, M. ... & Montorsi, F. (2009).** Benign prostatic hyperplasia and its aetiologies. *European Urology Supplements*, 8(13) :865-871.
- Brohi, R.D.; Wang, L.; Talpur, H.S.; Wu, D., Khan, F.A.; Bhattarai, D.; Rehman, Z.U.; Farmanullah, F.; Huo, L.J.(2017).** Toxicity of nanoparticles on the reproductive system in animal models: a review. *Front Pharmacol.* 8:606
- Brown, S. D.; Nativo, P.; Smith, J. A.; Stirling, D.; Edwards, P. R.; Venugopal, B.; Flint, D. J.; Plumb, J. A.; Graham, D. & Wheate, N. J. (2010).** Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin. *Journal of the American Chemical Society*, 132(13): 4678–4684.
- Brune, D.; Samsahl, K.; Wester, P. (1966).**A comparison between the amounts of As, Au, Br, Cu, Fe, Mo, Se and Zn in normal and uraemic human whole blood by means of neutron activation analysis. *Clin Chim Acta.* 13:285-91.
- Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; & Whyman, R. (1994).** Synthesis of thiol-derivatised gold nanoparticles in a two-phase liquid–liquid system. *Journal of the Chemical Society, Chemical Communications*, (7): 801-80
- Bunz, U. H.; & Rotello, V. M. (2010).** Gold nanoparticle-fluorophore complexes: sensitive and discerning "noses" for biosystems sensing. *Angewandte Chemie (International ed. in English)*, 49(19): 3268–3279.

References

- Calzolari, L.; Franchini, F.; Gilliland, D.; & Rossi, F. (2010).** Protein--nanoparticle interaction: identification of the ubiquitin--gold nanoparticle interaction site. *Nano letters*, 10(8):3101–3105.
- Carlson, C.; Hussain, S.M.; Schrand, A.M.(2008).**unique cellular interaction of silver nanoparticles: sizedependent generation of reactive oxygen species. *J Phys Chem B*, 112(43):13608-13619.Karpenko N.
- Carriere, M.; Sauvaigo, S.; Douki, T.; Ravanat, J.L.(2017).** Impact of nanoparticles on DNA repair processes: current knowledge and working hypotheses. *Mutagenesis*. 32(1):203-213.
- Chaudhary, J.; Sandler, I. and skinner, M.K. (2004).** Identification of a noval sertoli cell gene product SERT that influences follicle stimulating hormone action. *Gene.*, 324: 79-88
- Chen, L.; Zheng, L., Lv, Y.; Liu, H.; Wang, G.; Ren, N., Liu, D.; Wang, J.; Boughton, R.I.(2010).** Chemical assembly of silver nanoparticles on stainless steel for antimicrobial applications. *Surf Coat Technol.* 204(23):3871–3875
- Chen, W.H.; Lei, Q.; Luo, G.F.; Jia, H.Z.; Hong, S.; Liu, Y.X.; Cheng, Y.J.; Zhang, X.Z.(2015).** ational design of multifunctional gold nanoparticles via host-guest interaction for cancer targeted therapy. *ACS Appl Mater Interfaces*, 7:17171-80.
- Chen, S.J.; Chang, H.T.(2004).** Nile red-adsorbed gold nanoparticles for selective determination of thiols based on energy transfer and aggregation. *Anal. Chem*,76:3727–3734.
- Chen, Y. H.; Tsai, C. Y.; Huang, P. Y., Chang, M. Y., Cheng, P. C., Chou, C. H., ... & Wu, C. L. (2007).** Methotrexate conjugated to gold nanoparticles inhibits tumor growth in a syngeneic lung tumor model. *Molecular pharmaceutics*, 4(5) :713-722.

References

- Cheng, Y.; Samia, A. C.; Meyers, J. D.; Panagopoulos, I.; Fei, B. & Burda, C. (2008).** Highly efficient drug delivery with gold nanoparticle vectors for in vivo photodynamic therapy of cancer. *Journal of the American Chemical Society*, 130(32): 10643-10647.
- Chithrani, B.D.; Ghazani, A.A.; Chan, W.C. (2006).** Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Lett.* 6:662-8
- Cho, W.; Cho, M.; Jeong, J.; Choi, M.; Han, B.S.; Shin, H.; Hong, J.; Chung, B.H.; Jeong, J.; Cho, M.(2010).** Size-dependent tissue kinetics of PEG-coated gold nanoparticles. *Toxicol Appl Pharm*, 245:116-23
- Cho, E. C.; Zhang, Q.; & Xia, Y. (2011).** The effect of sedimentation and diffusion on cellular uptake of gold nanoparticles. *Nature nanotechnology*, 6(6): 385–391.
- Chou, L. Y.; Ming, K. & Chan, W. C. (2011).** Strategies for the intracellular delivery of nanoparticles. *Chemical Society Reviews*, 40(1), 233-245.
- Cigorruga, S. B.; Sorrell, S.; Bator, J.; Catt, K. J. & Dufau, M. L. (1980).** Estrogen dependence of a gonadotropin-induced steroidogenic lesion in rat testicular Leydig cells. *The Journal of clinical investigation*, 65(3): 699–705.
- Circu, M.L.; Aw, T.Y. (2008).** Glutathione and apoptosis. *Free Radical Res.* 42, 689–706.
- Clasadonte, J.; and Prevot, V. (2018).** The special relationship: glia–neuron interactions in the neuroendocrine hypothalamus. *Nature Reviews Endocrinology*, 14(1): 25.
- Cormier, S.; Lomnicki, S.; Backes, W. & Dellinger, B.(2006).** Origin and health impacts of emissions of toxic by-products and fine

References

- particles from combustion and thermal treatment of hazardous wastes and materials. *Environ Health Perspect*, 114 :810-817.
- Cummins, J.M.; Temple-smith, P.D. and Renfree, M.B. (1986).** Reproduction in male- the epididymis. *Am. J. Anat*, 177: 385-40
- Dadhich, P.; Ramasamy, R.; Scovell, J.; Wilken, N. & Lipshultz, L. (2017).** Testosterone versus clomiphene citrate in managing symptoms of hypogonadism in men. *Indian Journal of Urology: IJU: Journal of the Urological Society of India*, 33(3):236.
- Dagar, G.; Bagchi, G.(2020).** Nanoparticles as potential endocrine disruptive chemicals. In: *Nano Bio Medicine*. Singapore: Springer p. 411–429.
- Dahlman-Wright, K.; Cavailles, V.; Fuqua, S. A.; Jordan, V. C.; Katzenellenbogen, J. A.; Korach, K. S. ... & Gustafsson, J. Å. (2006).** International union of pharmacology. LXIV. Estrogen receptors. *Pharmacological reviews*, 58(4): 773-781.
- De Grava Kempinas, W. and Klinefelter, G. R. (2015).** Interpreting histopathology in the epididymis. *Spermatogenesis*, 4(2): e979114.
- De Nunzio, C.; Presicce, F. and Tubaro, A. (2016).** Inflammatory mediators in the development and progression of benign prostatic hyperplasia. *Nature reviews urology*, 13(10):613-626.
- Dekrester, D.M. (2002).** General structure of male reproductive system. In *endocrinology of male reproductive system*. Appleton and Lange. New York. Chapter 1: 2-17.
- Del Vento, F.; Vermeulen, M.; Ucakar, B.; Poels, J.; Des Rieux, A.; Wyns, C. (2019).** Significant benefits of nanoparticles containing a necrosis inhibitor on mice testicular tissue autografts outcomes. *Int J Mol Sci*. 20(23):5833.
- Demenev, V.A.; Shchinova, M.A.; Ivanov, L.I.; Vorob'eva, R.N.; Zdanovskaya, N.I.; Nebaykina, N.V.(1996) // Vopr. Virusol. V.**

References

- 41: 107–110. (in Russian)234. Zhao Z., Wakita T., Yasui K. // J. Virol. 2003. V. 77. P. 4248–4260.
- Derfus, A. M.; Chan, W. C. & Bhatia, S. N. (2004).** Probing the cytotoxicity of semiconductor quantum dots. *Nano letters*, 4(1): 11-18.
- Descazeaud, A.; Weinbreck, N.; Robert, G.; Vacherot, F.; Abbou, C.; Labrousse, F. ... & de la Taille, A. (2011).** Transforming growth factor β -receptor II protein expression in benign prostatic hyperplasia is associated with prostate volume and inflammation. *BJU international*, 108(2 Pt 2):E23-8.
- Dulkeith, E.; Ringler, M.; Klar, T. A.; Feldmann, J. ; Muñoz Javier, A. & Parak, W. J. (2005).** Gold nanoparticles quench fluorescence by phase induced radiative rate suppression. *Nano letters*, 5(4): 585–589.
- Duncan, D. B. (1955).** Multiple range and multiple F tests. *biometrics*, 11(1):1-42.
- Druart, X. and de Graaf, S. (2018).** Seminal plasma proteomes and sperm fertility. *Animal reproduction science*, 194:33–40.
- Dykman, L.A.; Staroverov, S.A.; Bogatyrev, V.A.; Shchy-ogolev, S.Yu.(2010)** // *Nanotechnologies in Russia*. V. 5: 748–761.
- Dykman, L. A. and Khlebtsov, N. G. (2011).** Gold nanoparticles in biology and medicine: recent advances and prospects. *Acta naturae*, 3(2): 34-55.
- Dykman, L. A. (2020).** Gold nanoparticles for preparation of antibodies and vaccines against infectious diseases. *Expert review of vaccines*, 19(5):465-477.
- Erdely, A.; Liston, A.; Salmen-Muniz, R.; Hulderman, T.; Young, S.H.; Zeidler-Erdely, P.C.; Castranova, V.; Simeonova, P.P. (2011).**

References

- Identification of systemic markers from a pulmonary carbon nanotube exposure. *J Occup Environ Med.*53(6Suppl.):S80–S86.
- Esworthy, R.S.; Ho, Y.S.; Chu, F.F.(1997).** The Gpx1 gene encodes mitochondrial glutathione peroxidase in the mouse liver. *Arch. Biochem. Biophys.* 340: 59-63.
- Fang, S. B.; Tseng, W. Y.; Lee, H. C.; Tsai, C. K.; Huang, J. T. & Hou, S. Y. (2009).** Identification of Salmonella using colony-print and detection with antibody-coated gold nanoparticles. *Journal of microbiological methods*, 77(2): 225-228.
- Foldbjerg, R.; Dang, D.A.; Autrup, H.(2011).** Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. *Arch Toxicol.* 85:743-50.
- Fraser, B.; Peters, A. E.; Sutherland, J. M.; Liang, M.; Rebourcet, D.; Nixon, B. & Aitken, R. J. (2021).** Biocompatible Nanomaterials as an Emerging Technology in Reproductive Health; a Focus on the Male. *Frontiers in physiology*, 12, 753686. Giljohann DA, Seferos DS, Prigodich AE, Patel PC, Mirkin CA. J.
- Frens, G. (1973).** Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nature physical science*, 241(105) :20-22.
- Fukui, H., Horie, M., Endoh, S., Kato, H., Fujita, K., Nishio, K., ... & Iwahashi, H. (2012).** Association of zinc ion release and oxidative stress induced by intratracheal instillation of ZnO nanoparticles to rat lung. *Chemico-biological interactions*, 198(1-3), 29-37.
- Gandaglia, G.; Briganti, A.; Gontero, P.; Mondaini, N.; Novara, G.; Salonia, A. ... & Montorsi, F. (2013).** The role of chronic prostatic inflammation in the pathogenesis and progression of benign prostatic hyperplasia (BPH). *BJU international*, 112(4): 432-441.

References

- Ganong, W. F. (2010).** Review of medical physiology. 23rd Ed. Lange medical Books/ McGraw Hill. United States of America .
- Giljohann, D. A.; Seferos, D. S.; Patel, P. C.; Millstone, J. E.; Rosi, N. L.; & Mirkin, C. A. (2007).** Oligonucleotide loading determines cellular uptake of DNA-modified gold nanoparticles. *Nano letters*, 7(12):3818-3821.
- Gopalan, R.C.; Osman, I.F.; Amani, A.; De Matas, M.; Anderson, D. (2009).** The effect of zinc oxide and titanium dioxide nanoparticles in the Comet assay with UVA photoactivation of human sperm and lymphocytes. *Nanotoxicology*,3(1):33-39.
- Grace, A.N. and Pandian, K. (2007)// J. Bionanosci. V. 1. P. 96–105.**
- Gu, H.; Ho, P.L.; Tong, E.; Wang, L.; Xu B.(2003) // Nano Lett. V. 3. P. 1261–1263.**
- Guo, S.; Huang, Y.; Jiang, Q.; Sun, Y.; Deng, L.; Liang, Z. ... & Liang, X. J. (2010).** Enhanced gene delivery and siRNA silencing by gold nanoparticles coated with charge-reversal polyelectrolyte. *ACS nano*, 4(9): 5505-5511.
- Gupta, H.; Singh, D.; Vanage, G.; Joshi, D. & Thakur, M. (2018).** Evaluation of histopathological and ultrastructural changes in the testicular cells of Wistar rats post chronic exposure to gold nanoparticles. *Indian Journal of Biotechnology*, 17. 9-15.
- Guyton, A. C. and Hall, J. E. (2006).** Text book of Medical physiology. 11th edition W.B.Saunders; company ,Philadelphia.
- Guz, J.; Gackowski, D.; Foksinski, M.; Rozalski, R.; Zarakowska, E.; Siomek, A.; Szpila, A.; Kotzbach, M.; Kotzbach, R.; Olinski, R.(2013).** Comparison of oxidative stress/DNA damage in semen and blood of fertile and infertile men. *Plos One*. 8:e68490

References

- Habas, K.; Demir, E.; Guo, C.; Brinkworth, M. H. and Anderson, D. (2021).** Toxicity mechanisms of nanoparticles in the male reproductive system. *Drug metabolism reviews*, 53(4): 604–617.
- Hansson, G.C.(2012).** Role of mucus layers in gut infection and inflammation. *Curr Opin Microbiol.* 15(1):57–62.
- Hassan, A. A.; Abdoon, A. S. S.; Elsheikh, S. M.; Khairy, M. H.; Gamaleldin, A. A., & Elnabtity, S. M. (2019).** Effect of acute gold nanorods on reproductive function in male albino rats: histological, morphometric, hormonal, and redox balance parameters. *Environmental Science and Pollution Research*, 26(16): 15816-15827.
- Hickman, C.P.; Roberts, L.S. and Larson, A. (1993).** Integrated principles of zoology. Baltimore:100-150.
- Hong, R., Han, G.; Fernández, J. M.; Kim, B. J.; Forbes, N. S. & Rotello, V. M. (2006).** Glutathione-mediated delivery and release using monolayer protected nanoparticle carriers. *Journal of the American Chemical Society*, 128(4):1078–1079.
- Hooker, C.W. (1970).**The intratubular tissue of testes. part 1. Chapter 8.: 483-556. printed in U.S.A.
- Hostetler, M.J.; Wingate, J.E.; Zhong, C.J.; Harris, J.E.; Vachet, R.W.; Clark, M.R.; Londono, J.D.; Green, S.J.; Stokes, J.J.; Wignall, G.D.; Glish, G.L.; Porter, M.D.; Evans, N.D; and Murray, R.W. (1998)** Alkanethiolate Gold Cluster Molecules with Core Diameters from 1.5 to 5.2 nm: Core and Monolayer Properties as a Function of Core Size. *Langmuir*, 14, 17-30.
- Hsin, Y.H.; Chen, C.F.; Huang, S.; Shih, T.S.; Lai, P.S.; Chueh, P.J.(2008).** The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. *Toxicol Lett*, 179:130-9.

References

- Hu, M.; Chen, J.; Li, Z. Y.; Au, L.; Hartland, G. V.; Li, X. ... & Xia, Y. (2006).** Gold nanostructures: engineering their plasmonic properties for biomedical applications. *Chemical Society Reviews*, 35(11):1084-1094.
- Huang, X.; Kang, B.; Qian, W.; Mackey, M. A.; Chen, P. C.; Oyelere, A. K. ... & El-Sayed, M. A. (2010).** Comparative study of photothermolysis of cancer cells with nuclear-targeted or cytoplasm-targeted gold nanospheres: continuous wave or pulsed lasers. *Journal of biomedical optics*, 15(5), 058002.
- Hubbs, A.F.; Mercer, R.R.; Benkovic, S.A. (2011).** Nanotoxicology-a pathologist's perspective. *Toxicol Pathol* 39:301-324.
- Hulkoti, N.I. and T.C. Taranath. 2014.** Biosynthesis of nanoparticles using microbes-a review. *Colloids and Surfaces B: Biointerfaces* 121: 474-483.
- Huo, Q.; Colon, J.; Cordero, A.; Bogdanovic, J.; Baker, C. H.; Goodison, S. & Pensky, M. Y. (2011).** A facile nanoparticle immunoassay for cancer biomarker discovery. *Journal of Nanobiotechnology*, 9(1), 1-12.
- Hussein, A.M.; AL-Sammarae, N.S. and Sadik, A.H.(1997).** The cyclic events of spermatogenesis and sperm the testes of adult rat. *Iraqi.J.Vet.Sci*, 1(6,7):116-120.
- Hudson, D. L.; Guy, A. T.; Fry, P.; O'Hare, M. J. ; Watt, F. M., & Masters J. R. (2001).** Epithelial cell differentiation pathways in the human prostate: identification of intermediate phenotypes by keratin expression. *Journal of Histochemistry & Cytochemistry*, 49(2), 271-278.

References

- Hussein, M. M.; Ali, H. A.; Saadeldin, I. M. & Ahmed, M. M. (2016).** Querectin alleviates zinc oxide nanoreprotoxicity in male albino rats. *Journal of biochemical and molecular toxicology*, 30(10):489-4.
- Ighodaro, O. M. and Akinloye, O. A. (2018).** First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria journal of medicine*, 54(4), 287-293.
- Islam, N. U.; Jalil, K.; Shahid, M.; Rauf, A.; Muhammad, N.; Khan, A.; ... & Khan, M. A. (2019).** Green synthesis and biological activities of gold nanoparticles functionalized with *Salix alba*. *Arabian Journal of Chemistry*, 12(8), 2914-2925.
- Jaclyn, E.; Canas, B.Q.; Shibin, L.; Jonathan, D. (2011).** Acute and reproductive toxicity of nano-sized metal oxides (ZnO and TiO₂) to earthworms (*Eisenia fetida*) J. Environ. Monit. Advance. Article.
- Jain, P. K.; Lee, K. S.; El-Sayed, I. H. & El-Sayed, M. A. (2006).** Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. *The journal of physical chemistry. B*, 110(14), 7238–7248.
- Johnston, H.; Baker, P.J.; Abel, M.; Charlton, H.M.; Jackson, G.; Fleming, L.; Kumar, T. R. And O'shanghnessy, P.J. (2004).** Regulation of sertoli cell number and activity by follicle stimulating hormone and androgen during postnated development in the mouse. *Endocrinal*, 145 (1): 318-329. 162.
- Joshi, H.M.; Bhumkar, D.R.; Joshi, K.; Pokharkar, V.(2006).** Sas-try M. // *Langmuir*. V. 22: 300–305.
- Kalthoff, K. (1996).** Analysis of Biological development. McGraw-Hill, INC. Printed in U.S.A. Chapter 3: 45-54.

References

- Kalynovskyi, V. Y.; Pustovalov, A. S.; Grodzyuk, G. Y.; Andriushyna, N. S. & Dzerzhynsky, M. E. (2016).** Effect of gold and silver nanoparticles on the morpho-functional state of the epididymis and prostate gland in rats. *Regulatory Mechanisms in Biosystems*, 7(2), 106-111.
- Kasperek, K.; Iyengar, G.V.; Kiem, J.; Borberg, H.; Feinendegen, L.E.(1979).** Elemental composition of platelets. Part III. Determination of Ag, Au, Cd, Co, Cr, Cs, Mo, Rb, Sb, and Se in normal human platelets by neutron activation analysis. *Clin Chem.* 25:711-5.
- Kennedy, L. C.; Bear, A. S.; Young, J. K.; Lewinski, N. A.; Kim, J.; Foster, A. E. & Drezek, R. A. T. (2011).** Cells Enhance Gold Nanoparticle Delivery to Tumors. *Vivo. Nanoscale Res. Lett.* 6, 283.
- Khan, I.; Saeed, K. and Khan, I. (2019).** Nanoparticles: properties, applications and toxicities. *Arab J Chem.* 12(7):908–931.
- Khanna, P.; Ong, C.; Bay, B.H.; Baeg, G.H. (2015).** Nanotoxicity: an interplay of oxidative stress, inflammation and cell death. *Nanomaterials (Basel).* 5(3):1163–1180
- Khlebtsov, N. and Dykman, L. (2011).** Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies. *Chemical Society Reviews*, 40(3):1647-1671.
- Kim, B.; Han, G.; Toley, B. J.; Kim, C. K.; Rotello, V. M. & Forbes, N. S. (2010).** Tuning payload delivery in tumour cylindroids using gold nanoparticles. *Nature nanotechnology*, 5(6), 465–472.
- Kim, C. K.; Ghosh, P.; Pagliuca, C.; Zhu, Z. J.; Menichetti, S. & Rotello, V. M. (2009).** Entrapment of hydrophobic drugs in nanoparticle monolayers with efficient release into cancer cells. *Journal of the American Chemical Society*, 131(4):1360–1361.

References

- Knez, J. (2013).** Endocrin e-disrupting chemicals and male reproductive health. *Reprod Biomed Online*. 26(5):440–448.
- Knol, B. W. (1991).** Stress and the endocrine hypothalamus pituitary testis system: A review. 13(2) :104-114.
- Koga, K.; Izumi, G.; Mor, G.; Fujii, T.; Osuga, Y. (2014).** Toll-like receptors at the maternal-fetal interface in normal pregnancy and pregnancy complications. *Am J Immunol*. 72(2):192-205.
- Kosasa, T.S. (1981).** Measurement of Human Luteinizing Hormone. *Journal of Reproductive Medicine*, 26, 201-6.
- Kovalev, I.E.; Polevaya, O.Y.; Biokhimicheskie, osnovy, k.(1985)** immuniteta nizkomolekulyarnim khimicheskim soed-ineniyam (*Biochemical Foundations of Immunity to Low-molecular Chemical Compounds*). Moscow: Nauka, . 304 p.
- Kuiri-Hänninen, T.; Sankilampi, U.; and Dunkel, L. (2014).** Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. *Hormone research in paediatrics*, 82(2): 73-80.
- Kumar, H.; Bhardwaj, K.; Dhanjal, D. S.; Nepovimova, E.; Şen, F.; Regassa, H.; Singh, R.; Verma, R.; Kumar, V.; Kumar, D.; Bhatia, S. K. & Kuča, K. (2020).** Fruit Extract Mediated Green Synthesis of Metallic Nanoparticles: A New Avenue in Pomology Applications. *International journal of molecular sciences*, 21(22):8458.
- Kumari, Y., Kaur, G.; Kumar, R.; Singh, S.K.; Gulati, M.; Khursheed, R.; Clarisse, A.; Gowthamarajan, K.; Karri, V.; Mahalingam, R.; Ghosh, D.; Awasthi, A.; Kumar, R.; Yadav, A.; Kapoor, B.; Singh, P.; Dua, K.; Porwal, O.(2019).** *Adv. Colloid Interface Sci.* 274, 102037.

References

- Kyprianou, N.; Tu, H. & Jacobs, S. C. (1996).** Apoptotic versus proliferative activities in human benign prostatic hyperplasia. *Human pathology*, 27(7): 668-675.
- Langer, C.; Jürgensmeier, J.M.; Bauer, G.(1996).** Reactive oxygen species act at both TGF--dependent and -independent steps during induction of apoptosis of transformed cells by normal cells. *Exp. Cell Res.* 222:117–124.
- Lasagna-Reeves, C.; Gonzalez-Romero, D.; Barria, M. A.; Olmedo, I.; Clos, A.; Ramanujam, V. S. ... & Soto, C. (2010).** Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice. *Biochemical and biophysical research communications*, 393(4), 649-655.
- Lee, K. L. and Peehl, D. M. (2004).** Molecular and cellular pathogenesis of benign prostatic hyperplasia. *The Journal of urology*, 172(5): 1784-1791.
- Li, W.Q.; Wang F.; Liu, Z.M.; Wang, Y.C.; Wang, J.; Sun, F.(2013).** Gold nanoparticles elevate plasma testosterone levels in male mice without affecting fertility. *Small.* 9:1708-14.
- Li, C.; Taneda, S.; Taya, K.; Watanabe, G.; Li, X.; Fujitani, Y. ... & Suzuki, A. K. (2009).** Effects of in utero exposure to nanoparticle-rich diesel exhaust on testicular function in immature male rats. *Toxicology letters*, 185(1), 1-8.
- Li, X.; Guo, J.; Asong, J.; Wolfert, M. A. & Boons, G. J. (2011).** Multifunctional surface modification of gold-stabilized nanoparticles by bioorthogonal reactions. *Journal of the American Chemical Society*, 133(29), 11147-11153.
- Lindgren, I.; Giwercman, A.; Axelsson, J.; and Giwercman, Y. L. (2012).** Association between follicle-stimulating hormone receptor polymorphisms and reproductive parameters in young men from the

References

- general population. *Pharmacogenetics and genomics*, 22(9): 667-672.
- Liu, Y.; Li, X.; Xiao, S.; Liu, X.; Chen, X.; Xia, Q.; Lei, S.; Li, H.; Zhong, Z. & Xiao, K. (2020).** The Effects of Gold Nanoparticles on Leydig Cells and Male Reproductive Function in Mice. *International journal of nanomedicine*, 15: 9499–9514
- Liu, W.; Halverson, J.; Tian, Y.; Tkachenko, A. V. & Gang, O. (2016).** Self-organized architectures from assorted DNA-framed nanoparticles. *Nature chemistry*, 8(9): 867-873.
- Lynch, I.; Cedervall, T.; Lundqvist, M.; Cabaleiro-Liu, W.; Halverson, J., Tian, Y.; Tkachenko, A. V. & Gang, O. (2016).** Self-organized architectures from assorted DNA-framed nanoparticles. *Nature chemistry*, 8(9): 867-873.
- Latayia Aaron, B.S.; Omar Franco, M.D.; Ph.D.; and Simon W.Hayward, Ph.D.(2016).** Review of Prostate Anatomy and Embryology and the Etiology of BPH.
- Lago, C.; Linse, S.; Dawson, K.A.(2007).** The nanoparticle–protein complex as a biological entity; a complex fluids and surface science challenge for the 21st century. *Adv Colloid Interface Sci*; 134–135:167–174.
- Lynch, I.; Dawson, K.A.(2008).** Protein-nanoparticle interactions. *Nano Today*. 3:40–47.
- Madkour, L. H.(2019).** Introduction to nanotechnology (NT) and nanomaterials (NMs). In *Nanoelectronic Materials* (pp. 1-47). Springer, Cham.
- Maitra, S. K. and Mitra, A. (2008).** Testicular functions and serum titers of LH and testosterone in methyl parathion-fed roseringed parakeets. *Ecotoxicology and environmental safety*, 71(1):236-244.
- Majoumouo, M.S.; Sharma, J.R.; Sibuyi, N.R.; Tincho, M.B.; Boyom, F.; Meyer, M.(2020).** *Molecules*. 25, 1–18.

References

- Mancini, A.; Milardi, D.; Bianchi, A.; Summaria, V. & De Marinis, L. (2005).** Increased estradiol levels in venous occlusive disorder: a possible functional mechanism of venous leakage. *International journal of impotence research*, 17(3), 239–242.
- Mangalampalli, B.; Dumala, N. & Grover, P. (2017).** Acute oral toxicity study of magnesium oxide nanoparticles and microparticles in female albino Wistar rats. *Regulatory Toxicology and Pharmacology*, 90:170-184.
- Manin, O.I.; Nikolaev, V.A.; Kolomiitsev, A.A.; Lebedenko, I. (2007).** Comparative toxicological evaluation of domestic golden alloys for soldering. *Stomatologiya*, 86(1):64–67.
- Manke, A.; Wang, L.; Rojanasakul, Y. (2013).** Mechanisms of nanoparticle-induced oxidative stress and toxicity. *Biomed Resint.* 942916.
- Massarsky, A.; Trudeau, V.L.; Moon, T.W. (2014).** Predicting the environmental impact of nanosilver. *Environ Toxicol Pharmacol.* 38(3):861–873.
- Mathias, F.T.; Romano, R.M.; Kizys, M.M.; Kasamatsu, T.; Giannocco, G.; Chiamolera, M.I.; Dias-da-Silva, M.R.; Romano, M.A. (2015).** Daily exposure to silver nanoparticles during prepubertal development decreases adult sperm and reproductive parameters. *Nanotoxicology.* 9(1):64–70
- McLachlan, R. I.; O'Donnell, L. and Meachem, S. J. (2002).** The hormonal regulation of spermatogenesis in primates and man Insights for the development of the male contraceptive. *J. Androl*, 23:149–162.
- Mcneilly, A. S.; Crawford, J. L.; Taragnat, C.; Nicol, L.; and Mcneilly, J. R. (2003).** The differential secretion of FSH and LH: regulation

References

through genes, feedback and packaging. reproduction-cambridgesupplement, 463-476.

- Mc Mahon, A. P.; Aronow, B. J.; Davidson, D. R.; Davies, J. A.; Gaido, K. W.; Grimmond, S. ... & Zhang, P. (2008).** GUDMAP: the genitourinary developmental molecular anatomy project. *Journal of the American Society of Nephrology*, 19(4) :667-671.
- Medley, C. D.; Smith, J. E.; Tang, Z.; Wu, Y.; Bamrungsap, S. & Tan, W. (2008).** Gold nanoparticle-based colorimetric assay for the direct detection of cancerous cells. *Analytical chemistry*, 80(4): 1067-1072.
- Mehanna, E. T.; Kamel, B.; Abo-Elmatty, D. M.; Elnabtity, S. M.; Mahmoud, M. B.; Abdelhafeez, M. M. & Sabry S Abdoon, A. (2022).** Effect of gold nanoparticles shape and dose on immunological, hematological, inflammatory, and antioxidants parameters in male rabbit. *Veterinary world*, 15(1): 65–75.
- Meng, J.; Holdcraft, R.W.; Shima, J.E.; Griswold, M.D.; Braun, R.E.(2005).** Androgens regulate the permeability of the blood-testis barrier. *Proc Natl Acad Sci U S A*. 102:16696-700
- Menon, S.; Rajeshkumar, S. & Kumar, V. (2017).** A review on biogenic synthesis of gold nanoparticles, characterization, and its applications. *Resource-Efficient Technologies*, 3(4): 516-527.
- Mie, G. J. A. P. (1908).** Articles on the optical characteristics of turbid tubes, especially colloidal metal solutions. *Ann. Phys*, 25(3):377-445.
- Mikuz G. (2019).** Ectopias of the kidney, urinary tract organs, and male genitalia. *Ektopien der Niere, Harnwege und männlichen Geschlechtsorgane. Der Pathologe*, 40(Suppl 1), 1–8.

References

- Mohamed, M. B.; Volkov, V.; Link, S. & El-Sayed, M. A. (2000).** Thelighting'gold nanorods: fluorescence enhancement of over a million compared to the gold metal. *Chemical Physics Letters*, 317(6): 517-523.
- Moretti, E.; Terzuoli, G.; Renieri, T.; Iacoponi, F.; Castellini, C.; Giordano, C., Collodel, G.(2013).** In vitro effect of gold and silver nanoparticles on human (spermatozoa. *Andrologia*. 45:392-6.
- Morgan, A. M.; Ibrahim, M. A. & Noshay, P. A. (2017).** Reproductive toxicity provoked by titanium dioxide nanoparticles and the ameliorative role of Tiron in adult male rats. *Biochemical and biophysical research communications*, 486(2), 595–600.
- Morgan, A. M.; Abd El-Hamid, M. I. and Noshay, P. A. (2015).**Reproductive toxicity investigation of titanium dioxide nanoparticles in male albino rats. *World J. Pharm. Pharm.Sci.*, 4(10): 34-49.
- Morishita, Y.; Yoshioka, Y.; Satoh, H.; Nojiri, N.; Nagano, K.; Abe, Y. ... & Tsutsumi, Y. (2012).** Distribution and histologic effects of intravenously administered amorphous nanosilica particlesin the testes of mice. *Biochemical and biophysical research communications*, 420(2):297-301.
- Mukherjee, P.; Bhattacharya, R.; Wang P., Wang L.; Basu S.; Nagy J.A., Atala A.; Mukhopadhyay D., Soker S.(2005) // Clin. Cancer Res. V. 11. P. 3530–3534.**
- Mukherjee, P.; Bhattacharya, R.; Bone, N.; Lee, Y. K.; Patra, C. R.; Wang, S., Lu, L., Secreto, C., Banerjee, P. C., Yaszemski, M. J., Kay, N. E. & Mukhopadhyay, D. (2007).** Potential therapeutic application of gold nanoparticles in B-chronic lymphocytic leukemia (BCLL): enhancing apoptosis. *Journal of nanobiotechnology*, 5, 4.

References

- Nakanishi, J.; Nakayama, H.; Shimizu, T.; Ishida, H.; Kikuchi, Y.; Yamaguchi, K. & Horiike, Y. (2009).** Light-regulated activation of cellular signaling Society, 131by gold nanoparticles that capture and release amines. *Journal of the American Chemical* (11): 3822-3823.
- Nazar, M.; Talebi, A.R.; Hosseini, S.M.; Abbasi, A.; Khoradmehr, A.; Danafar, A.H. (2016).** Acute and chronic effects of gold nanoparticles on sperm parameters and chromatin structure in Mice. *Int J Reprod Biomed (Yazd)*. 14:637-42.
- Negahdary, M.; Arefian, Z.; Dastjerdi, . HA.; Ajdary, M. (2015).** Toxic effects of Mn₂O₃ nanoparticles on rat testis and sex hormone. *J Nat Sci Biol Med*. 6(2):335–339.
- Newsholme, E. and Leech, T. (2010).** “ Functional Biochemistry in Health and Disease”. Wiley-Blackwell Publishing.p.398.
- Nishino, T.; Wedel, T.; Schmitt, O.; Buhlmeyer, K.; Schonfelder, M.; Hi Schulz, T.; Kühnel, W. and Michna, H. (2004).** Androgen dependent morphology of prostate and seminal vesicle in the Hershberger Assay: *Evaluation of immune-histochemical and morphometric parameters*. *Ann. Anat.*, 189:247-253.
- Niska, K.; Zielinska, E.; Radomski, M.W.; Inkielewicz-Stepniak, I. (2018).** Metal nanoparticles in dermatology and cosmetology: *interactions with human skin cells*. *Chem Biol Interact*. 295:38–51.
- Nkansah, P.O. (2013).** In vitro and in vivo testing of ultra –small gold nanoparticles as a novel drug delivery platform for enhancing the brain penetration and receptor binding affinity of central nervous system drugs. Doctoral Thesis submitted to University of Michigan.
- Noorafshan, A. and Karbalay-Doust, S. (2012).** Curcumin protects the seminal vesicles from metronidazole-induced reduction of secretion in mice. *Acta Medica*, 55:32-36. rtreiter, C.;

References

- Noruzi, M. (2015).** Biosynthesis of gold nanoparticles using plant extracts. *Bioprocess and biosystems engineering*, 38(1): 1-14. of joints aided by an etanercept-conjugated gold nanoparticle contrast agent—an ex vivo preliminary rat study *Nanotechnology* 19 095101
- Olugbodi, J.O. ; David, O.; Oketa, E.N. ; Lawal, B. ; Okoli, B.J. ; Mtunzi F. (2020).** Silver nanoparticles stimulates spermatogenesis impairments and hematological alterations in testis and epididymis of male rats. *Molecules*. 25(5):1063.
- Omar, A. I. & Kamar, S. S. (2021).** Does repeated gold-nanoparticles administration affect pars distalis hormonal and folliculo-stellate cells in adult male albino rats?. *Folia histochemica et cytobiologica*, 59(2): 95-107.
- Orabi, S. H. ; Mansour, D. A. ; Fathalla, S. I. ; Gadallah, S. M. ; ELDin, A. A. G. & Abdoon, A. S. S. (2020).** Effects of administration of 10 nm or 50 nm gold nanoparticles (AuNPs) on blood profile, liver and kidney functions in male albino rats. *Indian Journal of Biochemistry and Biophysics (IJBB)*, 57(4), 486-493.gggg.
- Osman, D.I. and Plöen, L. (1986) :** Fine structure of epididymal spermatozoain the camel (*C. dromedaries*). *Anim. Reprod. Sci.* 10: 177-184
- Paciotti, G.F. ; Kingston D.G.I. ; Tamarkin L.(2006) // Drug Dev. Res. V. 67. P. 47–54.**
- Paciotti, G.F. ; Myer L. ; Weinreich D. ; Goia, D. ; Pavel, N. ; McLaughlin, R.E. ; Tamarkin L.(3004) // Drug Deliv.V. 11. P. 169–183.192.**
- Page, S. T.; Lin, D. W.; Mostaghel, E. A.; Hess, D. L.; True, L. D.; Amory, J. K.; Nelson, P. S.; Matsumoto, A. M.; Bremner,Reference104 W. J. (2006).** "Persistent Intraprostatic Androgen Concentrations after Medical Castration in Healthy

References

- Men". *Journal of Clinical Endocrinology & Metabolism*. 91 (10):3850–6. Palmieri, B., & Sblendorio, V. (2007). Oxidative stress tests: *overvi* .
- Park, E.J. ; Bae, E. ; Yi, J. ; Kim, Y. ; Choi, K. ; Lee, S.H. ; Yoon, J. ; Lee, B.C. ; Park, K. (2010).** Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles. *Environ Toxicol Pharmacol*. 30:162-8.
- Patel, P.C.; Giljohann, D.A., Daniel, W.L.; Zheng, D.; Pri-godich, A.E.; Mirkin, C.A.(2011).** // *Bioconjug. Chem*. V. 21. P. 2250–2256.
- Pawar, K.; Kaul, G.(2012).** Toxicity of titanium oxide nanoparticles causes functionality and DNA damage in buffalo (*Bubalus bubalis*) sperm in vitro. *Toxicol Ind Health* ; 30: 520-533.
- Payne, A. H.; Hardy, M.P. and Russell, L.D. (1996).** The Leydig cell. Cache River press: 1-802.
- perez-Labrada, F.; Lopez-Vargas, E.R.; Ortega-Ortiz, H.; Pliego, G.; Benavides-Mendoza. A.; Juarez-Maldonado, A.(2019).** Responses of tomato plants under saline stress to foliar application of copper nanoparticles. *Plants*. 8(6):151.
- Perrault, S. D.; Walkey, C.; Jennings, T.; Fischer, H. C. & Chan, W. C. (2009).** Mediating tumor targeting efficiency of nanoparticles through design. *Nano letters*, 9(5): 1909-1915.
- Pineda, M.H. and Dooley, M.P. (2003).** Mc Donald's veterinary Endocrinology and reproduction. 5th ed. *A black well publishing company* : 239-265 .
- Pinho, A.R.; Rebelo, S.; Pereira, M.L. (2020).** The impact of zinc oxide nanoparticles on male (In) fertility. *Materials*. 13(4): 849.
- Pissuwan, D.; Niidome, T.; Cortie, M.B.(2011).** // *J. Control. Re-lease*. V. 149: 65–71.

References

- Podsiadlo, P.; Sinani, V. A.; Bahng, J. H.; Kam, N. W. S.; Lee, J. & Kotov, N. A. (2008).** Gold nanoparticles enhance the anti-leukemia action of a 6-mercaptopurine chemotherapeutic agent. *Langmuir*, 24(2):568-574.
- Popa, S. M.; Clifton, D. K. and Steiner, R. A. (2008).** The role of kisspeptins and GPR54 in the neuroendocrine regulation of reproduction. *Annu. Rev. Physiol*, 70: 213-238.
- Pow, D.V.; Crook, D.K.(1993) // J. Neurosci. Meth. V. 48. P. 51–63.**
- Rajasekar, T.; Karthika, K.; Muralitharan, G.; Maryshamya, A.; Sabarika, S.; Anbarasu, S.; Revathy, K.; Prasannabalaji, N.; & Kumaran, S. (2020).** Green synthesis of gold nanoparticles using extracellular metabolites of fish gut microbes and their antimicrobial properties. *Brazilian journal of microbiology : (publication of the Brazilian Society for Microbiology)*, 51(3):957–967.
- Rana, A.; Yadav, K.; Jagadevan, S. (2020).** A comprehensive review on green synthesis of nature-inspired metal nanoparticles: Mechanism, application and toxicity. *Journal of Cleaner Production*, 272, 122880.
- Rao, P.V. and Gan, S.H. (2015).** Recent advances in nanotechnology-based diagnosis and treatments of diabetes. *Current Drug Metabolism*, 16: 371-375.
- Rastrelli, G.; Maggi, M. and Corona, G. (2018).** Pharmacological management of late-onset hypogonadism. *Expert review of clinical pharmacology*, 11(4):439-458.
- Richthoff, J.; Spano, M.; Giwercman, Y.L. et al.(2002).** The impact of testicular and accessory sex gland function on sperm chromatin integrity as assessed by the sperm chromatin structure assay (SCSA). *Human Reproduction* 17: 3162–3169.

References

- Rosemary, M.J.; MacLaren, I.; Pradeep, T.(2006)** // *Langmuir*. V. 22. P. 10125–10129.
- Rosi, N. L.; Giljohann, D. A.; Thaxton, C. S.; Lytton-Jean, A. K.; Han, M. S. & Mirkin, C. A.(2006)**. Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. *Science*, 312(5776): 1027-1030.
- Saake, R.G. (1982)**. Components of semen quality. *J. Anim. Sci. Ss.*, 1: 1-13.
- Saha, B.; Bhattacharya, J.; Mukherjee, A.; Ghosh, A.K.; Santra, C.R.; Dasgupta, A.K.; Karmakar, P.(2007)** // *Nanoscale Res. Lett.* V. 2. P. 614–622.
- Saleh, R.A.; Agarwal, A.; Nada, E.A. et al. (2003)** .Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. *Fertility and Sterility*, 79 (Suppl. 3), 1597–1605.
- Sastry, M.S. and Gupta, S.S. (2011)**. Male reproductive toxicity of DMPA on seminal vesicle of Indian Palm Squirrel, *Funambulus pennant*. *Int. J. Sci. Nat.*, 2 (4):764-768.
- Savic, I.; Berglund, H.& Lindström, P. (2005)**. Brain response to putative pheromones in homosexual men. *Proceedings of the National Academy of Sciences of the United States of America*, 102(20): 7356 -7361
- Sau, T. K. and Rogach, A. L. (2012)**. Colloidal synthesis of noble metal nanoparticles of complex morphologies. *Complex-Shaped Metal Nanoparticles*, 7-90.
- Schrand, A. M.; Rahman, M. F.; Hussain, S. M.; Schlager, J. J.; Smith, D. A., & Syed, A. F. (2010)**. Metal-based nanoparticles and their toxicity assessment. *Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology*,2(5), 544–568.

References

- Selvaraj, V. & Alagar, M. (2007).** Analytical detection and biological assay of antileukemic drug 5-fluorouracil using gold nanoparticles as probe. *International journal of pharmaceutics*, 337(1-2): 275-281.
- Shalviri, A.; Cai, P.; Rauth, A.M.; Henderson, J.T., Wu, X.Y. (2012).** Evaluation of new bi-functional terpolymeric nanoparticles for simultaneous in vivo optical imaging and chemotherapy of breast cancer. *Drug Deliv Transl Res.* 2(6):437–453.
- Sharma, S.; Hanukoglu, A.; Hanukoglu, I.(2018).** Localization of epithelial sodium channel (ENaC) and CFTR in the germinal epithelium of the testis, Sertoli cells, and spermatozoa. *J Mol Histol.* 49:195-208.
- Sharma, R. K. and Agarwal, A. (1996).** Role of reactive oxygen species in male infertility . *Urology*, 48(6) : 835 – 850.
- Shen, J.; Yang, D.; Zhou, X.; Wang, Y.; Tang, S.; Yin, H.; Wang, J.; Chen, R.; Chen, J. (2019).** Role of autophagy in zinc oxide nanoparticles-induced apoptosis of mouse LEYDIG cells. *Int J Mol Sci.* 20(16):4042.
- Shiosaka, S.; Kiyama, H.; Wanaka, A. & Masaya, T. (1986).** A new method for producing a specific and high titre antibody against glutamate using colloidal gold as a carrier. *Brain research*, 382(2): 399-403.
- Sikka, S. C.; Rajasekaran, M.and Hellstrom, W. J. (1995).** Role of oxidative stress and antioxidants in male infertility. *J. Androl.*,16:464-481.
- Simoni, M.; Gromoll, J. and Nieschlag, E. (1997).** The folliclestimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology . *Endocrine reviews*, 18(6):739-773.
- Singh, B.N.; Prateeksha, Gupta, V.K.; Chen, J.; Atanasov, A.G.(2017)** .Organic nanoparticle-based combinatory approaches for gene therapy. *Trends Biotechnol.* ; 35:1121-4

References

- Sleiman, H.K.; Romano, R.M.; Oliveira, C.A.; Romano, M.A. (2013).** Effects of prepubertal exposure to silver nanoparticles on reproductive parameters in adult male Wistar rats. *J Toxicol Environ Health A*. 76(17):1023–1032.
- Sonavane, G.; Tomoda, K. & Makino, K. (2008).** Biodistribution of colloidal gold nanoparticles after intravenous administration: effect of particle size. *Colloids and Surfaces B: Biointerfaces*, 66(2): 274-280.
- Sopjani, M.; Foller, M.; Gulbins, E. & Lang, F. (2008).** Suicidal death of erythrocytes due to selenium compounds, *Cell Physiol Biochem*, 22 :387–394.
- Soto, K. M.; Mendoza, S.; López-Romero, J. M.; Gasca-Tirado, J. R., & Manzano-Ramírez, A. (2021).** Gold nanoparticles: synthesis, application in colon cancer therapy and new approaches-review. *Green Chemistry Letters and Reviews*, 14(4), 665-678.
- Soto, K. M.; Quezada-Cervantes, C. T.; Hernández-Iturriaga, M.; Luna-Bárceñas, G.; Vazquez-Duhalt, R.; & Mendoza, S. (2019).** Fruit peels waste for the green synthesis of silver nanoparticles with antimicrobial activity against foodborne pathogens. *Lwt*, 103, 293-300.
- Souza, M. R.; Mazaro-Costa, R. & Rocha, T. L. (2021).** Can nanomaterials induce reproductive toxicity in male mammals? A historical and critical review. *The Science of the total environment*, 769:144354.
- Stefan, M.; Melnig, V.; Pricop, D.; Neagu, A.p.; Mihasan, M.; Tartau, L. & Hritcu, L. (2013).** Attenuated effects of chitosan-capped gold nanoparticles on LPS-induced toxicity in laboratory rats. *Materials science & engineering. C, Materials for biological applications*, 33(1): 550–556.

References

- Suvarna, K. S.; Layton, C. and Bancroft, J. D. (2013).** Bancroft's Theory and Practice of Histology Techniques. 7th ed. Elsevier Health Sciences.
- Sylvestre, J. P.; Kabashin, A. V. E.; Sacher, M.; Meunier, J. H. T.; Luong, (2004).** —Stabilization and size control of gold nanoparticles during laser ablation in aqueous cyclodextrins, *J. Am. Chem. Soc.* vol.126, pp.7176-7177, 200.
- Taha, M. N. (2017).** The Effect of Copper Nanoparticles on Seminal Vesicles and Testosterone Hormone in Male Albino Mice. *Ibn AL-Haitham Journal For Pure and Applied Science*, 29(2), 310-319.
- Taylor, U.; Barchanski, A.; Garrels, W., Klein, S.; Kues, W.; Barcikowski, S.; Rath, D. (2012).** Toxicity of gold nanoparticles on somatic and reproductive cells. *Adv Exp Med Biol.* 733: 125–133
- Teimouri, M.; Khosravi-Nejad, F.; Attar, F.; Saboury, A. A.; Kostova, I.; Benelli, G. & Falahati, M. (2018).** Gold nanoparticles fabrication by plant extracts: synthesis, characterization, degradation of 4-nitrophenol from industrial wastewater, and insecticidal activity—a review. *Journal of Cleaner Production*, 184: 740-753..
- Thakur, M.; Gupta, H.; Singh, D.; Mohanty, I. R.; Maheswari, U.; Vanage, G. & Joshi, D. S. (2014).** Histopathological and ultra structural effects of nanoparticles on rat testis following 90 days (Chronic study) of repeated oral administration. *Journal of nanobiotechnology*, 12:42.
- Tietz, N.W.(1995).** Clinical guide to laboratory tests. 3rd ed. Philadelphia, WB Saunders Co.
- Tjionas, G. A.; Epstein, J. I.; Williamson, S. R.; Diaz, M.; Menon, M.; Peabody, J. O. ; Gupta, N. S.; Parekh, D. J.; Cote, R. J.; Jorda, M., & Kryvenko, O. N. (2015).** Average weight of seminal vesicles: An adjustment factor for radical prostatectomy specimens weighed

References

- with seminal vesicles. *International Journal of Surgical Pathology*, 23(8), 617–622
- Tsai, M.; Lu, Z.; Wang, J.; Yeh, T. K.; Wientjes, M. G.; & Au, J. L. S.** (2007). Effects of carrier on disposition and antitumor activity of intraperitoneal paclitaxel. *Pharmaceutical research*, 24(9), 1691-1701.
- Tornøe, C.W.; Agersø, H.; Senderovitz, T.; Nielsen, H.A.; Madsen, H.; Karlsson, M.O. & Jonsson, E.N.** (2007). Population pharmacokinetic/pharmacodynamic (PK/PD) modelling of the hypothalamic-pituitary-gonadal axis following treatment with GnRH analogues. *British journal of clinical pharmacology*, 63 6, 648-64 .
- Toderas, F.; Baia, M.; Maniu, D. & Astilean, S.** (2008). Tuning the plasmon resonances of gold nanoparticles by controlling their size and shape. *Journal of optoelectronics and advanced materials*, 10(9):2282-2284.
- Tsuji, T.; Iryo, K.; Watanabe, N.; Tsuji, M.** (2002). —Preparation of silver nanoparticles by laser ablation in solution: Influence of laser wavelength on particle size. *Appl. Surf. Sci. vol.202:80-85.*
- Turkevich, J.; Stevenson, P.C. & Hillier, J.** (1951). A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discussions of The Faraday Society*, 11, 55-75.
- Uehara, N.** (2010). Polymer-functionalized gold nanoparticles as versatile sensing materials. *Analytical Sciences*, 26(12):1219-1228.
- Van de Broek, B.; Devoogdt, N.; D'Hollander, A.; Gijs, H. L.; Jans, K.; Lagae, L.; ... & Borghs, G.** (2011). Specific cell targeting with nanobody conjugated branched gold nanoparticles for photothermal therapy. *ACS nano*, 5(6):4319-4328.

References

- Van Gelder, M.M.; van Rooij, I.A.; Miller, R.K.; Zielhuis, G.A. de Jong-van, D.B.L.; Roeleveld, N.(2010).** Teratogenic mechanisms of medical drugs. *Hum Reprod Update*. 16:378-94.
- Velikorodnaya, Y.I.; Pocheptsov, A.Y.; Sokolov, O.I.; Bogatyrev, V.A.; Dykman, L.A. (2015).**Effect of gold nanoparticles on proliferation and apoptosis during spermatogenesis in rats. *Nanotechnologies in Russia*. 10:814-9.
- Verma, A. and Stellacci, F. (2010).** Effect of surface properties on nanoparticle-cell interactions. *Small (Weinheim an der Bergstrasse, Germany)*, 6(1): 12–21.
- Verma, A.; Uzun, O.; Hu, Y.; Hu, Y.; Han, H. S.; Watson, N. ... & Stellacci, F. (2008).** Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. *Nature materials*, 7(7): 588-595.
- Vines, T. and Faunce, T.(2009).** Assessing the safety and cost-effectiveness of early nanodrugs. *J Law Med*.16:822-45.
- Villers, A. (1994).** Anatomy of the prostate: insight into benign prostatic hyperplasia anatomy and pathogenesis. *Progress in clinical and biological research*, 386, 21–30.
- Wang, A.Z.; Bagalkot, V.; Vasilliou, C.C.; Gu, F.; Alexis, F.; Zhang, L.; Shaikh, M.; Yuet, K.; Cima, M.J.; Langer, R. (2008).** Superparamagnetic iron oxide nanoparticle–aptamer bioconjugates for combined prostate cancer imaging and therapy. *ChemMedChem*. 3(9):1311–1315.
- Wang, R.; Song, B.; Wu, J.; Zhang, Y.; Chen, A.; & Shao, L. (2018).** Potential adverse effects of nanoparticles on the reproductive system. *International journal of nanomedicine*, 13, 8487–8506.
- Wang, D. C. & Wang, J. Q. (2018).** Zhonghua nan ke xue = *National journal of andrology*, 24(4), 360–363.

References

- Waterman, M.R. and Keeney, D.S.(1992).** Genes involved in androgen biosynthesis and the male phenotype. *Horm Res.* 38(5–6):217–221.
- Wells, P.G.; Bhuller, Y.; Chen CS, Jeng, W., Kasapinovic, S.; Kennedy J.C.; Kim, P.M.; Laposa, R.R.; Mc Callum ,G.P.; Nicol, C.J.(2005).** Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. *Toxicol Appl Pharmacol.* 207:354-66.
- White, G. (1976).** Reproduction in the male. In-veterinary physiology. Chapter 26. Printed in U.K. : 671-698
- Wilson, R.(2008).** The use of gold nanoparticles in diagnostics and detection. *Chem Soc Rev.* 37:2028-4.
- Wilson, H. R.; Piesco, N. P.; Miller, E. R. & Nesbeth, W. G. (1979).** Prediction of the fertility potential of broiler breeder males. *World's Poultry Science Journal*, 35(2): 95-118.
- Wiwanitkit, V.; Sereemasapun, A.; Rojanathanes, R.(2009).** Effect of gold nanoparticles on spermatozoa: the first world report. *Fertil Steril.* 91:e7-e8.
- Wright, C.; Milne, S.; Leeson, H.(2014).** Sperm DNA damage caused by oxidative stress: modifiable clinical, lifestyle and nutritional factors in male infertility. *Reprod Biomed Online.* 28:684-703.
- Xia, Q.; Huang, J.; Feng, Q.; Chen, X.; Liu, X.; Li, X.; Zhang, T.; Xiao, S.; Li, H.; Zhong, Z.; , Xiao K. (2019).** Size- and cell type-dependent cellular uptake, cytotoxicity and in vivo distribution of gold nanoparticles. *Int J Nanomed*;14:6957–6970. doi:10.2147.
- Xin Lee, K.; Shameli, K.; Miyake, M.; Kuwano, N.; Bt Ahmad Khairudin, N. B.; Bt Mohamad, S. E. & Yew, Y. P. (2016).** Green synthesis of gold nanoparticles using aqueous extract of *Garcinia mangostana* fruit peels. *Journal of Nanomaterials*, 2016.

References

- Yahyaei, B.; Nouri, M.; Bakherad, S. et al.(2019)** Effects of biologically produced gold nanoparticles: toxicity assessment in different rat organs after intraperitoneal injection. *AMB Expr* 9, 38 .
- Yan, S.Q.; Xing, R.; Zhou, Y.F.; Li, K.L.; Su, Y.Y.; Qiu, J.F.; Zhang, Y.H.; Zhang, K.Q.; He, Y.; Lu, X.P. et al. (2016).** Reproductive toxicity and gender differences induced by cadmium telluride quantum dots in an invertebrate model organism. *Sci Rep.* 6(1):3418.
- Yang, L.; Kuang, H.; Zhang, W.; Aguilar, Z. P.; Wei, H. & Xu, H. (2017).** Comparisons of the biodistribution and toxicological examinations after repeated intravenous administration of silver and gold nanoparticles in mice. *Scientific reports*, 7(1): 3303.
- Yauk, C.; Polyzos, A., Rowan-Carroll, A.; Somers, C. M.; Godschalk, R. W.; Van Schooten, F. J.; Berndt, M. L.; Pogribny, I. P.; Koturbash, I.; Williams, A.; Douglas, G. R. & Kovalchuk, O. (2008).** Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location. *Proceedings of the National Academy of Sciences of the United States of America*, 105(2):605–610.
- Yeh, Y. C.; Creran, B. & Rotello, V. M. (2012).** Gold nanoparticles: preparation, properties, and applications in bionanotechnology. *Nanoscale*, 4(6): 1871-1880.
- Zhang, J.; Wang, L.; Zhang, H.; Boey, F.; Song, S. & Fan, C. (2010a).** Aptamer-based multicolor fluorescent gold nanoprobe for multiplex detection in homogeneous solution. *Small*, 6(2): 201-204.
- Zhang, X. D.; Wu, H. Y.; Wu, D.; Wang, Y. Y.; Chang, J. H.; Zhai, Z. B.; Meng, A. M.; Liu, P. X.; Zhang, L. A. & Fan, F. Y. (2010b).** Toxicologic effects of gold nanoparticles in vivo by different administration routes. *International journal of nanomedicine*, 5, 771–781.

References

- Zhang, X.; Zhang, Q.; Zhang, Z.; Na, Y. & Guo, Y. (2006).** Apoptosis profiles in benign prostatic hyperplasia: close associations of cell kinetics with percent area density of histologic composition. *Urology*, 68(4):905-910.
- Zhang, J. L.; Yuan, K.; Wang, M. Q.; Yan, J. Y.; Wang, Y. & Zhang, G. D. (2018).** Seminal vesicle abnormalities following prostatic artery embolization for the treatment of benign prostatic hyperplasia. *BMC urology*, 18(1): 92.
- Zhao, P.; Li, N. & Astruc, D. (2013).** State of the art in gold nanoparticle synthesis. *Coordination Chemistry Reviews*, 257(3-4): 638-665.
- Zilber, L. A. and Friese, W. W. (1929).** Über die antigene Eigenschaften kolloidaler Metalle. *Zh Eksp Biol*, 11, 128-135.
- Zuber, M. X.; Simpson, E. R. and Waterman, M. R. (1986).** Expression of bovine 17 α -hydroxylase cytochrome P-450 cDNA in nonsteroidogenic (COS 1) cells. *Science*, 234(4781): 1258-1261.

الخلاصة :

بينت الدراسة الحالية التأثيرات السمية الناتجة عن جسيمات الذهب النانوية، السمية النانوية على الجهاز التناسلي الذكري لذكور الجرذان (Au NPs) Gold nanoparticles (*Rattus norvegicus*) دراسة نسيجية و فسيولوجية وقياس المعايير الهرمونية مثل هرمون التستوستيرون Testosterone hormone (T) والهرمون اللوتيني (LH) Luteinizing hormone والهرمون المحفز للجريبات (FSH) Follicle stimulating hormone وهرمون الاستراديول Estradiol hormone (E) وقياس مستوى مضادات الاكسدة الكلية (T.A.O) Total antioxidant كما شملت دراسة التغيرات النسيجية لكل من الخصى testes والبربخ epididymis والبروستات prostate و الحوصلة المنوية vesicle seminal كما تضمنت النتائج دراسة Histomorphometric للخصى، قياس قطر النبيبات المنوية وتجويف النبيبات المنوية وسمك طبقة الظهارة الجرثومية للنبيب المنوي. والتغيرات الوزنية للجسم والاعضاء تم اجراء هذه الدراسة في البيت الحيواني التابع لكلية العلوم/جامعه بابل ووفقا لذلك تمت المعاملة حيث اجريت هذه الدراسة في جامعة بابل في مختبرات الانسجة التابعة لكلية العلوم ومختبرات مؤسسة الفاضل/بابل ومستشفى الحله التعليمي العام حيث شملت الدراسة ٤٢ من ذكور الجرذ الابيض المتعافي البالغة جنسيا التي تراوحت معدل اعمارها ما بين (١٠-١٢) اسبوع تراوحت معدل اوزانها ما بين (٢٠٠-٢٣٠) غرام. تمت الدراسة خلال المدة من شهر تشرين الثاني ٢٠٢١ لغاية نيسان ٢٠٢٢، وزعت الجرذان عشوائيا الى (٦) مجاميع بواقع سبعة جرذان في كل مجموعة وعى النحو التالي:

- المجموعة الاولى: مجموعة السيطرة السالبة شملت مجموعتين كل مجموعة (٧ جرذان) حقنت داخل الصفاق بالمحلول الملحي الفسلجي، لمدة ٣٠ و ٦٠ يوم.
- المجموعة الثانية: شملت (٧ جرذان) حقنت داخل الصفاق $40\mu\text{g}/\text{kg}$ من وزن الجسم من Au NPs المخففة في الماء المقطر لمدة ٣٠ يوم.
- المجموعة الثالثة: شملت (٧ جرذان) حقنت داخل الصفاق $80\mu\text{g}/\text{kg}$ من وزن الجسم من Au NPs المخففة في الماء المقطر لمدة ٣٠ يوم.
- المجموعة الرابعة: شملت (٧ جرذان) حقنت داخل الصفاق $40\mu\text{g}/\text{kg}$ من وزن الجسم من Au NPs المخففة في الماء المقطر لمدة ٦٠ يوم.

الخلاصة

• المجموعة الخامسة: شملت (٧ جردان) حقنت داخل الصفاق $80\mu\text{g}/\text{kg}$ من وزن الجسم من Au NP s المخففه في الماء المقطر لمدة ٦٠ يوم ملاحظة: الحقن كان كل ١٥ يوم اي مرتين بالشهر حيث كانت مدة الحقن كاملة ٤ مرات خلال ال ٦٠ يوم, حقنت بجسيمات الذهب حجم $20-50\text{nm}$.

بعد اتمام الحقن داخل الصفاق تم التضحية بها بعد ٣٠ و ٦٠ يوم حيث ان الحقن بجسيمات الذهب النانوية اظهرت تأثيرات نسيجية وفسولوجية ولكن بسمية اقل من بقية المعادن وهذا ما يميز الذهب النانوي عن غيره من المواد النانوية باعتباره مادة خاملة كيميائيا ولكن هذا لا يمنع ان تكون هناك سمية عند المعاملة على الامد البعيد اذ اظهرت النواتج انخفاضا معنويا ($p \leq 0.01$) ($p \leq 0.05$) للهرمونات (testosterone hormone, Follicle Stimulating hormone, Luteinizing, hormone) ومضادات الاكسدة الكلية لكن حسب مدة التعرض وتغيرات نسيجية متفاوتة حيث كانت اكثر تأثيرا بعد الحقن لمدة ٦٠ يوم مسببا تنكس وتقرح الخلايا الجرثومية والخلايا الخلالية (لايدك) واختراق الحاجز الدموي للخصية مسببا انخفاضا في هرمون التستوستيرون وارتفاع هرمون الاستراديول (Esradiol hormone) مسبب متلازمة فرط الاروماتيز. اما التغيرات الوزنية فقد كان هناك ارتفاع في وزن الجسم ولكن لم يصل الى المعنوية اما بالنسبة للأعضاء فان التغيرات الوزنية متباينة حيث انخفض وزن الخصيتين والبرابخ والحوصلة المنوية وارتفاع وزن البروستات مسبب تضخم البروستات الحميد مقارنة مع مجموعة السيطرة. نستنتج من هذه الدراسة ان جسيمات الذهب النانوية لها تأثير اقل سمية على الجهاز التناسلي الذكري, ويكون اكثر تأثيرا عند التركيز $80\mu\text{g}/\text{kg}$ لمدة ٦٠ يوم وتبقى الآثار الضارة معتمدة على التركيز و مدة التعرض.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم للبنات- قسم علوم الحياة

**دراسة نسيجة وفسولوجية عن تأثيرات جسيمات الذهب
النانونية على بعض الاعضاء التكاثرية في ذكور الجرذان
(*Rattu norvegicus*)**

رسالة مقدمة الى

مجلس كلية العلوم للبنات - جامعة بابل عن استيفاء جزئي لمتطلبات نيل

شهادة ماجستير في علوم الحياة

من قبل الطالبة

نادية كامل محمد الماشطة

بكالوريوس علوم الحياة/ كلية العلوم/ جامعة الكوفة (٢٠٠١)

بأشراف

أ.م.د. منار محمد حسن