

Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Babylon
College of Science
Department of Biology



Effect of *Hibiscus rosa_sinensis* Flowers Ethanollic Extract carried on Nanoparticles on Male Albino Rats Fertility

A Thesis

Submitted to the Council of the College of Science

University of Babylon

in Partial Fulfilment of the Requirements for the Degree of
Master of Sciences /Biology

By

Samer Habib Kareem Habib Al-Rehemy

(B.SC-Babylon university-2017)

Supervised by

Prof. Dr. Faris Naji A. Alhady

Prof. Dr. Adnan Mansour Jasim

2022 A.D

1444 A.H

Summary

Summary

This study was conducted in Department of biology/ College of Science / University of Babylon, College of Veterinary Medicine/ Al-Qasim green university, Nanotechnology Department/ University of Technology in Baghdad and Veterinary hospital in babylon the period from the October 2021 to the June 2022.

This study aimed to test the effect of ethanolic extract of *Hibiscus rosa sinensis* flowers loaded on nanoparticles on male albino rats and find out the possibility of the use of this plant extract as a male antifertility , In addition to trying to obtain a half ED50 that is less than the half ED50 of the extract without nanoparticales (300mg/kg/BW) which was determined in previous studies .

Twenty adult male albino rats used as model for mammalian animals, divided to five groups (4 animal for each group)G1 (Negative Control) treated with tap water (0.2 ml per day), G2 (Positive Control) treated with Nanoparticles (300mg empty liposome per day),G3 treated with flower extract nanoparticles (100mg/kg per day),G4 treated with flower extract nanoparticles (200mg/kg per day) and G5 treated with flower extract nanoparticles (300mg/kg per day).The rats were housed under the same conditions of temperature and Photoperiodicity.

The result showed significant decrease($p < 0.05$)of epididymal sperm concentration in three group treated with *H.rosa* flower extract loaded on nanoparticles (100mg/kg/day,200mg/kg/day, 300mg/kg/day) compared to -ve control and +ve control , also the G5 was significant decrease ($p < 0.05$) compared to G3 ,there were

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insignificant differences ($p > 0.05$) between G3 and G4, also G4 and G5. Progressive sperm motility percent in G3 and G5 were decreased significantly ($p < 0.05$) compared to G4 and control groups. The nonprogressive motility percent were decreased significantly ($p < 0.05$) in all treated groups compared to control groups, also it was noticed that the more decreasing in nonprogressive sperm motility in G3 compared to other groups. While the results showed significant increase ($p < 0.05$) in immotile sperm motility present in all three treated groups compared to control groups there were insignificant differences ($p > 0.05$) between treated groups (G3, G4 and G5). Sperm viability percent was significant decrease ($p < 0.05$) in G5 compared to all studied groups, while G4 was significant decrease ($p < 0.05$) from G3 only and there were no significant differences ($p > 0.05$) compared with control groups, also the sperm viability percent was not significant differences ($p > 0.05$) between G3 and G1.

The results of hormonal assay of this study showed significant decrease ($p < 0.05$) in the level of Testosterone, LH and FSH in groups treated of extract with nanoparticles compared with control groups while there is significant increase ($p > 0.05$) in the level of Estrogen in groups G4 and G5 treated of extract with nanoparticles compared with G3 and control groups.

Also the results showed significant decrease ($p < 0.05$) in the level of IL-6 and TNF- α in groups treated of extract with nanoparticles compared with control groups.

It was concluded that the effective dose of *H.rosa* flower extract loaded on nanoparticles less than the effective dose of the same extract of *H.rosa* flower in studied parameters include: sperm concentration, progressive sperm motility percent, Testosterone and

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TNF- α , through the decreasing of mention parameters in addition to effect on studied parameters .

So that it was suggested the benefit using of nanoparticles to decrease the dosage and improvement the effect.

Acknowledgments

I thank Allah Almighty for His bounty as He allowed me to accomplish this work by His grace, to Him be praise first and foremost.

I would like to extend my thanks and gratitude to my Supervisors . prof . Dr. Faris Naji Aboud Al-Hadi and prof . Dr. Adnan Mansour Jasim for suggesting the idea of this study and extending a helping hand to me in my academic stage

I would also like to thank the presidency of the Department of biology at the College of Science and the College of Veterinary Medicine at Al-Qasim Green University for the assistance they provided me during the study period.

I also extend my thanks and gratitude to everyone who helped me in my academic career

SAMER 2022

Appendix

Appendix

Appendix (1)

Measurement the Rats LH ,FSH,testosterone,Estrogen, Interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNF- α):

The enzymes werer used in this assay include Rats LH ,FSH , testosterone,Estrogen, Interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNF- α) ,eliza kit with CAT . NO (KTE101032 ,SL0286Ra, SL0668Ra , , SL0271Ra, SL0411Ra and SL0722Ra) respectively, using ELISA kit by methoed of Sandwich-ELISA were determined according to as following :

1. All reagents were Prepared prior beginning assay procedure. It is recommended that all Standards and Samples be added in equivalent to the Micro Elisa Strip plate.
2. 50 μ l of each Rats LH , FSH, testosterone ,Estrogen, Interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNF- α), eliza kit, standards was added to standard wells
3. Testing samples 10 μ l of serum were added to sample diluent 40 μ l of each kit to assay samples wells while the blank well doesn't add anything.
4. 100 μ l of HRP-conjugate reagent was added to all well and then coated with an adherent strip and incubated for at least one hour at 37°C.

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5. Each well was perfused and washed, recurring the operation four times for a total of five washes. Following the final wash, any residue was eliminated .

6. 50 μ l of each Rats LH , FSH, testosterone ,Estrogen, Interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNF- α) chromogen sol A and 50 μ l of chromogen sol B was added to each well. Suavity was mixed and incubated for 15 minutes at 37°C. then Safekeeping from light.

7. 50 μ l of each Rats LH , FSH, testosterone ,Estrogen, Interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNF- α) Stop solution was added to all wells. The colour in the wells were transformed from blue to yellow.

8. Optical Density (O.D.) was read at 450 nm using a microtiter plate reader within 15 minutes.

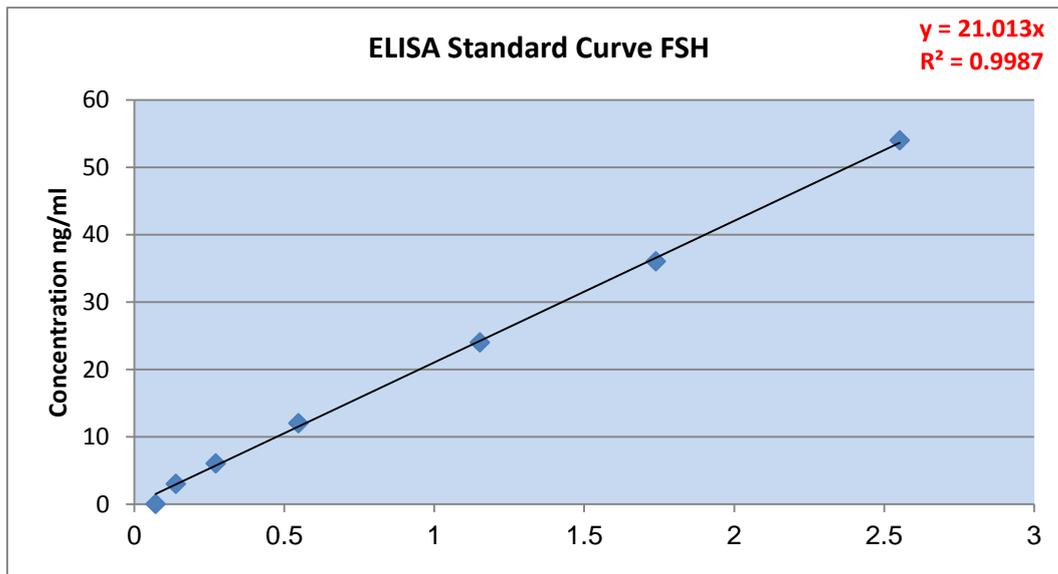
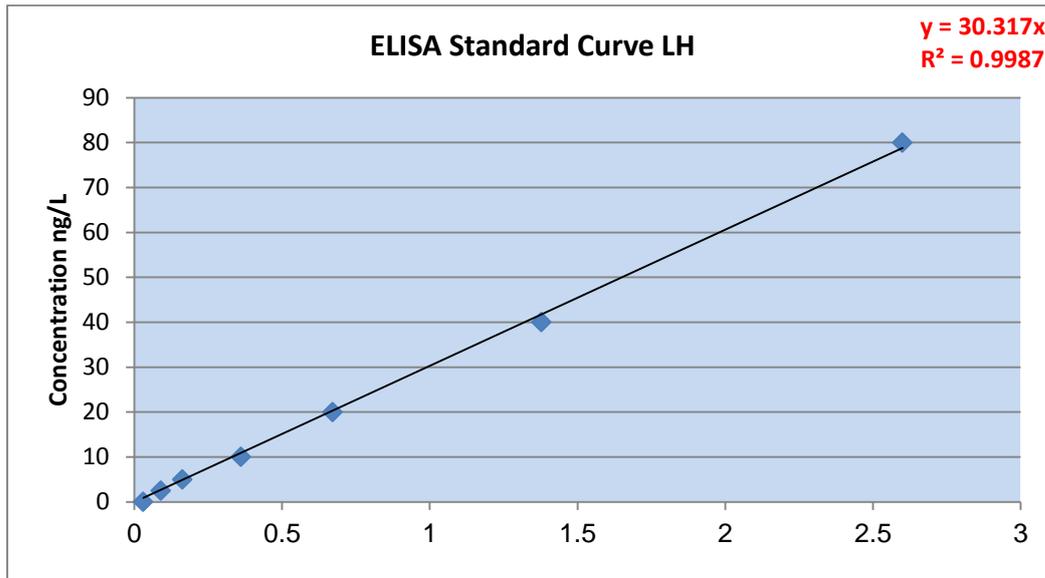
Appendix (2)

Calculations:

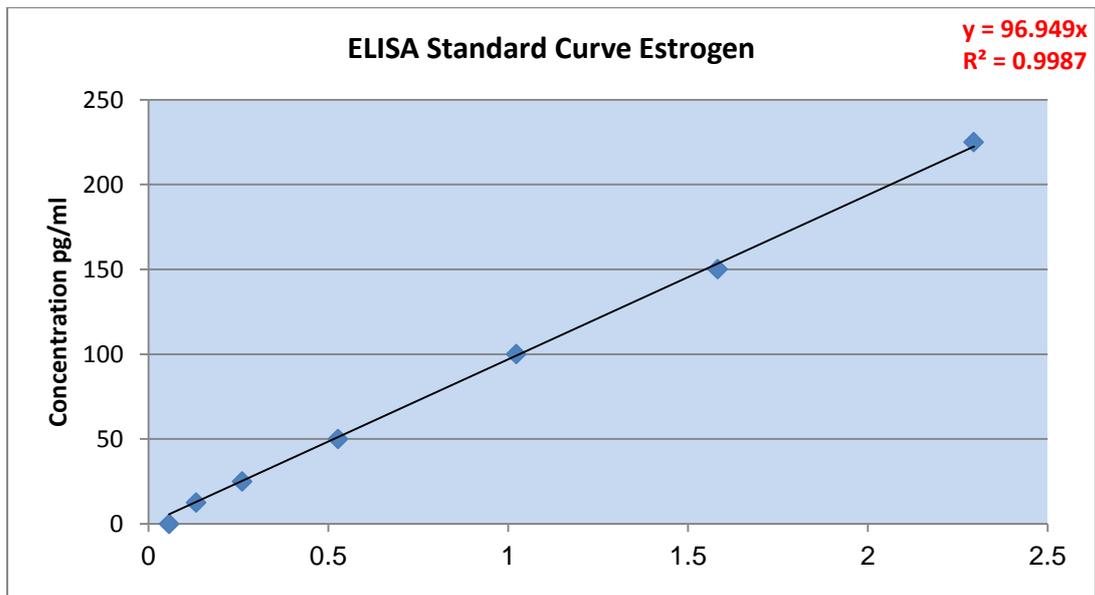
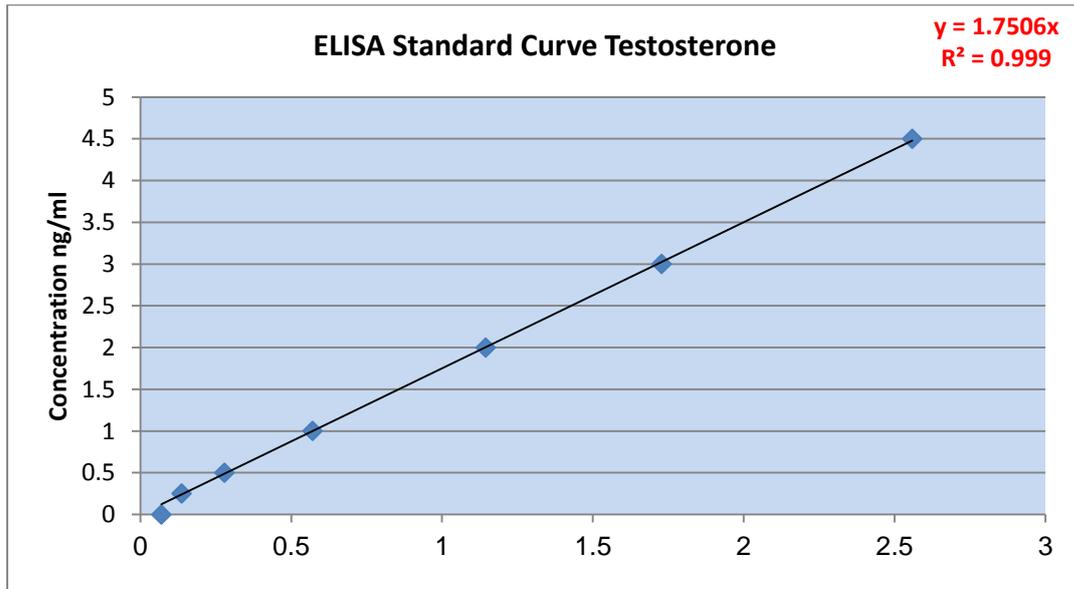
The standards curve were plotted for the absorbance versus the concentration of the standards, the absorbance value was entered on the y-axis and extended a horizontal line to the standard curve, the point of intersection was found, a vertical line was extended to the x-axis and the corresponding samples concentrations were read to each LH , FSH,

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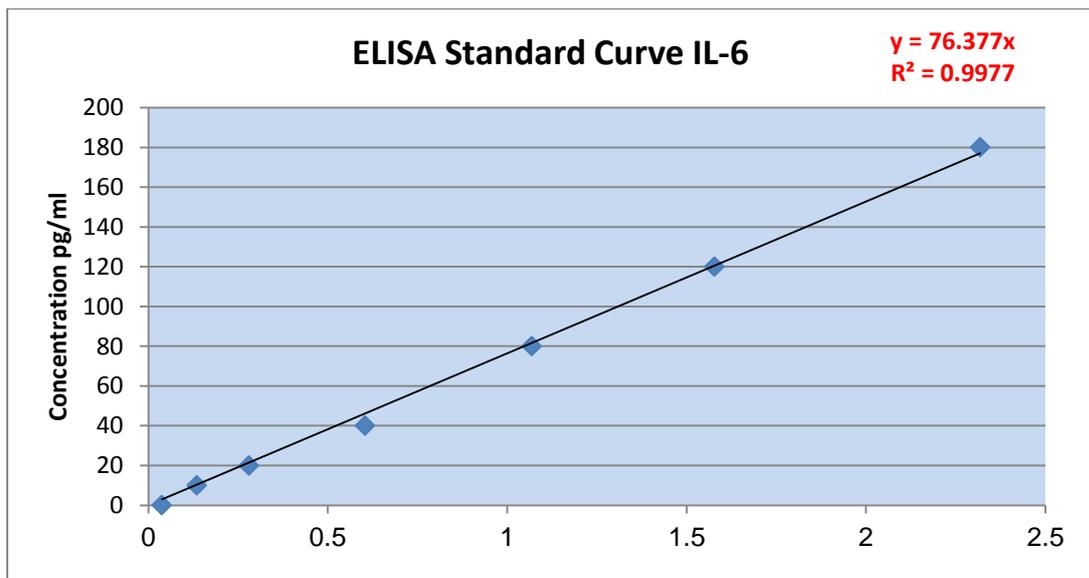
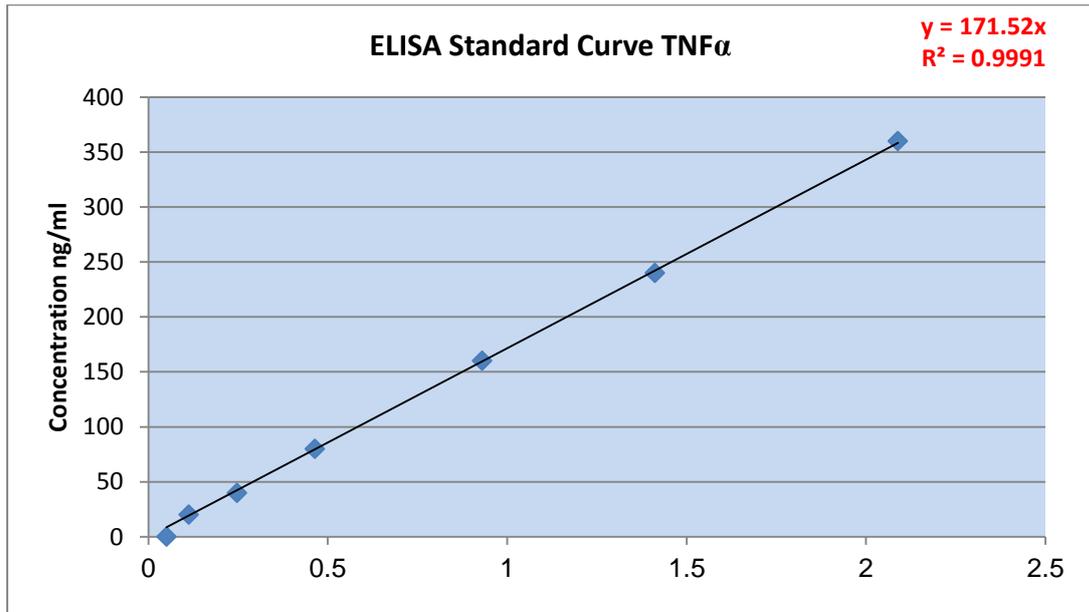
testosterone ,Estrogen, Interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNF- α) as shown in following figures



Appendix



Appendix



Appendix

Appendix (3)

Finding the ED₅₀ of ethanolic extract of *H. rosa* flowers loaded nanoparticles :

The effective dose (ED₅₀) was calculated through four studied parameters include: sperm concentration, sperm progressive motility percent , Testosterone concentration and TNF- α concentration by plotting the dose-response curve and extracting the median effective dose (MED₅₀) (Randolpha and Ciminera , 1985) .

1. Preparing a table that includes the required data as follows.

Observ.	X	Y	x ²	y ²	Xy
Sum	Σx	Σy	Σx^2	Σy^2	Σxy

2- Extract the values of (x) and (y)

$$X = \frac{\Sigma x}{n}$$

$$Y = \frac{\Sigma y}{n}$$

3. Extracting the variances of (x), (y) and (xy), as follows:

$$\frac{(\Sigma x)^2}{n}$$

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$$SS_x = \sum (x - \bar{x})^2 - \frac{(\sum x)^2}{n}$$

$$SS_y = \sum (y - \bar{y})^2 - \frac{(\sum y)^2}{n}$$

$$SS_{xy} = \sum [(x - \bar{x})(y - \bar{y})] = \sum xy - \frac{\sum x \sum y}{n}$$

4. Extracting the variance of linear regression (SS linear regression) according to the following law

$$SSL_r = \frac{(SS_{xy})^2}{SS_x}$$

5- Organizing the regression analysis table as follows.

Source	d.f	SS	MS	Cal.F	Tab.F
Total Variance (y)	n-1	SSY			
Linear Regression	1	SSLr	SSLr		
Deviation from linearity (error)	n-2				

6- Extracting the value of the correlation coefficient (r) according to the following law:-

$$r = \frac{SS_{xy}}{(SS_x)(SS_y)}$$

7- Determining the value of the correlation coefficient statistically according to the following law:

$$t = \sqrt{\frac{(1 - r^2)(n - 2)}{r - 0}}$$

8- Calculate the linear equation according to the following steps: -

A- Calculate the regression coefficient (Slope; b) according to the law

Appendix

$$b = \frac{SS_{xy}}{SS_x}$$

b- We substitute in the linear equation for the value of \hat{y} , b

$$\hat{y} = \hat{y} + b(x - \bar{x}) = \bar{y} + b(x - \bar{x})$$

C - Calculate the response value for each dose used using the previous linear equation:

when $x=0$ (control)	$Y_0 = a + bx_0$
when $x=1$	$Y_1 = a + bx_1$
when $x=2$	$Y_2 = a + bx_2$
when $x=3$	$Y_3 = a + bx_3$
when $x=4$	$Y_4 = a + bx_4$

D - We draw the graphic relationship between the doses used and the response that appeared in the previous step.

E - We extract the value of half the response according to the following law

$$\text{Response of (ED 50)} = \frac{\text{Max Response} + \text{Min Response}}{2}$$

F- We substitute the value of \hat{y} into the linear equation to extract the effective dose that leads to the half-response

$$\hat{y} = a + bx$$

I followed the same previous steps in determining all the criteria .

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List of Abbreviations :

Abbreviations	Meaning
ABPs	Androgen-Binding Proteins
AFM	Atomic Force Microscopy
AI	Atherogenic Index
APCs	Antigen-Presenting Cells
bbFGF	bovine basic Fibroblast Growth Factor
CRP	C-Reactive Protein
E1	Estrone
E2	Estradiol
E3	Estriol
E4	Estetrol
ED ₅₀	Effective Dose
ERs	Estrogen Receptors
FAO	Food and Agriculture Organization
FSH	Follice- Stimulating Hormone
FST	Forced induced Swimming Test
FTIR	Fiurier-Transform Infrared Spectroscopy
GC/MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
ICSH	Interstitial Cell–Stimulating Hormone
IGFs	Insulin-like Growth Factors
IL-6	Interleukin 6
LAM	Lactational Amenorrhoea Method
LDL	Low density Lipoprotein
LH	Luteinizing Hormone

mERs	membrane Estrogen Receptors
MgO-NPs	Magnesium Oxide Nanoparticles
NPS	Nanoparticles
OFT	Open Field Test
PAMPs	Pathogen-Associated Molecular Patterns
PRRs	Pattern Recognition Receptors
RAR	Retinoic Acid Receptor
SD	Standard Deviation
SEM	Scanning Electron Microscopes
TEM	Transmission Electron Microscopy
TIT	Tail Immersion Test
TLRs	Toll-Like Receptors
TNF- α	Tumor Necrosis Factor alpha
TST	Tail Suspension Test
WHO	World Health Organization
XRD	X-Ray Diffraction

DEDICATION

To my father the secret of my success and the reason for my existence .

To my mother, the source of my strength and the light of my path .

To the most wonderful person who embodied love in all its meanings, my wife, and she was the bond and the giving .

To my children, the sweetest of my life

To my brothers and sister my support and my pride

To my loyal friends who supported me.

SAMER

Certification Committee

We certify that we have read this thesis entitled "**Effect of *Hibiscus rosa_sinensis* Flowers Ethanolic Extract carried on Nanoparticles on Male Albino Rats Fertility**" and as examining committee, examined the student "**Samer Habib Kareem Habib Al-Rehemy**" in its content. In our opinion, it meets the standards of a thesis for the degree of Master of Science in Biology and accepted with "**Excellent**" degree.

Signature:

Name: Dr. Alauldeen subhi Al-sallami

Title: Professor

Address: Faculty of Science

University of Kufa

Date: / / 2022

(Chairman)

Signature:

Name: Dr. Hala Mohi Naji

Title: Assistant Professor

Address: College of Science

University of Babylon

Date : / / 2022

(Member)

Signature:

Name: Dr. Rawaa Safaa Abbas

Title: Assistant Professor

Address: College of Science

University of AL-Qassim green

Date: / / 2022

(Member)

Signature:

Name: Dr. Faris Naji A. Alhady

Title: Professor

Address: College of Science

University of Babylon

Date: / / 2022

(Member and Supervisor)

Signature:

Name: Dr. Adnan Mansour Jasim

Title: Professor

Address: College of Veterinary Medicine

University of AL-Qassim green

Date: / / 2022

(Member and Supervisor)

Approved for the college committee of graduate studies.

Signature:

Name: Dr. Mohammed Mansour Kadhum Alkafaji

Title: Professor

Date: / / 2022

(Dean of College of Science)

Certification

We certify that this thesis was prepared under our supervision at the Department of biology/ College of Science / University of Babylon, College of Veterinary Medicine/Al-Qasim green university, in partial fulfilment of the requirements for the degree of master of sciences in biology/zoology Accordingly, I recommend this study for discussion.

Supervisor

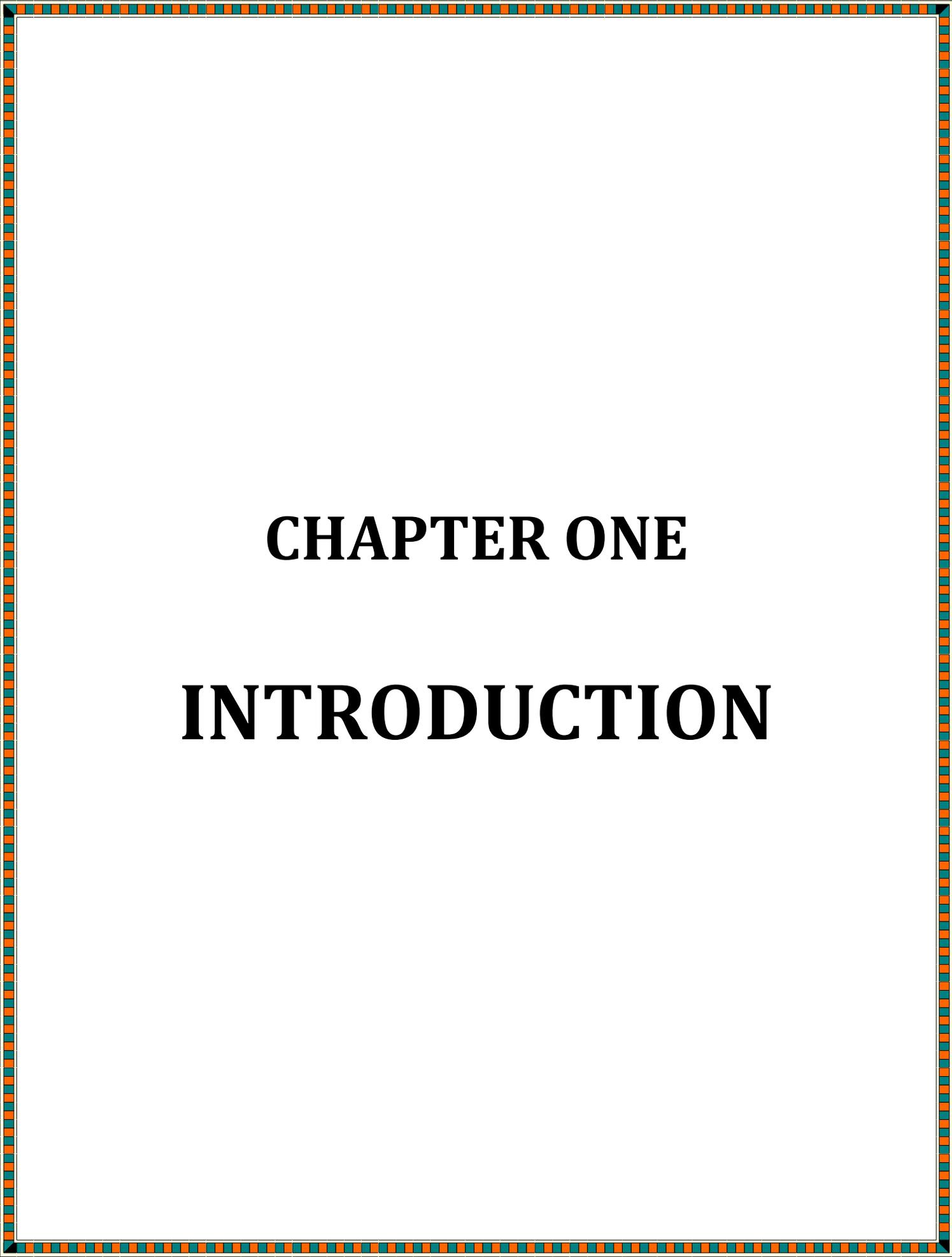
Prof. Dr. Faris Najj A. Alhady
College of Science
University of Babylon
/ / 2022

Supervisor

Prof. Dr. Adnan Mansour Jasim
College of Veterinary Medicine
Al-Qasim green university
/ / 2022

In view of the available recommendations, I forward this thesis for debate by the examination committee.

Asst .Prof. Dr. Adi Jassim Abd AL-Rezzaq
Head of Department of Biology
College of Science
University of Babylon
/ / 2022



CHAPTER ONE

INTRODUCTION

CHAPTER ONE

1.1 Introduction :

The growing rates of population in world's has a detrimental influence on the environment and economic growth, there is a global need to help individuals in family planning in impoverished nations (Acharya *et al.*, 2022). As a result, fertility regulation is a crucial and universal developmental element with a considerable influence on public health (Thakur *et al.*, 2017).

Approximately 90% of women used contraceptive globally. As a consequence, people interested in family planning highlight the significance of discovering techniques to inhibit male fertility that can be compared to female contraceptive techniques (Kwawukume *et al.*, 2022). Despite the tremendous progress that has been achieved in developing extremely efficient contraceptive techniques for females, research in developing strategies for preventing male fertility remains slow and limited (Gupta & Sharma, 2006; Sitruk-Ware *et al.*, 2013).

Numerous plant extracts have been discovered to be effective as contraceptives by decreasing male and female reproduction (Moroole *et al.*, 2022). Nevertheless, there are hardly any plant extracts that have been converted into contraceptives, and the modes of active and efficacy have not been adequately recognized due to the difficulties in determining the active elements of the extract to be used as a herbal contraceptive (Ghosh *et al.*, 2002). Several medicinal herbs have qualities that diminish male fertility, either by suppressing the spermatogenesis process, weakening sperm viability, or lowering sperm motility

(Olayemi, 2010; Abarikwu *et al.*, 2020). Others would result in aberrant hormone levels and alterations in testicular structure (Kumar *et al.*, 2012).

One of these plants is the powdered blossoms of the plant *Hibiscus rosa-sinensis* L. which possesses fertility-inhibiting effects demonstrated that rats administrated with the phenolic extract of *H. rosa-sinensis* flowers at a dose of 300 mg/kg for 60 days led to a massive drop in testes and epididymis weights, grade activity, epididymal sperm concentration, live and motile sperm percentage and substantial rise in the epididymal sperm abnormality as well as a fundamental lowering Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and Testosterone levels. (Lans, 2007; Al-Zubaide *et al.*, 2016) .

Hibiscus rosa-sinensis has traditionally been used as folk medicine to relieve constipation and improve circulation in illnesses such as hypertension, liver disease, nerve dysfunction, and tumor (Jadhav *et al.*, 2009). The aqueous-ethanolic extract of its aerial portions is supposed to relieve constipation and diarrhea (Kumar *et al.*, 2012). It is a blood cleanser and assists in the therapy of cystitis, as well as a natural source of vitamin C and a cure for genital infections such as syphilis and gonorrhoea (Sargam & Garg, 2013). This plant also has a number of useful characteristics, like antihypertensive (Kate & Lucky 2010), anti-inflammatory (Tomar *et al.*, 2010), and analgesic (Sawarkar *et al.*, 2009).

Additionally, *H. rosa-sinensis* is used to cure a variety of diseases since its leaves and blooms increase hair development, prevent premature greying, and treat scalp cases (Upadhyay & Upadhyay, 2011), hypolipidemic and anti-diabetic actions (Bhaskar & Nithya, 2012; Mamun *et al.*, 2013; Pethe *et al.*, 2017), anti-parasitic role (Nath & Yadav, 2016), wounds healing (Bhaskar & Nithya, 2012), cardiac protective impact and lowering blood pressure and beneficial for hypercholesterolemia therapy (Biswas *et al.*, 2014).

Many manufacturers have established the potential advantages of nanotechnology at both the high and low levels, and commercial goods are already being mass-produced in areas such as microelectronics, aircraft, and medicines (Dey *et al.*, 2022). Nanomaterials have been employed in a variety of scientific disciplines, such as medicine (due to their exceptional physiochemical features, which include small size, huge specific surface area, and distinctive optical characteristics), electronics, clean energy generation, space research, biotechnology, and environment (Knibbs & Morawska, 2012).

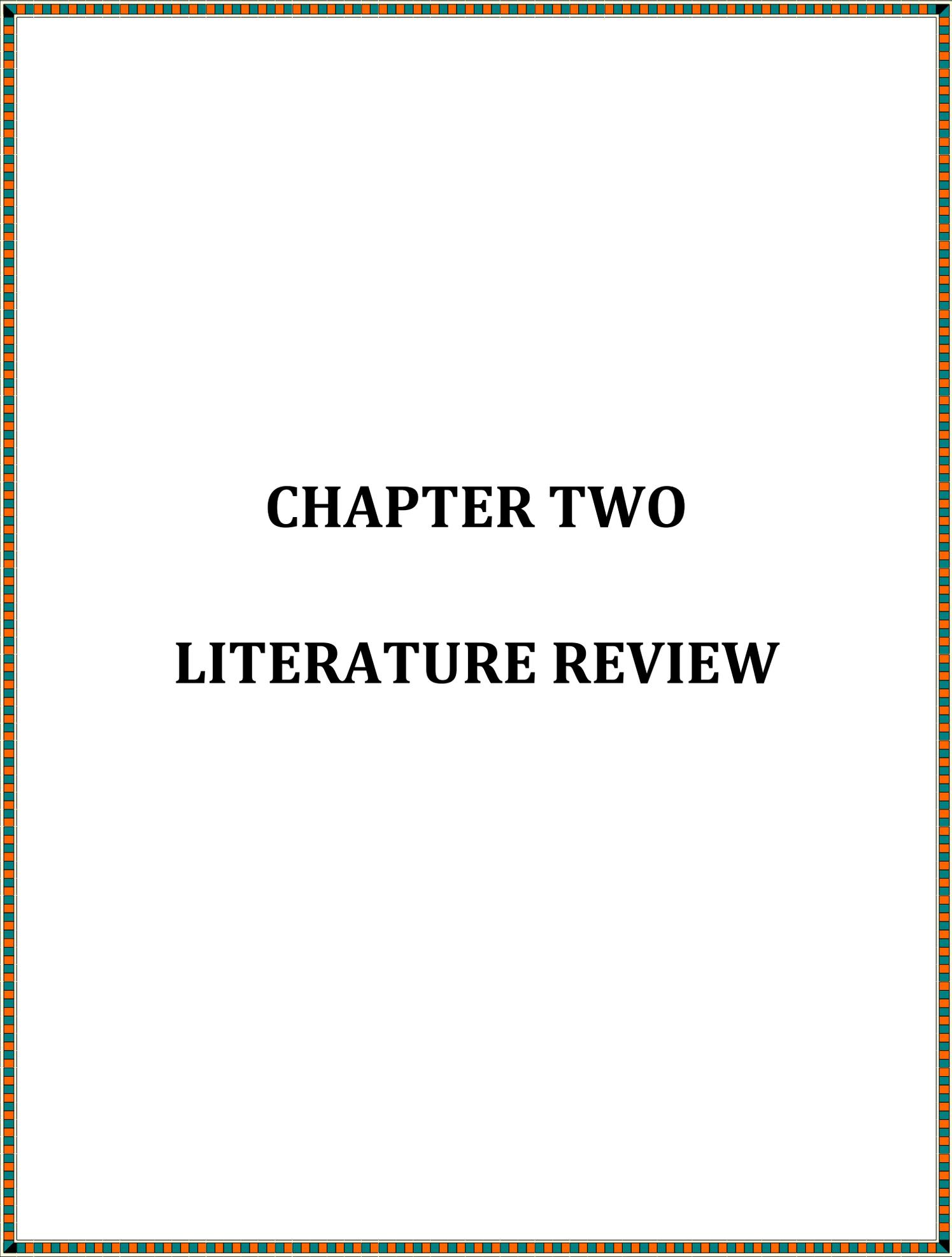
Nanoparticles (NPs) have the potential to enter cells and unite with them. Their DNA molecules are extremely tough to cope with, because of their immense complexity, small sizes and specific physical and chemical characteristics that distinguish them from bigger materials composed of the same elements. Various sorts of NPs have diverse characteristics and specific applications, due to the nature of the parent material and other considerations.

Despite recent advancements, some nanostructures might have toxic effects on cells, especially germ cells, and must be optimized with the right ingredients (Poddar & Kishore, 2022). Furthermore, the germ cells could impair fertilization and fetal development (Zakhidov *et al.*, 2010). Fertility and effective reproduction are critical to the survival of a species, and there is a growing necessity to raise attention due to NPs' induced reproductive toxicity, because the synthesis of engineered nanoparticles may raise the danger of disruption to the reproductive system (Hougaard *et al.*, 2015).

1.2 The aim of study :

The aim of this study is to study the effect of the ethanolic extract of *H.rosa sinensis* Linn loaded on nanoparticles on fertility of male albino rats, in addition to try to obtain effective dose (ED_{50}) of nanoparticles loaded with *H. rosa* flower extract less than the effective dose of extract without of nanoparticle which is estimated previously with Al-Zubaidi *et al.*, (2014), equal to 300 mg/kg.BW through estimation the following objective parameters:

- 1.Sperm parameters tests.
- 2.Hormonal assay: LH , FSH , Estrogen and Testosterone.
- 3.Immunological biomarkers assay : IL-6 and TNF- α level .



CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1. Pharmacognosy and Medicinal Plants:

The medicinal herbs are exploited to either maintain health or to treated a specific condition (Smith-Hall *et al.*, 2012). According to the Food and Agriculture Organization (FAO), over 50,000 medicinal plants were used worldwide in 2002, and the Royal Botanic Gardens stated that around 17,810 species of plants have a medicinal utilization in 2016, out from approximately 30,000 plants that are documented to be used in any way (Royal Botanic Gardens, 2016).

Herbal medicine is the world's oldest type of healthcare. Throughout history, all societies have employed herbs. Today, all of the world's civilizations have substantial knowledge of herbal medicine as a result of collected experience from previous generations. Plants are a rich source of secondary metabolites, which are employed in medications, agrochemicals, flavors, perfumes, colors, bio-pesticides, and food additives (Al-Snafi, 2018).

The World Health Organization (WHO) states that traditional medicine serves 80 percent of the world's population (Tilburt and Kaptchuk, 2008). Despite the safe image of herbal treatments, this posses dangers of toxicity and other

impacts on human health (Ekor, 2014). For thousands of years, medicinal herbs have been utilized to cure both mental and physical illnesses, as well as to promote individual health; poorer nations practise around 80% of medical therapies (Bent and Ko, 2004). However, herbal drinks, such as *Hibiscus*, seem to be well-known beverages because they have less side effects than synthetic medications. These herbal teas are also utilized as traditional treatments for a variety of diseases, including overweight (Iftikhar *et al.*, 2022).

The genus *Hibiscus* , include about 250 species distributed throughout the world (Hou *et al.*, 2005; Essa and Subramanian, 2007). There are about 40 species in India (Mudgal, 1974) and six species in Iraq , which are grown as garden plants (Chakravarty, 1976).

2.2. *Hibiscus Rosa-Sinensis* Linn profile:

Hibiscus rosa sinensis L. is one of medicinal plant and belongs to the family Malvaceae, it is a species of tropical and is considered a native to East Asia is a glabrous shrub that is frequently grown mostly in the tropics (Vyas *et al.*, 2012). The juice extracted from leaves and flowers are used as natural remedy to treat various diseases, and also used to make eyeliners and shoe polishes (Upadhyay and Upadhyay, 2011; Sivaraman and Saju, 2021).

2.2.1. Taxonomic classification:

Botanical: *Hibiscus*.

Name: *Rosa- sinensis L.*

Kingdom: Plantae

Sub kingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Malvales

Family: Malvaceae

Genus: *Hibiscus*

Species: *Rosa-Sinensis* (Mudgal, 1974).



Figure (2- 1) *Hibiscus rosa sinensis* L. plant grown in nurseries in Hilla .

2.2.2. Synonyms:

Although the plant is not connected to the real roses, the term ‘*Rosa sinensis*’ literally means ‘rose of China’ in Latin, it was first named by Carolus Linnaeus (Oguntoye *et al.*, 2014). The shrub with deep-red blossoms is said to be of Asian origin, thus the name *rosa-sinensis*, which means "rose of China." It is currently extensively farmed (Jadhav *et al.*, 2009).

Hibiscus arnottii Griff. as Mast.; *Hibiscus boryanus* DC.; *Hibiscus cooperi* auct.; *Hibiscus festalis* Salisb., *Hibiscus liliiflorus* Griff. as Mast., *Hibiscus rosiflorus* Stokes and *Hibiscus storckii* Seem (Al-Snafi, 2018).

2.2.3. Common names:

The common names of this plant in Arabic are Bent EL-Kunsil, Ward El-Jemal, Khatmah Siniyah, Hab misk Seni, Pooq Seni, while in Chinese are Zhu jin, Da hong hua, Fo sang, Fu sang, in English are China-rose, Chinese hibiscus, Hawaiian *Hibiscus*, Tropical *Hibiscus*, Shoe-flower plant, in French are *Hibiscus de Chine*, *Hibiscus rose de Chine*, Rose de Chine, in German is chinesischer Roseneibisch, in Italian is Rosa della Cina, in Japanese are Aka-bana, Fusou, Haibisukasu, in Portuguese is rosa-da-China, and in Spanish-speaking nations they are quite varied among others as oceano pacífico, tulipán, cayena, clavel japonés, cucarda, rosa de china, rosella, poppy, carnation, flor de avispa, sinesia, papo, pavona, and blood of Christ (Nwachukwu and Mbagwu, 2008; Al-Snafi, 2018).

2.2.4. Distribution:

Hibiscus rosa-sinensis is widely assumed that it originated in tropical Asia and South China (Al-Snafi, 2018). It was cultivated for a long time in China, Japan, and the Pacific islands. Currently, it is mainly spread all across Africa, the

Americas, Asia, and different European countries, in which it is grown like a decorative plant because of its lovely colors and green vegetation, and also for pharmaceutical purposes because of its historical and contemporary medicinal benefits (Valdivi e and Mart inez, 2022).

This plant is prevalent in the tropics and is grown as a house plant throughout the world (Kumar *et al.*, 2009).

2.2.5. Planting conditions:

This plant develops leaves all year in subtropical and tropical regions with average temperatures of 26  C (13–34  C), which does not occur in continental or cold climates. It may also be found at altitudes ranging from sea level to 1200 m above sea level. It could be cultivated on soils that are neutral or slightly basic (ideal pH between 5.5 and 6), with good soil fertility and not susceptible to flood, with a minimum annual rainfall of 900 mm (Bastida *et al.*, 2008).

Among the most common pests that attack this plant; are mealybugs, spiders, whiteflies, caterpillars, and aphids, although without inflicting irreversible harm (Cu ellar and Arrieta Herrera, 2010). The leaves of *Hibiscus Rosa-sinensis* are alternate and oval, although they can vary and even have jagged edges, dark green coloring, with a shiny appearance, abundant mucilage, and high protein content (Leiva *et al.*, 2008).

2.2.6. Morphology:

The height of the plant is around 7-2 feet. It is available in various colors, but red flower is preferred for medicinal purposes (Jadhav *et al.*, 2009b; Upadhyay and Upadhyay, 2011). The plant's characteristics are extremely typical, such as cylindrical roots that are 5-15 cm long, simple ovate leaves that are coarsely serrated at the tip, and pediculate flowers with 5 red petals. This plant develops enormous blooms on bushy hedges; the flowers are dark crimson in color and are not generally fragrant (Kumar *et al.*, 2012).

The blooms are often large, noticeable, and showy trumpet-shaped flower with five or more petals. They are multicolored blooms that range from white through purple, crimson, pink, and even yellow. The petals range in width from 4 to 15 cm. Flowers also have nectarines, which are composed of many densely packed glandular hairs and are generally positioned at the sepals, and the fruit is a capsule made up of dry five-lobes, it contains numerous seeds in each of its lobes, which are then released at the time of maturation, when the capsule splits open (Alarcon-Aguilar *et al.*, 2007; Vitullo *et al.*, 2009).

2.3. Phytochemical Components in *H.rosa sinensis* L .

The phytochemical findings indicate that the flowers of *Hibiscus rosa-sinensis* comprised phenolic compounds, tannins, quinines, flavonoids, alkaloids,

terpenoids, saponins, and anthraquinones (Menon *et al.*, 2014; Kumari *et al.*, 2015), in addition to glycosides, protein, carbohydrates, mucilage, free amino acids, reducing sugars, essential oils, and steroids, which played a role to the plant's medicinal functionality (Sobhy *et al.*, 2017; Missoum, 2018).

Hibiscus rosa sinensis blooms additionally consist of variety of compounds, including anthocyanin, cyclopropanoids, methyl stercolate, malvalate, and beta-sitosterol, with cyanidin 3-sophoroside being the flower's most prevalent anthocyanin (Khare, 2008), calcium oxalate, vitamin B1, B2, and C. (Nair *et al.*, 2005). In addition to Ascorbic acid, Thiamine, Niacin, Riboflavin, Quercetin, Kaempferol, Apigenidine, and Camphoral, existed in the *H. rosa* plant (Shukla and Mishra, 2001; Ghani, 2003), and Galic acid (Borde *et al.*, 2011).

Earlier research employing High Performance Liquid Chromatography (HPLC) methods revealed that the *H. rosa sinensis* flower's phenolic extract contains the following compounds: Acetylsalicylic acid, Chlorogenic acid, Coumarin, Lutein, Cynidin, and Quercetin (Al-Zubaidi *et al.*, 2014).

Quercetin and Lutein comprise flavonoids as glycosides, and flavonoids have anti-oxidant and anti-cancer properties (Chon *et al.*, 2009; Al-Saily *et al.*, 2018; AL-Azawi R. S., & Al-hady, 2019). *Hibiscus Rosa* plant noticed in a variety of shapes, and the blooms are brightly coloured in red, white, pink, and yellow.

Red-hued blossoms are utilized in medicinal applications (Adhirajan *et al.*, 2003), since they contain a high concentration of Anthocyanin, which may be responsible for anti-oxidant benefits (Gauthaman *et al.*, 2006).

2.4. Pharmacological Properties:

Hibiscus Rosa-sinensis is a prominent medicinal plant, with varied effective and safe pharmacological activity (Ghaffar *et al.*, 2012; Arullappan *et al.*, 2013; Al-Snafi, 2018). It is recognized as a potential medicinal herb with vast variety of pharmacological properties that, due to its efficacy and safety, might be employed in number medical purposes (Al-Snafi, 2018).

It is well acknowledged that the leaves and petals of this plant have numerous therapeutic applications, making it a valuable one. (Upadhyay and Upadhyay, 2011). Traditionally, *H. Rosa* is used as folk medicine for many conditions such as hypertension, liver disease, nerve disorder, and cancer, to alleviate constipation and promote circulation (Jadhav *et al.*, 2009). *Hibiscus rosa sinensis* has several beneficial properties, including antihypertensive (Kate and Lucky 2010), anti-inflammatory (Tomar *et al.*, 2010), and analgesic (Sawarkar *et al.*, 2009).

Moreover, this plant is used to treat many disease, since its leaves and flowers are used to promote hair growth, prevent the premature graying, and treat scalp disorders (Upadhyay and Upadhyay, 2011), hypolipidemic and anti-diabetic effects (Moqbel *et al.*, 2011; Agrawal *et al.*, 2012; Bhaskar and Nithya, 2012; Mamun *et al.*, 2013; Pethe *et al.*, 2017; Zaki *et al.*, 2017), anti-parasitic effect (Nath and Yadav, 2016), wounds healing (Bhaskar and Nithya, 2012), cardiac protective effect and reduce blood pressure and useful for treatment of hypercholesterolemia (Biswas *et al.*, 2014).

The Leaves of *H.Rosa-sinensis* could be utilized to provoke blood circulation and so aid to manage menstruation, as well as a diuretic. *H. Rosa-sinensis* fruits might be employed to treat sprains and wounds (Sargam and Garg, 2013; Al-Snafi, 2018). Furthermore, its extract can be utilized to treat peptic ulcers and leukaemia (Atherton & Blaser, 2009; Arullappan *et al.*, 2013).

Its aerial parts' aqueous-ethanolic extract is thought to relief constipation and diarrhea (Kumar *et al.*, 2012). It acts as a blood purifier and aids in the cure of cystitis, and is an excellent natural source of vitamin C and a cure for venereal diseases as syphilis and gonorrhoea (Sargam and Garg, 2013).

2.4.1. Antimicrobial effect:

The Leaves of *H. rosa-sinensis* showed antibacterial properties against *Staphylococcus aureus*, *Staphylococcus aerogenes*, *Bacillus subtilis*, *E. coli*, and *Shigella dysenteriae* (Hemarana *et al.*, 2014; Tiwari *et al.*, 2015; Udo *et al.*, 2016) and antifungal properties against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Candida glabrata*, and *Candida parapsilosis* (Hemarana, *et al.*, 2014; Sobhy *et al.*, 2017). Leaves of *Hibiscus Rosa-sinensis*, demonstrated also antiparasitic properties in vivo and in vitro against *Hymenolepis diminuta* (Nath and Yadav, 2016).

Ruban and Gajalakshmi (2012) investigated the antibacterial activity of *H. Rosa sinensis* flowers against two gram - positive organisms (*Staphylococcus aureus* and *Bacillus subtilis*) and two negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The cold extract had the greatest inhibition zone against *E. coli* and *B. subtilis* (17.00 2.91 and 14.50 1.71) mm, followed by hot extraction against *E. coli* and *Salmonella sp.* (11.66 and 10.60) mm, respectively. Methanol extract had the greatest inhibition zone against *E. coli* and *B. subtilis* (18.86 and 18.00) mm, respectively, whereas ethanol extract had the greatest inhibition zone against *Salmonella sp.* (20.40) mm. The flower's crude protein

exhibited the greatest inhibitory zone against *Salmonella* sp. and *E. coli* (16.55 and 14.30) mm, respectively (Ruban and Gajalakshmi, 2012).

2.4.2. Antioxidant effect:

The leaves of this plant are also highly antioxidant activity (Agrawal *et al.*, 2012; Ghaffar, and El-Elaimy, 2012; Divya *et al.*, 2013; AL-Azawi and Al-hady, 2019). *Hibiscus rosa* leaves have abundant phenolic compounds , promoting excellent antioxidant activity (Garg *et al.*, 2012; Ghaffar and El-Elaimy, 2012; Prasad, 2014; Prasanna, 2017).

2.5. Cardio protective role:

Some authors (Khandelwal *et al.*, 2011; Missoum, 2018) studied the cardioprotective benefits of *H. rosa-sinensis* dried pulverized flower (150 - 200 mg/ kg orally) on isoproterenol-induced myocardial damage in the rats. They noticed a considerable rise in the baseline amount of thiobarbituric acid reactive compounds among both dosages of *H. Rosa sinensis*. There was a substantial rise in superoxide dismutase, reduced glutathione, and catalase levels in the 250 mg/kg treatment group but not in the 125 and 500 mg/kg treated groups. As a result, *H. rosa sinensis* (250 mg/kg) increased endogenous antioxidant molecules in the rat heart while also protecting the myocardium from isoproterenol-induced myocardial damage.

2.6. Effect of *Hibiscus rosa-sinensis* on reproduction:

Hibiscus rosa sinensis ethanol extract was discovered to trigger implantation loss, which might be due to anti-zygotic, blast cytotoxic, or anti-implantation activities. (Vasudeva and Sharma, 2008). The extract of the *H. Rosa-sinensis* was exploited to induce abortions and to provoke the placental discharge after animal parturition (Nivsarkar *et al.*, 2005). In recent research, it is necessary to determine the exact reproductive age in which consuming *H. Rosa- sinensis* causes the hormonal alterations and reproductive harm in females (Valdivi  and Mart nez, 2022).

The effect of Antiandrogens on male albino mice fertility and reproductive system was described by (Al-Hady, 1989b). Furthermore, (Al-Zubaidi *et al.*, 2016) investigated the effect *H. rosa* flowers' phenolic extract on estrogen level and fertility of male albino rats. The treatment of rats with *H. Rosa-sinensis* flowers' phenolic components for 60 days at a dosage of 300 mg/kg resulted in a substantial drop in testicular and epididymal weights, sperm motility percent, grade activity, epididymal sperms concentration and live sperm percentage. While a significant elevation in the epididymal sperm abnormality.

The influence of *H. Rosa-sinensis* extracts in chloroform, ethyl acetate, and ethanol on the spermatogenesis and sperm variables in mice was examined.

Subcutaneous administration of 125 mg/kg bw of these substances for three days resulted in substantial reduction in sperm count, viability and testis weight (Sharawy and Ibrahim, 2014).

The effectiveness of an aqueous extract of *H. Rosa-sinensis* flower extract on the maternal-fetal outcome in diabetic pregnant rats was studied. In comparison to the non-diabetic group, the diabetes group had lower high density lipoprotein cholesterol, higher atherogenic index (AI) and coronary artery risk index (CRI), and a higher preimplantation loss rate. Even though *H. Rosa-sinensis* treatment had a negative impact on reproductive and cardiac activities, the diabetic medicated patients exhibited higher fetal and maternal weights, a lower atherogenic index (AI) and coronary artery risk index (CRI), and a lower preimplantation loss rate when compared with the untreated diabetic cases (Afiune *et al.*, 2017).

The same extract but with different concentration of *H. Rosa-sinensis* (100 mg/kg from day 0-7 of pregnancy, 200 mg/kg from day 8-14 and 400 mg/kg from day 15-20) was investigated on oxidative stress and biochemical features in non-diabetic and diabetic pregnant rats. After administration of *H. Rosa-sinensis* extract, non-diabetic and diabetic rats showed no glycemic changes. The therapy using *H. rosa-sinensis* in diabetic group was able to decrease the triglycerides and ALT levels compared to diabetic nontreated animals (Silva *et al.*, 2015).

2.7. Anti-inflammatory effect of *Hibiscus Rosa-sinensis*:

2.7.1 Inflammatory proteins:

2.7.1.1 Tumor necrosis factor alpha (TNF- α):

TNF- α is a cytokine that belongs to the TNF superfamily of transmembrane proteins with a homologous TNF- α domain. TNF- α is a cell signaling molecule produced by the immune system. As part of an inflammatory response, macrophages (a kind of white blood cell) produce TNF- α to notify other immune system cells when they identify an infection (Sethi and Hotamisligil, 2021).

2.7.1.2 Interleukin 6 (IL-6) :

IL-6 is an interleukin that functions as both a pro-inflammatory and anti-inflammatory cytokine and myokine. The IL-6 gene in humans is responsible for its production (Ferguson-Smith *et al.*, 1988). Macrophages produce IL-6 in response to certain microbial compounds known as pathogen-associated molecular patterns (PAMPs). These PAMPs bind to a collection of innate immune system detecting molecules known as pattern recognition receptors (PRRs), which includes Toll-like receptors (TLRs). These are found on the cell surface and within the cell, and they trigger intracellular signaling cascades that lead to the generation of inflammatory cytokines. Fever and the acute phase response are both mediated

by IL-6. In the bone marrow, IL-6 stimulates the creation of acute phase proteins as well as the generation of neutrophils. It promotes the proliferation of B cells while suppressing the expansion of regulatory T cells (Tanaka *et al.*, 2014).

2.8. Effect of *Hibiscus Rosa-sinensis* on inflammation:

The ethanolic extract of *Hibiscus Rosa-sinensis* leaves has a potent anti-inflammatory effect in edematous syndrome caused by carrageenan injection, which was associated with a reduction in polymorphonuclear leukocyte infiltration (Raduan *et al.*, 2013). The hydroalcoholic extract of the leaves of *H. Rosa-sinensis* significantly reduced ulcers, oxidative stress, and other damage in the colon in a study with male Wistar rats, where colitis was induced by injecting 2 mL of 4 percent acetic acid intrarectally, corroborating the anti-inflammatory properties of this medicinal plant (Kandhare *et al.*, 2012). Consequently, *H. Rosa-sinensis* extract cause the reduction in the levels of tumor necrotic factors and interleukines, with no effect on C-reactive protein, which means that *H. Rosa-sinensis* could act as anti-inflammatory substance and prevent the inflammatory storm which is happened when we use nano particles as carriers of drugs (Kandhare *et al.*, 2012).

2.8.1. Antidiabetic and hypolipidemic effects:

The antidiabetic effect of ethyl acetate fraction of *H. Rosa-sinensis* petals was evaluated in induced diabetes at a dose of 25 mg/kg bw and compared with

metformin. The elevated levels of serum glucose (398.56 ± 35.78) and glycated haemoglobin (12.89 ± 1.89) in diabetic rats were significantly decreased (156.89 ± 14.45 and 6.12 ± 0.49 , respectively) by ethyl acetate fraction of *H. rosa-sinensis* administration. The glycogen content was restored by modulating the activities of glycogen metabolising enzymes, and hepatotoxicity marker enzyme levels in serum were corrected. It influenced the expression of marker genes involved in the glucose homeostasis signalling pathway considerably. The biochemical findings were corroborated by histopathological investigation of the liver and pancreas (Pillai and Mini, 2016).

The antidiabetic, hypolipidemic, antioxidant and histopathological effects of *Hibiscus rosa-sinensis* were investigated in alloxan induced diabetes in rats. Hydroalcoholic extract of flower of *H. rosa-sinensis* (HEFHR, 50-200 mg/kg bw) possessed significant and sustained antidiabetic activity, comparable with the hypoglycemic effect of glibenclamide and sulphonylurea. Flower extract of *H. Rosa-sinensis* was more efficacious in lipid lowering effect and in antioxidative activity than glibenclamide. After a 28-day therapy with flower extract, the size of the islets grew dramatically, as did necrosis and atrophy; there was also an increase in the number and diameter of cell islets compared to the diabetes group (Pethe *et al.*, 2017).

The extract of *H. Rosa-sinensis* has a beneficial impact on the lipid profile measures, and this extract had the ability to protect the liver, because of its free radical scavenging activity and the presence of natural antioxidants (AL-Azawi and Al-hady, 2019).

2.8.2. Anticancer effects:

The in vitro cytotoxic activity of the crude petroleum ether, ethyl acetate and methanol extracts of the leaf and stem of *H.Rosa-sinensis* (20 - 100 µg/ml) was evaluated against leukaemic cancer cell line (K562).

At dosages that had no impact on nontransformed cells, aqueous extracts of *H.Rosa-sinensis* flowers suppressed melanoma cell proliferation in a dose-dependent manner. Furthermore, these extracts included low molecular weight growth inhibiting chemicals with a molecular weight of less than 3 kD (Goldberg *et al.*, 2017).

According to the findings of a study conducted by(Al-Saily *et al.*, 2018), the phenolic extract of *H.Rosa-sinensis* flowers was inhibits the growth of cancer cells represented by Tira-1 and RAW264.7 without affecting the normal cells represented by WRL-68 by observing the IC₅₀ for each cell line .

2.8.3. Healing Effect:

The aqueous and ethanolic extracts of *H.Rosa-sinensis* leaves have a large healing effect in laboratory mice (Ali *et al.*, 2014; Mondal *et al.*, 2016). Using an excisional wound healing model in rats, different concentrations of n-butyl alcohol extract of *H.Rosa-sinensis* red flowers or recombinant bovine basic fibroblast growth factor (bbFGF) were administered twice daily for 9 days to investigate the efficacy and possible mechanism of the n-butyl alcohol extract of *H.Rosa-sinensis* red flowers. Histopathology was assessed on day 9 using eosin and hematoxylin staining, Masson's trichrome staining, and immunohistochemistry for VEGF, TGF-1, and CD68. The n-butyl alcohol extract of *H. pylori* modulates the immune system. In mice, the carbon removal of *rosa-sinensis* red flowers was studied. Extract of *H.* in N-butyl alcohol the red blooms of *Rosa sinensis* speed wound healing by increasing macrophage activity, speeding angiogenesis, and collagen fibre deposition, all of which are mediated by VEGF and TGF-1. (Shen *et al.*, 2017).

2.8.4. Anti-obesity effect:

No study is available in the literature on the anti-obesity effect of *H.rosa-sinensis* tea on rat body weight reduction according to (Iftikhar *et al.*, 2022), while AL-Azawi and Al-hady,(2019) demonstrated that *H. rosa-sinensis* extract caused

a distinct reduction in body weight. The anti-obesity efficacy of *Hibiscus sabdariffa* aqueous extract was established by a considerable weight loss in treated and non-treated rats, which was dosage dependent (Omar *et al.*, 2018).

Iftikhar *et al.*, (2022) assumed that, none of the previous studies have reported the effects of *Hibiscus rosa sinensis* tea/extract on the kidney and liver indices, and concluded that selected herbal infusions may have a therapeutic potential and can be used as antiobesity agents, while AL-Azawi and Al-hady, (2019) reported that the treatment by phenolic extract of *H. rosa-sinensis* hadn't affected liver and renal function tests. Further studies should investigate the effects of individual phenolic acids and flavonoids on the biochemical parameters in obesity and elucidate the action mechanism at the molecular level. The consumption of ginger and hibiscus teas separately and in combination at dosages of 250 and 500 mg/kg body weight resulted in a significant decrease in weight gain and oxidative stress in rats fed a high-fat and high-sugar diet. The 500 mg/kg dosage had more dramatic effects, equivalent to the anti-obesity medication (orlistat) (Iftikhar *et al.*, 2022).

2.9. Toxicity of Ethanolic Extracts:

The *H. rosa-sinensis* leaves' ethanolic extracts cause toxicity in mice after giving a dosage of 800 mg/kg of body weight for 2 weeks, as noticed by a rise in

bilirubin, urea, creatinine, alanine transferase, and aspartate amino transferase. Histological sections of the liver reveal dilated sinusities, nuclear apoptosis, and inflammation in the capillaries, as well as disarray of the tubules and glomeruli of the kidney, as well as thickening of the organ's interstitial spaces (Nath and Yadav, 2015). However, it was recorded that the phenolic extract of *H. rosa-sinensis* has a protective role in the liver, and the treatment by this extract in mice had no effect on the levels of liver function enzymes such as GOT, GPT, and Bilirubin (AL-Azawi and Al-hady, 2019).

In mice, administration of *H. rosa-sinensis* flower methanolic extract at dosages of 100, 200, 400, and 800 mg/Kg resulted in no notable changes in behavior, respiration defecation, impairment in food intake and water consumption, skin affect, postural abnormalities, and hair yellowing or loss (Meena *et al.*, 2014). Dosing of animals up to 500 mg/kg of all extracts caused no toxicity in rats. No remarkable alterations in levels of liver enzymes and no histologically lesions in the organs (Raduan *et al.*, 2013).

The multi- parameters cytotoxicity role of *H. rosa-sinensis* flowers' phenolic extract were observed on Tera-1 cell line showing a notable reduction in nuclear intensity, valid cell count, and mitochondrial membrane potential and a significant increase in membrane permeability and cytochrome C releasing, therefor the phenolic extract of *H. rosa-sinensis* have anti-tumor activity,

especially on testicular cancer cells (AL-Saily and Al-hady, 2018). On contrary, the genotoxic potential of the methanolic flower extract of *H. rosa-sinensis* was studied using micronucleus assay in Balb/c mice, and it showed no genotoxic activity in the micronucleus test. The rate of micronuclei in groups of animals given *H. rosa-sinensis* showed no differences in comparison to the negative control (vehicle) (Meena *et al.*, 2014).

2.10. Hormonal assay :

2.10.1 Luteinizing hormone (LH):

Luteinizing hormone is considered a hormone generated by gonadotropic cells in the anterior pituitary gland. Ovulation and the creation of the corpus luteum in females is triggered by an intense spike in LH (“LH surge”). LH increases Leydig cell testosterone production in males, where it was formerly known as interstitial cell–stimulating hormone (ICSH). It works in conjunction with follicle-stimulating hormone to have a synergistic effect (FSH) (Nedresky & Singh, 2022).

Al-Zubaidi *et al.*,(2016) found that the treatment of male albino rats with the phenolic components of *H. Rosa sinensis* flowers at a dosage of 300 mg/kg for 60 days resulted in significant decrease in LH.

2.10.2 Follicle-stimulating hormone (FSH) :

Follicle-stimulating hormone is considered as a gonadotropin, a glycoprotein polypeptide hormone (Cahoreau *et al.*, 2015). The anterior pituitary gland's gonadotropic cells create and emit FSH, which controls the body's development, growth, pubertal maturation, and reproductive activities. In the reproductive system, luteinizing hormone (LH) and FSH act in tandem (Santi *et al.*, 2020).

FSH boosts the development of primordial germ cells among males and females. FSH causes Sertoli cells to release androgen-binding proteins (ABPs) in men, which is controlled by the anterior pituitary's negative feedback mechanism. FSH stimulates Sertoli cell activation promotes spermatogenesis and inhibin B production. FSH stimulates follicular development in females, with granulosa cells being the primary target. FSH levels drop near the end of follicular phase, while inhibin B levels rise in tandem. This appears to be crucial in ovulation selection, as only the most mature follicles are selected. There is a little increase in FSH near the end of the luteal phase, which appears to be important for the onset of the following ovulatory cycle (Ulloa-Aguirre *et al.*, 2018).

FSH causes primary spermatocytes to divide for the first time, resulting in secondary spermatocytes. FSH stimulates the Sertoli cells in the testes to produce androgen-binding protein by binding to FSH receptors on their basolateral

membranes, and is necessary for the commencement of spermatogenesis (Al-Zubaidi *et al.*, 2016; Oduwole *et al.*, 2018) investigated that the treatment of male rats with the phenolic extracts of *H. Rosa sinensis* flowers for 60 days at a dosage of 300 mg/kg resulted in significant decrease in FSH.

2.10.3. Testosterone :

Males' principal sex hormone and anabolic steroid is testosterone. In humans, testosterone is important for the development of male reproductive tissues like the testes and prostate, and secondary sexual traits like increased muscle and bone mass and body hair growth (Mooradian *et al.*, 1987; Al-Hady, 1989a). Furthermore, testosterone has a role in both men and women's health and well-being, including mood, behavior, and osteoporosis prevention (Bassil *et al.*, 2009). Low testosterone levels in males can cause issues such as weakness and bone loss (Tuck and Francis, 2009).

Al-Zubaidi *et al.*, (2016) found that the treatment of male albino rats with the phenolic components of *H. Rosa sinensis* flowers at a dosage of 300 mg/kg for 60 days resulted in significant decrease in LH,FSH and Testosterone .

Al-Saily ,(2018) found that the treatment of male albino rats with the phenolic components of *H. Rosa sinensis* flowers at a dosage of 300 mg/kg for 60 days resulted in significant decrease in testosterone.

In a recent study by (AL-Azawi and Al-hady, 2020), found a substantial reduction in Testosterone and Progesterone levels in rats under investigation in comparison with control groups for 30 and 60 days of medication, and marked lowering in the expression of the progesterone and androgen receptors in the testicular cells therapy cases with phenolic extracts of *H. Rosa-sinensis* flowers at a dose of 300mg / kg /day of body weight for 60 days compared to control groups.

2.10.4. Estrogen :

Estrogen, often known as oestrogen, is considered a sex hormone that is involved in the development and control of the female reproductive system and also secondary sexual characteristics. Estrone (E1), estradiol (E2), and estriol (E3) are considered the three main endogenous estrogens with estrogenic hormonal action (E3). The most powerful and widely used estrane is estradiol. Estetrol (E4), a different estrogen, is only created during pregnancy (Fuentes & Silveyra, 2019). In both women and men, estrogens circulate in smaller quantities than androgens. Despite the fact that male have lower estrogen levels than females, estrogens play an essential physiological function in men (Alemany, 2021) .

Estrogens, as with all steroid hormones, move easily through cell membranes. They attach to and activate estrogen receptors (ERs) once within the cell, modulating the expression of several genes (Zhao, 2020). Estrogens also

adhere to and trigger membrane estrogen receptors (mERs), which are involved in fast signaling (Prossnitz *et al.*, 2007). Estrogens are utilized as pharmaceuticals as well as natural hormones, such as in menopausal hormone treatment, hormonal birth control, and feminizing hormone treatment for non-binary people and transgender women (Unger, 2016).

Aqueous extracts of *H. Rosa-sinensis* leaves administered to mice during days 1 and 6 of gestation hindered implantation in the endometrium because of the rise in superoxide anionic radicals, resulting in an exaggerated reduction in the enzyme superperoxidase dismutase, which leads to a decrease in estrogenic activity (Nivsarkar *et al.*, 2005). *Hibiscus Rosa-sinensis* roots have been evaluated for anti-implantation and estrogenic activities (Vasudeva and Sharma, 2008).

2.11. Male Contraception:

For several decades, contraception has allowed men and women to carefully organize their families and select whether or not to have children (Dominiak *et al.*, 2021). Men's participation in contraceptive knowledge concerns is critical, since contraception and family-planning decisions are nearly typically shared by both couples resulting in the widespread concept of birth control, which is a key component of modern progress and is required for women's integration into economic and social life (Aldabbagh and Al-Qazaz, 2020).

For centuries, humans have utilized traditional or natural methods of male contraception such as withdrawal, male condom, rhythm (periodic abstinence), and lactational amenorrhoea method (LAM), in addition to prolonged abstinence, douching or folk practices (Hatcher *et al.*, 2011; UN, 2015). Contrary to women, males have less contraceptive options. In reality, no male-directed agent has been created since the condom in the 18th century (Youssef, 1993), with male sterilization (vasectomy) following in the late 19th century (Drake *et al.*, 1999), together providing the whole menu of male contraceptive alternatives today.

Modern male contraceptive techniques used today are classified as hormonal or non-hormonal. Male hormonal contraception is based on the use of exogenous male hormones to inhibit a man's own testosterone levels and, as a result, sperm production, and present in the form of injections, implants, or pills (Thirumalai & Page, 2019). Non-hormonal strategies inhibit sperm maturation or hinder transport of sperm through the male reproductive system by targeting sperm or testes-specific proteins, such as Reversible Inhibition of Sperm Under Guidance (RISUG), Vasalgel, EP055, Indenopyridines, Lonidamide derivatives, and Retinoic acid receptor (RAR) antagonists (Thirumalai & Page, 2019).

Other non-hormonal substances that look promising as male contraceptives have yet to undergo pre-clinical testing in non-human primates (Thirumalai & Page, 2019). It has also been hypothesized that ultrasound with frequencies ranging

from 8 Hz to 22 kHz might be used as a method of contraception for males by lowering sperm cell mobility and viability and delaying their passage into the epididymis. This looks to be a low-cost and reversible technique (Sewak *et al.*, 2017).

Although male contraceptives have not been on the market in over two centuries, there has been substantial research in this field during the last 40 years. Over the previous decade, the pharmaceutical industry's lack of enthusiasm and investment has hampered progress (Dominiak *et al.*, 2021).

Numerous countries are attempting to regulate fertility through the use of herbal medicines (Chowdhury *et al.*, 2001). Fertility regulation, which includes contraception and infertility management, is an important aspect of reproductive health (Al-Hady, 1989; Al-Zubaidi *et al.*, 2016; Al-Saily *et al.*, 2018). Most medicinal herbs have qualities that diminish male fertility, either by inhibiting spermatogenesis, lowering sperm viability, or affecting sperm motility (Paul *et al.*, 2006). Other extracts may induce hormonal imbalances and alterations in testicular structure (Kumar *et al.*, 2012). One of these species, *Hibiscus Rosa-sinensis* L. flower extract, has fertility inhibiting characteristics (Al-Zubaidi *et al.*, 2016; Al-Saily *et al.*, 2018; AL-Azawi, and Al-hady, 2020).

2.12. Nanoparticles (NP):

The term 'nano' originated from a Greek word that means 'dwarf,' and is usually used in conjunction with other words like nanometer, nanobots, nanotechnology, and so on. Nanotechnology is the science and technology of very small objects (less than 100 nanometers) with novel chemical and physical characteristics, as well as increased reactivity and solubility (Vert *et al.*, 2012). Nanotechnology is a significant subject of nanoscience that promotes molecular, atomic, and macromolecular technologies (Tatli Seven *et al.*, 2018). Pharmaceutical companies are becoming more interested in nanotechnology because it offers benefits such as changed release methods and the opportunity to create novel compounds that were previously unavailable (Lee *et al.*, 2010).

Since the early 1990s, nanomaterials have been explored and employed in drug delivery systems, which are characterized as "coloidal systems of the submicron size that may be created from many of materials in various compositions." Liposomes, phytosomes, polymeric nanoparticles, metallic nanoparticles, micelles, solid-liquid nanoparticles, semiconductor nanoparticles, and others which are examples of nanomaterial vectors (Ram *et al.*, 2011). A nanomaterial is defined by the International Organization for Standardization (ISO) as "a nano-object having all three exterior dimensions in the nanoscale, which is

about 1 to 100 nm." (Boverhof *et al.*, 2015). Nanomaterials behave in a completely different manner than bulk systems due to two key characteristics. Surface-related properties and quantum properties are two types of characteristics that can be distinguished. Nanomaterials are frequently employed to carry and deliver medications, DNA, vaccinations, and diagnostic tools into particular cells and tissues in a variety of forms (M Ram *et al.*, 2011). Because of their tiny size and vast surface area, they have unique chemical and physical properties when compared to their raw materials (Vaidyanathan *et al.*, 2010).

Nanomaterials have been increasingly prevalent in several aspects of human life, including chemical industries, food technology, cosmetics, diagnostic and therapeutic domains, and so on, in recent decades (Sierra *et al.*, 2016). Chemical reactivity, electrical conductivity, melting temperature, fluorescence, and magnetism are all impacted by particle sizes up to 100 nm. This is owing to nanoparticles' greater surface area relative to larger-scale particles of equivalent mass, which allows for more interaction with the surrounding materials and hence changes in their reactivity (Colvin, 2003). Nanomaterials are extensively applied due to their small size, orientation, and physical qualities, which have been demonstrated to alter the performance of any other material that comes into touch with these microscopic particles (Panigrahi, 2013).

The distribution of phytoconstituents can be enhanced with the use of a unique delivery technique that involves putting the medication material into an appropriate carrier. This can help with solubility, stability, toxicity protection, pharmacological action, and long-term delivery (Babu *et al.*, 2019).

Different biological nanomaterials have been generated as a result of developments in synthetic chemistry, and they may be utilized for various biological treatments, including medication delivery, cancer diagnostics, illness management, and imaging agents (Jamieson *et al.*, 2007).

2.12.1. Classification of Nanoparticles in Medicine :

Synthetic polymer molecules, liposome compounds, micellar nanoparticles, protein nanoparticles, nanocrystals, and many more nanotechnology-based products and clinical trials have been authorized by the FDA since the 1990s. Although regulatory procedures for nanomedicines, as well as safety and toxicity evaluations, are still being developed, nanomedicine has already transformed how to identify and administer pharmaceuticals in living organisms (Patra *et al.*, 2018).

2.12.2. Liposome nanoparticles:

Liposome comes from two Greek words: "lipo" (fat) and "some" (cell-like). (Anwekar *et al.*, 2011). Liposomes are a colloidal tiny spherical self-closed

structure, formed of an interior watery compartment enclosed by one or more lipidic multiple concentric bilayers utilized for encapsulating vaccines, medicines, enzymes, or other compounds to target cells or organs (Salimi, 2018). These vesicles are relatively nontoxic, biodegradable and practically non-immunogenic material (Asili *et al.*, 2011). Dr. Alec D. Bangham and collaborators initially produced liposomes in England in 1961, demonstrating that when phospholipids are hydrated in an aqueous solution, they spontaneously form encapsulated structures (Bangham *et al.*, 1965). It was generally understood in the 1960s that lipid vesicles might be used to entrap medicines and dyes for systemic and local uses, as well as drug targeting (Shen *et al.*, 2003).

Due to their ability to encapsulated and effectively bring both lipophilic and hydrophilic drug components (Figure 2.1), they have been attracting more and more attention as a drug carrier for drug delivery systems of active ingredients and many different compounds in biological, pharmaceutical, medical, and nutritional fields (Fielding, 1991). Hydrophilic compounds are contained in the aqueous chamber, which ranges in diameter from 0.03 to 10 μm , whilst lipophilic elements are incorporated into the membrane (Chorilli *et al.*, 2004). Liposomes varied in diameter from around 20 nm to several micrometers and are made up of one or more concentric membranes with a thickness of about 4 nm.

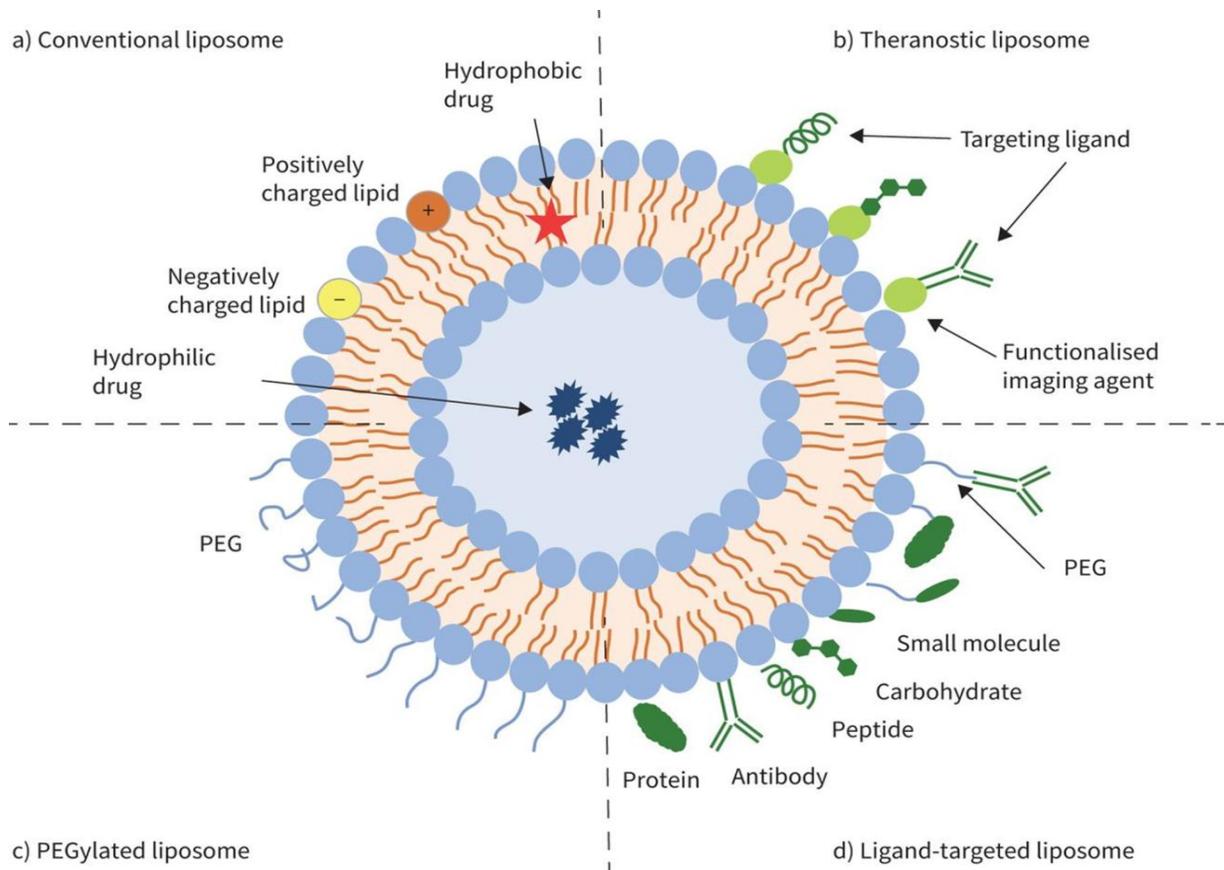


Figure (2.2) Structure of liposomes for drug delivery. a) Conventional liposome. b) Theranostic liposome. c) PEGylated liposome. d) Ligand-targeted liposome. PEG: polyethylene glycol. Reproduced and modified from (Chalmers *et al.*, 2021).

Liposomes, which are amphipathic molecules with a water soluble hydrophilic head fraction and a lipid soluble hydrophobic tail portion, have been made from a range of synthetic and naturally occurring phospholipids (Figure 2.1). They commonly include cholesterol in various ratios, ranging from 1:1 to 2:1 molar ratios of cholesterol to phosphatidyl-choline. The integration of cholesterol

with its hydroxyl group inside the lipid bilayer membrane improves liposome stability in bodily fluids, lowers membrane permeability to water soluble compounds, and increases the fluidity or microviscosity of the bilayer. The high solubility of cholesterol in phospholipid liposomes has been linked to both hydrophobic and particular head group interactions, however there is no conclusive evidence for cholesterol organization in the bilayer (Anwekar *et al.*, 2011).

Liposome properties are regulated by a variety of parameters, including lipid content, surface charge, particle size, and manufacturing process (Samad *et al.*, 2007). The particle size, lamellarity, and surface charge of liposomes may all be used to classify them. Liposomes are categorized as anionic, cationic, or neutral depending on their surface charge. Liposomes are classified as oligo-lamellar (few bilayer), unilamellar (single bilayer), multi-lamellar (multiple bilayer), small, big, or enormous depending on number of lamellae and particle size (Bonifacio *et al.*, 2014). Liposomes might be synthesized from a variety of phospholipid compositions or manufactured in a variety of ways, with changes in particle size, size distribution, surface electrical potential, number of lamellae, encapsulation efficiency, and surface modifications (Dwivedi & Verma, 2013).

Liposomes are commonly utilized as drug delivery nanocarriers because of their capacity to carry drugs to target areas while reducing systemic exposure. They are classified as per their kind (conventional, theranostic, PEGylated, or

ligand-targeted), size, lamellarity, and surface charge (Figure 2.1) (Chalmers *et al.*, 2021).

Liposome formulations are biocompatible and biodegradable, as they are made up of mammalian cell membrane-like elements, and they can enter biofilms and intracellular regions such as macrophages. Liposomes are appealing for drug delivery for a variety of reasons, including their pharmacological inactivity, ability to self-assemble, large aqueous center to carry large drug "payloads," controlled drug release, and potential for improved pharmacokinetics and reduced toxicity; they are particularly useful for drugs that require penetration of cell membranes (Chalmers *et al.*, 2021).

2.12.3. Phytosome nanoparticles:

The term phytosome is derived from two Greek words: "phyto" (plant) and "some" (cell-like). It is also known as pharماسome and herbosome (Upase *et al.*, 2019). The phytosome, also known as the phyto-lipids delivery system, is a TM technology developed by Indena S. P. of Italy that enhances the bioactivity of selected phytomedicines by incorporating phospholipids into standardized plant extracts or water soluble phytochemicals to generate lipid compatible molecular complexes that strengthen their absorption and usage by integrating phospholipids

into standardized plant extracts or water soluble phytochemicals to produce lipid compatible molecular complex (Tripathy *et al.*, 2013).

Phytosomes are a small structure that reflects a mixture of natural active compounds and phospholipid(s) that increases absorption of herbal extracts or isolated active components (Singh *et al.*, 2020). In an aprotic solvent, stoichiometric concentrations of phospholipids react with phytoconstituents to produce this complex (Kareparamban *et al.*, 2012). Its forms a bridge between the conventional and novel delivery system (Gandhi *et al.*, 2012). The phospholipids used in the preparation of phytosome nanoparticles are phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol; however, phosphatidylcholine derived from soybean is widely used due to its therapeutic value in cases of liver diseases, alcoholic steatosis, drug-induced liver damage, and hepatitis. Phospholipids are often used as natural digestion aids and transporters for fat- and water-soluble compounds (Upase *et al.*, 2019).

Physical size, membrane permeability, entrapment ratio, chemical composition, and the quantity and purity of the precursor chemical components are just few of the parameters that might influence the unique originality of phytosome nanoparticles in their physical condition (Kumar *et al.*, 2019). In fact, nutraceuticals are more sophisticated herbal preparations that typically contain bioactive phyto-constituents of plant extract such as terpenoids, glycosides,

flavonoids, or else as nutraceuticals. They are incapable of transitioning from a hydrophilic to a lipid-friendly state of the outer cell membrane (Karimi *et al.*, 2015). It can prevent digestive fluids and gut bacteria from destroying the beneficial components of medicinal plants or nutraceuticals (Gandhi *et al.*, 2012). Pharmacokinetics and pharmacodynamics investigations in experimental animals and human volunteers have confirmed that phytosomes have a higher bioavailability than non-complexed botanical derivatives (Keshwani *et al.*, 2016).

Like liposomes, phytosomes include both lipophilic and hydrophilic domains, allowing them to entrap both lipophilic and hydrophilic drugs (Pathan & Bhandari, 2011). Phytosomes are thought to be more stable than liposome nanoparticles, which solely encapsulate these elements in the aqueous domain (Figure 2.1) (Verma *et al.*, 2013).

Through its preparation in different compositions such as solutions, suspensions, syrups, emulsions, creams, lotions, gels, pill, capsule, powder, granules, chewable tablet, and aqueous microdispersion, the phytosome has obtained important role in various disciplines such as pharmaceuticals, cosmeceuticals, and nutritional supplements (Choudhary and Sekhon, 2011).

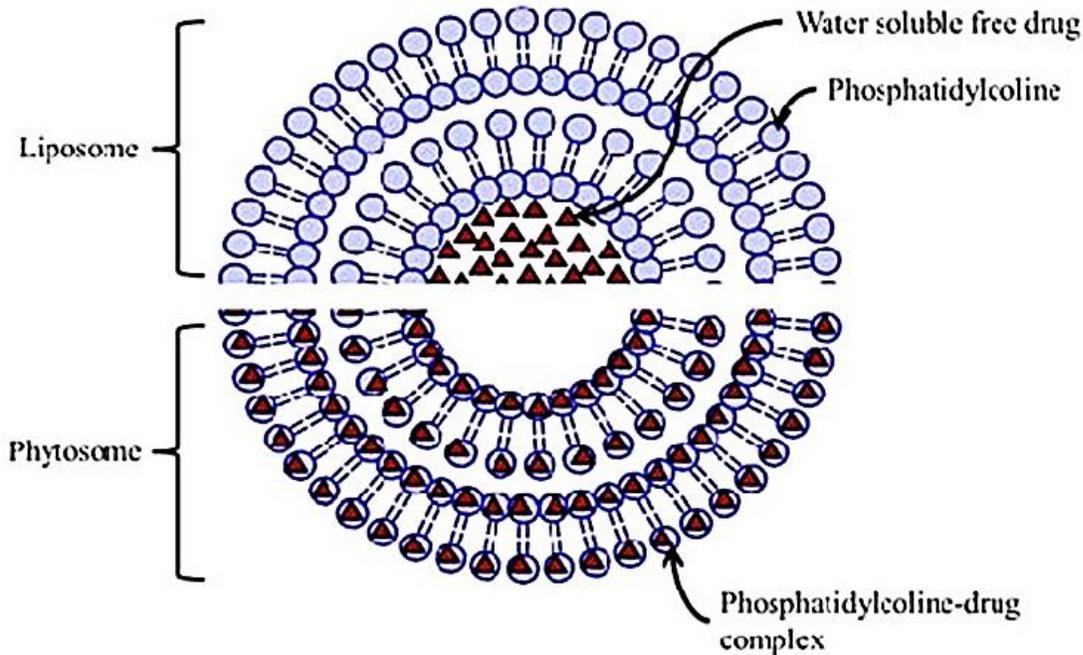


Figure (2.3) Difference between liposome and phytosome. The molecular organization of phytosomes (lower segment) liposome (upper segment). The figure modified from (Awasthi *et al.*, 2011).

2.13. Toxicity of Nanoparticles on the Reproductive System:

Females are more susceptible to nanoparticle toxicity than males, and poisoning in this group may have an impact on reproduction and embryonic development. Furthermore, different kinds of nanoparticles have harmful effects on male germ cells, embryonic development, and the female reproductive system. These effects are linked to nanoparticle composition, concentration, delivery method, and animal type. As a result, it is critical to comprehend the effects of nanoparticles on animal development and reproduction. Many research have

looked at the impacts of nanoparticles on primary and secondary target organs, with a focus on the clinical, cellular, and molecular effects of nanoparticles on female and male reproductive systems in vivo and in vitro (Brohi *et al.*, 2017).

2.14. Nanoparticle Mediated Immunotoxicity:

The basic purpose of immune system is to defend the body against potentially harmful small objects. Innate and adaptive immunity are two main categories of the immune system. When in touch with foreign bioactive agents like bacterial and viral strains, innate immunity produces a non-specific immune response. Antigen-presenting cells (APCs), which are triggered by distinct receptors that function as pathogen identification agents, activate the acquired immune system. On contrary, the adaptive immune system is responsible for manufacturing antibodies in response to antigens and follows a complex process. Nanoparticles' surface features and compositional qualities aid immune system detection. As a result, these properties may be employed as handles to control the interplay of immune systems and nanoparticles. There have been multiple instances of negative interactions between the immune system and nanoparticles as a result of immunostimulation or immunosuppression, which might lead to inflammatory or autoimmune diseases (Figure 2.3) (Ray *et al.*, 2021) .

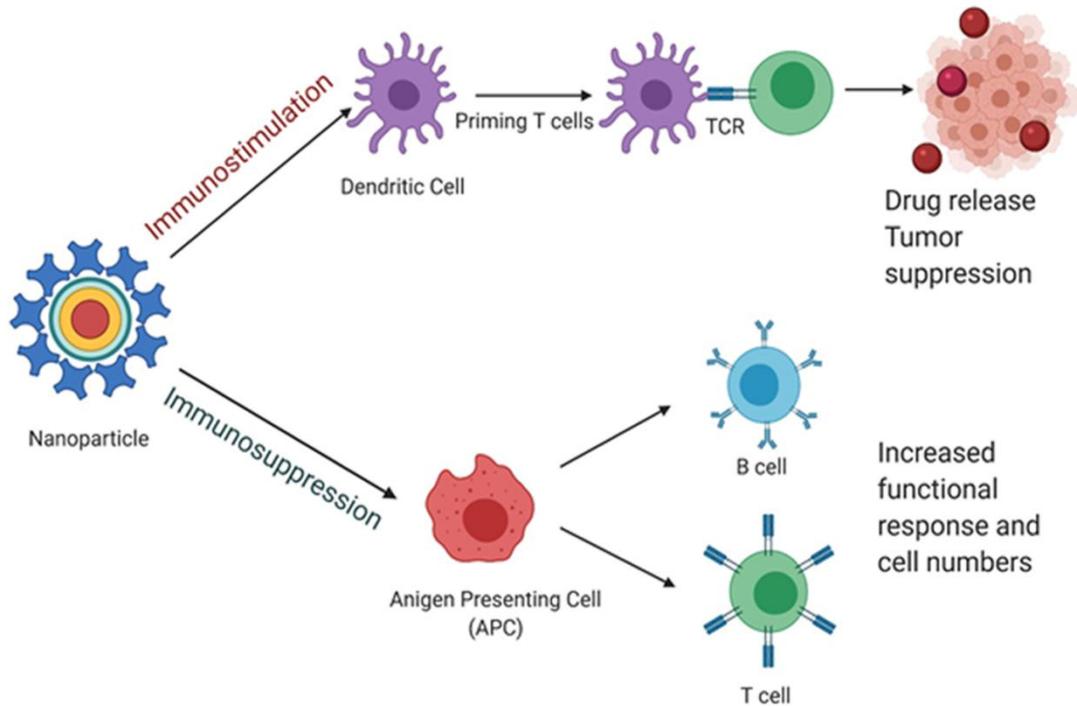
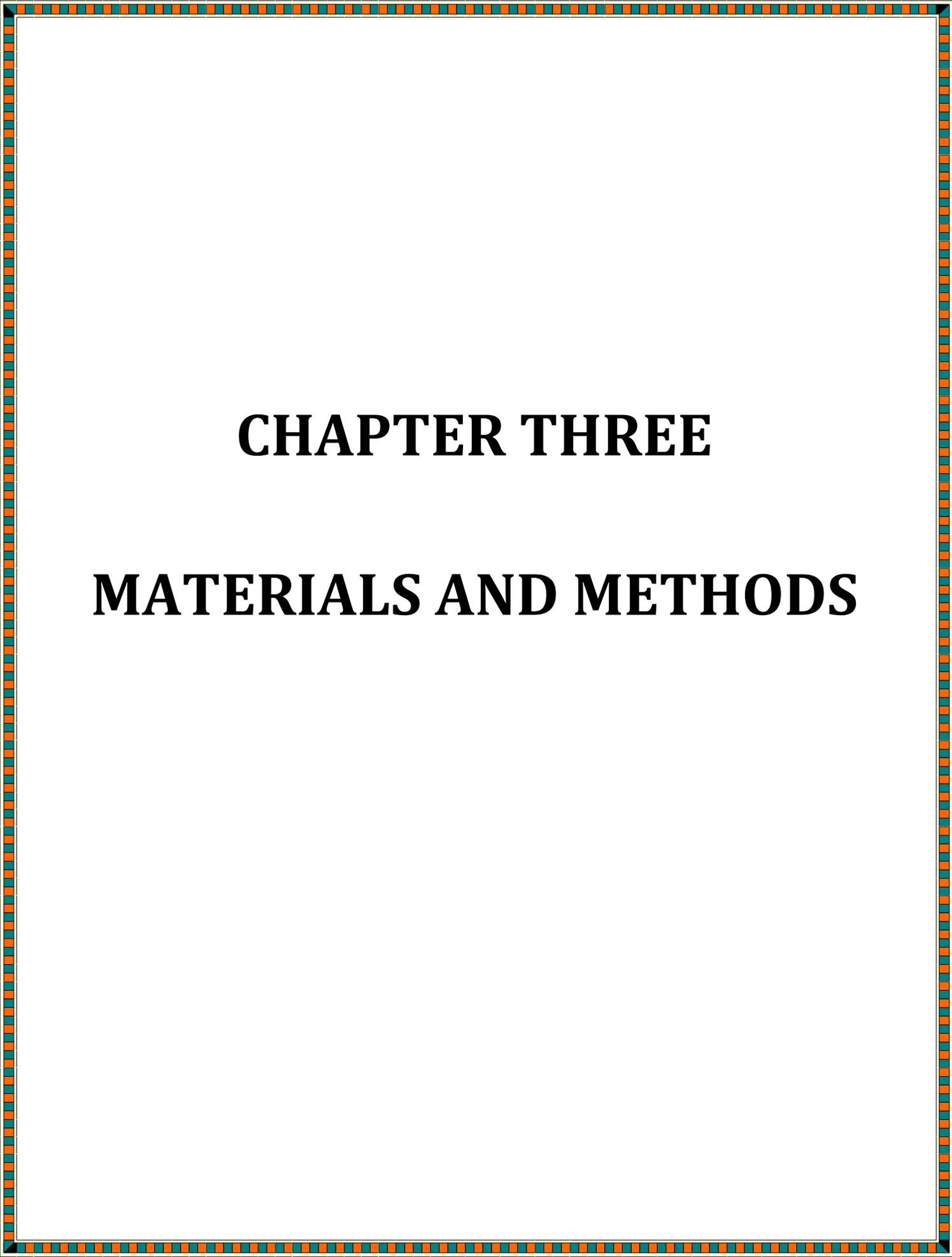


Figure (2.4) The two major ways of interaction between nanoparticles and the immune system. TCR (T-cell receptor) These modalities are: 1- dendritic cell immunostimulation followed by T-cell priming, resulting in drug release into tumor cells; and 2- APC immunosuppression, resulting in increased T and B-cell numbers and functioning. Modified from (Ray *et al.*, 2021).



CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

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3.1. Materials

3.1.1. Equipment and Apparatuses

Table (3-1): Instruments and their origins.

NO.	Apparatuses Name	Manufacture	Origin
1	Enzyme Linked Immune Sorbent Assay (ELISA)	Organ on Teak micro well system reader 230 s	Belgium
2	Electron Sensitive Balance	Denver	Canada
3	Balance	Shimadzu company	Japan
4	Centerfuge	GMBH	Germany
5	Cool centrifuge	Hettich	Germany
6	Cylinder	HBG	England
8	Hote plate	R-labinco	Germany
9	Imbalance	Kern	Germany
10	Incubator	Binder	Germany
11	Insulin syringe	Q-ject	China
12	Micro cuvettes	Biotech	England
13	Micropipete	Winlab	Germany

14	Microscope	Olympus	Germany
15	Oven	Memmert	Germany
16	PH meter	Misonix	Romani
17	Sonicator	Newtown	Germany
18	Uv spectrophotometer	Jenway	England
19	Water bath	Optima	Japan
20	GCMS	Schimadzo	Japan
21	Rotary evaporator	Hahnvapor	Korea
22	Transmission electron microscope	Jeol ltd	Japan
23	Zeta sizers	Malvern	Uk
24	Vortex	Quality Lab System	England
25	Rotary Evaporator	Orem Scientific Ltd	Switzerland
26	Dissection Set		Germany
27	Blender	National MX-T76N, Deluxe Super	Japan

3.1.2. Chemical and Biological Materials :

Table (3-2): Chemicals with their supplier which were used in study

NO.	Name of solution	Company	Orgin
1	Acetic acid		Belgium
2	Tween 80	Ran reac	Spain
3	Chloroform		England

4	Formalin	BDH	England
5	Cholesterol	Biolabo	France
6	Deionized water		Iraq
7	Formaldehyde		Iran
8	Ketamine	Kepto	Holland
9	Methanol	Pan Reac	Spain
10	Vitamine ETPGS	MEC	USA
11	Vorapaxar	MEC	USA
12	Xylazine	VMD	Belgium
13	Eosin Stain	Fluka AG , Buchs	Switzerland
14	Ethanol alcohol (96%)	BDU chemical Ltd	England
15	Physiological Normal Salin	BDU chemical Ltd	England

3.2. Methods :

3.2.1 Preparation of eosin stain:

1 g of eosin were dissolved in 100 ml of distilled water (Luna, 1968).

3.2.2 Preparation Formalin solution:

It was prepared the Formalin solution by taking 10 ml of a 40% formalin solution and completing the volume to 100 ml with distilled water to get a 10% formalin solution (Bancroft, J. D., Stevens, A., & Turner, 1982).

3.2.3. Plant Collection

The flowers of *Hibiscus rosa-sinensis* L were collected from nursery and agricultural areas in Babylon and Karbala province during the period from October to November 2021. The flowers were collected in the flowering stage after they were well cleaned and washed with water and left to dry in the air at room temperature for two weeks. After that, the dried flowers were blended with a blender, until used.

3.2.4. Preparation of Crude ethanolic compounds of *H.rosa* flowers:

The *Hibiscus rosa-sinensis* Linn flowers were air-dried under the shade, then grind into powder and used for further studies, Five grams of fine powder of flowers was extracted with 100 ml of ethanol (70%) and 100 ml of acetone in a round bottom flask with a magnetic stirrer for 10 hours at room temperature. The flowers extract was then filtrated by Whatman filter paper then the extract was placed in the oven at 40 °C until removed all solvent. After grinding it, Ribereau- Gayon (1972).Sodium azide is added to prevent bacterial growth. The extract then kept in the refrigerator until used.

3.2.5. Preparation Nanoparticles (Phytosome) :

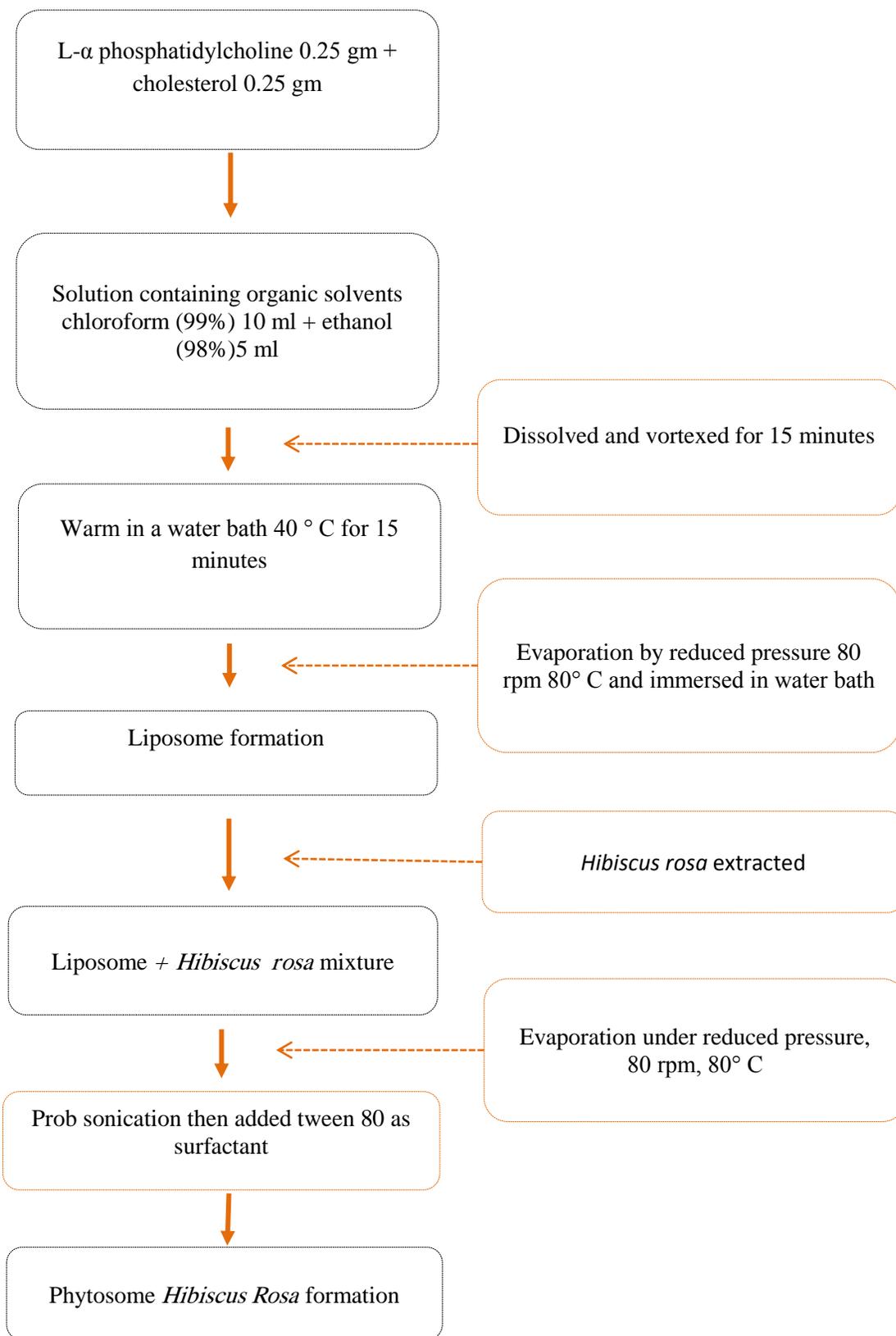
Phytosome preparation were performed in the College of Veterinary Medicine / University of AL-Qassim green and set as follows:

The liposomes were prepared by dried thin lipid film technique as described previously by (Ramana *et al.*, 2007). The lipid phase components include L- α phosphatidylcholine 0.25 gm and cholesterol 0.25 gm was dissolved in 15 ml of mixed organic solvents chloroform: ethanol (2:1) mixing in the 25 ml glass test tube, vortexed for 30 minutes, 1500 rpm.

The mixture was kept warm in a water bath at 40°C for 15 minutes and transferred to a round bottom flask connected with a vacuumed rotary evaporator machine at; 80 rpm, 40° C attached to a vacuum pump. The round-bottom flask was immersed in a thermostatic water bath at 40 °C for 2 hours to affirm the dryness of the thin lipid film deposited on the inner walls of the round bottom glass flask. The empty liposome was formed and suspended by a phosphate buffer.

3.2.6. Phytosome Load on *Hibiscus rosa-sinensis* L.

The 400 mg of prepared extract was added to the 800 mg of empty liposome and vortexed at 1500 rpm for 30 minutes and then phytosome solution was sonicated for 5 minutes using a probe sonicator (Hielscher sonicator, ultrasonic processor up 100H [100 W, 30 kHz, 0.8 cycles, 80% amplitude]), and tween 80 was added until final concentration 5% while stirring the solution and it was further sonicated for 5 minutes rejoined with the previous set of rotary evaporator under 80 rpm, 80 °C for 30 min, as described in figure (3.1).



(Fig. 3.1) Flowchart of preparation of phytosomal formulations (Ramana *et al.*, 2007).

3.3. Gas chromatography-Mass spectrometry (GC/MS) analysis:

Agilent 5975C Series Gas Chromatography/Mass Selective Detector (GC/MS) was used in the current study in Nanotechnology Department/ University of Technology in Baghdad as shown in figure (3.2). The analysis uses a compact silica column packed with DB-WAX (100% dimethyl polysiloxane, 30 m x 0.25 mm ID x 2.5 μ m). The oven temperature was set at 60 °C for 10 min, then raised at 20 °C/min to 230 °C and held at 250 °C for 10 min. The carrier gas, helium, was set to a linear velocity of 30 cm/sec (Agilent Technologies Co; Ltd. USA).



(Fig. 3.2) Gas chromatography-Mass spectrometry (GC/MS) with triple-axis High energy diode (HED) Electron Multiplier (EM) detector used in the present study.

3.4. Characterization of the nanoparticules ethanolic extract of the *H. rosa* flowers

3.4.1. Long term stability study :

This part conducted by assessment the long-term stability of Phytosome of *H. rosa* -NPs destined by nano-precipitation method, these were stored at RT, 4 ± 2 °C for two months. Samples were analyzed after these periods to determine particle size value in accordance with the method already described.

3.4.2. Particle size distribution and zeta size :

A Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) was used to determine the distribution of particle size (mean diameter and polydispersity index), as shown in figure (3.3). Briefly, 1 ml of Phytosome of *H. rosa* loading suspension was prepared by 30 s sonication in MilliQ water. The suspension above (10 μ l) with MilliQ water was diluted to 1 ml and analysis was performed. The mean diameter of three determinations has been calculated for each sample.



(Fig. 3.3) Malvern Zetasizer Nano ZS used in the present study.

3.4.3. Fourier-transform infrared spectroscopy (FTIR) :

Functional groups at the surface of the prepared materials were investigated using FTIR spectroscopy (Panayotov and Yates, 2007). FTIR spectra were recorded using (Shimadzu ft-IR 8400S) in the range from 4000 to 400 cm using KBr disc to characterize the Nano products surface properties.

3.4.4. X-ray Diffraction (XRD) :

Paul Scherrer equation used to determination of size of crystals in the form of powder. The Scherrer equation can be written as:

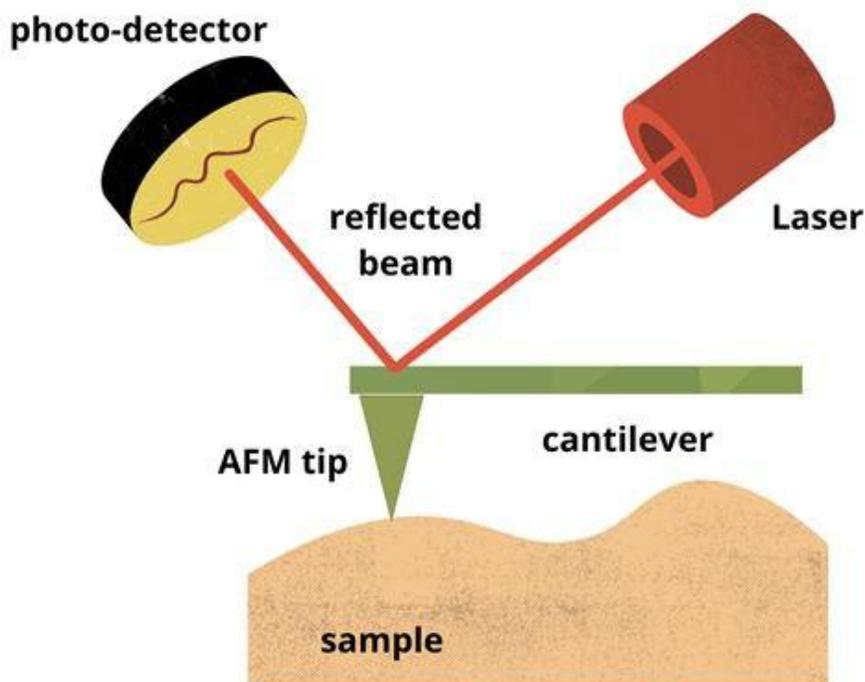
$$D_p = (0.94 \times \lambda) / (\beta \times \cos\theta)$$

Where, D_p = Average Crystallite size, β = Line broadening in radians, θ = Bragg angle, λ = X-Ray wavelength (Saw *et al.*, 2018).

3.4.5. Atomic force microscopy (AFM) :

Morphological investigation of the prepared materials in this study was performed using atomic force microscopy. This device unable to probe surface of the studied samples in three dimensions. The AFM machine has a probe that is used to scan sample surface (Egerton, 2005). This technique provide us with image in three dimensions for each sample, depending on the properties of the sample (contact or non- contact) mode is used detector tracks and records these beam changes, as illustrated in figure (3.4). AFM images were obtained in this work using the SPM model AA 300 of Angstrom Advanced INC USA. The samples were pre-prepared by

adding few drops of prepared materials that were pre dispersed in acetone via using sonication technique for 20 minute.



(Fig. 3.4) Schematic illustrations showing basic principles of AFM
(Image Credit: Ilamaran Sivarajah).

3.4.6. Scanning electron Microscopes (SEM) :

Surface morphology of the prepared materials was investigated using SEM. This technique shows the relative homogeneity of the investigated samples and provide image in one dimension of the scanned sample (Egerton, 2005), using finely focused beam of electrons from an electron source which may be thermionic like a Tungsten filament (in some regular SEM), or many be an field emitting source (in FE-SEM). SEM images that used in this study was, FE-SEM Hitachi model S-4160, Nano-Electronic Lab, University of Tehran, Iran.



(Fig. 3.5) Scanning electron Microscopes FE-SEM Hitachi model S-4160, used in the present study.

3.5. Animals of experimental :

This study was conducted in the University of Babylon / College of science/ department of biology, Al-Qassim green university/ College of veterinary medicine, veterinary hospital in Babylon and university of technology in Baghdad for the period from October 2021 to June 2022.

Twenty (20) animals were taken from the Albino rat male, aged between 2-3 weeks and their weight ranges between 120-250 gm they were placed in the animal house at the University of Babylon. They were divided into 5 groups (4 animals for

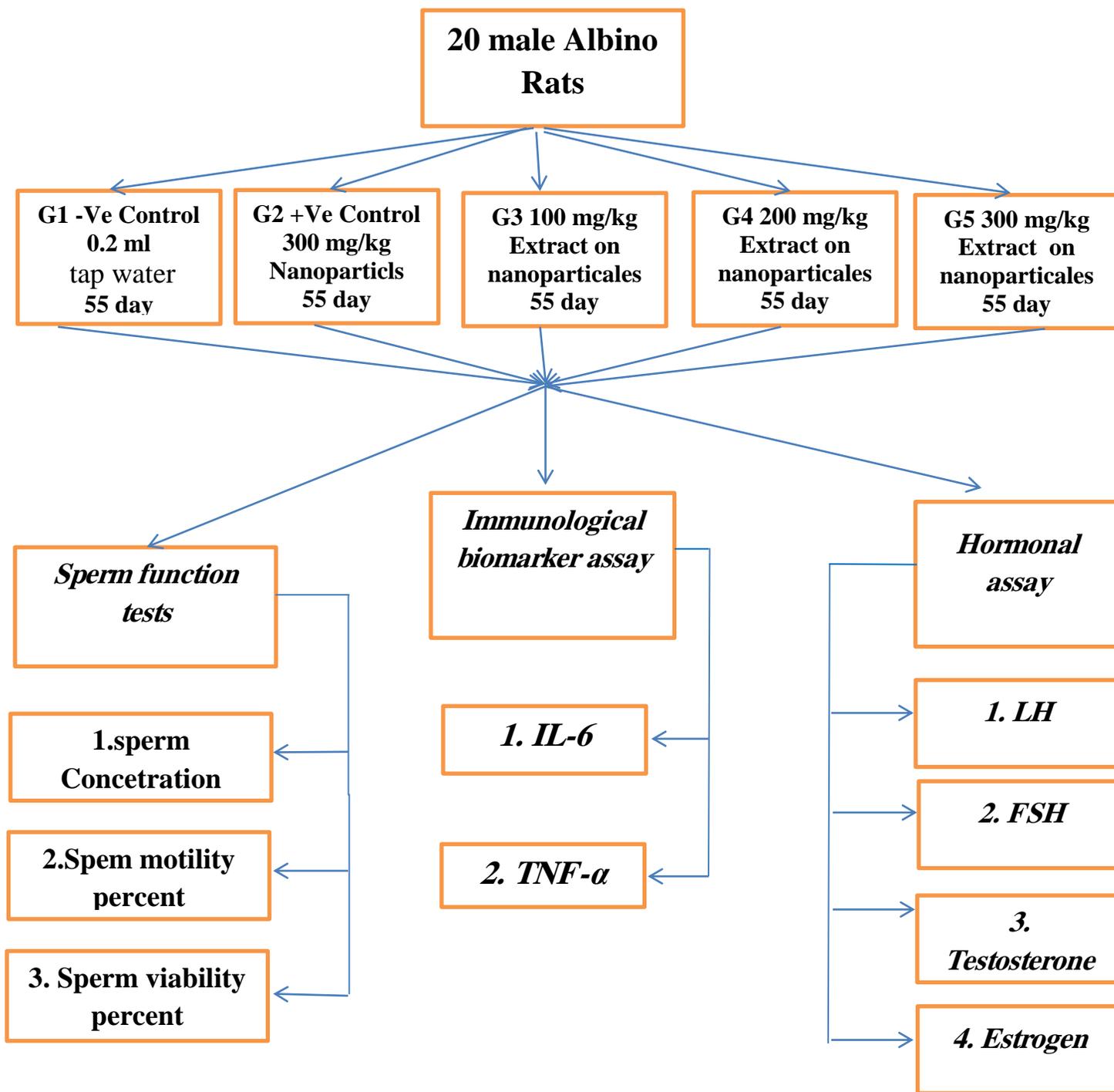
each group). They were dosed for 55 day consecutively at a rate of one dose per day using a 5ml wine syringe modified for this purpose.

After the end of the dosing period, the animals were anesthetized by chloroform. The abdominal cavity was opened with a sharp scalpel, then the left epididymis was removed from them for the purpose of calculating sperm concentration, sperm motility percent and sperm viability percent after aspirate epididymis sperm from the tail and put it in normal saline.

By drawing the dose-response curve for the above three criteria, then finding the effective dose for each criterion, and then calculating the arithmetic average for three doses as follows:

- Group 1 (Negative Control): treated with tap water (0.2 ml per day).
- Group 2 (Positive Control): treated with Nanoparticles (300mg/kg empty liposome per day).
- Group 3: treated with flower extract nanoparticles (100mg/kg per day).
- Group 4: treated with flower extract nanoparticles (200mg/kg per day).
- Group 5: treated with flower extract nanoparticles (300mg/kg per day).

3.5.1. Experimental Design :



(Fig. 3.6) A diagram demonstrating the experimental design of the present study.

3.6. Sperm parameters study :

3.6.1. Microscopic examination :

One drop was taken from each well mixed sample after complete gluing, and the drop was placed on a warm slide and covered with a standard cover slip. It was examined first under 40 X.

The following sperm parameters were measured:-

3.6.1.1. Sperm concentration in the epididymis :

The sperm concentration was estimated from the average number of sperm in ten random fields and the average number was multiplied by a factor of 10^6 (Hinting, 1988).

3.6.1.2.Sperm motility percent & Grade activity in the epididymis :

Ten field in each sample were tested to evaluate the sperms motility then the average number of sperm with progressive and non-progressive and immotile sperm percent with in a limited space were recorded. (Hinting, 1989).

3.6.1.3.Sperm viability percent in the epididymis :

The percentage of sperm viability was calculated using Eosin dye (concentration of 0.5%). The basis of the action of this dye is to place a drop of this dye on a glass slide, then a drop of semen is placed and the two drops are mixed on the glass slide and then covered with the cover slide. The dead sperm will dye their

entire head with the dye, since the cell membrane of the dead sperm has lost the ability to permeate the dye into the cell and made it red, while the living sperm will dye its outer membrane only with the dye without the dye entering the cell because the membrane prevents its passage.

At least sperm in ten fields were counted in each segment, and then the percentage of sperm viability was calculated according to the following equation : -
$$\% \text{ of sperm viability} = \text{number of viability sperm} / \text{total number of sperm} \times 100$$

(Acosta *et al.*, 1986).

3.7. Finding the effect of the effective dose (ED₅₀) of ethanolic extract of *H. rose* flowers on the fertility on male albino rats :

The effective dose value ED₅₀ of the ethanolic extract of *Hibiscus rosa-sinensis* loaded on nanoparticles was calculated by sperm concentration, prograssive sperm motility percent .testosterone level and TNF- α level. It was estimate the ED₅₀ according to method of draw a dose-response curve in (Appendix 3) .

3.8. Hormonal and Immuno assay :

3.8.1. Samples:

The rats were autopsied after they were anesthetized with chloroform The blood was drawn by stab of the heart by 5 ml medical wine syringes, then the blood was placed in dry, sterile tubes, free of anticoagulant, and the blood was left for an hour to take enough time to clot.

Then the blood was transferred to a centrifuge to separate it at a speed of 5000 rpm for 5 minutes to obtain the serum. The serum that was placed in special tubes (Eppendorf tube) and kept in the refrigerator at a temperature of -20°C until hormones were measured.

3.9. ELIZA Tests (Hormonal assay):

The level of testosterone, Estrogen, LH and FSH was measured using the Eliza /enzyme-linked immunosorbent assay (ELISA) technique manufactured by the company (Abbkin Scientific Co; Ltd. China and Sunlong Biotech Co; Ltd. China) and using a special measurement kit for each hormone (Appendix).

3.9.1. LH:

Estimation with ELIZA kit (Abbkin Scientific Co; Ltd. China) the procedure the LH found in the (Appendix).

3.9.2. FSH:

Estimation with ELIZA kit (Sunlong Biotech Co; Ltd. China) the procedure the FSH found in the (Appendix).

3.9.3. Testosterone:

Estimation with ELIZA kit (Sunlong Biotech Co; Ltd. China) the procedure the testosterone found in the (Appendix).

3.9.4. Estrogen:

Estimation with ELIZA kit (Sunlong Biotech Co; Ltd. China) the procedure the Estrogen found in the (Appendix).

3.10. Immunological biomarkers assay:

The level of TNF- α and IL-6 was measured using the Eliza /enzyme-linked immunosorbent assay (ELISA) technique manufactured by the company (Sunlong Biotech Co; Ltd. China) and using a special measurement kit for each proteins (Appendix).

3.10.1. TNF- α :

Estimation with ELIZA kit (Sunlong Biotech Co; Ltd. China) the procedure the TNF- α found in the (Appendix).

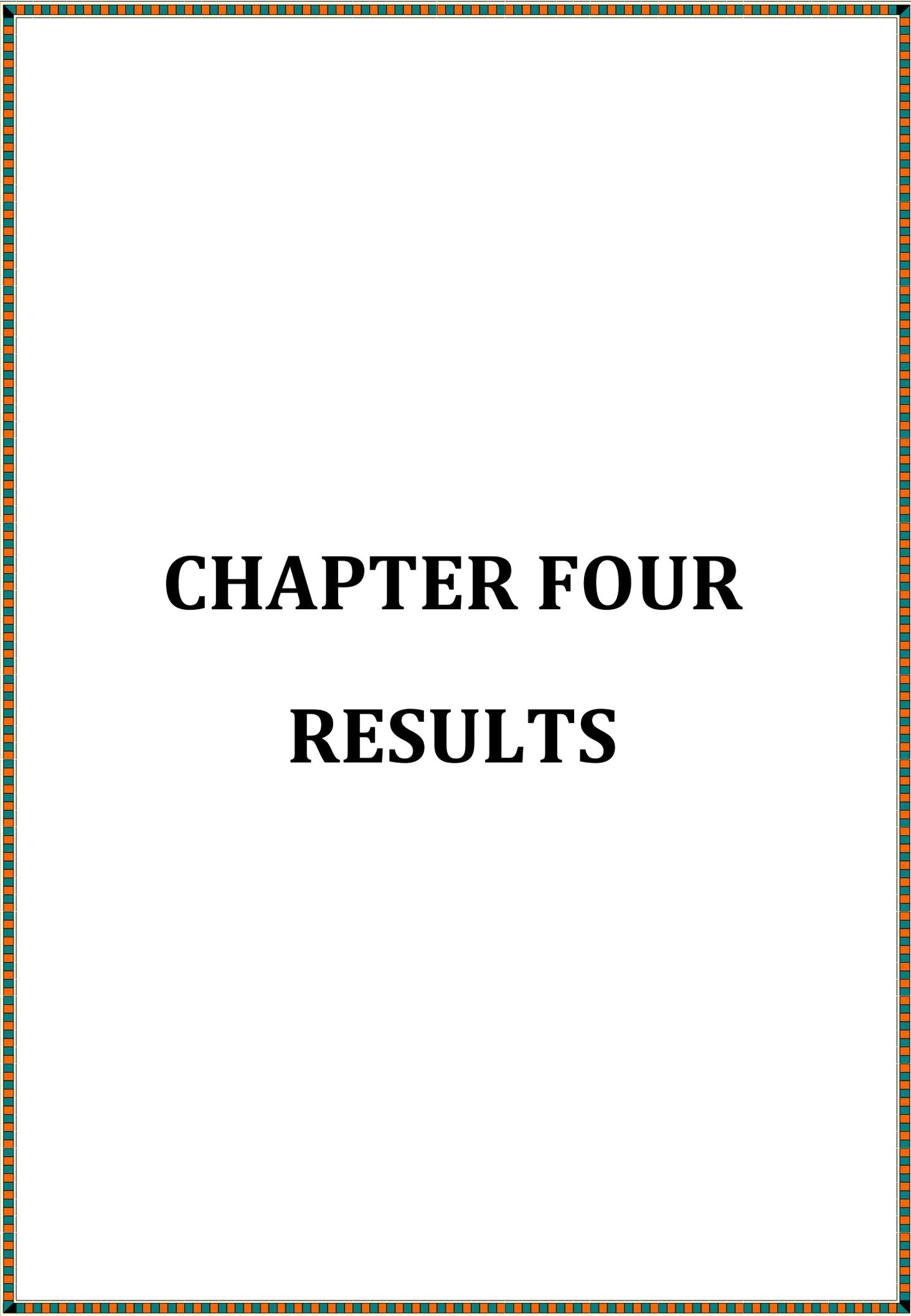
3.10.2. IL-6:

Estimation with ELIZA kit (Sunlong Biotech Co; Ltd. China) the procedure the IL-6 found in the (Appendix).

3.11. Statistical analysis :

The SPSS were used to infer the significance and analysis of variance (ANOVA), and the least significant coefficient (LSD) was used to compare the results, the Correlation Coefficient test (Scheffler, 1980), in addition to the standard

statistical methods used in determining the mean and standard deviation (SD) (Al-Ukaeli, S. A. and Al-Shaeb, 1998).



CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

4.1. Separation of the components of the ethanolic extract by Gas Chromatography-Mass Spectrometry (GC-MS):

The results pertaining to GC-MS analysis led to the identification of a number of compounds from the GC fraction of the ethanolic extract of *Hibiscus rosa* flowers. The results of the present study were tabulated and The results revealed that the presence of many active compounds such as (Palmitic acid, ethyl ester/ Ascorbic acid/ 9-Octadecenoic acid, methyl ester/ Methyl stearate/ Oleic acid/ 9, 12-Octadecadienoic acid/ Eicosane/ Heptadecane/ 1-1,5-dimethylhexyl/ Fumaric acid/ Butanedial/ Stigmasterol, and Beta-Sitosterol) (Fig 4.1) and (Table 4.1) .

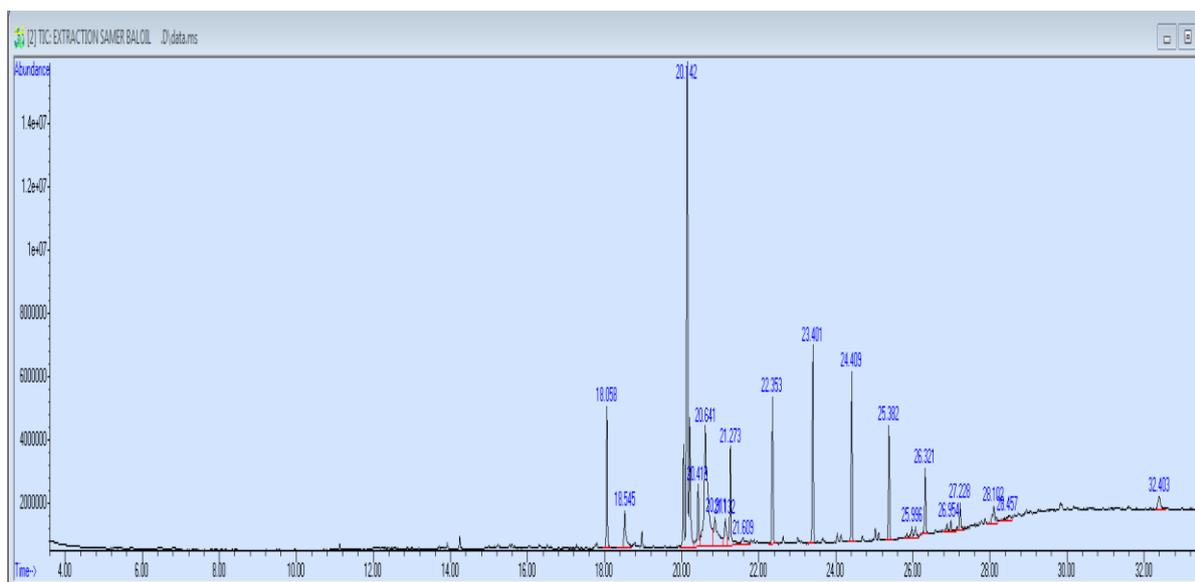


Fig (4.1): GC-MS analysis and fractionations of the ethanolic extract of *H. rosa* flowers.

Table (4.1): Components detected in the plant of ethanolic extract of *H.rosa* flowers MW: Molecular Weight, RT: Retention Tim and activity.

No	Compounds	Molecular Formula	RT	Area % nm	Activity
1	Palmic acid, ethyl ester	$\text{CH}_3(\text{CH}_2)_{14}\text{CO}_2\text{CH}_3$	18.054	4.82	Antioxidant, antimicrobial, and anti-inflammatory activities
2	Ascorbic acid	$\text{C}_6\text{H}_8\text{O}_6$	18.540	2.65	Activation of immune system, antioxidant, cofactor in many enzymatic reactions.
3	9-Octadecenoic acid, methyl ester	$\text{C}_{19}\text{H}_{34}\text{O}_2$	20.147	29.87	Antioxidant and antimicrobial activities
4	Methyl stearate	$\text{C}_{19}\text{H}_{38}\text{O}_2$	20.417	2.92	Detergents, emulsifiers, wetting agents, stabilizers, resins, lubricants.
5	Oleic acid	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	20.644	14.98	Improve heart conditions by lowering cholesterol and reducing inflammation.
6	9,12-Octadecadienoic acid	$\text{C}_{18}\text{H}_{32}\text{O}_2$	21.129	1.81	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocide, insectifuge(cide), antihistaminic, antieczemic, antiacne, 5- α reductase inhibitor, antiandrogenic, antiarthritic, anti-coronary.
7	Eicosane	$\text{C}_{20}\text{H}_{42}$	22.348	5.7	anti-microbial, anti-inflammatory, proliferation, migration and collagen synthesis
8	Heptadecane	$\text{CH}_3(\text{CH}_2)_{15}\text{CH}_3$	25.380	4.22	Unknown
9	1-(1,5-dimethylhexyl)	$\text{C}_{11}\text{H}_{22}$	25.9	1.61	Pesticides
10	Fumaric acid	$\text{HO}_2\text{CCH}=\text{CHCO}_2\text{H}$	26.955	1.22	Treat a variety of skin disorders, including psoriasis cutaneous sarcoidosis.
11	Butanedial	$\text{C}_4\text{H}_6\text{O}_2$	28.099	1.44	An emollient and a humectant and helps retain skin moisture by creating a barrier on the skin.
12	Stigmasterol	$\text{C}_{29}\text{H}_{48}\text{O}$	28.455	1.57	Used in the manufacture of progesterone and corticoids
13	Beta-Sitosterol	$\text{C}_{29}\text{H}_{50}\text{O}$	32.4	1.2	β -sitosterol is used to produce estrogens, contraceptives, diuretics, and male hormones

4.2. Characterization of the ethanolic extract of the *H.rosa* flowers *sinensis* :

The average particle diameter of *H. rosa*–*sinensis* L. loaded Phytosome NPs using solution of different emulsifier, such as vitamin E TPGS and Tween 80 to characterization suitable particle size using particles sizers was showed in figure (4.2a-b-c-d). As well as the Table (4.2) summarized the effective of average particle diameter (161.4, 83.5, 119.9 and 69.0 nm) of Hibiscus–NPs with polydispersity index value around (0.226, 0.120, 0.319 and 0.03). The morphology of the *H. rosa* - NPs was also confirmed in terms of crystallite size utilizing scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The particle size was negligible elevated (Table 4.1) around 100 nm with a slightly change in PDI value, whereas this suggested the satisfactory stability of *H. rosa* loaded phytosome NPs during storage.

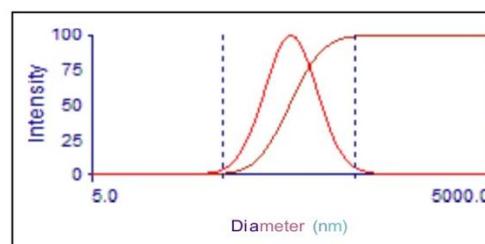
Table (4.2) also displayed the quantities of *H. rosa* encapsulated in the nanoparticles, which was measured by UV-vis spectrophotometry at the absorbance peak of *H. rosa* that is 250 nm. After *H. rosa* loading quantity and encapsulation efficiencies of different *H. rosa* loaded NP formulations formulated by fixed volume ratio of lecithin: cholesterol were 2: 1 was more suitable as well as that tween 80:2% is perfect emulsifier to reduced particle size than tween 5% and TPGS. The elevated loading efficiency content of *H. rosa* loaded nanoparticle was observed as

10.53 to 6.89 with encapsulation efficiency was more than 98 as illustrated in Table (4.2).

Table (4.2): Effect of emulsifier and organic solvent on particles size, loading efficiency, encapsulation efficiency of liposome loaded *Hibiscus rosa – sinensis* L.

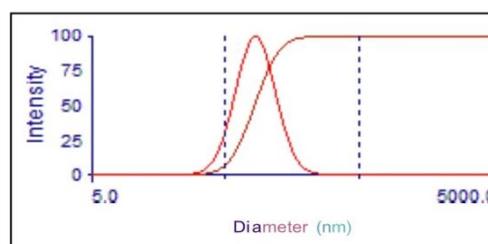
Ref	Organic solvent Methanol :chloroform	Emulsifier	Particle size nm	Loading efficiency	Encapsulation efficiency
2B-0	1:1	Vitamin E TPGS	161.4	7.72	70
2B-1	2:1	Tween 80 5%	83.5	7.02	64
2B-2	1:1	Tween 80 5%	119.9	8.7	81
2B-3	2:1	Tween 80 2%	69.0	9.85	95

Effective Diameter: 161.4 nm
 Polydispersity: 0.226
 Baseline Index: 7.9/100.00%
 Elapsed Time: 00:01:30



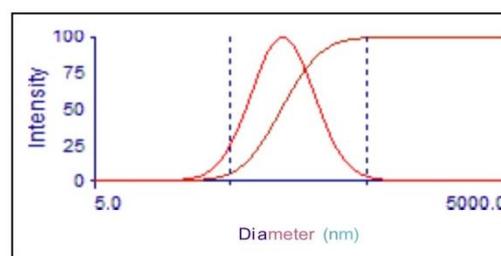
(figure 4-2a)

Effective Diameter: 83.5 nm
 Polydispersity: 9.6/ 98.82%
 Baseline Index: 9.6/98.82%
 Elapsed Time: 00:00:45



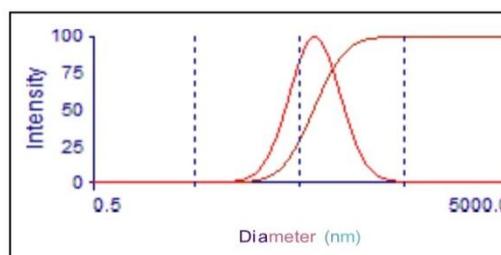
(figure 4-2b)

Effective Diameter: 119.9 nm
Polydispersity: 0.319
Baseline Index: 0.0/ 88.30%
Elapsed Time: 00:01:30



(figure 4-2c)

Effective Diameter: 69.0 nm
Polydispersity: 0.0363
Baseline Index: 8.7/ 93.94%
Elapsed Time: 00:01:30



(figure 4-2d)

Fig (4.2): particle size and dispersion index PDI determined by particle sizers.

4.2.1. Particle size and zeta potential analysis:

Liposomes can be made with desired sizes and zeta potentials, so they are well suited for establishing trapping in a salinity gradient and to perform characterization. This study used liposomes with four different lipid compositions ratios that are either 1:1 with Vitamin E TPGS (Fig 4.3) , 2:1 tween 5% (Fig 4.4) or 1:1 with tween 5% (Fig 4.5) and 2:1 with tween 2% (Fig 4.6) consequently, different zeta potentials (8, 29, 1.6 and 38 mV).

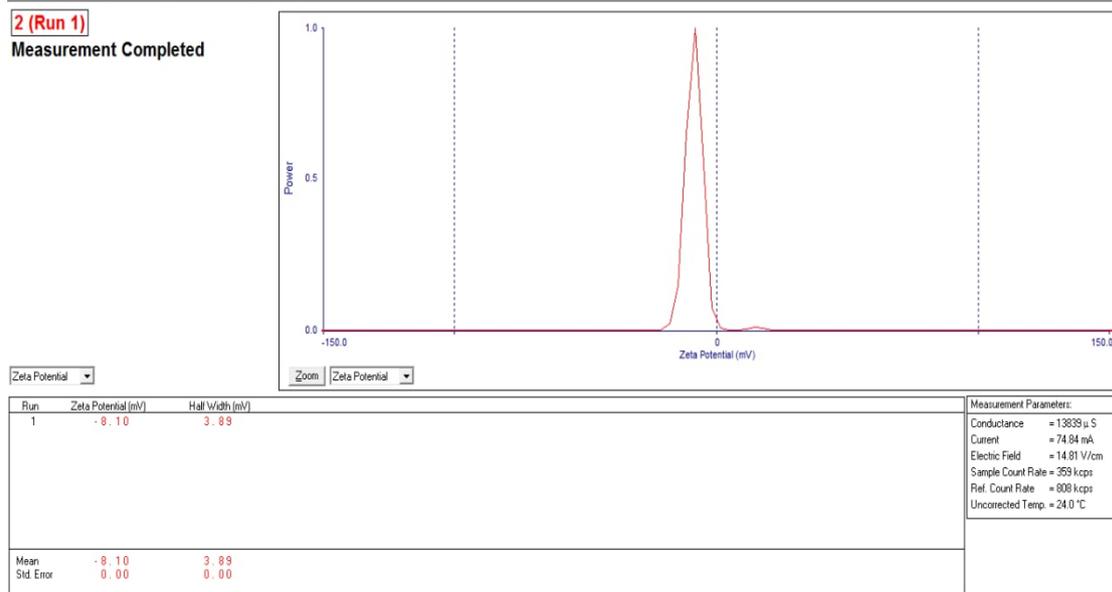


Fig (4.3): Zetapotential liposomes with four different lipid compositions ratios 1:1cholesterol to lecithin with TPGS.

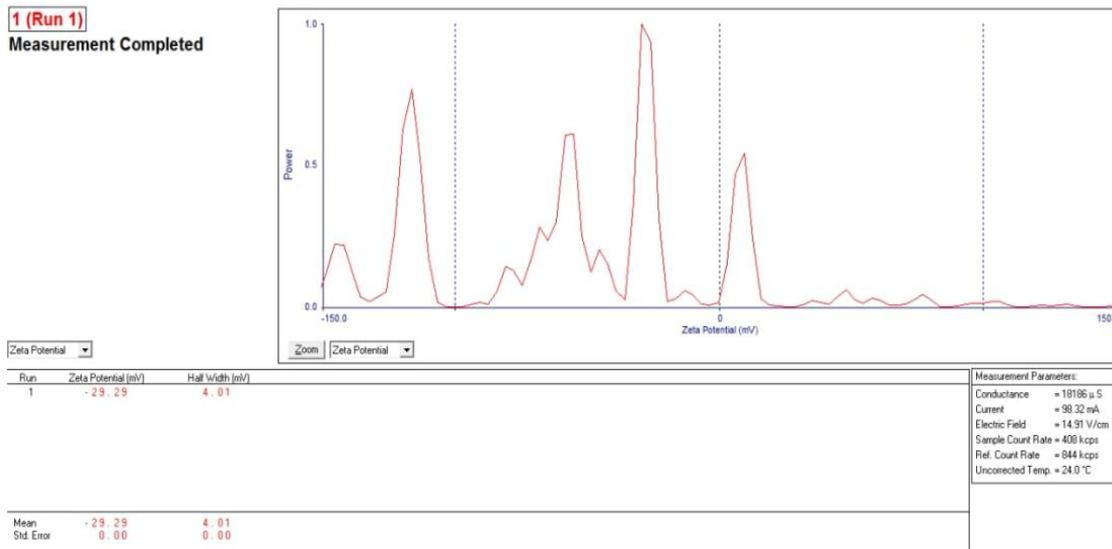


Fig (4.4): Zetapotential liposomes with four different lipid compositions ratios 1:2 cholesterol to lecithin with Tween 80 5% .

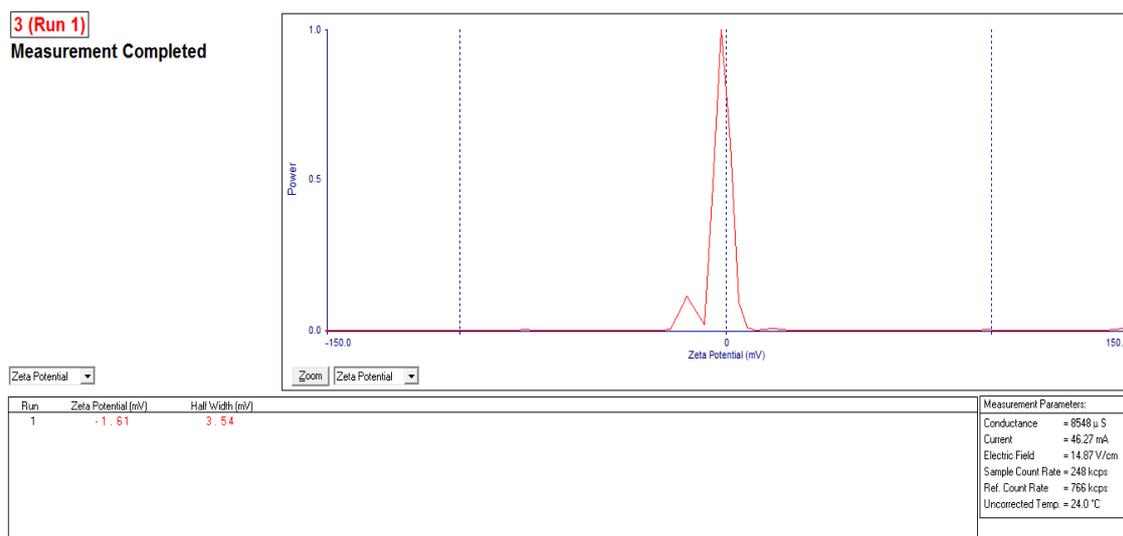


Fig (4.5): Zetapotential liposomes with four different lipid compositions ratios 1:1 cholesterol to lecithin with Tween 80 5% .

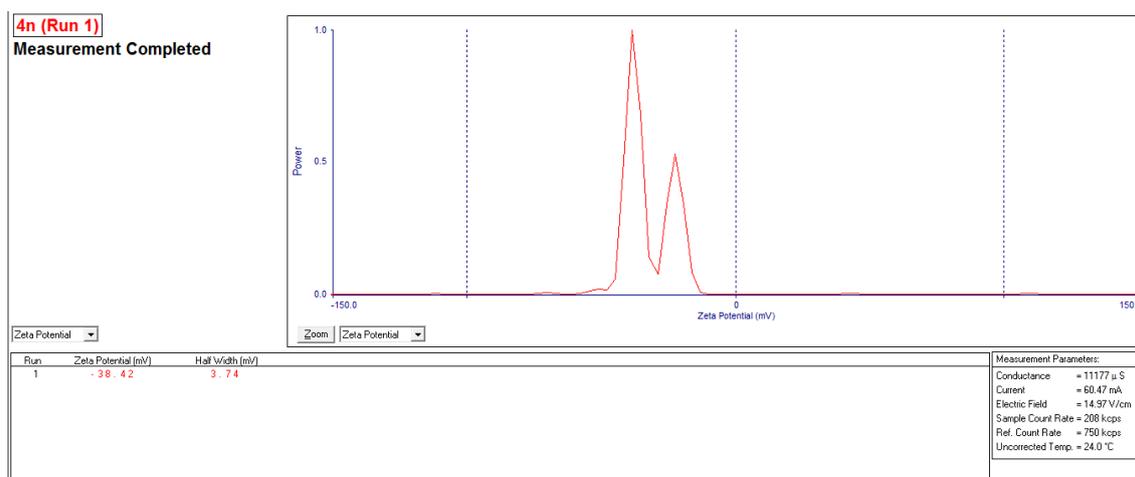


Fig (4.6): Zetapotential liposomes with four different lipid compositions ratios 1:2 cholesterol to lecithin with Tween80. 2%

4.2.2 Fourier-Transform Infrared Spectroscopy (FTIR):

The FTIR spectra for prepared ethanolic extract of *H.rosa* flower loaded on nanoparticles are represented in (Fig 4.7). The data in figure show peak values present in the flower extract as obtained by FTIR

analysis and the probable functional groups, utilized to predict the chemical and biological activities of biomolecules. Following descriptions are FTIR results that could be interpreted for effective use of the plant products. Exhibited the characteristic bands at 1398.57 cm^{-1} indicating the presence of N-H stretching aliphatic primary amine group, at 2916.37 cm^{-1} presence of strong broad N-H stretching amine salt.

The extract of flower had peak value at 2850.79 cm^{-1} indicating the presence of alkane (C-H) group, carbonyl (C=O) group functions at peak value 1732.08 cm^{-1} and presence of alkane (C-H) group, at 1639.49 cm^{-1} for carbonyl (C=O) group, at 1616.35 cm^{-1} indicating alkene (C=C) group. On the other aspect strong - N-O stretching nitro compound was appear at 1573.91 and 1546.91 cm^{-1} . Moreover, the carboxylic acid O-H was confirmed at 1408.04 and 1327.03 cm^{-1} . While strong - C-O for primary alcohol and sulfoxide was recorded at 1056.99 and 1033.85 respectively. The presence of esters (S-OR) group at 879.54 and 771.53 cm^{-1} .

The FTIR nanoparticles of *H. rosa* loading with liposome, showed there were many active compound listed in (Fig 4.8) marked by new functional group formed by correlation and interference between liposome and extract that indicate to encapsulation of nanomaterial of extract inside liposome and formation phytosome noted to stability of nanomaterial via FTIR that show generation and disappear number of peak noted in extract

alone. *H. rosa* flower loading liposome showed the characteristic bands for N—H group (ammonium ions) at 3248.13 and 2854.65 cm^{-1} as well as band at 2927.14 cm^{-1} indicating presence of C—H bond (alkyl, methyl) functions groups. Strong - N-N stretching bond was recorded at 2137.13.

A carbon–nitrogen double bond C=N bond confirms at peak 1631.78 cm^{-1} , C-H bending alkane methyl group at 1469.76 cm^{-1} . Characteristic fingerprint for phytosome of *H. rosa* was strong -C-O stretching secondary alcohol at 1099.43 and 1014.56 cm^{-1} that Methanol extract of leaf bands were observed at peak value 3338.93 cm^{-1} showing presence of amines (N-H) group, at 1684.74 cm^{-1} indicating alkene (C=C) group, and function of phosphine at peak value 1080.90 cm^{-1} with (PH₃) bending.

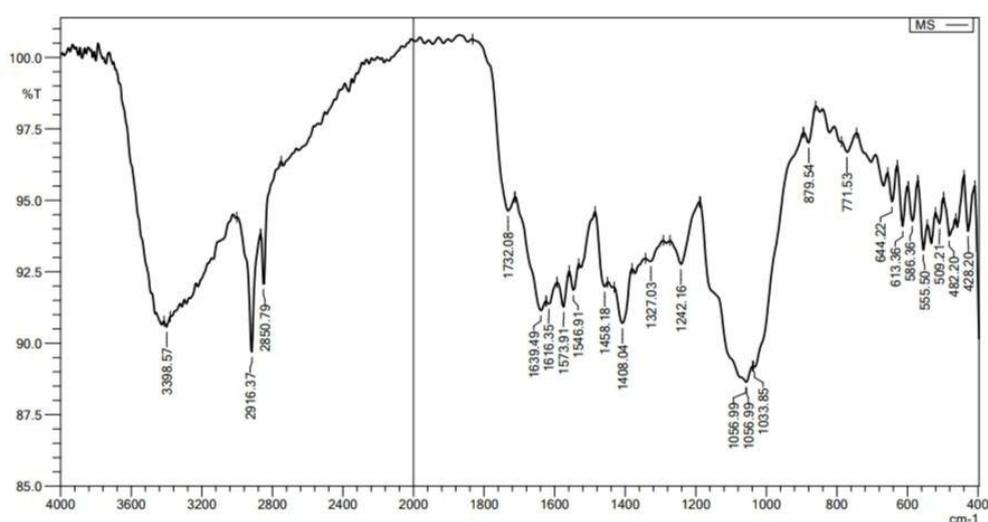


Fig (4.7): FTIR spectrum for ethanolic extract of *H.rosa* flower.

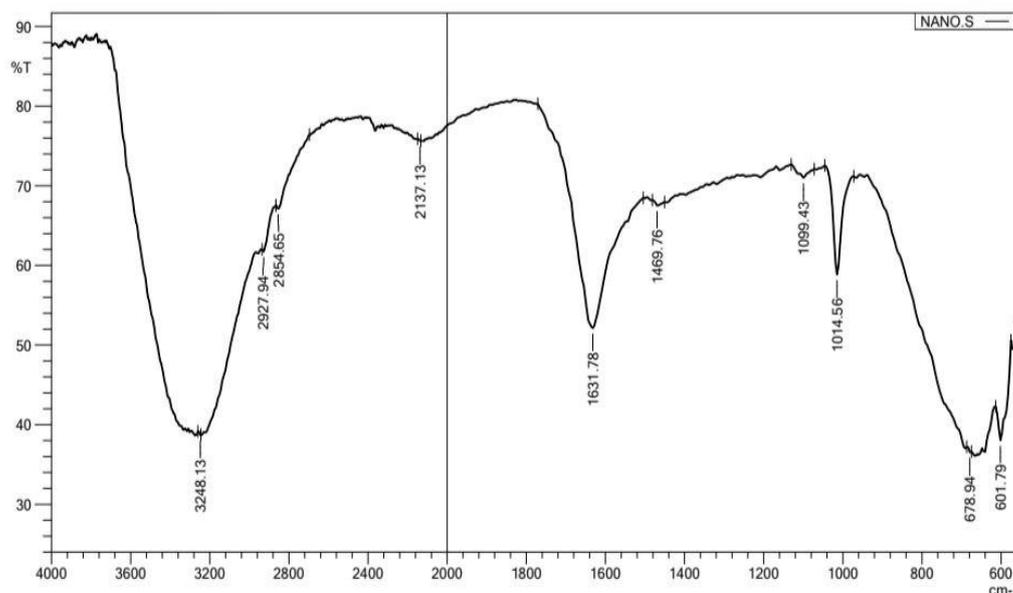


Fig (4.8): FTIR spectrum for ethanolic extract of *H.rosa* flower loaded on nanoparticles (phytosome).

4.2.3 X-ray Diffraction (XRD):

The present data of XRD recorded three peaks 2-Theta at 23.2927, 31.5598 and 29.2063 with their FWHM 0.57360, 0.49050 and 0.70380 to recorded size at 14.77, 17.59 and 12.19 nm respectively (Figure 4.9). On the other aspect, strongest 3 peaks in (Figure 4.10) recorded three peak 2-Theta at 23.2497, 29.2399, and 35.2885 with their FWHM 0.50750, 0.55330 and 51070 to recorded size at 85.02, 15.59 and 17.06 nm respectively. The strong peak at 23.371620 with FWHM 0.393600 to recorded mean crystal size for phytosome of *H.rosa* at 21.54 nm (Fig 4.11).

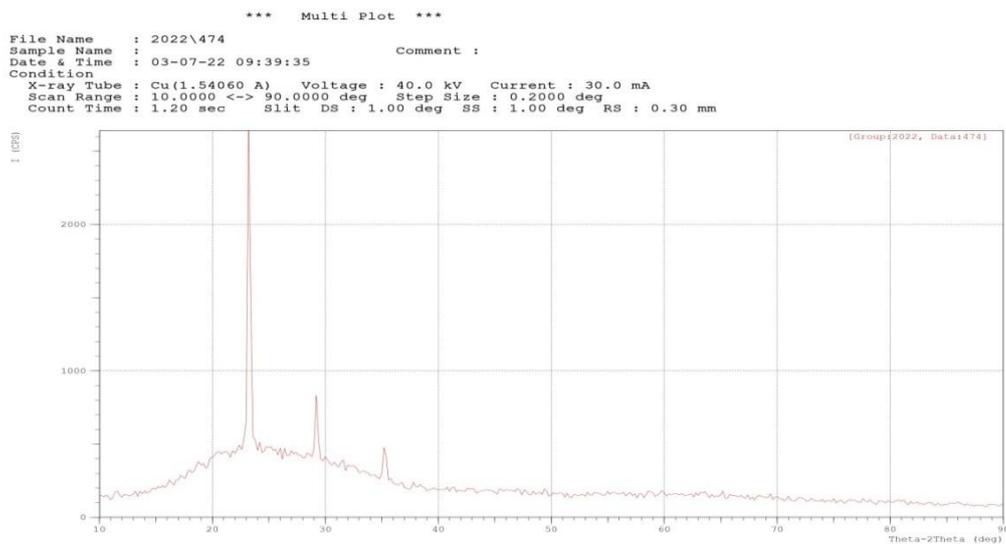


Fig (4.9): XRD of *H.rosa* phytosome appear round to oval shape of nanoparticles with particle size around 100 nm. Theta at 23.2927, 31.5598 and 29.2063 .

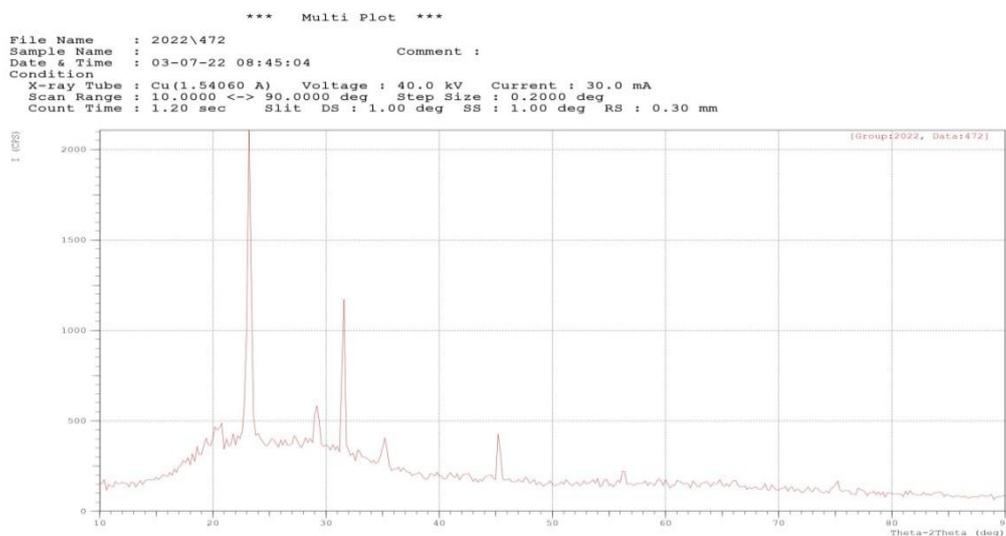


Fig (4.10): XRD of *H.rosa* phytosome appear round to oval shape of nanoparticles with particle size around 100 nm. at 23.2497, 29.2399, and 35.2885 .

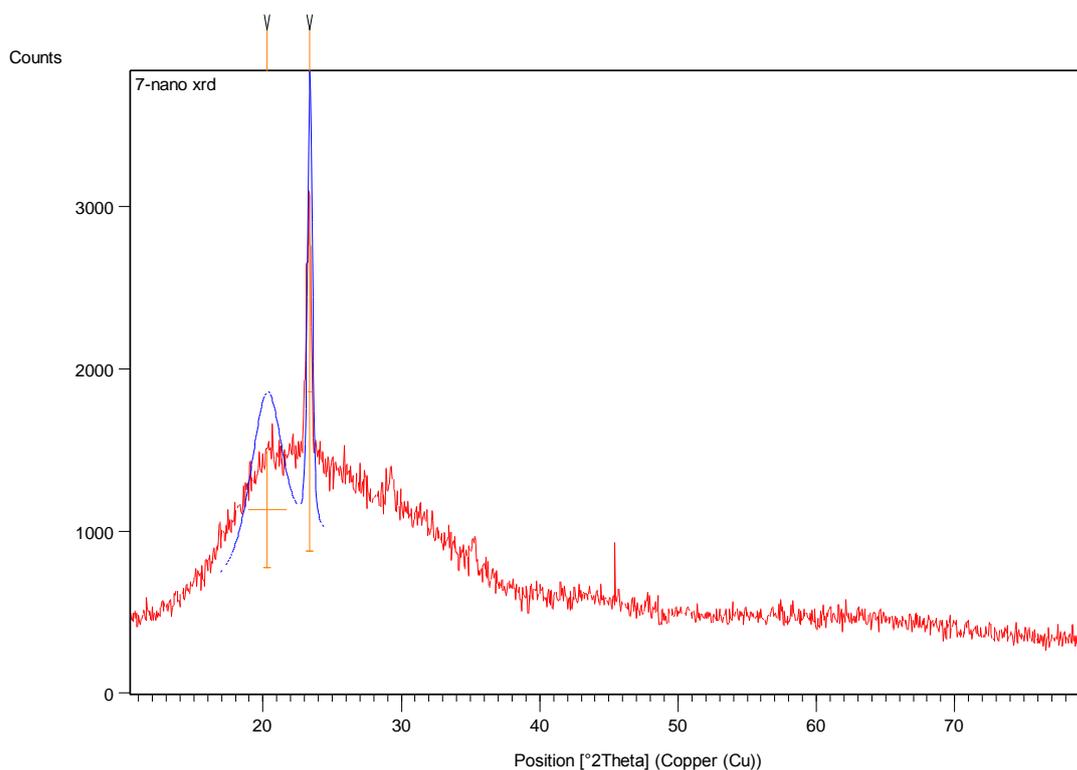


Fig (4.11): XRD of *H.rosa* phytosome appear nanoparticles with particle size under 100 nm.

4.2.4. Atomic Force Microscope (AFM):

The outer surface of the ethanolic extract of *H. rosa* flower loaded on nanoparticles was studied (Figure 4.12) shows a two-dimensional image, showing molecular assemblies with semi-spherical shapes to oval. The height of the molecular assemblies is observed, which is about 88 nm. The nanoparticles were found to be within 48–88 nm (Figure 4.12) showed the pictographs of the morphology and size of three-dimensional analysis graph gained from AFM.

The obtained average size from the AFM pictographs in the contact mode from the line analysis measurement by utilizing the SPMLab programmed Veeco diInnova software was ~ 88 nm for *H.rosa* flowers loaded on nanoparticles.

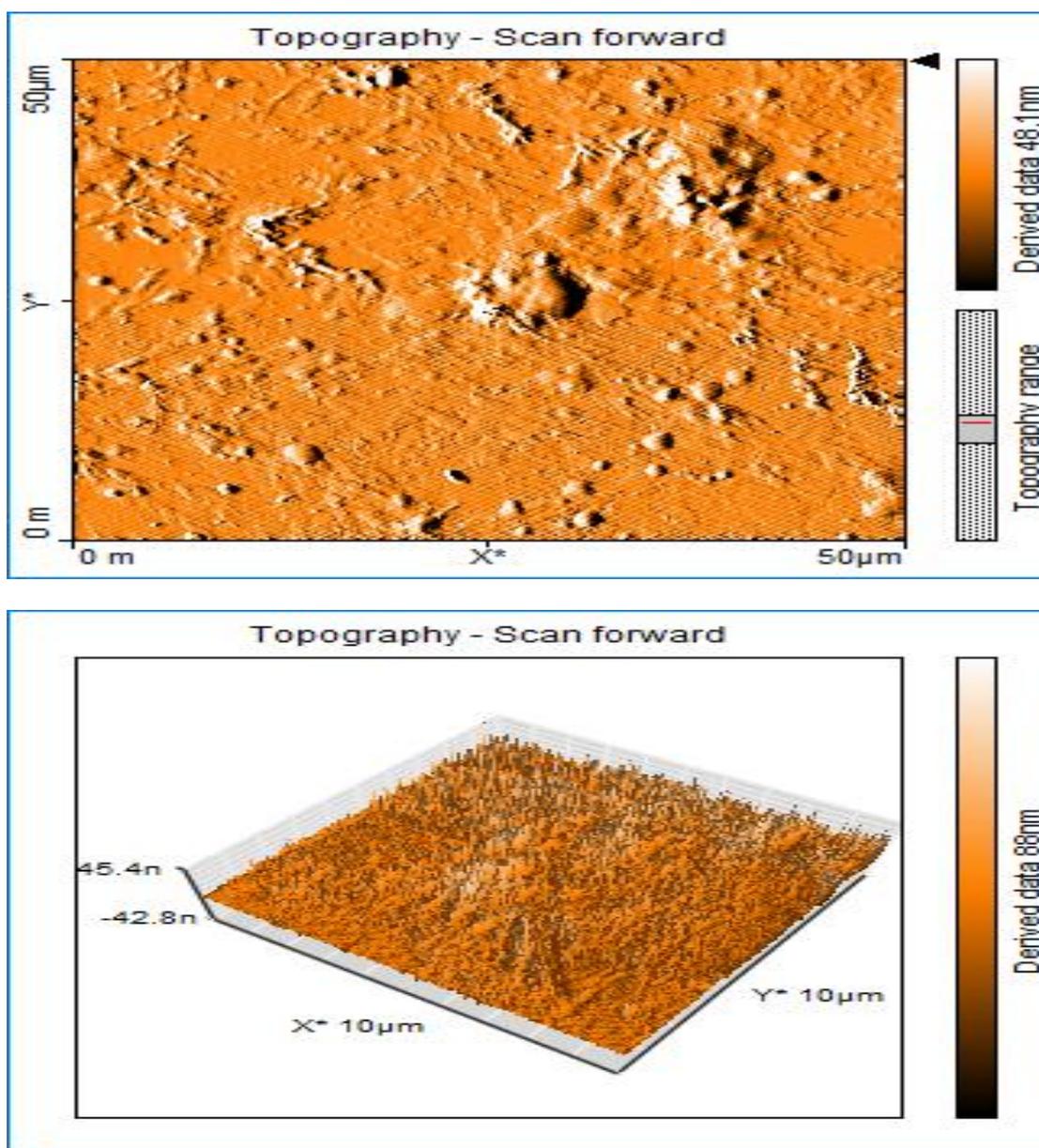


Fig (4.12): AFM 2D views in FTIR spectrum for ethanolic extract of *H.rosa* flower loaded on nanoparticles (phytosome).

4.2.5. Scanning electron Microscopes (SEM):

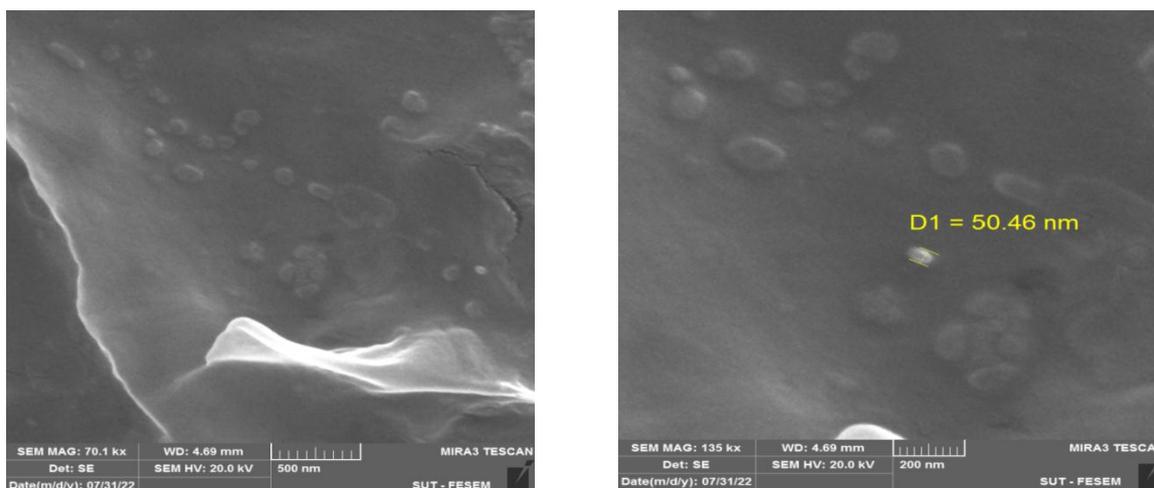


Fig (4.13): SEM of *H.rosa* phytosome appear round to oval shape of nanoparticles with particle size around 100 nm.

4.3. Sperm parameters tests:

4.3.1. Sperm motility and Sperm count:

The results of sperm function test for the animals treated with ethanolic extract of *H.rosa* flower loaded on nanoparticles(phytosome).Showed significant decrease ($p<0.05$) of epididymal sperm concentration in three group treated with nanoparticles loaded by *H.rosa* flower extract (100 mg/kg/day, 200 mg/kg/day, 300 mg/kg/day) of body weight for 55 days compared to negative control group and +ve control group that received empty liposome, the G5 that received 300mg/kg/day of *H. rosa sinensis* loaded with phytosome recorded more significant decline ($p<0.05$) in comparison to G3 and G4 that received phytosome of *H. rosa sinensis* at dose 100 and 200 mg respectively(Fig 4.14)

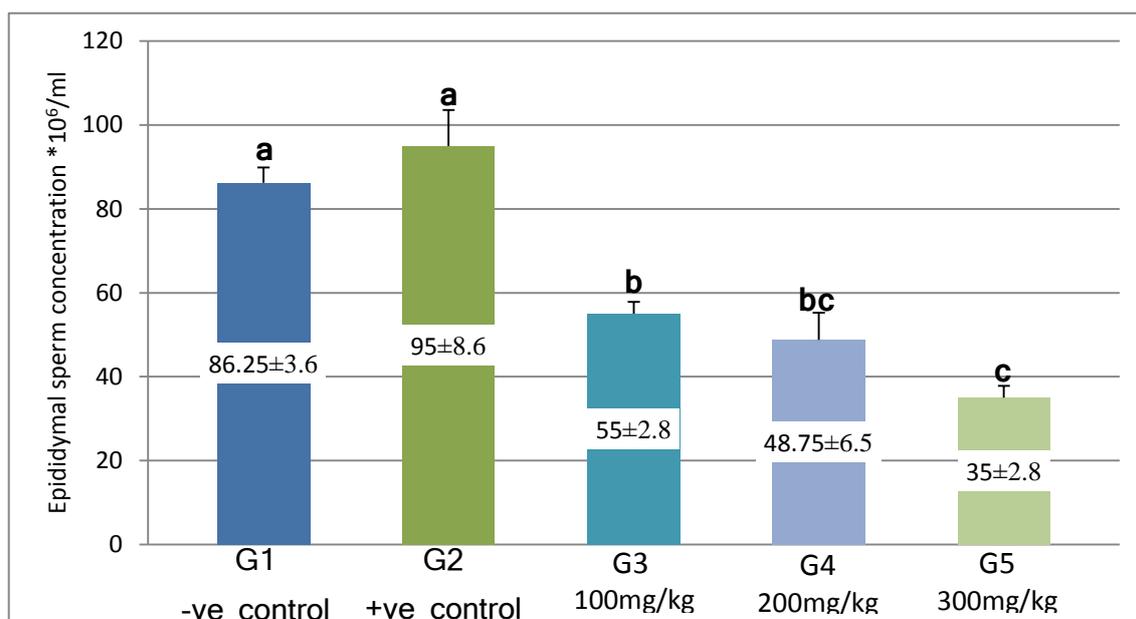


Fig (4.14): Epididymal sperm concentration in rats treated with *H.rosa* nanoparticles.(mean ±14.14) .

The present study showed that the progressive sperm motility percent in G3 and G5 had significant decrease ($p < 0.05$) compared to G4, and control groups, also G4 recorded significant decrease ($p < 0.05$) compared to -ve control and +ve control (Figure 4.15).

On the other aspect, current data showed that non-progressive motility percent were significant decrease ($p < 0.05$) in rats that received *H.rosa* loaded liposome with graded dose (100,200 and 300 mg/kg) compared to negative and positive control groups, also it was noticed that the more significant decreasing ($p < 0.05$) in non-progressive sperm motility in G3 compared with G4 and G5. While there was no significant difference ($p > 0.05$) between negative and positive control groups (Figure 4.16). The current study reported that empty

liposome has no influence on motility of sperm and recorded data mimic with negative.

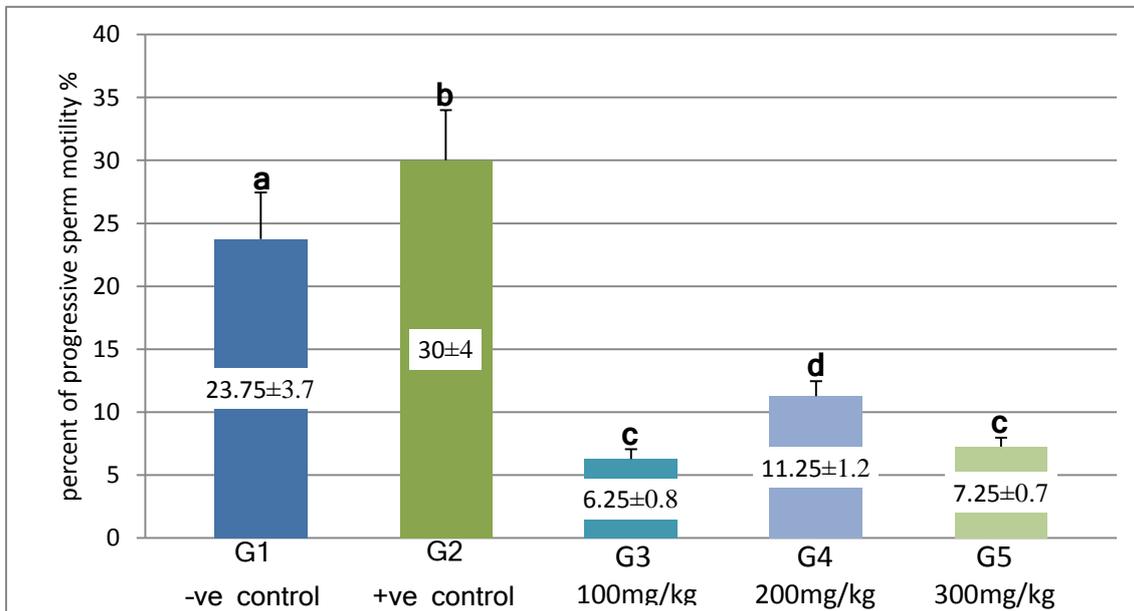


Fig (4.15): Epididymal progressive sperm motility percent in rats treated with *H.rosa* nanoparticles.(mean \pm 3.41).

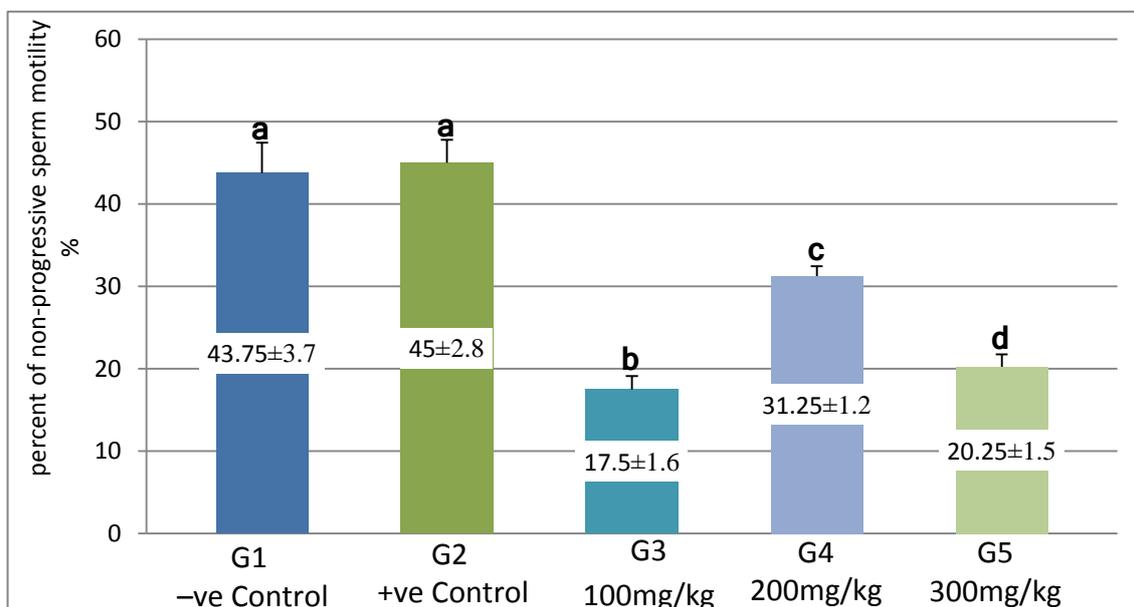


Fig (4.16): Epididymal non-progressive sperm motility percent in rats treated with *H.rosa* nanoparticles.(mean \pm 6.05).

Finding of the current study revealed that immotile sperm value between treated groups of *H.rosa* loaded with liposome showed a significant elevation ($p<0.05$) comparison with negative and empty liposome (Figure 4.17).

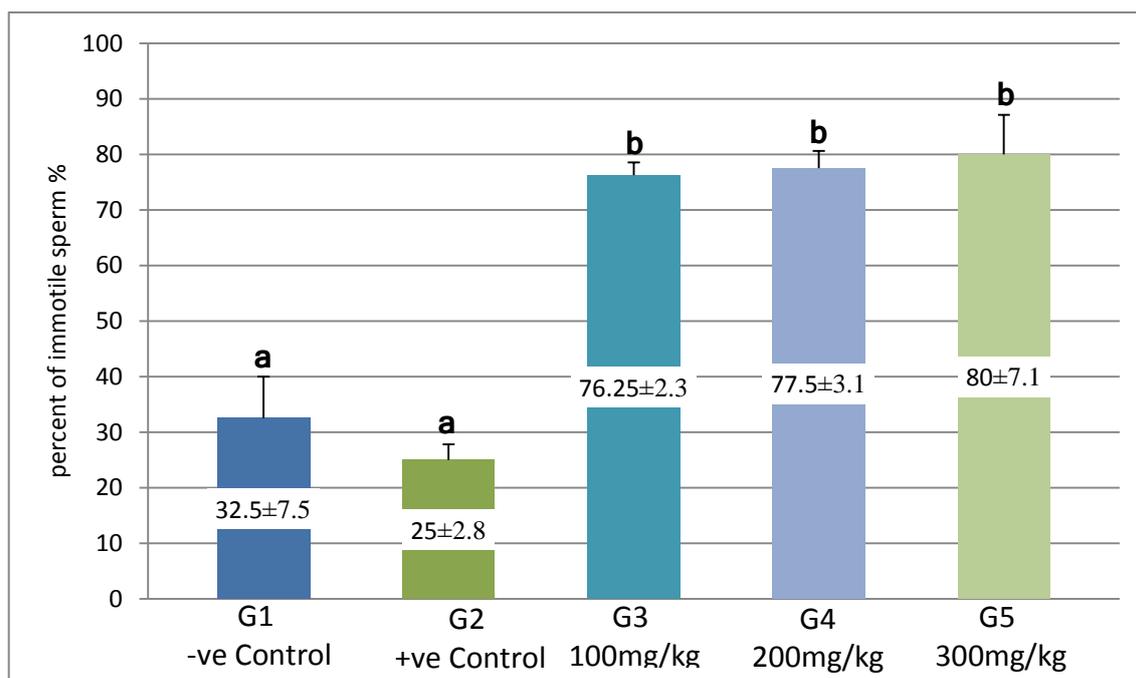


Fig (4.17): Epididymal immotile sperm motility percent in rats treated with *H.rosa* nanoparticles.(mean \pm 13.99).

In addition the animals which were received *H. rosa* nanoparticles at dose 300 mg/kg showed clear reduction in the percentages of sperm viability comparison with low dose (100, 200 mg/kg) that recorded mean value that no different from negative and positive control group (Fig 4.18).

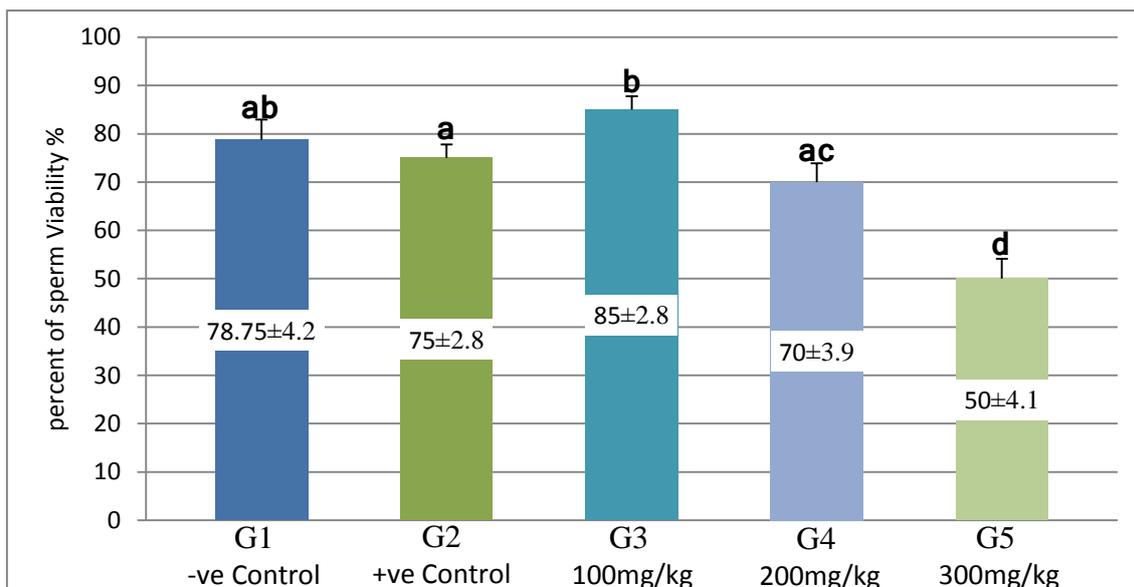


Fig (4.18): Epididymal sperm viability percent in rats treated with *H.rosa* nanoparticles.(mean \pm 9.16).

4.4. Hormonal and Immuno-assay :

4.4.1 Hormonal assay:

The result showed signification decrease ($p < 0.05$) of LH in G4 and G5 of treated groups compared to G3 and both control groups (G1, G2), while there was no signification decreased ($p > 0.05$) between G3 and control groups (Figure 4.19).

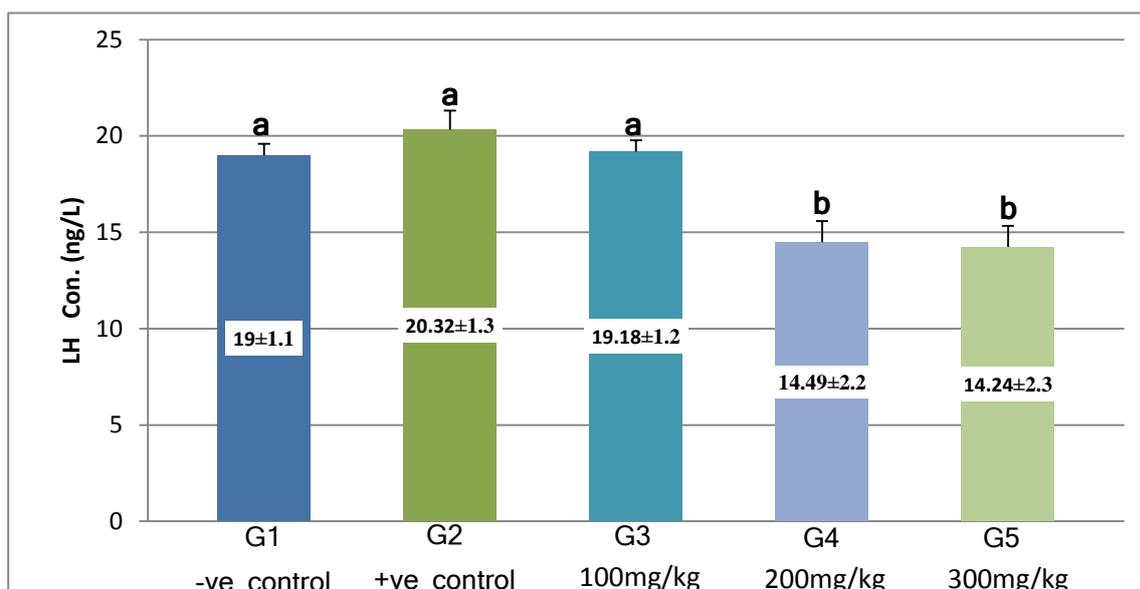


Fig (4.19): changes in LH hormone level in rats treated with *H.rosa* nanoparticles.(mean \pm 2.147).

result of showed The FSH was significantly decrease ($p < 0.05$) in G4 and G5 of treated animals compared to G3 and control groups (-ve and +ve), while FSH in G1 was significantly decreased ($p < 0.05$) that control +ve received empty liposome (Figure 4.20).

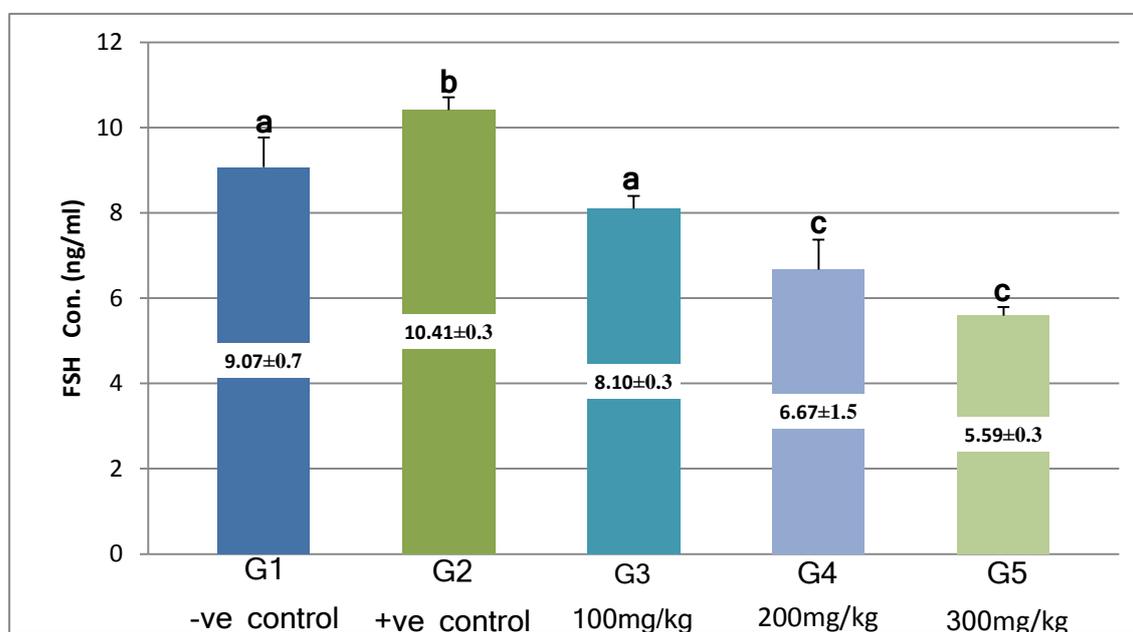


Fig (4.20): changes in FSH hormone level in rats treated with *H.rosa* nanoparticles.(mean \pm 1.116).

The results showed that Testosterone hormone was a significantly decreased ($p < 0.05$) in G5 which were treated with 300mg/kg of ethanolic extract of *H. rosa* loaded with nanoparticles compared to all other studied groups .Also, G3 and G4 were significantly decrease ($p < 0.05$) of Testosterone compared to control groups (Figure 4.21) .

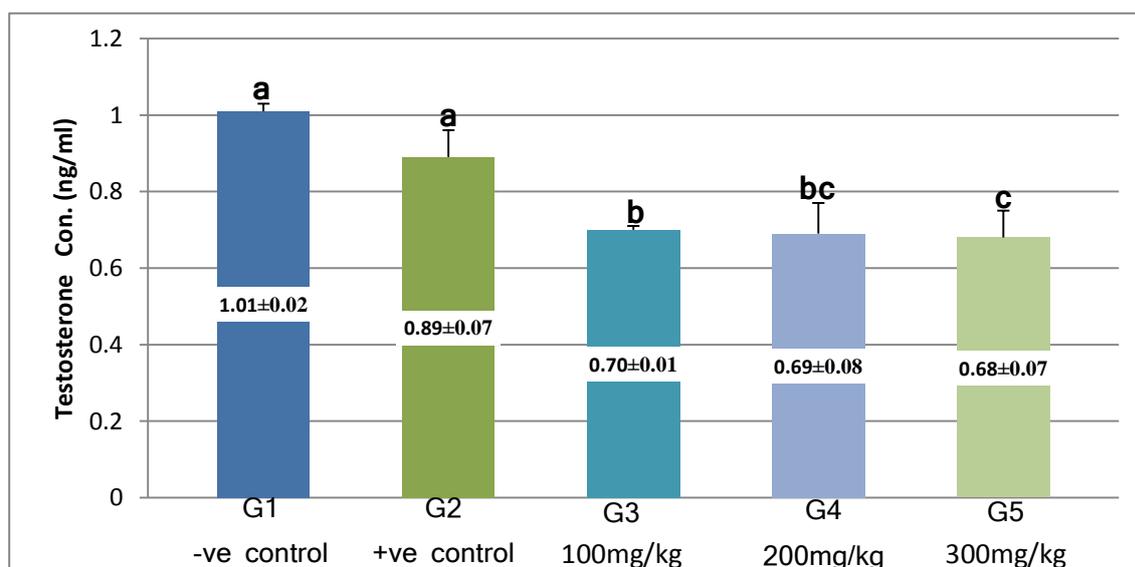


Fig (4.21): changes in Testosterone hormone in rats treated with *H.rosa* nanoparticles .(mean \pm 0.142).

The results of Estrogen hormone revealed a significant increase($p > 0.05$) of Estrogen level in G4 and G5 compared with G3 and control groups, while there was no significant differences ($p > 0.05$) between G3 and control groups, also there was no significant differences($p > 0.05$) between G1,+ve control and -ve control groups (Figure 4.22).

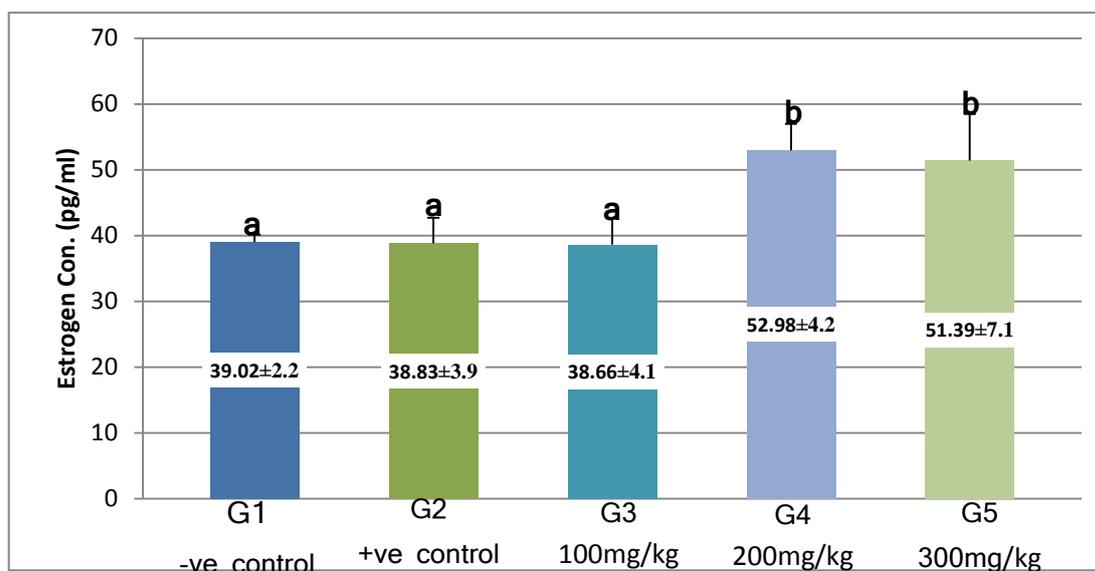


Fig (4.22): changes in Estrogen hormone level in rats treated with *H.rosa* nanoparticles.(mean \pm 5.673).

4.4.2. Immunological biomarkers assay:

The result of this study showed a significant decrease ($p < 0.05$) of TNF- α in G5 groups compared to other treated groups and control groups. Also, a significant decrease ($p < 0.05$) of TNF- α in G3 and G4 compared to control groups, while the G2 recorded signification increase ($p < 0.05$) compared to negative control (Figure 4.23).

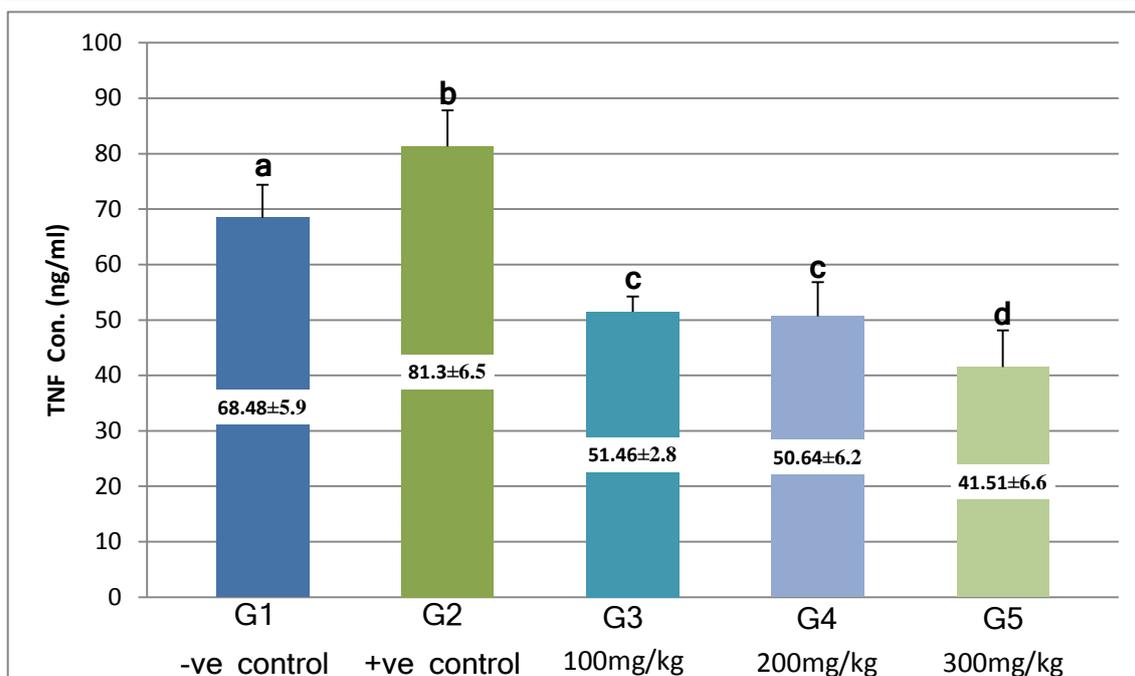


Fig (4.23): Level TNF- α in rats treated with *H.rosa* nanoparticles.(mean \pm 7.581).

Interluikin-6 recorded significant decrease ($p < 0.05$) in G5 compared to control groups and G3, while it was significant decrease ($p < 0.05$) in G5 compared to G4. The IL-6 was significantly increased ($p < 0.05$) in +ve control compared to negative control, while it was not significant differences ($p > 0.05$) between same group (+ve control) and G3 (Figure 4.24).

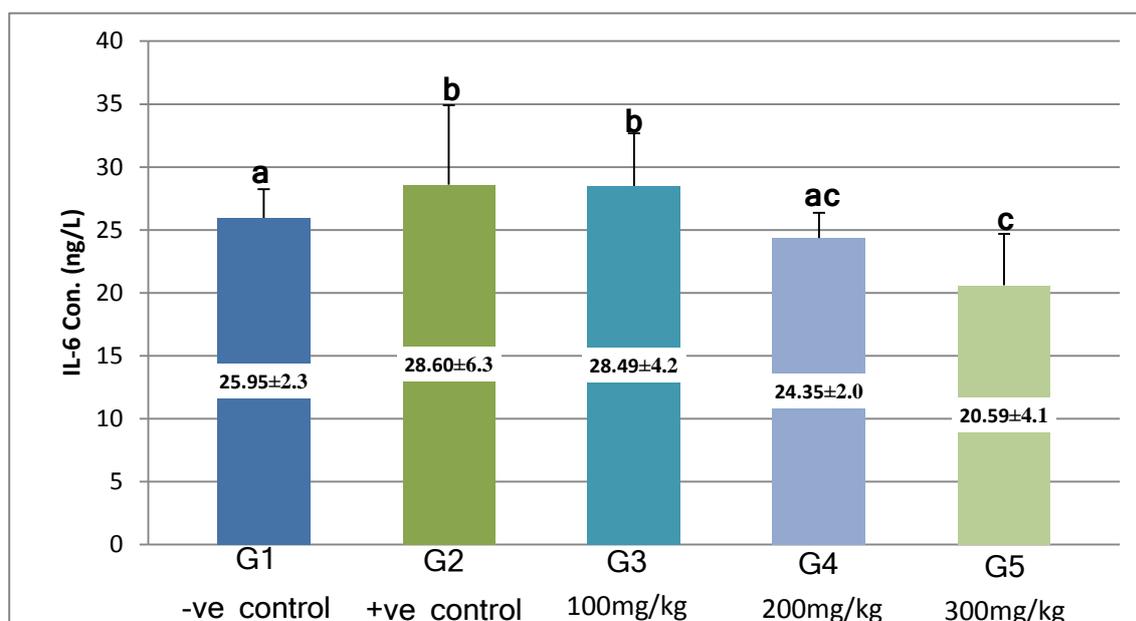


Fig (4.24): Level IL-6 in rats treated with *H.rosa* nanoparticles.(mean \pm 4.111).

4.5. Finding the ED₅₀ of the ethanolic extract of the *H.rosa* flowers loaded on nanoparticles:

The effective dose (ED₅₀) was calculated through four studied parameters include: sperm concentration, sperm progressive motility percent, Testosterone concentration and TNF- α concentration (Figures 4.25, 4.26, 4.27 and 4.28, respectively). The ED₅₀ equal to 96.605 mg/kg. BW.

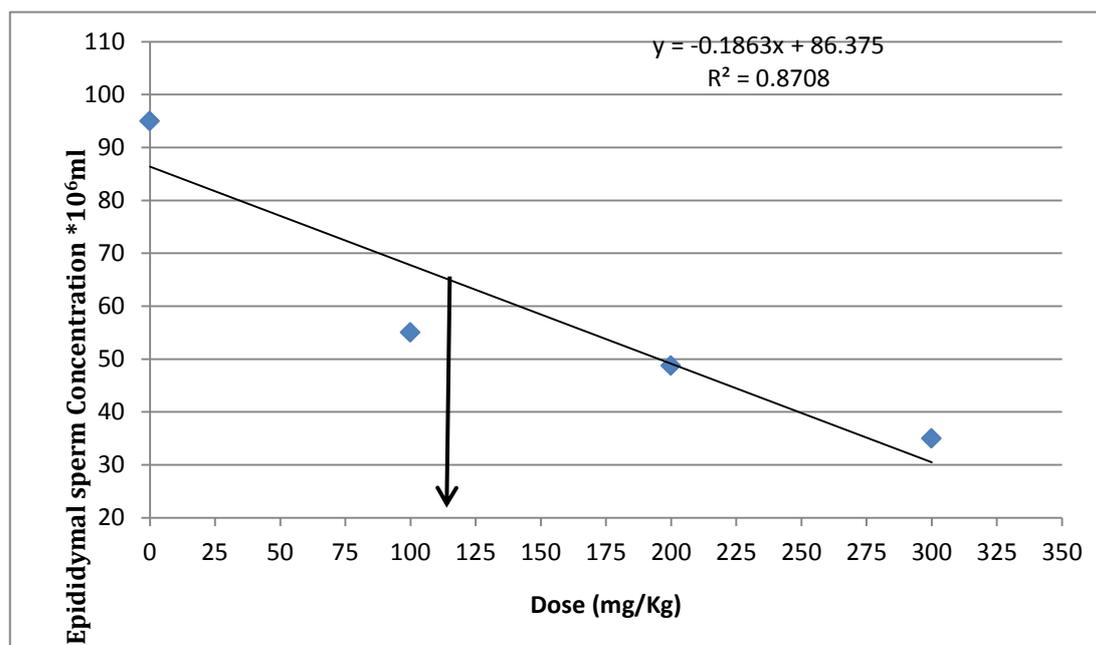


Fig (4.25): Effect of ED₅₀ of ethanolic extract of the *H.rosa* flowers loaded on nanoparticles on sperm concentration (effective dose 114.73 mg/Kg).

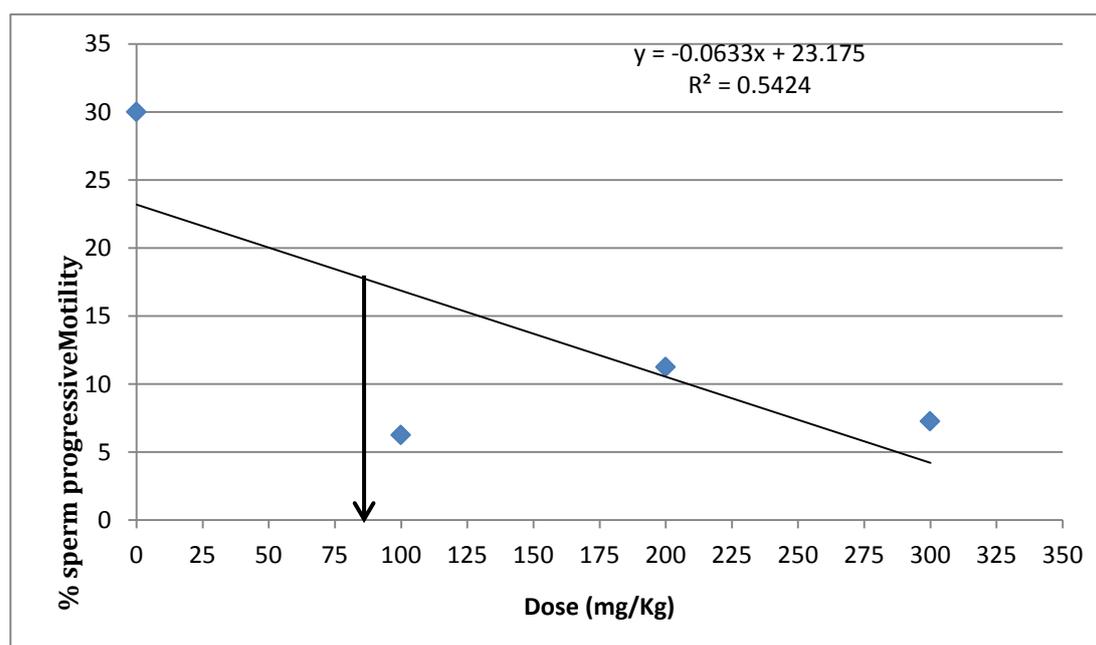


Fig (4.26): Effect of ED₅₀ of ethanolic extract of the *H.rosa* flowers loaded on nanoparticles on sperm progressive motility percent (effective dose 79.78 mg/Kg).

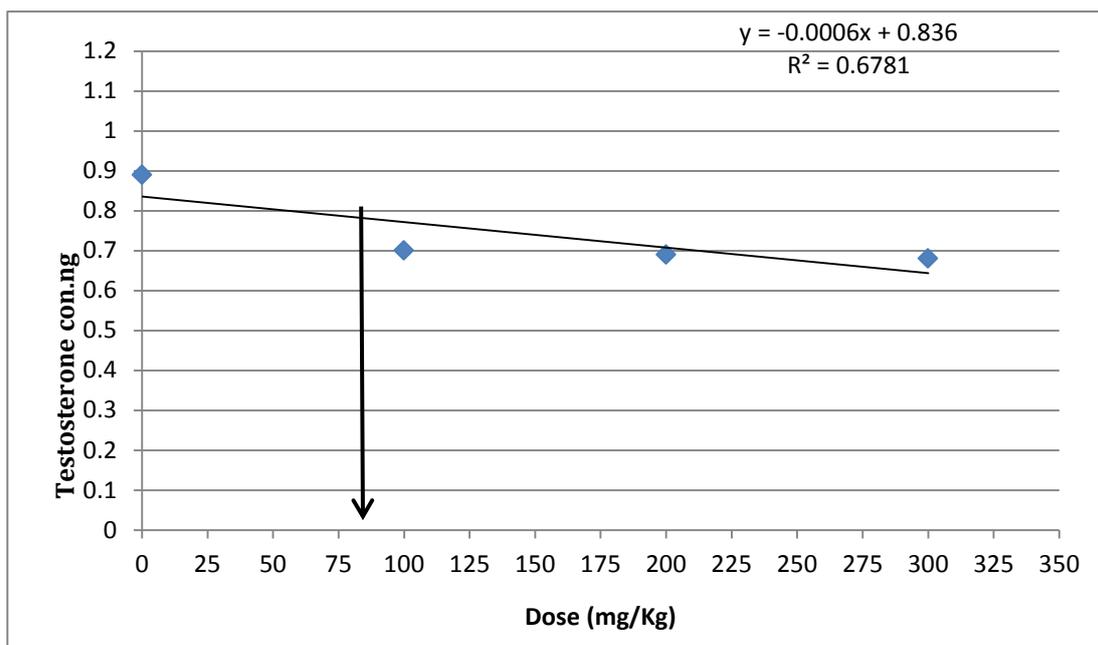


Fig (4.27): Effect of ED₅₀ of ethanolic extract of the *H.rosa* flowers loaded on nanoparticles on Testosterone level (effective dose 85 mg/Kg).

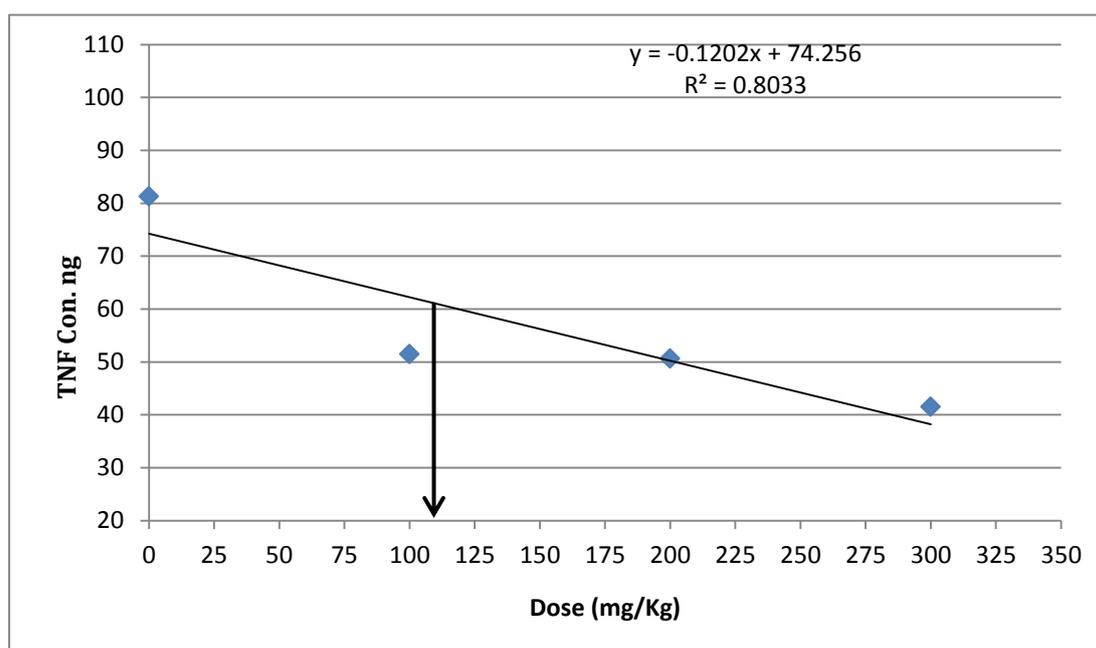


Fig (4.28): Effect of ED₅₀ of ethanolic extract of the *H.rosa* flowers loaded on nanoparticles on TNF level (effective dose 106.91 mg/Kg).

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

5.1. Separation of ethanolic extract of *Hibiscus rosa* flowers components using GC/MS technology :

The results pertaining to GC/MS analysis led to the identification of thirteen active compounds of the ethanolic extract of *Hibiscus rosa* flowers. In addition to presence of Beta-Sitosterol a natural phytosterol of steroidal nature that has important bioactive compound as contraceptive as well as p glycoprotein inhibitors (Yalcin, 2020). This effect was attributed to Stigmasterol that confirmed by GC/MS our data, which is similar to the composition of steroid hormones such as estrogen, and thus it affects in some way the process of hormonal regulation in the body and leads to an increase the levels of the estrogen hormone which subsequently affected the fertility rats.

Moreover, the present study was concord with previous study confirmed (Rassem, *et al.*, 2017), that GC-MS analysis of Hibiscus flowers (methanolic extract) showed many compounds as Propanal, (Ethanimidic acid, 1-Propanol, 2-methyl-(1.57%, ethyl ester (31.43%), Hexadecanoic acid, methyl ester (2.99%), (S)-(2.72%),1,3,5-Triazine-2,4,6-triamine (2.48%), 2,3-dihydroxy (12.58%), Ethylenediamine (6.71%), O-Methylisourea hydrogen sulfate (4.06%), Ethene, ethoxy- (3.63%), N-Formyl- β -alanine (2.36%), (Z)6,(Z)9-Pentadecadien-1-ol (1.70%), Butanedial (1.65%), Propanamide, N-ethyl- (10.69%), 7-Formylbicyclo (4.1.0) heptanes (2.80%), 2-Butanamine and Methanecarbothiolic acid (1.08)).

Hibiscus rosa sinensis Linn are flavones, containing, quercetin-3-sophorotrioside, cyaniding-3,5-diglucoside, and other constituent are cyanidin chloride, ascorbic acid, riboflavin, taraxeryl acetate, β -sitosterol, thiamine, hentriacontane, malvalic acids and cyclic acids sterculic (Sankaralingam *et al.*, 2022). These compounds were shown to have antioxidant, cancer preventive, pesticide, hypocholesterolemic, dermatitogenic, and anemia genic and antioxidant (Missoum, 2018; Sankaralingam *et al.*, 2022).

5.2. Synthesis and characterization of *Hibiscus rosa-sinensis*

L. loaded on Phytosopme :

The average particle diameter of *H. rosa-sinensis* L. loaded on Phytosopme nanoparticles (NPs) using solution of different emulsifiers, such as vitamin E TPGS and Tween 80, to characterize suitable particle size using particles sizers. Also summarized the effective of average particle diameter (161.4, 83.5, 119.9, and 69.0 nm) of *Hibiscus* –NPs with polydispersity index value around 0.226, 0.120, 0.319 and 0.03, and favorable zeta potential (-28 ± 2).

FTIR spectra of *H. rosa* flower loading on nanoparticles that there were many active compounds marked by a new functional group formed by correlation and interference between liposome and extract, which indicates to encapsulation of nanomaterial of extract inside liposome and formation phytosome noted to the stability of nanomaterial via FTIR that showed generation and disappear a number of peaks noted in extract alone.

According to one study (Muruganantham *et al.*, 2009), FTIR spectral analysis of medicinal plant parts like leaf, stem and roots of *Eclipta alba* Hassk and *Eclipta prostrate* Linn. reported the presence of

carboxylic acids, amines, amides, sulphur derivatives. The FTIR analysis results of *A. lanata* stem validated the presence of amide, alcohols, phenols, amines, alkanes, ketones, primary amines, nitro compounds, alcohols, carboxylic acids, esters, ethers, alkyl halides and aliphatic amines (Yamunadevi *et al.*, 2012) .

Phytosomes were extruded through membranes with different pore sizes to produce subpopulations with different diameters (161.4, 83.5, 119.9 and 69.0 nm). The present study was in recorded with a study by (Gibis *et al.*, 2014), who confirmed that z-potential of the liposomes changed from -29 to +78 mV. The zeta potential, which depends on the surface charge, is important for the stability of nanoparticles in suspension and is also the major factor in the initial adsorption of nanoparticles onto the cell membrane. After adsorption, the endocytotic uptake rate depends on the particle size. The zeta potential and size thus affect nanoparticle toxicity (Rasmussen *et al.*, 2020).

The extract *H. rosa* loaded on phytosome has a negative zeta potential with stabilizing agents. This confirmed that the surface charge of liposome had a negative charge, mainly due to the phosphate groups (polar heads) of phosphatidylcholine (Lu *et al.*, 2011). Olatunde *et al.*, (2020) recorded a negative zeta potential when encapsulated ethanolic coconut husk extract, were encapsulated in liposome. Generally, a stable phytosome system shows a positive or negative zeta potential higher than 30 mV.

With respect to ZP, this parameter is an indirect measure of the surface electrical charge, which translates the particle stability in dispersion. Values lower than -25 mV and higher than 25 mV indicate physical stability of the dispersions over time The decrease of the percentage of surfactant and increase of the percentage of lipid phase

contributed to the increase of the ZP values (Pimentel-Moral *et al.*, 2019).

The crystalline-amorphous state of materials can be evaluated by XRD. In common, the material in crystalline state appear a series of sharp peaks, while amorphous materials produce broad and non-defined peaks (Caparino *et al.*, 2012). XRD analysis revealed the crystalline nature of the extracts within the liposome, which indicated better solubility in aqueous medium. The outer surface of the ethanolic extract of *H. rosa* flower loaded on nanoparticles was studied using AFM showed a two-dimensional image, showing molecular assemblies with semi-spherical shapes to oval. The height of the molecular assemblies is observed, which is about 88 nm.

The nanoparticles were found to be within 48–88 nm. AFM was employed to characterize liposome-loaded drugs or active compounds. AFM has been utilized to investigate the efficacy of ultrasound-mediated drug delivery (Duan *et al.*, 2020). AFM is generally used to obtain information on the size, shape, and surface morphology of nanoparticles, and produce a three-dimensional contrast image. The zeta potential (size distribution kinetics) is an indicator of their surface charge property and reflects their electrical potential. refer that importance of a zeta potential above (+/-) 30 mV indicated a stable systems, as surface charge prevents particle aggregation and fusion. The zeta potential also indicates whether the charged bioactive is incorporated in the core or adsorbed onto the surface translate Takagi *et al.*, (2020) .

5.3. Sperm parameters :

The results of the current study indicated that oral administration of ethanolic extract of *H. rosa* loaded on phytosome at a dose of 200 and 300 mg/kg of body weight for 55 days had a significant negative effect on

sperm parameters in the tail of the epididymis. As there was a significant decrease in the average of epididymal sperm concentration, motile sperm percent, and in the percentage of viability sperm in treated males compared to the control group. These findings agreed with previous studies, which found that oral administration of the aqueous extract of *H. rosa sinensis* flowers to male mice at a dose of 300 and 500 mg/kg of body weight caused a significant decrease in the concentration of sperm in the testes and epididymis and a decrease in the percentage of fertility (Mishra *et al.*, 2009; Missoum, 2018). Also, the administration of turpentine extract of the flowers *H. rosa sinensis* at a dose of 175 mg/kg of body weight, made the sperm parameters and sex hormones decreased and the percentage of male fertility in rats decreased (Sivaraman & Saju, 2021).

Furthermore, El-Nabarawi *et al.*, (2018) found that the treatment of male albino rats with the phenolic components of *H. rosa sinensis* flowers at a dosage of 300 mg/kg for 60 days resulted in a significant decrease in testosterone level, substantial drop in testicular and epididymal weights, sperm motility percent, grade activity, epididymis sperms concentration, and live sperm percentage, with a significant elevation in the epididymal sperm abnormality. This effect of extract and phytosome may be attributed to the decrease in the secretion of FSH and LH hormones from the anterior lobe of the pituitary gland, which leads to the inhibition of the production of the testicular lipotropic hormone, which affects the process of sperm development, and then a decrease in the concentration of sperm inside the seminiferous tubules and the fertility of animals (Qasim & Al-Mayali, 2019). The production of sperm and testicular testosterone hormone in the testicle is mainly regulated by the FSH and LH hormones,

which are released from the pituitary gland, which are the main regulators of the process of spermatogenesis (AL-Murshidi, 2018).

In treated animals, because of the inhibition of the androgen is responsible for the initiation and completion of the process of spermatogenesis during the normal maturation of the developing or developing sperm (Shetty *et al.*, 2021), and that initiating the process of spermatogenesis and maintaining it quantitatively and qualitatively naturally requires the presence of adequate levels of hormones, so that any decrease associated with severe abnormalities in the sperm and may lead to a state of oligospermia or asthenia. Androgen has an important role in the last stages of spermatogenesis via stimulating the conversion of round to elongated spermatoblasts during the spermatogenesis cycle, in addition to the fact that androgen deficiency impairs the process of sperm liberation (Al-Saily *et al.*, 2018; Hernawati *et al.*, 2019).

Perhaps the reason for the decreasing of the concentration of sperm in the testes and epididymis due to the effect of the extract on Sertoli cells responsible for incubating spermatoblasts and their differentiation with the effect of FSH, which stimulates the maturation of sperm in the seminiferous tubules by enhancing the production of androgen-binding protein by Sertoli cells by FSH receptors on the basal lateral membranes, which are important for the initiation of spermatogenesis (El-Nabarawi *et al.*, 2018).

The anti-fertility activity of the *H. rosa* flower plant may be attributed to the presence of phytoestrogen compounds such as β -Sitosterol, Stigmasterol, Quercetin, Apigenidine, and Kaempferol, which are confirmed in the GC-MS that mimic functions of hormones in the reproductive system in mammals due to the similarity of their structural structure with estrogen (Nandhini *et al.*, 2021). This enables it to bind

with estrogen receptors, causing misregulation of the latter and thus affecting the function of the reproductive system. These compounds have been linked to fertility in mammals by affecting sexual development and reproductive system function (Alrabie *et al.*, 2022). It is now known that some plant estrogens such as genistein have the ability to bind to estrogen receptors α and β and change the transcription of genes that respond to estrogen (Routledge *et al.*, 2000).

5.4. The level of reproductive hormones :

The results of the current study showed a significant decrease in the level of LH, FSH and Testosterone, while a significant increase in the level of estrogen was noted. The decreasing of LH and FSH may be attributed to the inhibitory effect of ethanolic extract of *H. rosa* flower loaded on phytostome. These findings agreed with some studies which indicated that the effect of various plant extracts in inhibiting the secretion of these hormones (Benie *et al.*, 2003; Yakubu *et al.*, 2008; Thakur *et al.*, 2009; Bala *et al.*, 2014).

A recent study by (AL-Azawi & Al-hady, 2020) showed a significant decrease in the level of testosterone and progesterone in treated rats compared with control groups for 30 and 60 days of treatment, and a significant decrease in the expression of the androgen receptors and progesterone receptor in the testicular cells treatment group with phenolic compounds of *H. rosa-sinensis* flowers at a dose of 300 mg/kg/day of body weight for 60 days compared to control groups.

In addition, Al-Zubaide, (2014) revealed that the treatment of male rats with phenolic compounds of flower *H. rosa-sinensis* with a dose of 300 mg/kg/day of body weight caused a significant decrease in the level of LH in the serum of treated male albino rats with the elevation of estradiol. Moreover, AL-dulaimy, (2010) found that the treatment of male

rats with terpins extract of *H. rosa-sinensis* flowers at a dose 175 mg/kg of body weight, caused a significant decrease in sperm parameters and sexual hormones. Also, these results are consistent with the results of other study (AL-Azawi & Al-hady,2020), which were revealed that the treatment of albino male rats with a dose of 300 mg/kg/day of body weight of phenolic compounds of *H. rosa-sinensis* flowers for 30 and 60 days caused a significant decrease in the level of testosterone and progesterone.

The reason for the low level of the testosterone hormone may be attributed to the fact that the extract of *H. rosa* flower has the effect of reducing the level of cholesterol in the blood and LDL (Sachdewa & Khemani, 2003). Cholesterol derived from LDL molecules is the main compound and has essential role in the process of synthesis of testosterone hormone, which is synthesized and produced in Leydig cells. Recent study reported that *H. rosa* has lowered blood glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides, blood pressure in dose-dependent responses (Anand *et al.*, 2015; Jeffery & Richardson, 2021).

(Shi *et al.*, 2017). The 17 β -Hydroxysteroid dehydrogenase (17 β -HSD) enzymes, which catalyze the reversible reactions between androstenedione and testosterone, and between estrone and estradiol, are believed to play crucial roles in the arrangement of the tissue levels of bioactive steroids. It was revealed that 17 β -reduction and oxidation of androgens and estrogens are catalyzed by different isozymes (Bertelloni *et al.*, 2009), which may be phytosome of *H. rosa* flower that reduces the activity of this enzyme.

It is possible that the decrease in the level of the testosterone hormone in this study is due to the effect of the ethanolic extract *H. rosa* loaded phytosome on the Leydig cells producing the testosterone hormone directly. These results were in agreement with study by (Al-Zubaide *et al.*, 2015), who showed a significant decrease in the number of Leydig cells and the diameter of their nuclei. It also, agreed with previous studies of (Aladakatti & Ahamed, 2006; Mohammad *et al.*, 2009, Munir *et al.*, 2017) which showed that some plant extracts have a negative effect on the diameters of Leydig cells and the diameters of their nuclei and numbers, which causes a decrease in the production of the testicular lipid hormone. In general, these reports indicated that the dose of NP (e.g., size, shape, chemical composition, surface area and surface charge) plays an essential role in limitation the effect of NPs on spermatogenesis (Luisetto *et al.*, 2003).

Recent advances in drug formulations, such as drug particle micronization (<50 μm), nanomaterials, and drug delivery like phytosome have improved the bioavailability of contraceptives via a considerable increase in solubility, small size, and containing absorption enhancers (e.g., surfactants as Tween 80) that penetrate the BTB, leading to accumulation of NPs in the Sertoli and germ (e.g., spermatocytes) cells without causing testicular injury (Morishita *et al.*, 2012).

Mostrom & Evans, (2011) Other studies showed the effect of these plant compounds in the process of steroid generation due to the possibility of binding to estrogen receptors. It was shown that exposure of adult male mice to a high dose of plant estrogen compounds caused a decrease in DHT levels. The results of the current study showed a significant increase in the estrogen level in the serum of rats received phytosome of *H. rosa* flower at 100,200,300, which may be attributed to

the conversion of testosterone hormone to estrogen or to the presence of phytoestrogen compounds in the loaded extract. The extract of *H. rosa* flower has been confirmed by many studies to possess anti-implantation and anti-spermatogenic activities (Murthy *et al.*, 1997).

Vasudeva & Sharma ,(2008) reported that the roots of *H. rosa-sinensis* were tested for their anti-implantation and estrogenic properties. The loss of implantation caused by ethanol extract may be due to antizygotic, blastocytotoxic, or anti-implantation activity as well as a dose at 400 mg/kg caused a significant increase in uterine weight in immature rats versus control as well as uterotrophic potency via the increased thickness of the endometrium thus changes were mimic to ethinyl estradiol the effect returned to the presence of steroids, amino acids and carbohydrates in the ethanol extract (Al-Snafi, 2018; Putra *et al.*, 2020; Sharma *et al.*, 2021).

5.5. The level of immunological proteins :

The current study indicated a change and reduction in the average of the immunological parameters (TNF-a and IL-6), which indicates that the extract of *H. rosa* flower loaded on nanoparticales possesses anti-inflammatory purity especially at 200 and 300 mg/kg.

Alkaloids, steroids, flavonoids, polyphenols have been proven antioxidant, anti-inflammatory and immunomodulatory active principles. These phytoconstituent of *H. rosa* may have synergistically contributed to the attenuation of ROS. Our results were confirmed by (Aziz *et al.*, 2021), who reported that the phytochemical screening of *H. rosa-sinensis* L. of flower and leaf ethanol extracts showed the presence of flavonoids and saponins that had anti-pyretic activity via inhibition of non-selective

inhibitors of COX enzyme, act on both COX-1 and COX-2, TNF- α and PGs synthesis.

The oral administration of *H. rosa* extract showed significant analgesic and anti-inflammatory effects when exposed to the acetic acid test, tail immersion test (TIT), carrageenan-induced edema, formalin-induced paw licking, and dextran-induced edema which might be due to the presence of anthocyanin, flavonoids, and alkaloids (Rasheed *et al.*, 2010). Begum, *et al.*, (2018) reported that the polyphenolic compounds had a potential therapeutic anti-inflammatory effect via suppressing the NF-kappaB p65 activation by inhibiting IkappaB α degradation (mast cell stabilization), that suppress the Tumor Necrosis Factor-Alpha and Interleukin (TNF- α , and IL-6).

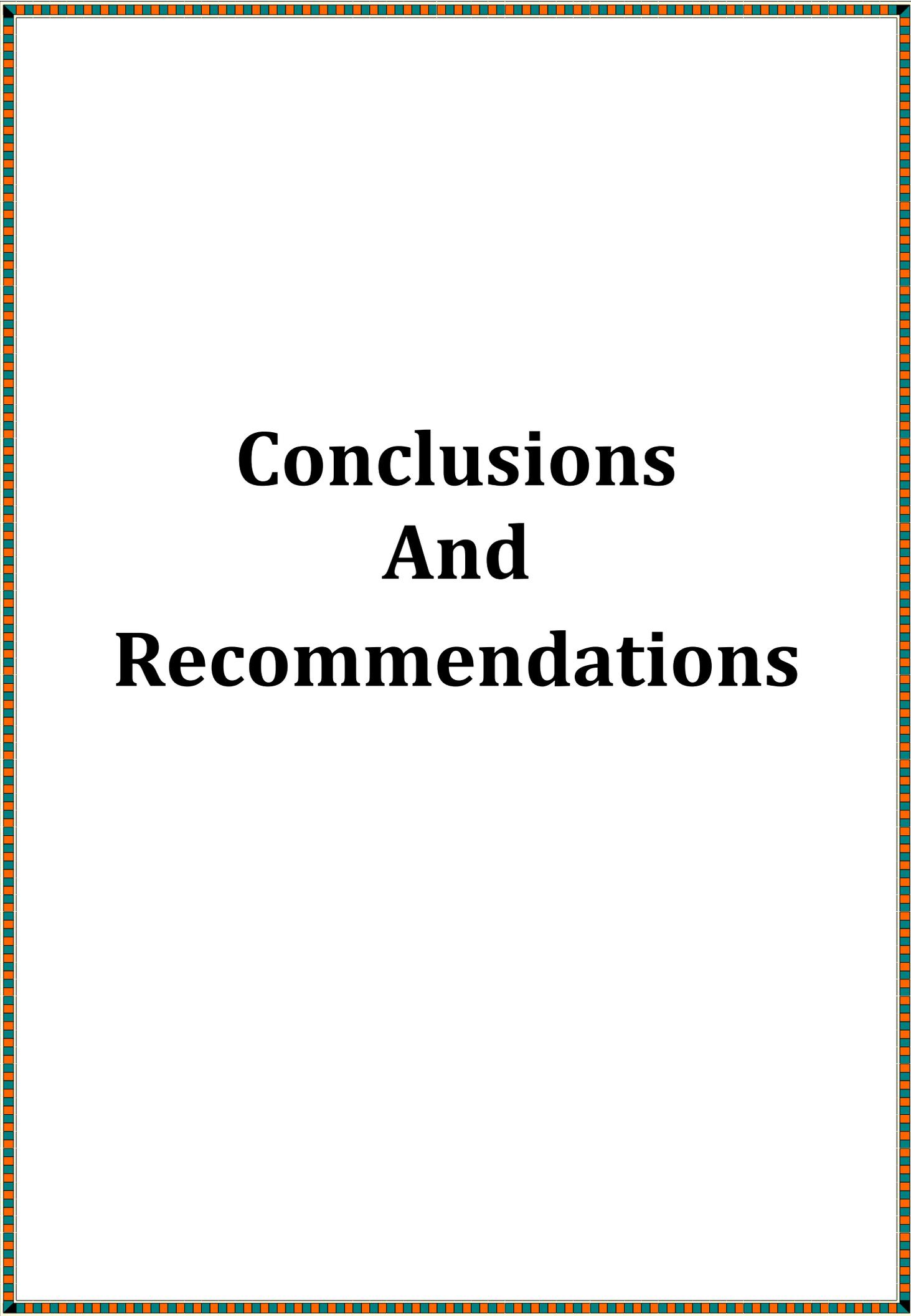
5.6. Effect of ED₅₀ of ethanolic extract of *Hibiscus rosa* flowers loaded nanoparticles:

Currently, there is universal interest in the use of eco-friendly and cost-effective drug delivery systems to improve drug performance. In the current study, it was shown that the effective dose (ED₅₀) of ethanolic extract of *H. rosa* flower loaded on nanoparticles (NPs) was 96.605 mg/kg. BW compared to the ED₅₀ of *H. rosa* flower extract which was approximately 300 mg/kg.BW (Al-Zubaide, *et al.*, 2015).Therefore, the benefit of nanoparticle is decreasing the effective dose. These results were in accordance with Vijayanand *et al.*, (2018), who administrated *H. rosa sinensis* extract loaded solid lipid nanoparticles (HSLNs) at various doses to Swiss albino mice, and reported a significant greater antidepressant activity when compared with native crude extract of *Hibiscus*. Moreover, at all other lower doses tested, the HSLNs

demonstrated similar, if not superior antidepressant activity compared to the crude drug extract.

In this study, it was shown that the ED₅₀ approximately 100 mg/kg. BW, so that of nanoparticles loaded with *H. rosa* while ED₅₀ of *H. rosa* flower extract approximately 300 mg/kg. BW. Consequently, the benefit of nanoparticle using caused decreasing the effective dose of extraction without using nanoparticles.

In addition, Kainat *et al.*, (2021) introduced an ecofriendly protocol for biosynthesized NPs were found to be excellent antioxidant and biocompatible nanomaterial as they are non-toxic and had biocompatible nature. In the same line, the plant extracts are rich in antioxidants that act as both reducing and stabilizing agents appear to be useful for creating metallic nanoparticles (Flieger *et al.*, 2021). Recently, Lu *et al.*,(2022) biosynthesized silver NPs immobilized *H. rosa-sinensis* extract, and demonstrated its notable anti-liver cancer activities against common liver cancer cell lines, which probably were linked to their antioxidant activities due to the presence of many antioxidant compounds with related bonds (Shahriari *et al.*, 2022).



Conclusions And Recommendations

Conclusions and Recommendations

Conclusions

It was concluded from the results which were obtained by the treated of Albino rats with *H.rosa* flower extracted loaded on nanoparticles :

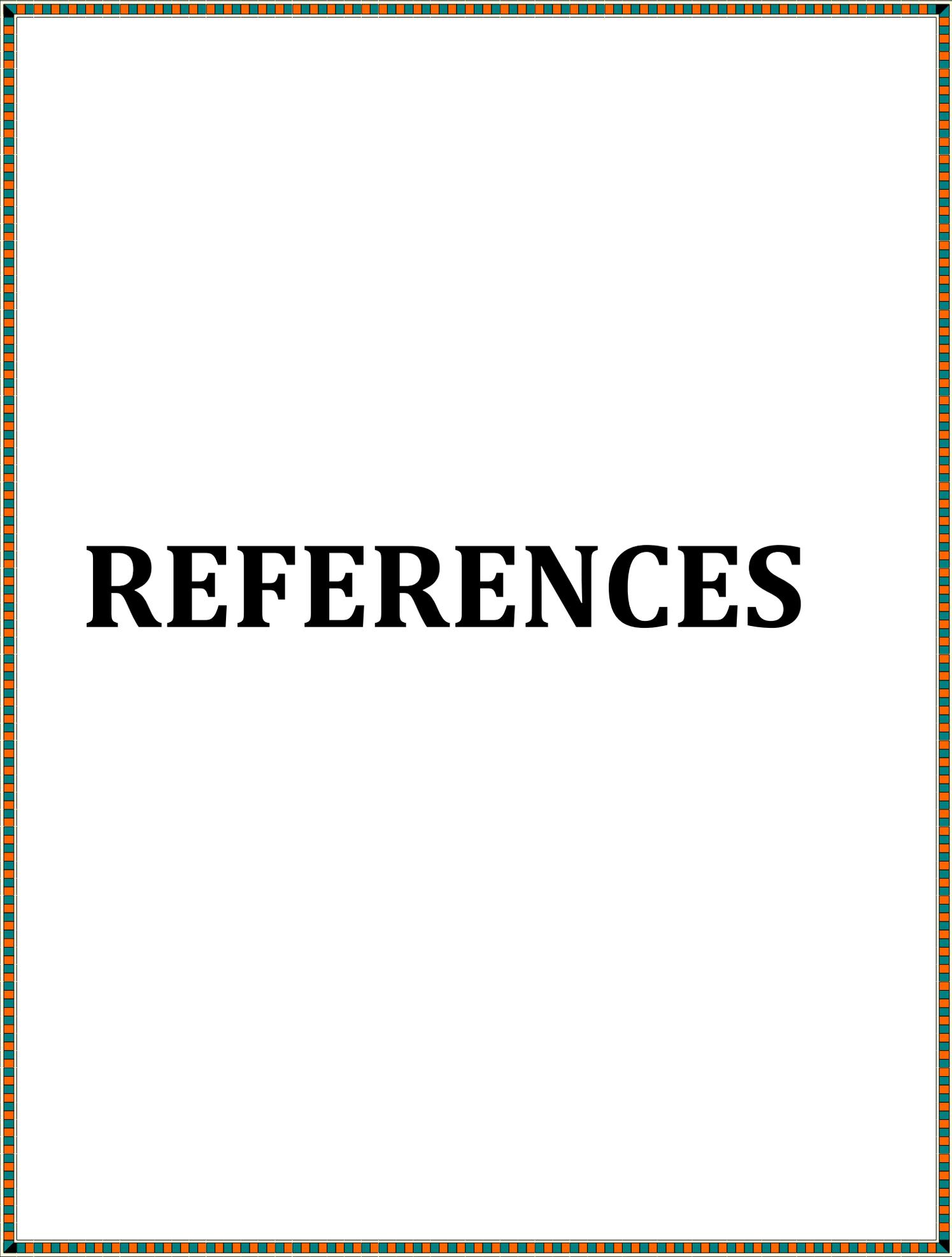
1. ED50 for extract of *H.rosa* flower loaded with nanoparticles 1/3 ED50 of extract alone .
2. The ethanolic extract of *H.rosa* flowers contains contraceptives, so it can be used as an anti-fertility .
3. The ethanolic extract of *H.rosa* flower extract loaded on nanoparticles caused significant decreases in sperm function test and LH,FSH and testosterone, while elevation of estrogen .
4. The *H.rosa* flower extract has a potential effect of immunological parameters IL-6 and TNF- α .

Conclusions and Recommendations

Recommendations

By knowing the results of the current study, it can be recommended to conduct more future studies of the medical importance of the ethanolic extract of the *H.rosa* flower loaded on nanoparticles (Phytosome) in its impact on fertility and reproduction, including: -

1. Comparative study between different nanoparticles used with the same extract .
2. Study the effect of *H.rosa* flower extract loaded with nanoparticles on Liver function test ,Renal function test,Lipid profile and body weight .
3. Study cytotoxic effect of extract with nanoparticules on some cancer cell lines .
4. The possibility of using the extract on more advanced animals, up to humans .



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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(هُوَ الَّذِي جَعَلَ الشَّمْسَ ضِيَاءً وَالْقَمَرَ نُورًا وَقَدَرَهُ مَنَازِلَ
لِتَعْلَمُوا عَدَدَ السِّنِينَ وَالْحِسَابِ ۗ مَا خَلَقَ اللَّهُ ذَلِكَ
إِلَّا بِالْحَقِّ ۗ يُفَصِّلُ الْآيَاتِ لِقَوْمٍ يَعْلَمُونَ (٥))

صدق الله العلي العظيم

سورة يونس (الآية ٥)

الخلاصة

اجريت هذه الدراسة في جامعة بابل /كلية العلوم /قسم علوم الحياة ، وجامعة القاسم الخضراء/كلية الطب البيطري ،والجامعة التكنولوجية /قسم النانوتكنولوجي والمستشفى البيطري في بابل للمدة من شهر تشرين الاول 2021 الى شهر حزيران 2022 .

الهدف من هذه الدراسة هو اختبار تاثير المستخلص الايثانولي لازهار نبات زهرة الجمال المحمولة على الجسيمات النانوية على ذكور الجرذان البيض ومعرفة امكانية استخدام هذا المستخلص النباتي كمضاد للخصوبة عند الذكور.بالاضافة الى محاولة الحصول على جرعة نصف مؤثرة تكون اقل من الجرعة النصف مؤثرة للمستخلص بدون جزيئات نانوية والتي تم تحديدها بدراسات مسبقة (300 ملغم/كغم من وزن الجسم) .

تم استخدام 20 جرد بالغ ابيض كنموذج للتدييات وتم تقسيمهم الى 5 مجاميع (اربع حيوانات لكل مجموعة)المجموعة الاولى هي السيطرة السالبة الجرعة بماء الحنفية 0.2 مل لكل يوم والمجموعة الثانية هي السيطرة الموجبة الجرعة بالجزيئات النانوية (لايوسوم) الفارغة 300ملغم/كغم لكل يوم والمجموعة الثالثة والرابعة والخامسة جرعت بالمستخلص المحمول على الجزيئات النانوية 300,200,100 ملغم/كغم لكل يوم على التوالي .تم ايواء الفئران في نفس ظروف الحرارة والضوء .

اظهرت النتائج انخفاض معنوي ($p<0.05$) في تركيز النطف في البربخ في المجاميع الثلاثة الجرعة بالمستخلص الايثانولي لازهار نبات زهرة الجمال المحمولة على الجزيئات النانوية (100ملغم/كغم،200ملغم/كغم،300ملغم/كغم) لكل يوم مقارنة مع مجاميع السيطرة السالبة والموجبة ،كما اظهرت المجموعة الخامسة انخفاض معنوي ($p<0.05$) مقارنة مع المجموعة الثالثة،في حين لا يوجد اختلاف

معنوي ($p > 0.05$) بين المجموعة الثالثة والرابعة وايضا بين المجموعة الرابعة والخامسة .

سجلت النسبة المئوية للنطف ذات الحركة التقدمية بالمجموعة الثالثة والخامسة انخفاض معنوي ($p < 0.05$) مقارنة مع المجموعة الرابعة ومجاميع السيطرة ، اما نسبة النطف ذات الحركة غير التقدمية فقد سجلت انخفاضا معنويا ($p < 0.05$) بين المجاميع المجرعة مقارنة مع مجاميع السيطرة السالبة والموجبة ، كما لوحظ ان هنالك انخفاض معنوي كبير ($p < 0.05$) في نسبة النطف ذات الحركة غير التقدمية في المجموعة الثالثة مقارنة مع كل المجاميع . بينما اظهرت النتائج حدوث ارتفاع معنوي ($p > 0.05$) في نسبة النطف غير المتحركة في الجاميع الثلاثة المجرعة مقارنة مع مجاميع السيطرة حيث لا توجد فروق معنوية ($p > 0.05$) بين المجاميع المجرعة الثلاثة (G3, G4 and G5). سجلت النسبة المئوية لحيوية النطف انخفاضا معنويا ($p < 0.05$) في المجموعة الخامسة مقارنة مع جميع المجاميع الاخرى ، بينما سجلت المجموعة الرابعة انخفاض معنوي ($p < 0.05$) مقارنة مع المجموعة الثالثة ولا يوجد فرق معنوي ($p > 0.05$) مقارنة مع مجاميع السيطرة ، كذلك لا يوجد فرق معنوي ($p > 0.05$) في النسبة المئوية لحيوية النطف بين المجموعة الاولى والثالثة .

كما بينت الدراسة الحالية من خلال فحص مستوى الهرمونات وجود انخفاض معنوي ($p < 0.05$) في مستوى هرمون محفز الجريبات والهرمون اللوتيني وهرمون الشحمون الخصوي ، بينما اظهرت الدراسة الحالية ارتفاع معنوي ($p > 0.05$) في مستوى هرمون الاستروجين، وايضا وجود انخفاض معنوي ($p < 0.05$) في مستوى $IL-6$, $TNF-\alpha$.

نستنتج من هذه الدراسة بان الجرعة نصف المؤثرة لمستخلص ازهار نبات زهرة الجمال المحمولة على الجزيئات النانوية (96.605 ملغم/كغم) وتمثل ثلث الجرعة نصف المؤثرة لمستخلص ازهار نبات زهرة الجمال من خلال حسابها من قيم تركيز النطف والنسبة المئوية للنطف ذات الحركة التقدمية وهرمون الشحمون الخصوي

و $TNF-\alpha$ ، من خلال نقصان المعايير المشار اليها اضافة الى تاثير المستخلص المحمل على الجزيئات النانوية على بقية المعايير المدروسة .



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

تأثير المستخلص الايثانولي لأزهار نبات *Hibiscus rosa-sinensis* المحمولة على الجزيئات النانوية على خصوبة ذكور الجرذان البيض

رسالة مقدمة الى
مجلس كلية العلوم – جامعة بابل
وهي جزء من متطلبات نيل درجة ماجستير في العلوم
علوم الحياة

من قبل

سامر حبيب كريم حبيب الرهيمي

بكالوريوس علوم حياة/جامعة بابل/2017

بإشراف

أ.د.عدنان منصور جاسم

أ.د.فارس ناجي عبود الهادي

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