

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



**The Effect of *Mentha Spicata* Leaves on Some Physiological,
Immunological Parameters and Aggressive Behavior in Albino
Female Rats Treated with Testosterone**

A Thesis

**Submitted to the Council of the College of Science,
The University of Babylon as a Partial Fulfillment of the
Requirements for the Degree of Master of Science / Biology**

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(B.Sc. In Biology/ University of Babylon, 2020)

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(يَرْفَعُ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ
وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ).

صِدْقَةُ اللَّهِ الْعَظِيمَةِ

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Dedication

To:

To: who supported me with everything he had, to lead me to the path of success..... to my pride and the crown of my head **my dear father.**

To who was credited with bringing me to this moment..... to my pure angel and the smile of my heart..... **my dear mother.** To the companions of the path, childhood, life partners, and beautiful memories, to those with whom I knew sincere brotherhood and true love..... **my beloved brothers.**

And to everyone who believes that the head of wisdom the fear from Allah.

ZAHRAA 2024

Acknowledgments

First and foremost I thank Almighty God the Merciful , who gave me the ability to accomplish this work. I am strongly grateful with deep appreciation to my supervisor Prof .Dr. Alaa Jawad Hassan and Assist. Prof Dr. Halla Abdul Hadi Abdul Ghani for suggesting and supervising this work and for their valuable advices and scientific assistance during the course of investigation and writing of this thesis. And would like to extend my sincere thanks and appreciation to Prof .Dr. Alaa Tariq Shaker and Asst. Prof. Dr. Fadia Hameed Mohammed and for their continuous support and constant encouragement.

Express my thanks and gratitude to the dean of the college of science I want to thank the staff of the Department of Biology in the College of Science at the University of Babylon Furthermore, much appreciation is dedicated to the Head of the Biology Department, Assist. Prof. Dr. Adi Jasim Abd AlRazzaq who offered a permission to use all the required equipments and the necessary materials for the successful completion of the present work.

ZAHRAA 2024

Summary

The present study was performed to investigate the effects of an alcoholic extract of *Mentha spicata* leaves on physiological and immunological parameters and aggressive behavior in female rats treated with testosterone propionate.

The current study was conducted in the animal house of the Department of Biology / College of Science / University of Babylon during the period from November (2022) to (March) 2023. Thirty female rats (*albino wistar*) divided into six groups (5 rats for each group) were used in the present study as follows : **The first group (I)** rats received sesame oil (0.1 ml) by dosage as a control group daily for 60 days. **The second group (II)** rats are injected subcutaneously with testosterone propionate(TP) (6 mg/kg). **The third group (III)** rats received alcoholic extract of mentha leaves (100 mg/kg) orally. **The fourth group (IV)** rats injected with TP (6 mg/kg) after that received alcoholic extract of mentha leaves (200 mg/kg) orally. **The fifth group (V)** rats injected with TP (6 mg/kg) and received alcoholic extract of mentha leaves (400 mg/kg) orally. **The sixth group (VI)** rats injected with TP (6 mg/kg)and received alcoholic extract mentha leaves (600 mg/kg) orally at the same time . All groups of rat treated for 60 days daily. All these animals were anesthetized by chloroform and sacrificed on day 60 , and after that blood samples were obtained from all groups of rats for estimating lipid profile levels, some physiological (testosterone, dopamine levels) and immunological (TNF- α , IL-2 levels) parameters .

Summary

The results revealed that the rats treated with TP (6 mg/kg) in group II showed a significant increase ($p < 0.05$) (16.52 ng/ml) in the levels of the testosterone and dopamine hormones compared to the control group and the other groups, whereas, rats in groups (IV, V and VI) are treated with TP (6 mg/kg) and alcoholic extract of mint leaves (200, 400 and 600 mg/kg) respectively showed a significant decrease ($P < 0.05$) (4.82 ng/ml) in the levels of testosterone and dopamine hormone levels when compared to group II. The present study showed that there was a significant negative linear relationship between the level of the testosterone in the serum of female rats and the concentration of the alcoholic extract of mint leaves (*Mentha Spicata*).

The results showed a significant increase ($P < 0.05$) (56.19 pg/ml, 32.75 pg/ml) in the levels of TNF- α and IL-2 in the rats treated with TP group II (6 mg/kg) compared to the control group and the other groups, whereas, rats in the groups (IV, V and VI) treated with TP (6 mg/kg) and alcoholic extract of mint leaves (200, 400 and 600 mg/kg) respectively for 60 days showed a significant decrease ($P < 0.05$) (26.26, 18.72 pg/ml) in the levels of these cytokines when compared to group II. Furthermore, the results showed a significant increase ($P < 0.05$) in the levels of triglyceride (TG) (153.22 mg/dl), total cholesterol (TC) (116.65 mg/dl), low density lipoprotein (LDL) (83.41 mg/dl), and very low density lipoprotein (VLDL) (23.33 mg/dl) in group II treated with TP (6 mg/kg) compared to group III, while the level of high density lipoprotein (HDL) is significantly decreased ($P < 0.05$) (19.36 mg/dl) in the group II. The levels of TC (111.99 mg/dl), LDL (58.64

Summary

mg/dl), VLDL(16.46mg/dl) and TG(77.41 mg/dl) showed significant decrease ($P<0.05$) in group III compared to group II, whereas, the level of HDL is significantly increased ($P<0.05$) (57.90 mg/dl) in the G III. The results revealed that significant increase ($P<0.05$) of aggressive behavior in group II of rats in Number of threat and thrust (45.00), Number of attack (17.60) and Number of keep down (22.40) compared to the control group and other groups, while, there is a significantly decreased ($P<0.05$) of aggressive behavior of (Number of threat and thrust (8.40), Number of attack (5.60) and Number of keep down (1.20) in groups (IV, V and VI) compared to group II.

In conclusion, this study illustrated that the protective effects of alcoholic extract of *Mentha spicata* leaves a giants high levels of testosterone, dopamine, pro-inflammatory cytokines and lipid profiles and its role in reduced aggressive behavior in female rats exposed to testosterone propionate.

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List of Abbreviations

AML	alcoholic extract of mint leaves
CVD	Cardiovascular disease
DA	Dopamine
ELISA	enzyme linked immunosorbent assay
FST	forced-swim test
HDL	High density lipoprotein
HRP	Horseradish peroxidase
HIV	human immunodeficiency virus
IL-2	Interleukin-2
(LCAT)	Lecithin-cholesterol acyltransferase
LDL	Low density lipoprotein

NTs	Neurotransmitters
OD	optical density
OFC	Orbitofrontal cortex
PFC	Prefrontal cortex
PCOS	polycystic ovarian syndrome
SHBG	Sex hormones-binding globulin
TC	Total cholesterol
TG	Triglyceride
TP	testosterone propionate
TRT	testosterone replacement therapy
TNF- α	Tumor necrosis factor
VLDL	Very low density lipoprotein

Chapter One

Introduction

Introduction

Testosterone is a sex hormone created by males and females. In males, it is created and released from the Leydig cells in the testes, while in females produced by the placenta and ovaries (25%). In both sexes testosterone secreted by the adrenal cortex (25%) (Banihani, 2019). The testosterone levels differ significantly in both sexes, with males having higher testosterone levels than females. However, the typical serum testosterone levels in females are generally 10 to 20 times lower. Testosterone has physiological impacts on many body organs and reproductive tissues in women (Parish *et al.*, 2021).

The immune system is the body's main active defense against physical damage and pathogens. These reasons activate leukocytes that produce cytokines to support several kinds of inflammatory responses. Cytokines are known to produce a collection of sickness behaviors such as reductions in activity, food intake, and social interaction, along with improved sleep. Psychological stress can produce cytokine release, and growing evidence has shown a significant role for the immune system in regulating negative emotional states as well as personality (Takahashi *et al.*, 2018).

There is growing evidence that changed neuroimmune responses are implicated in the neurobiology of aggression, including the production of pro- and anti-inflammatory cytokines. Pathological levels of aggression, aggressive traits in humans, high states of anger and hostility have been associated with increased serum tumor necrosis factor α , interferon- γ , IL-1 β , IL-2, IL-6, C-reactive protein. Data found on the basis of animal models

have indicated that aggressive behavior is accompanied through increased immune responsiveness and cytokine production (Alperina *et al.*, 2019).

Testosterone plays an important role in lipid metabolism and glucose homeostasis. Inferior total testosterone predicts a high incidence of the metabolic syndrome. Contrary to the positive connotation seen between endogenous testosterone and HDL concentrations, meta-analyses of studies of testosterone replacement therapy (TRT) in hypogonadal men have generally demonstrated that exogenous testosterone lowers HDL; however, this effect is commonly related with a concomitant lowering of total cholesterol and LDL concentrations (Thirumalai *et al.*, 2015). HDL particles perform myriad functions, including immunomodulatory roles, the regulation of endothelial cell function and removal of cholesterol from the artery wall through reverse cholesterol transport. Interestingly, in-vitro findings suggest that testosterone could accelerate reverse cholesterol transport (Haddad *et al.*, 2007).

Aggression behaviour is a form of social interactions and its observed in all animal species, such as lizards, insects, frogs, fish, rodents, and most mammals including humans, and it can be defined as the performance of an action, from threatening gestures to biting, lateral attacks and fighting, against animals belonging to a different or same species (Regis *et al.*, 2015). Testosterone has an impact on the brain areas and may be the cause of some behavioral disorder between the sexes. Numerous studies have been conducted on testosterone's effects, particularly in models of anxiety and aggressive (Domonkos *et al.*, 2018).

Aggressive behavior is a complex social behavior and is commonly involved with intellectual disability and psychiatric disorders, it has received considerable research attention for more than five decades two types of aggression have been identified, the first sub-type with automatic control, and the interactive-impulsive sub-type. The second is considered impulsive, usually associated with anger, while the first is more objective and directed towards the goal. Neurotransmitters refer to molecules in the nervous system that function as signaling molecules on specific receptors for each neurotransmitter in the synaptic cleft. Examples include dopamine. These molecules are major cofactors in a wide variety of molecules (Trifu *et al.*, 2020).

Testosterone and dopamine have been implicated in the regulation of aggressive behavior, but it has remained challenging to assess the dynamic changes in these neurotransmitters while aggressive behavior is in progress (van Erp and Miczek, 2000). Cooper *et al.*(2014) found that mice showed social and aggressive behavior when determined levels of monoamine in four brain regions in these mice. exhibited a significant increase in levels of dopamine in these regions. Increased brain dopamine levels have been reported to be involved with increased aggression.

A study has shown that menthol in mint lowers testosterone levels in men, which ultimately leads to a decrease in libido and aggressive behavior. (Fahimi *et al.*, 2021). It is known that spearmint is beneficial in decreasing free testosterone levels and hirsutism in women with mild hirsutism with PCOS, and its adverse histopathological effects on kidney, liver and uterine tissue in animals are observed (Alaee *et al.*, 2020).

Certain studies displayed that female students receiving *M. spicata* in capsule form showed dysmenorrhea with lower severity compared with placebo. Additionally, the potentiating effect of the *M. spicata* extract with typical antidepressants looks promising and its potential as a supplementary agent for the management of depression (Abbasi-Maleki, *et al.*, 2017).

Aim of the study :

The aim of the current study was to determine the possible protective properties of alcoholic extract of mint leaves *Mentha spicata* against aggressive behavior in female rats undergoing from higher levels of testosterone as well as evaluation certain physiological and immunological parameters related with aggressive behavior . The aim of current study is achieved by using the following objectives :

1. Estimation the level of testosterone and dopamine.
2. Estimation the levels of IL-2 and TNF- α .
3. Estimation the levels of lipid profile.
4. Study the aggressive behavioral by using resident-intruder test.

Chapter Two

Literature

Review

2.1 : Definition of Testosterone

Testosterone is a sex hormone created by males and females. In males, it is created and released from the leydin cells in the testes, while in females produced by the placenta and ovaries (25%). In both sexes also testosterone secreted by the adrenal cortex (25%)(Banihani, 2019).

The testosterone levels differ significantly in both sexes, with males having higher testosterone levels than females. However, the typical serum testosterone levels in females are generally 10 to 20 times lower. Testosterone has physiological impacts on no reproductive and reproductive tissues in women (Parish *et al.*, 2021), in addition to a critical role in secondary sexual attributes development; such as increased bone mass, muscle and body hair in males. However, it is also of private interest in the studies of social and emotional behavior because it impacts the brain in some cases, like flight, fighting, and mating. Levels of testosterone positively participate in the sexual functions of women, and many studies have shown that testosterone can be efficient in the treatment of sexual dysfunction in women. (Eisenegger *et al.*, 2011).

Testosterone is present in blood either unbound (free) or bound with albumin proteins. Moreover, inactive testosterone is bound to globulin (sex hormones-binding globulin (SHBG), carrier proteins created in the liver that have a high affinity to the testosterone hormones. Free testosterone hormone is the active portion of the androgen groups and therefore free testosterone could connect more to the risk of detection aggressiveness

(Regis *et al.*, 2015). Increased active testosterone levels reflect lower SHBG levels and vice versa (Smith and Batur, 2021).

2.2: The biological effects of Androgens on females

Androgens are important to females and influence most organs of the body, such as the skeletal, muscular, bone, adipose tissue, immunological, neural, and hemopoietic systems, and cardiovascular, systems (Abdulhussein *et al.*, 2022). Interestingly, they also directly impact the reproductive system of females, breasts, and other systems. The active form of androgens such as testosterone and dihydrotestosterone are essential for women's health, emotionally, cognitively and physically (Smith and Batur, 2021).

A woman of reproductive age produces three to four times as much testosterone as estrogen every day from her ovaries. But these androgens levels decrease with age, about in the mid-30s onward, with no clear rise in the speed of decrease at menopause, due to the ova continuing to produce hormones (Donovitz ,2021). High levels of testosterone cause infertility in females and are problems of global proportions. The most common cause of infertility in women is polycystic ovaries, and it is one of the most common internal disorders, as it affects approximately 5% of women of childbearing age). Hyperandrogenic in females causes hirsutism, acne, menstrual irregularity, and metabolic abnormalities like insulin resistance, obesity, dyslipidemia, diabetes, cancer, in addition, coronary heart diseases and it has been associated with hypothalamus ovary axis alterations, (Morales-Ledesma *et al.*, 2017).

Johansen *et al.* (2020) have shown testosterone treatments improved sexual function in postmenopausal women who suffer from sexual desire disorders, she given testosterone either as an intramuscular injection and orally, many of the women in these experiments showed high testosterone levels in serum, and oral testosterone administration was associated with a defect lipid profile, and cause metabolism disorder in the liver, and thereby the negative impact on lipids.

Kelly and Jones, (2015) showed that Low levels of testosterone are involved with increased fat mass (adiposity). This morphological feature is linked with obesity metabolic dysfunction, impaired glucose control, energy imbalance, and decreased insulin sensitivity. According to Davis *et al.* (2015), “the observational data showed that low levels of testosterone free or bound with albumin and globulin in serum are involved with atherosclerotic carotid and cardiovascular diseases. Moreover, high levels of testosterone increase the risks of cardiovascular disease (CVD) in females.”Iwasa *et al.* (2018) and Mishra *et al.* (2019) they found that increased testosterone hormone levels in women, participate in the increased risk of cardiovascular diseases and hypertension development. However, Low levels of androgens have an adverse impact on cardiovascular health because testosterone improves vascular relaxation and improving blood flow through its impacts on resistance of the peripheral vessels.

The most powerful androgen impact on the hair follicle is mediated by dihydrotestosterone, which causes a shorter growth phase of the follicle and shrinkage, leading to hair loss. androgen receptors on the cheeks, chin and upper lips cause coarse hair to grow or excessive hair growth

(hirsutism). Acne is a more common syndrome with raised levels of testosterone in women because androgens stimulate the sebaceous gland of the hair to grow and release sebum (Tyagi *et al.*, 2017).

Both testosterone and estrogen have anti-inflammatory impacts, therefore will protect the nerve cells against injuries. Testosterone may modulate oxidative stresses and decrease the accumulation of the damaging amyloid-beta proteins inside the brain. Androgen (testosterone) receptors are dispersed through the central nervous system, and their activities affect heat regulation, libido, Mood, sleep, cognitive function, and visuospatial skill. Testosterone treatment does not appear to have any adverse cognitive impact, nor does it adversely impact well-being or mood (Johansen *et al.*, 2020 ; Smith and Batur, 2021).

Androgens also have receptors present on bone cells meaning that declining androgen levels, like in post-menopausal women, are involved with an increased risk of fractures in the spine and hip and decreased bone density. So, some benefits that showed improvement in muscle strength and muscle masses may be seen with androgen supplementation in women who have undergone hysterectomy (Valenzuela *et al.*, 2019).

The increased aromatase level in the breast tissues means that androgens are rapidly converted to estrogen, resulting in proliferative effects on the breast. Interestingly, experimental studies display the opposite, with testosterone appearing anti-proliferative impact as well as promoting apoptosis within the breast tissues. Testosterone acts to inhibit estrogen receptors alpha and suppress the growth of breast cancer cells. These

changes depend on the dose of testosterone, the type of breast cancer cells and the type of supplementation (Islam *et al.*, 2019 ;Johansen *et al.*, 2020).

2.3: The effect of hormones and neurotransmitters on aggressive behavior

Aggression behaviour is a form of social interactions and its observed in all animal species, such as lizards, insects, frogs, fish, rodents, and most mammals including humans, and it can be defined as the performance of an action, from threatening gestures to biting, lateral attacks and fighting, against animals belonging to a different or same species (Regis *et al.*, 2015).

The neurophysiological mechanism of aggression is same in all vertebrates, aggression as a social activity that occurs naturally in mammals in order to protect their area,, preserve resources, and increase the likelihood of fertile mating. However, when levels of aggression becomes excessive, harmful, disturbs social order, and has a cost that is significantly greater than its benefit, the behavior is no longer adaptive and is referred to as maladaptive or pathologic aggressiveness (Ishola, 2020).

The studies on rodents models have exhibited that testosterone promotes violence and aggressive behavior in female and male mammals animals that have given testosterone treatment. The hippocampus, the amygdala complex, and the prefrontal cortex (PFC) are three areas of the brain associated with aggressive behavior (Rankov *et al.*,2019). As well as the roles of inhibitory and excitatory neurotransmitters in the brain and their receptors in the control of aggressive behavior that focuses on the relationships between neuropeptides, neuromodulators and neurotransmitters

that implicate in aggression, such as dopamine , serotonin, oxytocin, vasopressin ,estrogen, testosterone, corticotrophin opioids, monoamine oxidase-A and neuronal nitric oxide synthase (Bambico *et al.*, 2015).

Testosterone has an impact on the brain areas and may be the cause of some behavioral disorder between the sexes. Numerous studies have been conducted on testosterone's effects, particularly in models of anxiety and aggressive (Domonkos *et al.*, 2018).

2.4: The relationship of dopamine with testosterone and aggressive behavior

Aggressive behavior is a complex social behavior and is commonly involved with intellectual disability and psychiatric disorders, it has received considerable research attention for more than five decades Two types of aggression have been identified, the first sub-type with automatic control, and the interactive-impulsive sub-type. The second is considered impulsive, usually associated with anger, while the first is more objective and directed towards the goal. Neurotransmitters refer to molecules in the nervous system that function as signaling molecules on specific receptors for each neurotransmitter in the synaptic cleft. Examples include dopamine. These molecules are major cofactors in a wide variety of molecules (Trifu *et al.*, 2020).

Testosterone, dopamine and serotonin have been implicated in the regulation of aggressive behavior, but it has remained challenging to assess the dynamic changes in these neurotransmitters while aggressive behavior is in progress (van Erp and Miczek, 2000).Testosterone is an androgen that has

been implicated in the development and maintenance of masculine characteristics in a variety of species. It has been documented that the females of most species are less aggressive and have far lower testosterone levels than do males; this is taken as evidence of a link between testosterone and aggression(Book *et al.*, 2001).

Cooper *et al.*(2014) found that mice showed social and aggressive behavior when determined levels of monoamine in four brain regions in these mice. exhibited a significant increase in levels of dopamine in these regions. Increased brain dopamine levels have been reported to be involved with increased aggression.

Aluja *et al.* (2015) studies in healthy men confirm the hypothesis that high testosterone levels impact risk-taking or impulsive behavior. In a later study, it has been exhibited that early-onset users under testosterone range are more impulsive and show deficits in behavioral disinhibiting, affective processing, and planning.

Senkal *et al.* (2021) showed that testosterone induces molecular changes in dopamine in the nigrostriatal pathway, thus increasing the effect of dopamine in the body thus, increased dopamine causes behavioral abnormalities. There is a lot of evidence indicating that testosterone can affect the release of dopamine from neurons. Studies have also demonstrated a link between the development of dopamine-related. Studies indicate that testosterone can increase oxidative stress in dopamine neurons (Holmes *et al.*, 2016).

2.5: Dopamine (DA)

It is catecholamine neurotransmitter that plays important role in the brain of mammalian as a neurotransmitter or as neuromodulator where it responsible for some functions including emotion, cognition, locomotor activity, food intake, endocrine regulation and in motivated behaviors (Ayano, 2016).

Dopamine also plays role in the hormone secretion, modulator vascular function, catecholamine release, renal function and gastrointestinal motility, Dopamine is one of the key neurotransmitters (NTs) convoluted with emotion and cognition. A high dopamine level shows cardio toxicity prominent to express heart rates, hypertension, heart failure, and drug addiction. However, a low-slung dopamine level may cause stress, Parkinson's disease, schizophrenia, Alzheimer's disease, and depression. It is apparent that dopamine measurements are essential for understanding its biological functions and associated biological processes and mechanisms (Liu and Liu, 2021).

Dopamine plays an important role in learning, control, motivation, and movement. Understanding the information that dopamine conveys is critical to determining how dopamine regulates different functions (Kim *et al.*, 2020). Growing research has also shown that dopamine acts as an important regulator of immune function. Many immune cells express dopamine receptors and other dopamine related proteins, enabling them to actively respond to dopamine and suggesting that dopaminergic

immunoregulation is an important part of proper immune function (Pan *et al.*, 2019) .

2.5.1: Dopamine Synthesis

It is synthesis in the brain (in the cerebral cortex) from the amino acid (tyrosine), which is conveyed in the brain by an active transport mechanism. Tyrosine is produced in the liver from phenylalanine through the action of phenylalanine hydroxylase. Tyrosine is then transported to dopaminergic neurons in the ventral tegmental area of the substantia nigra, midbrain and the arcuate nucleus of the hypothalamus where a series of reactions convert it to dopamine (Nataf ,2020). Dopamine belongs to catecholamine, which consists of norepinephrine, epinephrine, and dopamine. The formation process begins with the decarboxylation of dihydroxyphenylalanine, and then tyrosine is formed, and the path is completed to dopamine, adrenaline, and noradrenaline. Therefore, dopamine can bind to adrenergic receptors (alpha_1, beta 1,2) in addition to dopamine receptors (D1, D2, D3, D4, and D5) (Wagels *et al.*, 2020).

Dopamine is secreted into neurons from two areas of the brain in the brainstem The first: the black region (substantia nigra), dopamine secreted from this region have a role in controlling functional movement and learning abilities, and motivational behaviors such as the reward system (Gowda *et al.*, 2020) . When a defect occurs in this region or cells, this leads to Parkinson's disease, depression, or a psychological disorder. The second: the ventral tectorial region (Ventral tegmental). Dopamine produced by this

region is responsible for the reward or motivation system (Lehrer and Rheinstein 2022).

2.5.2: Risks of low and of increased dopamine secretion

Lack of dopamine secretion leads to Parkinson's disease as well as many mental and psychological diseases and disorders. Risks of increased dopamine secretion: There is no doubt that high levels of dopamine can make you at the top of your activity and this lasts for a very short period, as it quickly leads you to a more dangerous state, and this becomes a contributing factor to the feeling of hallucinations and delusions. Increasing it more than necessary leads to injury: Obesity Addictive schizophrenia (Tammina *et al.*, 2019).

2.6: Aggression and the immunity response

Evidence point to an immunosuppressive role of testosterone on different components of the immune system and also suggests a role of testosterone in the different phases of the immune response the negative effect of testosterone on the immune response has been evaluated in numerous preclinical studies therefore, there is increasing evidence that testosterone acts negatively on the immune response in both bacterial and viral infections, and this powerful immunosuppressive effect could explain the greater susceptibility of males to infections the lower incidence of autoimmune diseases in men than women, and the lower response to vaccines in men compared with women (Giudice *et al.*, 2021).

The immune system is the body main active defense against physical damage and pathogens. These reason activation of leukocytes that products cytokines to support several kinds of inflammatory responses. Cytokines are known to products an collection of sickness behaviors such as reductions in activity, food intake, and social interaction, along with improved sleep and anhedonia. Psychological stress can produce cytokine release, and growing evidence has shown an significant role for the immune system in regulating negative emotional states as well as personalit (Takahashi *et al.*, 2018).

There is growing evidence that changed neuroimmune responses are implicated in the neurobiology of aggression, including the production of pro- and anti-inflammatory cytokines. Pathological levels of aggression, aggressive traits in humans, high states of anger and hostility have been associated with increased serum tumor necrosis factor α , interferon- γ , IL-1 β , IL-2, IL-6, C-reactive protein. Data found on the basis of animal models have indicated that aggressive behavior is accompanied through increased immune responsiveness and cytokine production (Alperina *et al.*, 2019). Jabbar *et al.* (2020) Mice selectively bred for high levels of aggression displayed increased production of proinflammatory cytokines. Knockout of both TNF α -receptor-1 and TNF α -receptor-2 abrogates aggressive behaviors, while IL-6 knockout mice demonstrated shorter attack latency and increased frequency of aggressive behaviors in the resident-intruder test.

Zain (2019) emphasized the role of the immune system following the response to stress. Since then, Have learned that stress is a broad category including some aversive events which can elicit an aggressive response, and that the immune system interferes with normal and pathological brain

functioning and behavior (Vaeroy *et al.*, 2019). Pheromones and odors from the urine have been associated with aggressive behavior. It is currently accepted that aggressive behavior can be viewed as a strategy by humans and animals to cope with stress, implying that neurobiological mechanisms involved in stress responses should underlie both physiological and pathological aggression (Cui *et al.*, 2019).

Gogos *et al.* (2012) Discover testosterone playing a detrimental role in schizophrenia. For instance, testosterone levels sharply rise during adolescence, reach their peak, and then gradually decline as age. The concept of suicide is closely connected to mood disorder. However, only about half of those who make serious suicide attempts are depressed apart from psychiatric diagnoses, such as unipolar depressive disorder, anxiety disorder, bipolar disease, alcohol and/or substance abuse, borderline personality disorder, phenomena such as childhood abuse, grief, religious, political or socio-economic aspects, as well as physical disease like cancer, human immunodeficiency virus (HIV) and epilepsy are associated with suicidality. In order to predict and prevent suicidal behaviour, much effort has been spent to discover a public denominator for those who make suicide attempt (Fonseca *et al.*, 2020).

2.7: Effect the Testosterone on lipid profiles

Testosterone plays an important role in lipid metabolism and glucose homeostasis. Inferior total testosterone predicts a high incidence of the metabolic syndrome. Contrary to the positive connotation seen between endogenous testosterone and HDL concentrations, meta-analyses of studies

of testosterone replacement therapy (TRT) in hypogonadal men have generally demonstrated that exogenous testosterone lowers HDL; however, this effect is commonly related with a concomitant lowering of total cholesterol and LDL concentrations (Thirumalai *et al.*, 2015). HDL particles perform myriad functions, including immunomodulatory roles, the regulation of endothelial cell function and removal of cholesterol from the artery wall through reverse cholesterol transport. Interestingly, in-vitro findings suggest that testosterone could accelerate reverse cholesterol transport (Haddad *et al.*, 2007).

The effects of testosterone on plasma lipids have been a focus of attention. Lower endogenous testosterone levels are related with a proatherogenic lipid profile most of these studies have also demonstrated an converse relationship between testosterone levels and both plasma triglycerides and total cholesterol. Elevated levels of VLDL are linked with increased danger of atherosclerosis (Mohamed *et al.*, 2010).

2.8: Mint plant (*Mentha spicata*)

Taxonomy

- Kingdom-Plant
- Division-Magnoliophyta
- Class-Magnoliopsida
- Order-Lamiales
- Family-Lamiaceac
- Genus-Mentha

Spearmint is an aromatic herb or groundcover in the Lamiaceae (mint) family native to Europe. In its natural habitat it is found growing in moist fields and pond or lake margins. This upright perennial thrives in full sun in well-drained soils moist growing quickly 1 to 2 feet high and wide with bright green leaves and shoots. It can be distinguished from other mints by its almost hairless (glabrous) leaves that are attached to the stem with a very to almost non-existent petiole (sessile). The square stems can root wherever they touch the ground and it also spreads by rhizomes (Mahendran *et al.*, 2021). It is widely used for culinary purposes, and is known as one of the best mints for flavor. Use the flowers and leaves fresh or dried in teas, beverages, jellies, syrups, candies, ice creams, lamb dishes, and mint sauce. Best grown in a container as it spreads rampantly (Kanatt *et al.*, 2007).

This genus is a perennial herb with a pungent, pleasant aroma. It is spread almost globally around the continent of Europe, Africa, Asia, Australia and North America. It is a perennial plant and rarely a biennial plant, and it has stems that spread widely under and around the ground. Its stems are square, forked and erect. The leaves are arranged in opposite pairs, and their shape ranges from square to lanceolate. They are often smooth to the touch, with serrated edges. Leaves vary in color from dark green and gray green to violet, blue and sometimes light yellow. and its flowers are white to purple (Boukhebt *et al.*, 2011). Their family includes about 260 genera and more than 7000 species. Their characteristic features contain the stems which are quadrangular (square) in cross-section and the bisexual, zygomorphic bilaterally symmetrical flowers, composed of five united and deeply lobed petals and five united sepals, characteristically the lower petal

is larger than the others. The fruit is dry and woody, a schizocarp or drup. The distinctive strongly aromatic leaves are opposite with successive pairs at right angles (i.e., decussate) with margins entire or lobed. Many species of this family, such as mints, have significant commercial uses for the culinary, pharmaceutical, herbal, and ornamental industries (Mahendran *et al.*, 2021).(Figure ,1)



Figure(2.1): Illustrated the external feature of *Mentha spicata*

Spermint (*Mentha spicata*), a perennial herb belonging to the Lamiaceae family, is a sterile natural hybrid derived from a cross between *Mentha aquatic* x *Mentha spicata* species. It is native to Europe and it has become both cultivated and naturalized in many European countries and in North America (Saqib *et al.*, 2022). Altered mints are known for a reasonably high content of important oils (EO), which are deposited in the glandular trichomes, mostly placed on the adaxial surface of their leaves

(Kalembe and Synowiec 2019). Spearmint extract consists of menthone and menthol with several other secondary components, including polygon menthofuran limonene. Its chemical composition may vary with plant maturity and geographic area. Some reports have demonstrated that peppermint extract has some immunomodulatory properties (Yusuf *et al.*, 2022).

2.8.1: The active ingredients in *mentha* species

2.8.1.1: Essential oils

Essential oils are volatile and natural secondary metabolites characterized by a complex composition and a strong odor. They are commonly obtained by steam or hydro-distillation from different aromatic plants. Many species of *Mentha* are cultivated for essential oil production. mint oils are among the most important essential oils produced in the world. For example, Mint oil, composed of menthone, menthol, and menthyl acetate as major components; *M. gracilis*, *M. spicata*, and *M. viridis* produce mostly carvone-rich oil, although several compounds have been reported; *M. citrata* is a source of linalyl acetate and linalool; *M. pulegium* produces the called pennyroyal oil, that is a pulegone-rich oils (El-Shemy, 2017).

Mentha spicata L. typically contain of essential oils including Carvone, pulegone, piperitenone oxide, 1,8-cineole, piperitone, limonene, cis-piperitone oxide, , caryophyllene, piperitenone, and menthofuran as well as *Mentha spicata* also contain other Polyphenol Compounds such as Rosmarinic acid, salvianolic acids, flavanones, hydroxybenzoic acids,

caffeoylquinic acids, hydroxycinnamic acids, and flavones (Bahadori *et al.*, 2018 and Malekmohammad *et al.*, 2021).

2.8.1.2: Phenolic compounds

Phenolic compounds, secondary metabolites ubiquitously distributed in plants, consist of large groups of biologically active compounds. *Mentha* species have been reported to be composed of a range of compounds, including aglycon, cinnamic acids, acylated flavonoids and glycoside. Pérez *et al.*, (2014) exhibited that water extract from mint contains flavonoid derivatives, esters of phenolic acids and glycosidic flavonoids hydroxylated. *Mentha* is particularly rich in caffeic acid and its derivatives, rosmarinic acid, chlorogenic. As well as, seven salvianolic acids have been found in these plants, like salvianolic acid H/I, salvianolic acid B, salvianolic acid E, and isosalvianolic acid A (caffeate trimers) (Kapp, 2015). *Mentha* leaves are rich in flavonoids, especially in flavanones and flavones. Luteolin and its derivatives. The components luteolin-7-O-glucoside, eriocitrin, isorhoifolin, eriodictyol, naringenin-7-O-glucoside, apigenin and luteolin are identified in aqueous extract from *mentha* species (Brahmi *et al.*, 2015).

2.8.1.3: Other compounds

M. spicata leaves contain diacylglycerol, triacylglycerol, and free fatty acids like linolenic, linoleic, and palmitic acid also mints composed of triterpenoids and steroids. Moreover, various pigments are found in *mentha* xanthophylls, carotenes and chlorophylls. Among α -tocopherols, vitamins, and ascorbic acid are found in mints (El-Shemy, 2017).

2.9: Biological activities

One of the most important benefits of mint is that it acts as a carminative and antispasmodic, as well as relieves hirsutism and menstrual pain (Mekkaoui *et al.*, 2022). Among the specific benefits of boiled mint is helping to regulate muscle relaxation, and some soothing herbs that increase the number of patients suffering from stomach or stomach disorders (Soleimani *et al.*, 2022). One of the benefits of mint is also the treatment of allergies, because mint plants contain antioxidants and anti-inflammatory agents such as rosmarinic acid, which has the following benefits: Reduces asthma symptoms that cause difficulty breathing in some people. The mint family contains a group of plant compounds that have anti-allergic effects (Wani *et al.*, 2022).

Budiu *et al.* (2019) showed the mint also soothes cold symptoms, especially since it contains menthol, an aromatic decongestant that may help break up phlegm and mucus, making it easier to expel and thus open up the respiratory passages. Several studies have evidenced that mint extract can improve the digestive system, central nervous system, and respiratory system of the human body, and have anti-inflammatory antibacterial, antiviral, anticancer, antifungal, antioxidation and other effects (Paulus *et al.*, 2019). The abovementioned curing effect of mints are since of the occurrence of pharmaceutically valuable bioactive compound. The main volatile components of mint include menthol, menthone, menthyl acetate, menthofuran, and 1,8-cineol (Wei *et al.*, 2023) and also for alleviating hirsutism and menstrual pain The antioxidant, anticancer, inflammatory,

antifungal, antimicrobial, and antidiabetic properties of *Mentha spicata* have been shown in some studies (Alaee *et al.*, 2020).

2.9.1: Antioxidant activity

M. spicata -derived secondary metabolites, similar bioactive compounds, are well-known antioxidants that have antiviral, cardio-tonic, and immune-stimulatory properties. New or dry herbs are positively related to the prevention of several diseases, and possess antimicrobial activity, in addition to antibacterial and antifungal effects (Ilić *et al.*, 2022). *M. spicata* contain an antioxidant and anti-inflammatory agent, known as rosmarinic acid, whose effectiveness in relieving seasonal allergy symptoms is studied, so the detection of a promising natural remedy that could be developed for use and application (Imran *et al.*, 2021).

2.9.2: Antimicrobial activity

Mentha oil contain antimicrobial properties, and studies have established the efficiency of these properties on numerous strains of bacteria: *Escherichia coli*, *E. Coli*, *Staphylococcus aureus* (Valková *et al.*, 2021). Spearmint are among the most extensively consumed single-ingredient herbal teas or tisanes. In vitro and animal in vivo studies display their compounds with antimicrobial properties and health benefits throughout their passage through the digestive system (Soleimani *et al.*, 2022).

2.9.3: Insecticidal activity

Many spearmint species are grown for profitable purposes such as their use in food flavors, cosmetics and pharmaceuticals. Many studies have been carried out on the fungicidal and insecticidal activities of mint species (Mejdoub *et al.*, 2019). The recurrent uses of synthetic insecticide for periods has disrupted biological control by natural enemies and has led to outbreaks of other insect species and at times have caused in resistance of pesticides in insect pest (Pipariya *et al.*, 2023). In term of biological uses, mentha acts as antispasmodics and anti-platelets and insecticides (Jabbar and Kathem, 2019).

2.9.4: Cytotoxicity

Contemporary pharmacology research has showed that the entire herb of *M. spicata* possesses antioxidant, cytotoxic, antiallergenic, antiviral and antibacterial activities . The essential oil of *M. spicata* is reported to have antimicrobial and antioxidant activities (Sun *et al.*, 2014).

2.9.5: Anti-inflammatory properties

Mentha oil is used as an antifungal because of its anti-inflammatory, antioxidant and antimicrobial properties (Hejna *et al.*, 2021). The attendance of secondary phytoconstituents in a significant quantity imparts the therapeutic applications of mint leaves as anti-inflammatory agents, anti-cancer agents as well as for the treatment of allergic reactions(Shanmuganathan *et al.*, 2023).

2.9.6: Toxic and adverse effects

Its side effects are claimed to be usually slight and minimally poisonous (gastro esophageal reflux, heartburns, nausea, vomiting, allergic reactions and diarrhea). High doses could be hepatotoxic and nephrotoxic (rarely interstitial nephritis and acute renal failure) (Nath *et al.*, 2012). high concentration of aqueous extract of *Mentha spicata* have a side effect on the structure and function of liver tissue (Ali, 2017).

2.10: The effect of mint on both testosterone and dopamine levels

A study has shown that menthol in mint lowers testosterone levels in men, which ultimately leads to a decrease in libido. In addition, mint may reduce sperm production if consumed in excess, which causes impotence (Fahimi *et al.*, 2021). It is known that spearmint is beneficial in decreasing free testosterone levels and hirsutism in women with mild hirsutism with polycystic ovarian syndrome (PCOS), and its adverse histopathological effects on kidney, liver and uterine tissue in animals are observed (Alaee *et al.*, 2020). *M. spicata* has a lethal effect on the testis by causing a significant in experimental animals. Past studies showed structural changes in testicular tissues, morphological deformities and inhibition of spermatogenesis of different mammalian species treated with peppermint (Mohammed *et al.*, 2021).

A study displayed that female students receiving *M. spicata* in capsule form showed dysmenorrhea with lower severity compared with placebo. The ethanolic extract of *M. spicata* possesses antidepressant-like properties in the

forced-swim test (FST) in mice. This response might be attributed to interactions with the dopaminergic, noradrenergic, and serotonergic receptors. Additionally, the potentiating effect of the *M. spicata* extract with typical antidepressants looks promising and its potential as a supplementary agent for the management of depression (Abbasi-Maleki *et al.*, 2017).

Chapter Three

Materials and
Methods

3. Materials and methods

3. 1. Chemicals and instruments

Chemicals and Instruments that were used in this study are listed in tables (3.1 and 3.2) with their manufacturing companies and countries.

Table (3.1): Chemicals used during this study.

Chemical name	Supplying company and origin
Zinksulfat	Switzerland
Ethanol CH ₃ CH ₂ OH	Merck – Germany
Elisa kite of Testosterone, Dopamine ,TNF- α ,IL-2	Elabscience Japan

Table (3.2): Instruments and tools that used during this study.

Instruments	Supplying company and origin
Autoclave	Haramaya/ Japan
Centrifuge, Cooling centrifuge	Hettich – Germany
Digital Camera	Sony/Japan
Distillater	GFL – Germany
Electronic Sensitive Balance	Denevr INSTRUMENT /Germany
Elisa reader & washer	Biotek – USA
Incubator	Memmert – Germany
Refrigerator	Kiriazi –Egypt
Water path and shaker	GFL/Germany

3.2.Design of study

The study design of current study is illustrated in figure (3.1).

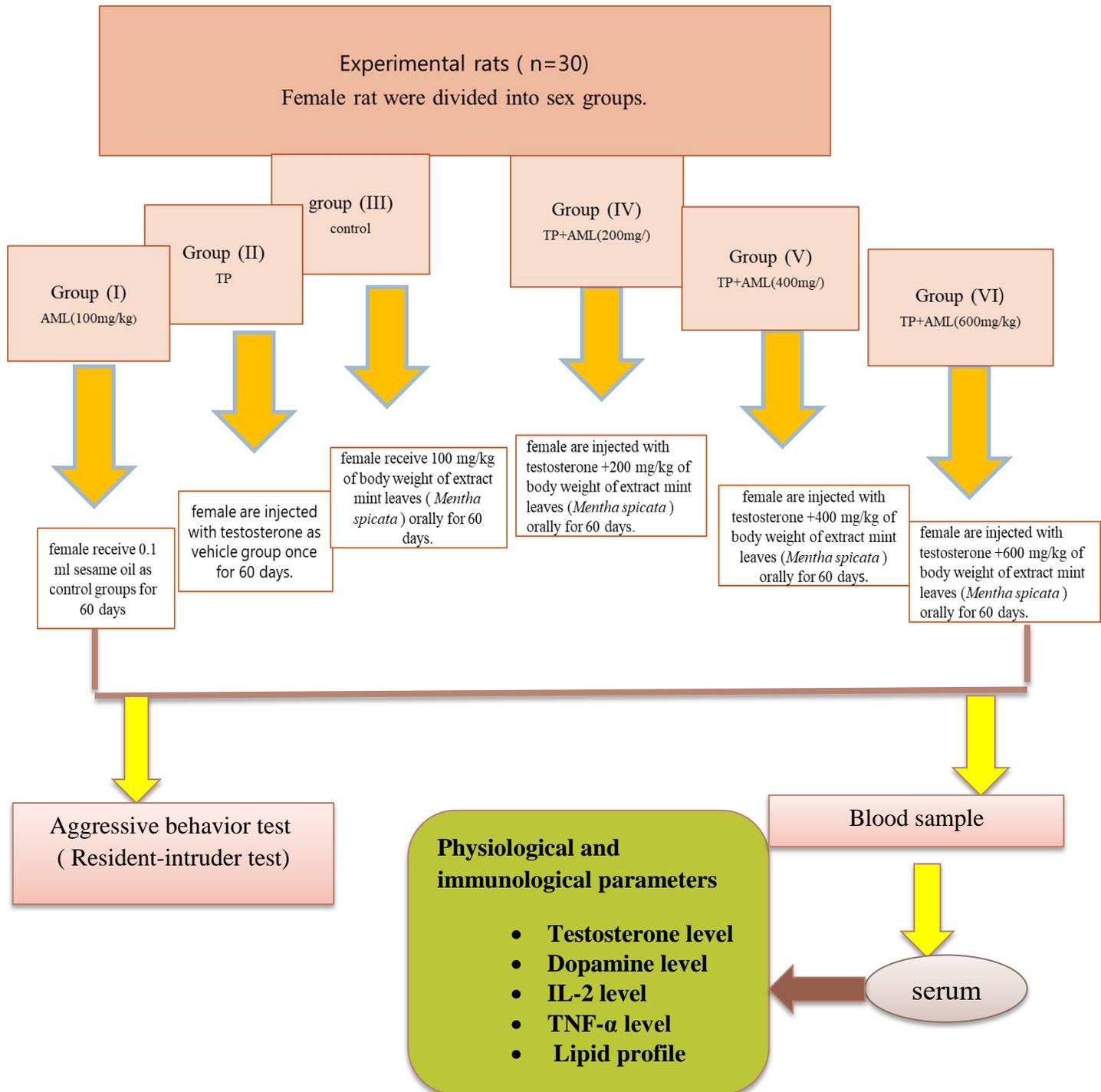


Figure (3.1): Experimental design of the study

3.3: Preparation of the alcoholic extract of *Mentha spicata*

The alcoholic extract of *Mentha spicata* was prepared by washing the mint leaves well, drying them at room temperature, after that they were milled well until get a like powder, and dissolved it in ethanol at a concentration =70%, then it was placed in the shaker for an hour, after that it was incubated in the water bath at a temperature 35°C for two hours. It is filtered by pieces of gauze and placed in a tray in the electric oven at a temperature of 45°C degrees in the electric oven until it hardens, then the pieces were milled (González-Montelongo, *et al.*, 2010)

3.4: Preparation of the testosterone propionate

Testosterone was purchased from the country of China through the FedEx company, and it was prepared as follows. Where take (6 mg/kg) of testosterone propionate powder, and the quantity is measured using an electronic scale. Dissolved its in 0.5 ml of sesame oil (Isayama *et al.*, 2017).

3.5: Experimental animals

In this study using thirty *Albino Westar* female rats in a different age from aged between five to thirteen weeks (weight range 250 -300 g)kept in house wood cages (70 cm x 60 cm x 45 cm) with wood chip bedding *ad libitum*. All experiments worked in animals house of the College of Science / University of Babylon from (16/11/2022-15/2/2023). Experimental animals are habituated to the colony rooms for two weeks before any experimental testing began for adaption.

3.6: Experimental design

Female rats are divided into six groups.

Group (I) : Negative Control group :female rats received 0.1 ml from sesame oil as control groups for 60 days.

Group (II): Positive control group: female rats were injected in subcutaneously with testosterone propionate TP(6 mg/kg) It is dissolved in 0.5 ml of sesame oil as vehicle group once for 60 days.

Group (III): female rats received 100 mg/kg of body weight of extract mint leaves (*Mentha Spicata*)AML as affective dose according to (Daneshbakhsh et al. 2018) orally for 60 days.

Group (IV) : female rats were injected in subcutaneously with testosterone propionate TP (6mg/kg)+ alcoholic extract of mint leaves (*Mentha Spicata*)AML (200 mg/kg) of body weight orally for 60 days.

Group (V) : female rats were injected in subcutaneously with testosterone propionate TP(6mg/kg)+ alcoholic extract of mint leaves (*Mentha Spicata*)AML (400 mg/kg) of body weight orally for 60 days.

Group (VI) : female rats were injected in subcutaneously with testosterone propionate TP(6mg/kg)+ alcoholic extract of mint leaves (*Mentha Spicata*)AML (600 mg/kg) of body weight orally for 60 days. Rat's body weight was estimated each 10 days during the experiment from 0, 10, 20, 30,40 50, and 60 days.

3.7: Blood collection

Animals in all groups are killed between 9:00 and 10:00 am. after 24 hours from the last treated dose and after the end of behavioral tests of all experiments, animals are anaesthetized by chloroform. Blood samples were collected from the rats by cardiac puncture and putting it in a gel tube and it was placed in a centrifuge at 3.000 rpm for 10 minutes to separate the serum. Serum samples were transferred into separate tubes and stored at -20° C until analysis following parameters (Hamza and El-Shenawy, 2017).

3.8: Study parameters

3.8.1 Biochemical assay

Serum testosterone, dopamine, TNF- α and IL-2 measured by using special kits of the enzyme linked immunosorbent assay (ELISA) supplied by Elabscience- USA company, according to the procedure of kits:

1. **Added sample:** 100 μ l of standard, blank, or sample per well were added. The blank well is added with reference stand and sample diluent. solutions are added to the bottom of micro plate well, avoid touching inside wall and foaming as possible. Mixed it gently and covered by the plate with sealer then Incubated for 90 minutes at 37° C.
2. **Biotinylated detection Ab:** The liquid of each well is removed, do not wash. Immediately added (100 μ l) of biotinylated detection Ab working solution to each well and Covered by the Plate with sealer. Gently tap the plate to ensure thorough mixing and Incubated for one hour at 37 °C.
3. **Wash:** Each well and wash were aspirated, and repeated the process three times. Washing by filling each well with wash buffer (approximately 350 μ l)

(a squirt bottle , multi-channel pipette, manifold dispenser or automated washer are needed). Complete removal of liquid at each step is essential, after the last wash, remove remained buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.

4. **HRP Conjugate:** 100 μ l of horseradish peroxidase HRP conjugate working solution was added to each well and Covered by the plate with sealer. Incubated for 30 minutes at 37 °C.
5. **Wash:** the wash processes were repeated for five times.
6. **Substrate:** 90 μ l substrate solution is added to each well. Covered by a new plate with sealer. Incubated for about 15 minutes at 37 °C, and protected the plate from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. When gradient appeared well, the user should terminate the reaction.
7. **Stop:** 50 μ l of stop solution was added to each well. Then, the color turns to yellow immediately. The order to add stop solution should be the same as the substrate solution.
8. **Optical density measurement:** The optical density (OD value) of each well is determined at once, using a micro-plate reader set (Spectrophotometrically at a wavelength of 450 nm., user should open the micro-plate reader in advance, preheat the instrument , and set the testing parameters, tables (3-8, 3-9 and 3-10).

3.8.2 Physiological parameters

As the OD values of the standard curve may vary according to the condition of the actual assay perform(e.g operator ,pipetting technique,

washing technique or temperature effects), the operator should establish a standard curve for each test. typical standard curve and data was provided below for reference only.

3.8.2.1 Concentration values of the standard curve of dopamine (DA).

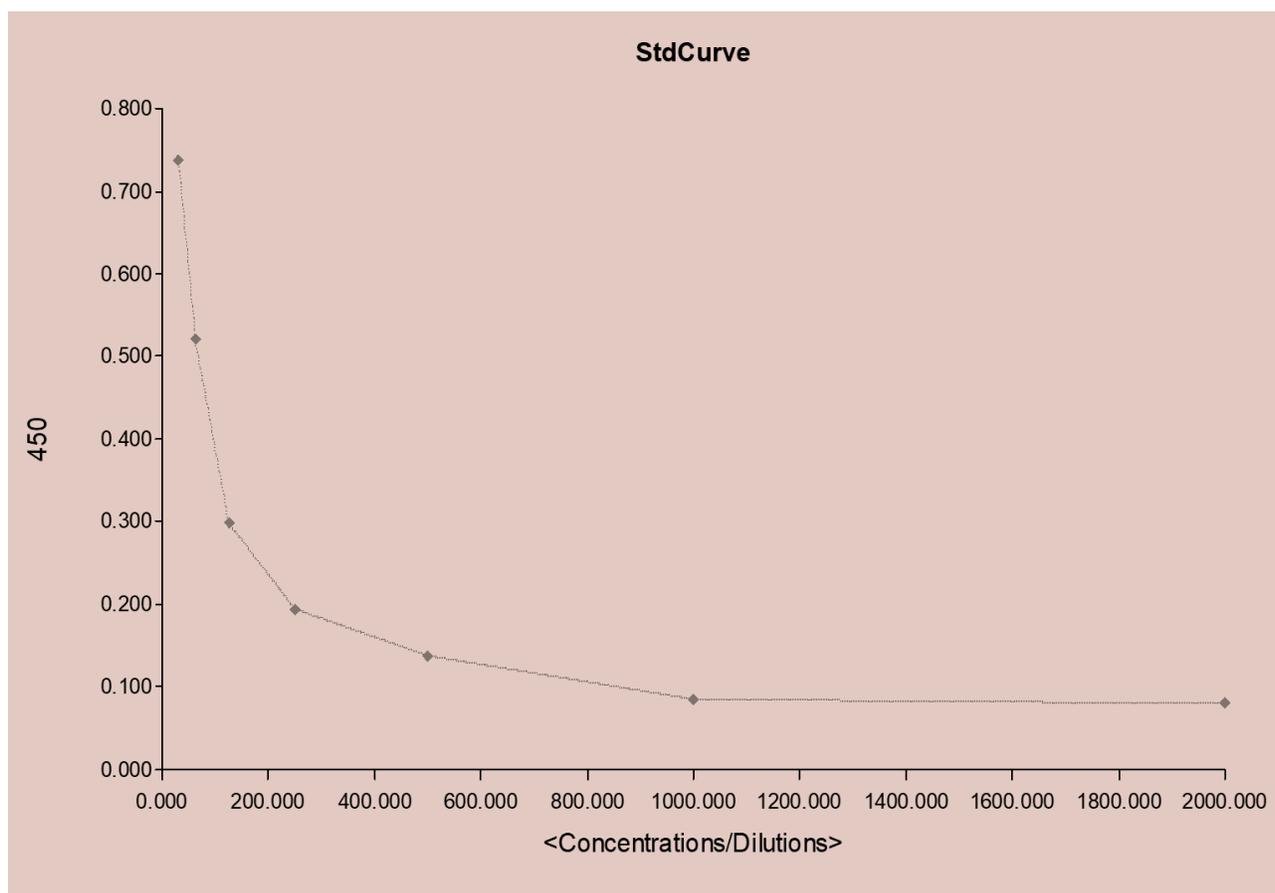


Figure (3.2): Standard curve for estimate the level of dopamine

3.8.2.2: Concentration values of the standard curve of testosterone (TP).

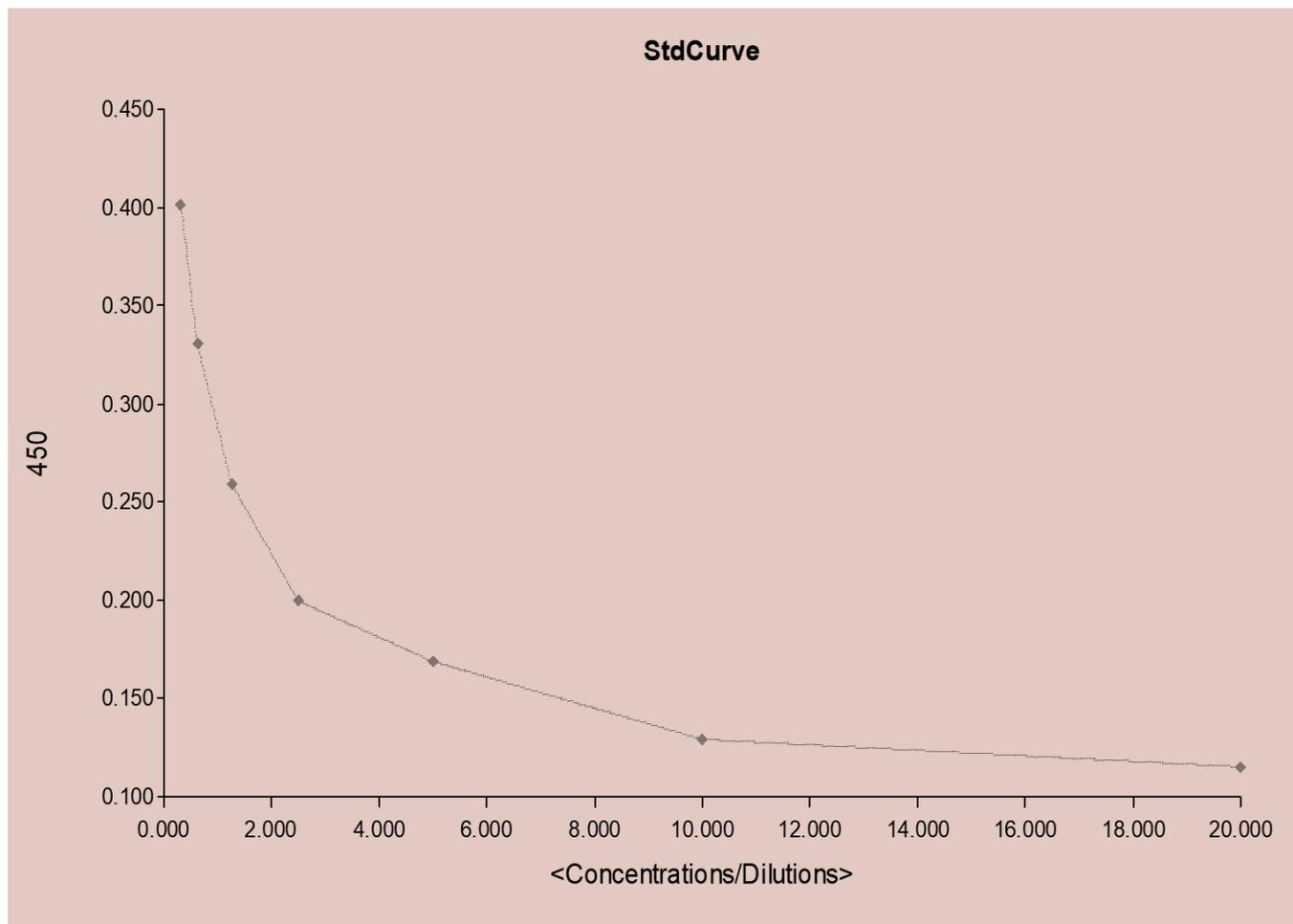


Figure (3.3) : Standard curve for estimate the level of testosterone

3.8.3: Immunological parameters

As the OD values of the standard curve may vary according to the condition of the actual assay perform (e.g operator ,pipetting technique, washing technique or temperature effects) , the operator should establish a standard curve for each test . typical standard curve and data is provided below for reference only.

3.8.3.1: Concentration values of the standard curve of IL-2.

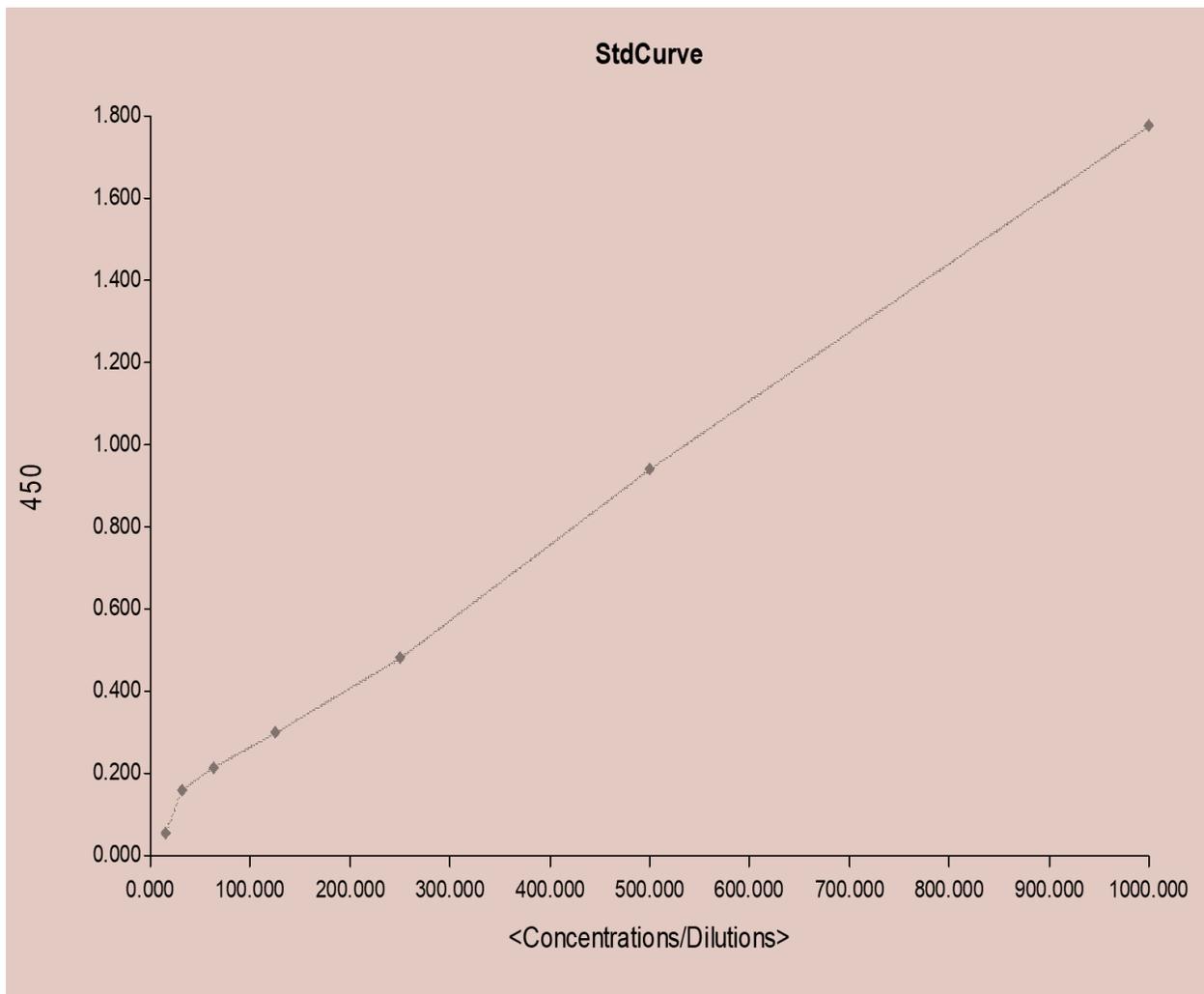


Figure (3.4): standard curve for estimate the level of IL-2

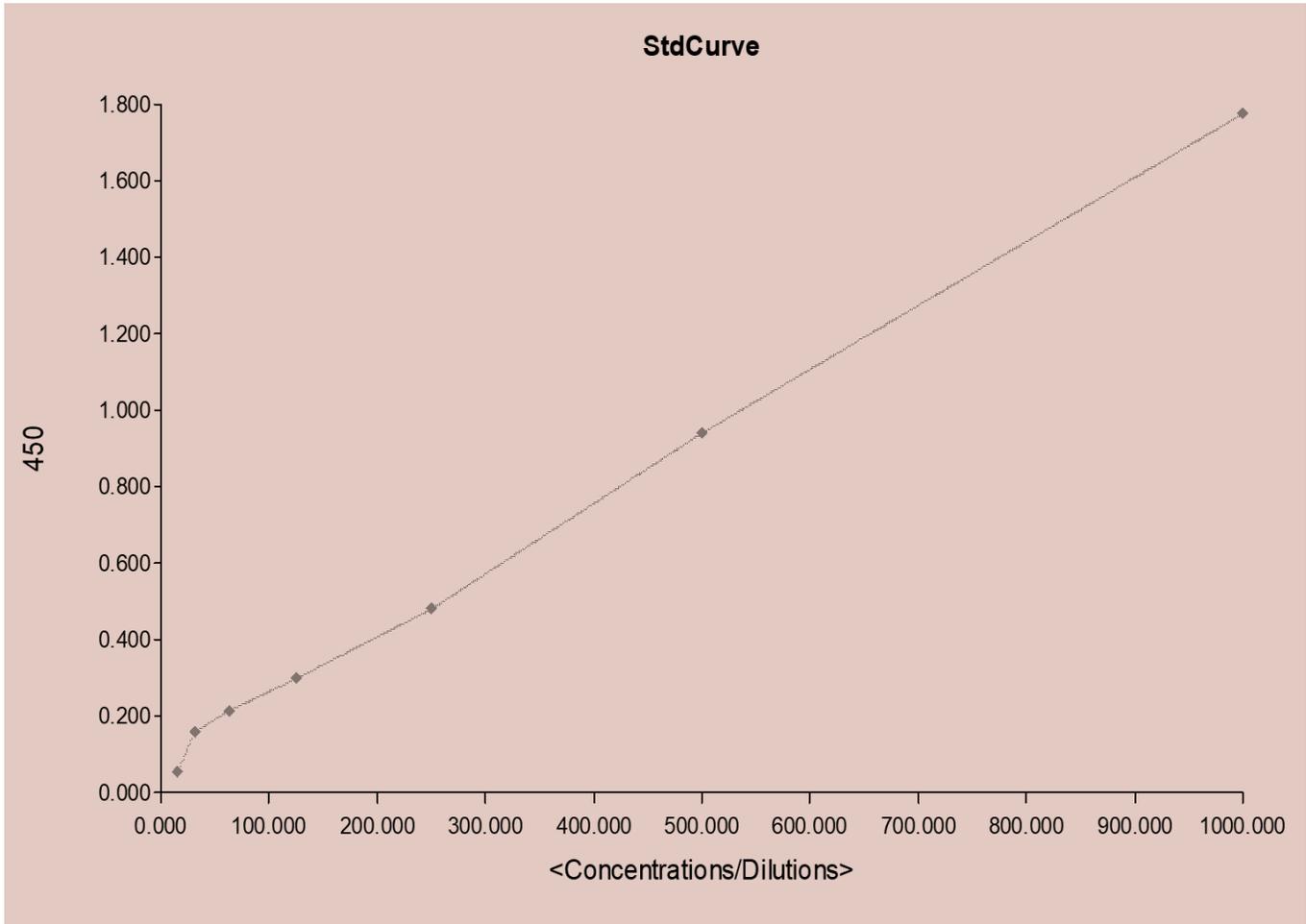
3.8.3.2: Concentration values of the standard curve of TNF.

Figure (3.5): Standard curve for estimate the level of TNF- α

3.9: Lipid profile assays .

Measurements are made of the serum's (TC) total cholesterol, (TG) triglycerides, HDL high density lipoprotein, (LDL) low density lipoprotein, and (VLDL) very low density lipoprotein. by using manual method according to the following source (Tietz, 1995).

3.10.4: behavioral study

3.10.4.1: Resident-intruder test

In this model, one of the rats (intruders) were allowed to establish a territory (the resident) in its home cage, another animal was placed into the residents' home cage and two animals were allowed to interact with each other for a fixed period of time. Determine in the resident rat duration and frequency of the following behavioral parameters: Attack latency: (the time between the introduction of the intruder and the first clinch attack) (Hobson, 2020). from the top of the cage (figure 3.7). Before initial this test, were put drops from 40% zinc sulphate in the nose of intruder male prior to encounters (Montag and Rathjen, 2023).



Figure(3.6):Resident- intruder test and isolated box

3.11: Statistical Analysis:

All statistical analysis for data and body weigh represent a mean \pm S.E. and parameters of behavior tests results represent a mean \pm S.D. Equal

variance between the groups was first checked using the Levene's test for homogeneity of variances and statistical analysis of the difference between the mean values was carried out using one-way analysis of variance (ANOVA) followed by the Duncan test for Post Hoc multiple comparisons. IBM SPSS statistical software version (23) for Windows. A value of $p < 0.05$ was considered statistically significant for all tests. Analysis correlation between parameters of groups done by Pearson test (O'Dea et al., 2021).

Chapter Four

Results

4.1.1: Body weight change

4.1.1.1: Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the body weight of female rats treated with testosterone propionate for 60 days.

The present results in table (4.1) showed a significant increase ($P < 0.05$) in levels of body weight in rats treated by testosterone propionate (TP), alcoholic extract of mint leaves (AML), and their combinations (TP + AML) for 60 days. Rats treated with testosterone propionate (6 mg/kg) in group II showed a significant increase ($p < 0.05$) of body weight compared to the control group and the other groups, also the results showed a significantly decreased ($P < 0.05$) in level of body weight in the groups IV, V and VI treated with testosterone propionate (6 mg/kg) and alcoholic extract of mint leaf (200, 400 and 600 mg/kg) compared to the group II.

Table (4.1):Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the body weight of female rats treated with testosterone propionate for 60 days. (n =5).

Body weight(day) Groups	0	10	20	30	40	50	60
Group I (control)	227.8±22.1	225.2±23.2	222.6±22.5	246.8±4.1	247.8±4.1	247.8±5.1	248.2±6.5
Group II (TP)	218.2±10.3	216.6±10.6	212.0±11.5	237.6±16.8 ^b	238.6±16.9 ^b	246.6±6.8 ^b	249.2±5.8 ^b
Group III (AML 100mg/kg)	238.4±9.5 ^b	236.6±9.8 ^b	233.8±9.7 ^b	218.4±17.0 ^a	220.4±18.5 ^a	222.8±15.7 ^b	226.4±16.4 ^b
Group IV (TP+AML200mg/kg)	241.6±16.0 ^b	240.2±15.5 ^b	237.0±15.2 ^b	242.6±3.4 ^b	243.6±3.3 ^b	243.8±4.6 ^b	241.8±6.9 ^b
Group V (TP+AML400mg/kg)	228.4±19.5 ^b	227.0±19.8 ^b	220.2±17.6	216.6±9.4 ^a	217.6±9.5 ^a	214.0±13.3 ^b	214.6±12.0 ^b
Group VI (TP+AML600mg/kg)	193.6±5.3 ^a	192.0±5.2 ^a	190.8±5.6 ^a	135.2±5.4 ^{ab}	136.0±6.1 ^a	135.4±8.6 ^a	135.8±4.7 ^a

TP : testosterone propionate. AML: alcohol extract of mint leaves. TP + AML: testosterone propionate + alcohol extract of mint leaves.

a: The mean is significantly different at the $P \leq 0.05$ as compared to group I (controls).

b: The mean is significantly different at the $P \leq 0.05$ as compared to group II.

4.1.2: Physiological study.

4.1.2.1: Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the testosterone and dopamine levels in serum of female rats

Table (4.2) Illustrated that the data of testosterone and dopamine levels in rats primed by testosterone propionate (TP), alcoholic extract of mint leaves(AML), and their combinations (TP + AML) for 60 days. Rats treated with testosterone propionate (6 mg/kg) in group II showed a significant increase ($p < 0.05$) in the level of testosterone hormone compared to the control group and the other groups, whereas, rats in the groups IV, V and VI treated with testosterone propionate (6 mg/kg) and alcoholic extract

of mint leave (200, 400 and 600 mg/kg) respectively for 60 days are showed a significant decrease ($P < 0.05$) in the level of testosterone hormone when compared to the group II. No significantly ($p > 0.05$) difference is noted between control group I and groups IV, V, VI of rats treated by TP + AML.

The changes in dopamine level of rats treated with TP, AML and their combinations (TP + AML) are shown in table (4.2). TP alone caused, a significant increase ($p < 0.05$) in the level of dopamine in group II of rats that are treated with testosterone propionate (6 mg/kg) compared to the control group and the other groups. Whereas, the rats in the groups IV, V and VI treated with TP + AML (200, 400, 600 mg/ kg) exhibited a significant ($p < 0.05$) decrease compared to group II. When, the rats in the group III affective dose (100mg/kg) treated with AML are showed significant increased ($P < 0,05$) in the level of dopamine compared to the groups V and VII treated with TP + AML(400,600mg/kg). Animals in the group IV showed a significant increased $P < 0,05$ in the level of dopamine compared to the groups V and VI treated with TP + AML(400,600mg/kg).

Table (4.2): Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the testosterone and dopamine levels in the serum of female rats treated with testosterone propionate for 60 days. (n =5).

Parameters Groups	Testosterone(ng/ml)	Dopamine (pg/ml)
	Mean \pm S.E	Mean \pm S.E
Group I (control)	4.74 \pm 0.58	125.73 \pm 13.70
Group II (TP)	16.52 \pm 1.20 ^a	607.59 \pm 62.88 ^a
Group III (AML 100mg/kg)	4.82 \pm 0.42 ^b	272.83 \pm 24.28 ^{ab}
Group IV (TP+ AML200mg/kg)	7.40 \pm 0.65 ^{ab}	263.49 \pm 20.49 ^{ab}
Group V (TP+ AML400mg/kg)	6.02 \pm 1.40 ^b	135.43 \pm 13.92 ^{bcd}
Group VI (TP+ ML600mg/kg)	6.81 \pm 1.161 ^b	131.334 \pm 2.786 ^{bcd}

TP : testosterone propionate. AML: alcohol extract of mint leaves. TP + AML: testosterone propionate + alcohol extract of mint leaves.

a: The mean is significantly different at the $P \leq 0.05$ as compared to group I (controls).

b: The mean is significantly different at the $P \leq 0.05$ as compared to group II.

c: The mean is significantly different at the $P \leq 0.05$ as compared to group III.

d: The mean is significantly different at the $P \leq 0.05$ as compared to group IV.

4.1.2.2: Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the levels of TNF- α and IL-2 in serum female rats.

The present results in table (4.3) showed a significant increase ($P < 0.05$) in levels of TNF- α and IL-2 in rats treated by testosterone propionate (TP), alcoholic extract of mint leaves (AML), and their combinations (TP + AML) for 60 days. Rats treated with testosterone propionate (6 mg/kg) in group II showed a significant increase ($p < 0.05$) in the level of TNF- α compared to the control group and the other groups, also the results showed a significantly decreased ($P < 0.05$) in level of TNF- α in the groups IV, V and VI treated with testosterone propionate (6 mg/kg) and

alcoholic extract of mint leave (200, 400 and 600) compared to the group II. No significantly ($p>0.05$) difference is noted between control group I and III, IV, and VI groups of rats treated by TP + AML.

The alterations in IL-2 level of rats treated with TP, AML are shown in table (4.3). TP alone caused, a significant increase ($p<0.05$) in the level of IL-2 in the II group of rats that treated with testosterone propionate (6 mg/kg) compared to the control group and other groups (III, IV and V). Whereas, the rats in the groups IV, V and VI treated with TP + AML (200, 400, 600 mg/ kg) showed a significantly decreased ($p<0.05$) compared to group II. No significantly ($p>0.05$) difference is noted between control group I and III, IV and V, groups of rats treated by TP + AML.

Table (4.3) : Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the TNF- α and IL-2 levels in the serum of female rats that treated with testosterone propionate for 60 days (n =5).

Parameters Groups	TNF- α (pg /ml) Mean \pm S.E	IL-2 (pg/ml) Mean \pm S.E
Group I (control)	29.58 \pm 1.07	20.76 \pm 2.97
Group II (TP)	56.19 \pm 6.36 ^a	32.75 \pm 4.36 ^a
Group III(AM L 100mg/kg)	26.26 \pm 2.63 ^b	18.72 \pm 3.31 ^b
Group IV(TP+ AML200mg/kg)	26.54 \pm 2.39 ^b	17.17 \pm 2.59 ^b
Group V (TP+AML400mg/kg)	17.96 \pm 3.09 ^{ab}	18.96 \pm 4.34 ^b
Group VI (TP+ ML600mg/kg)	22.22 \pm 3.55 ^b	26.99 \pm 4.11

TP : testosterone propionate . AML: alcohol extract of mint leaves. TP + AML: testosterone propionate + alcohol extract of mint leaves.

a : The mean is significantly different at the $P \leq 0.05$ as compared to group I (controls). **b**: The mean is significantly different at the $P \leq 0.05$ as compared to group II.

4.1.3: Lipid profile

4.1.3.1: Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the serum lipid profile.

The effect of alcoholic extract of mint leaves (*Mentha Spicata*) on the lipid profile of the female rat are shown in table (4.4) . Injection of testosterone propionate (6 mg/kg) into rats in the II group for 60 days led to increased ($P < 0.05$) in plasma cholesterol and VLDL compared to control and other groups. Whereas, rats in groups III affective dose (100mg/kg) treated with AML, IV and V treated with (TP+AML 200,400 mg/kg) the

levels of cholesterol and VLDL decreases ($P < 0.05$) compared to groups II, VII. Total cholesterol and VLDL decreased ($P < 0.05$) in the VI group treated with (TP+AML 600 mg/kg) compared to others groups. No significantly ($p > 0.05$) difference was noted between control group I and III, V groups.

Changes in the triglycerides of rats treated with TP, AML were shown in table (4.4). TP alone caused, a significant increase ($p < 0.05$) in the level of triglycerides in the II group of rats that are treated with testosterone propionate (6 mg/kg) compared to the control group and other groups .As well as III group affective dose (100mg/kg) treated with AML the level of triglycerides decreased ($p < 0.05$) compared to the VI group. No significantly ($p > 0.05$) difference was noted between control group I and IV, V, VI groups of rats treated by TP + AML.

The results show that there were statistically significant differences in HDL of rats treated with TP, AML. TP caused, a significant decreased ($p < 0.05$) in the level of HDL in the II group of rats that are treated with testosterone propionate (6 mg/kg) compared to the other groups. However, rats in group III affective dose (100mg/kg) treated with AML the level of HDL increase ($P < 0.05$) compared to other groups. No significantly ($p > 0.05$) difference was noted between control group I and II,IV, V and VI groups of rats treated by TP + AML.

The results of current study revealed that TP alone caused, a significant increased ($p < 0.05$) in the level of LDL in the II group of rats that are treated with testosterone propionate (6 mg/kg) compared to the control

and other groups. LDL decreased ($P < 0.05$) in III group affective dose (100mg/kg) treated with AML and IV, V and VI treated with (TP+AML 200,400,600 mg/kg) compared to the group II. No significantly ($p > 0.05$) difference is noted between control group I and III IV, V and VI groups of rats treated by TP + AML.

Table (4.4) : Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the serum lipid profile of the rats treated with testosterone propionate for 60 days. (n =5).

Parameters Groups	TC (mg/dl)	TG (mg/dl)	HDL(mg/dl)	VLDL(mg/dl)	LDL(mg/dl)
G I (control)	101.21± 1.23	57.43±4.45	29.15± 2.64	13.94± 2.20	48.72± 2.39
G II TP(6mg/kg)	153.22± 1.51 ^a	116.65±12.68 ^a	19.36± 2.58	23.33± 2.54 ^a	83.41± 4.94 ^a
G III (AML 100mg/kg)	111.99±11.41 ^b	77.41±6.11 ^{ab}	57.90± 6.82 ^{ab}	16.46± 1.84 ^b	58.64± 5.52 ^b
G IV (TP+AML200m g/kg)	124.89 ±5.41 ^{ab}	67.72±4.11 ^b	35.21± 3.73 ^{bc}	15.51± 1.51 ^b	58.48± 5.79 ^b
G V (TP+ AML 400mg/kg)	115.42 ±2.47 ^b	71.02±5.41 ^b	35.32± 4.23 ^{bc}	14.09± 1.09 ^b	65.64± 2.78 ^{ab}
G VI (TP+ AML 600mg/kg)	69.32± 4.11 ^{abc}	51.38±7.46 ^{bcd}	34.45± 3.69 ^{bc}	8.60± 1.59 ^{bc}	53.98± 5.39 ^b

TP : testosterone propionate . AML: alcohol extract of mint leaves. TP + AML: testosterone propionate + alcohol extract of mint leaves.

a : The mean is significantly different at the $P \leq 0.05$ as compared to group I (controls).

b: The mean is significantly different at the $P \leq 0.05$ as compared to group II.

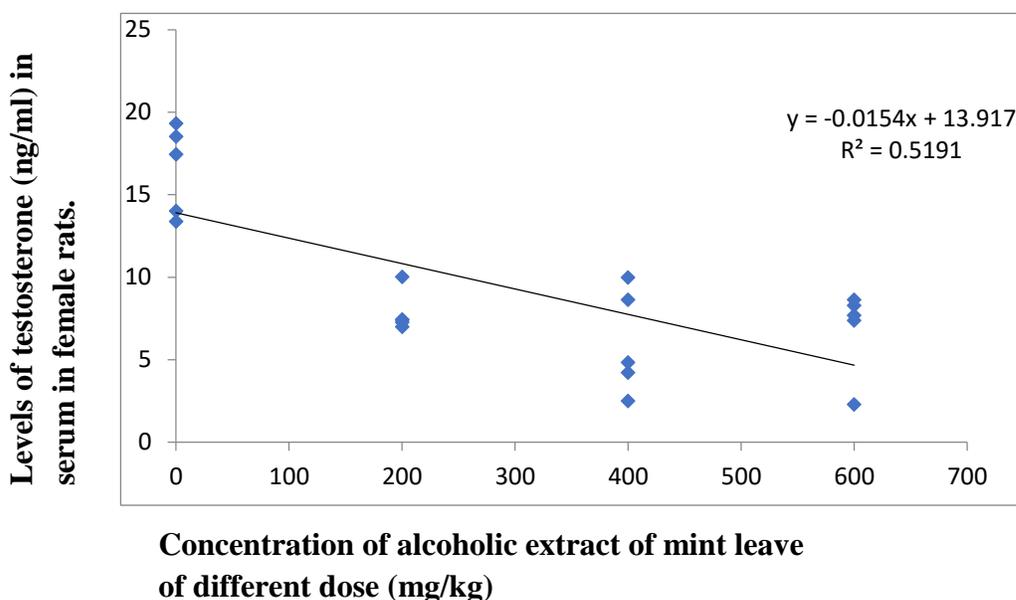
C : The mean is significantly different at the $P \leq 0.05$ as compared to group III.

d : The mean is significantly different at the $P \leq 0.05$ as compared to group IV.

4.1.4: Correlation between different doses of the alcoholic extract of mint leaves (*Mentha spicata*) and physiological parameters.

4.1.4.1 :Correlation between different doses of the alcoholic extract of mint leaves (*Mentha spicata*) and testosterone propionate in female rats treated with testosterone propionate .

The present study (Figure , 4.1) showed that there was a significant negative linear relationship between the level of the testosterone in the serum of female rats and the concentration of the alcoholic extract of mint leaves (*Mentha spicata*) (200,400 and 600 mg/kg).The equation is linear between them $y = -0.0154x + 13.917$ and correlation coefficient $r = -0.720$



Figure(4.1) : The relationship between testosterone levels and alcoholic extract of mint leaves (200,400,600 mg/kg).

4.1.4.2 Correlation between different doses of the alcoholic extract of mint leaves (*Mentha spicata*) and dopamine level in the serum of female rats suffer from higher testosterone after treated with testosterone propionate .

The current study showed that there was a significant negative linear relationship between the level of dopamine in the female rat and the concentration of the alcoholic extract of mint leaves (*Mentha spicata*) (200,400 and 600 mg/kg). The equation is linear between them $y = -0.7784x + 517.99$. and correlation coefficient $r = -0.847$. As shown in figure (4.2).

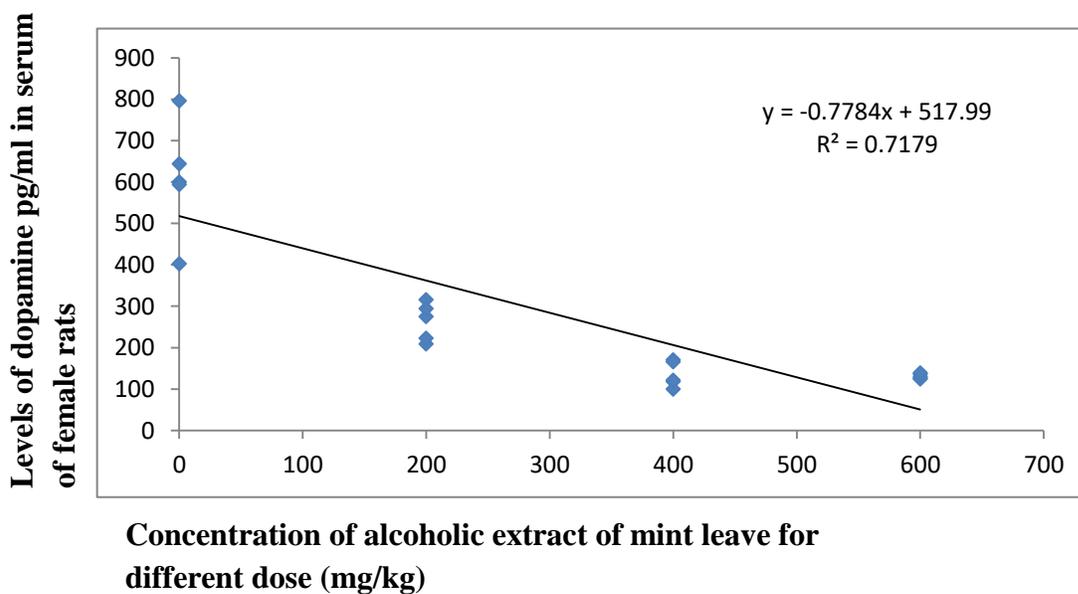


Figure (4.2) : The relationship between dopamine concentrations and alcoholic extract of mint leaves (200,400,600 mg/kg).

4.1.4.3 Correlation between the testosterone and dopamine levels in the female rats that suffer from higher testosterone after treated with testosterone propionate .

The results showed that there was a significant positive linear relationship between the levels of testosterone and dopamine hormones in the female rats. The equation is linear between them $y = 34.82x - 39.217$ and correlation coefficient $r = 0.810$. As noted in figure (4.3).

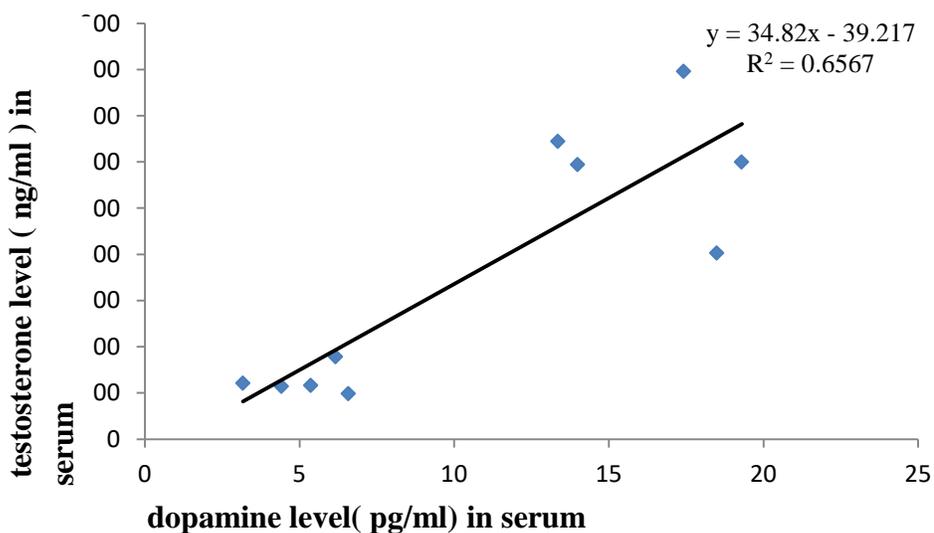


Figure (4.3) : The relationship between testosterone and dopamine levels.

4.1.4.4 Correlation between different doses of the alcoholic extract of mint leaves (*Mentha spicata*) and TNF- α level in the female rats suffer from higher testosterone after treated with testosterone propionate .

There was a significant negative linear relationship between the level of the TNF- α in the serum of female rats and the concentration of the alcoholic extract of mint leaves (*Mentha spicata*) (200,400 and 600 mg/kg) , as shown in figure (4.4). The equation is linear between them $y = -0.0153x + 28.67$. and correlation coefficient $r = -0.553$.

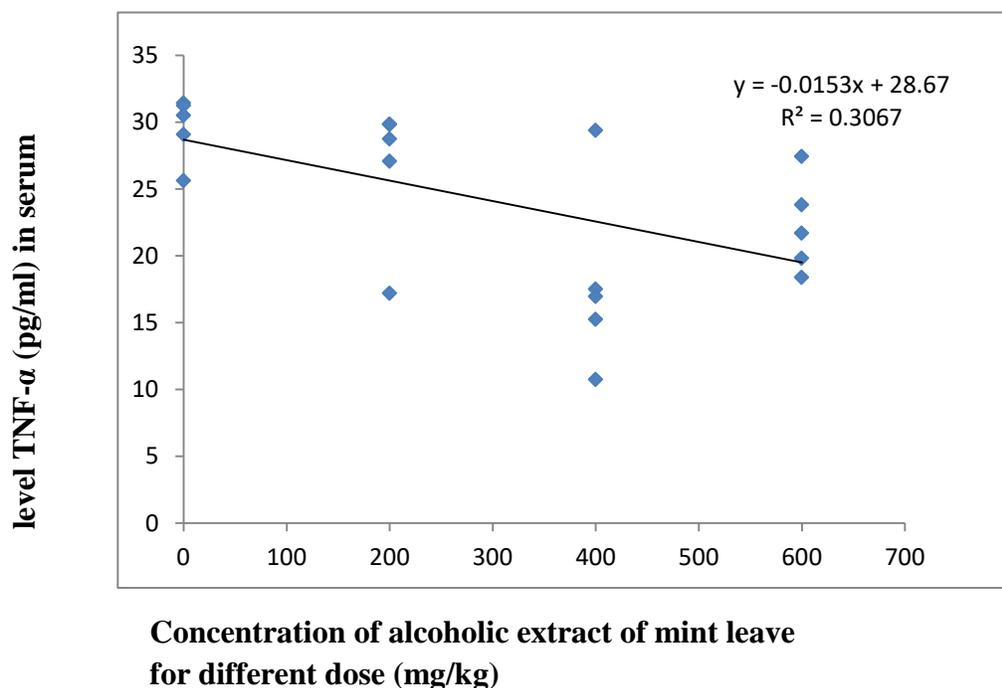


Figure (4.4) : The relationship between TNF- α concentrations and alcoholic extract of mint leaves (200,400,600 mg/kg).

4.1.4.5 Correlation between different doses of the alcoholic extract of mint leaves (*Mentha spicata*) and Trig level in the female rats suffer from higher testosterone after treated with testosterone propionate .

There was a significant negative linear relationship between the level of the Trig in the serum of female rats and the concentration of the alcoholic extract of mint leaves (*Mentha spicata*) (200,400 and 600 mg/kg) , as shown in figure (4.5). The equation is linear between them $y = -0.1033x + 105.76$. and correlation coefficient $r = -0.797$.

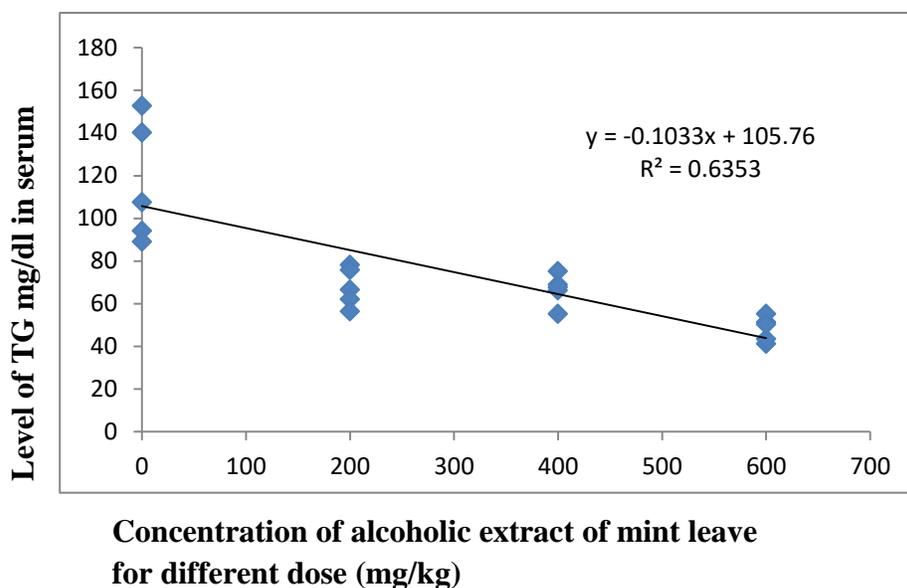


Figure (4.5) : The relationship between TG concentrations and alcoholic extract of mint leaves (200,400,600 mg/kg).

4.1.4.6 Correlation between different doses of the alcoholic extract of mint leaves (*Mentha spicata*) and cholesterol level in the female rats suffer from higher testosterone after treated with testosterone propionate .

The data revealed that a significant negative linear relationship between the level of the cholesterol in the serum of female rats and the concentration of the alcoholic extract of mint leaves (*Mentha spicata*) (200,400 and 600 mg/kg) , as shown in figure (4.6). The equation is linear between them $y = -0.1306x + 154.89$ and correlation coefficient $r = 0.939$

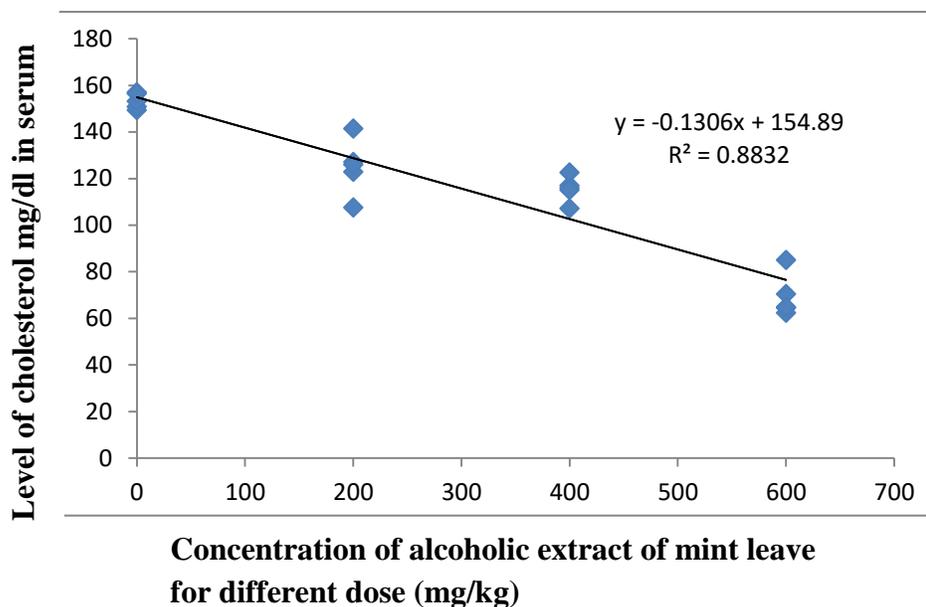


Figure (4.6) : The relationship between cholesterol concentrations and alcoholic extract of mint leaves (200,400,600 mg/kg).

4.1.4.7 Correlation between different doses of the alcoholic extract of mint leaves (*Mentha spicata*) and HDL level in the female rats suffer from higher testosterone after treated with testosterone propionate .

The data (Figure, 4.7) showed that there is a significant positive linear relationship between the level of the HDL in the serum of female rats and the concentration of the alcoholic extract of mint leaves (*Mentha spicata*) (200,400 and 600 mg/kg) , as shown in figure (4.10). The equation is linear between them $y = 0.0227x + 24.278$ and correlation coefficient $r = 0.512$.

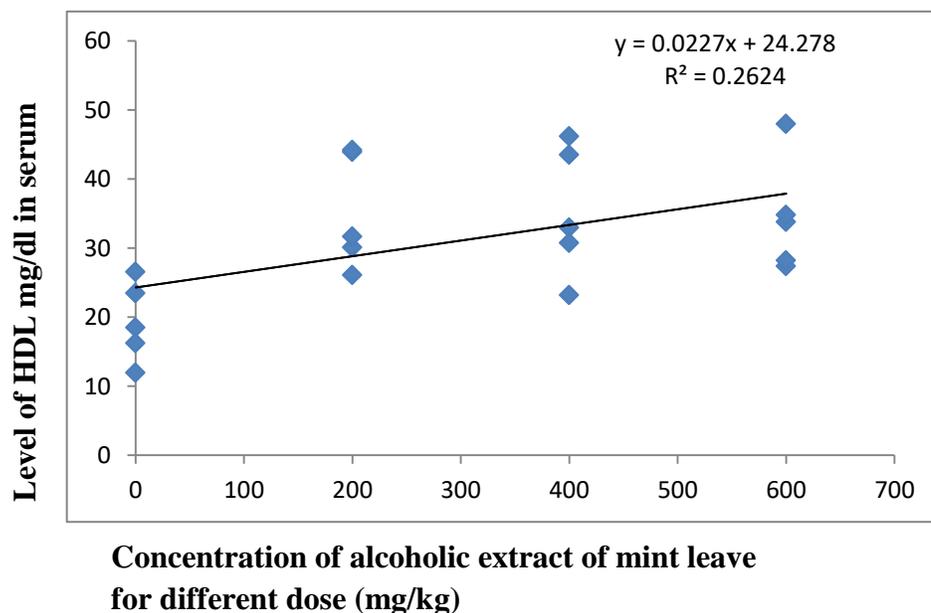


Figure (4.7) : The relationship between HDL concentrations and alcoholic extract of mint leaves (200,400,600 mg/kg).

4.1.4.8 Correlation between different doses of the alcoholic extract of mint leaves (*Mentha spicata*) and LDL level in the female rats suffer from higher testosterone after treated with testosterone propionate .

There is a significant negative linear relationship between the level of the LDL in the serum of female rats and the concentration of the alcoholic extract of mint leaves (*Mentha spicata*) (200,400 and 600 mg/kg) , as shown in figure (4.8). The equation is linear between them $y = -0.0648x + 109.17$. and correlation coefficient $r = 0.790$.

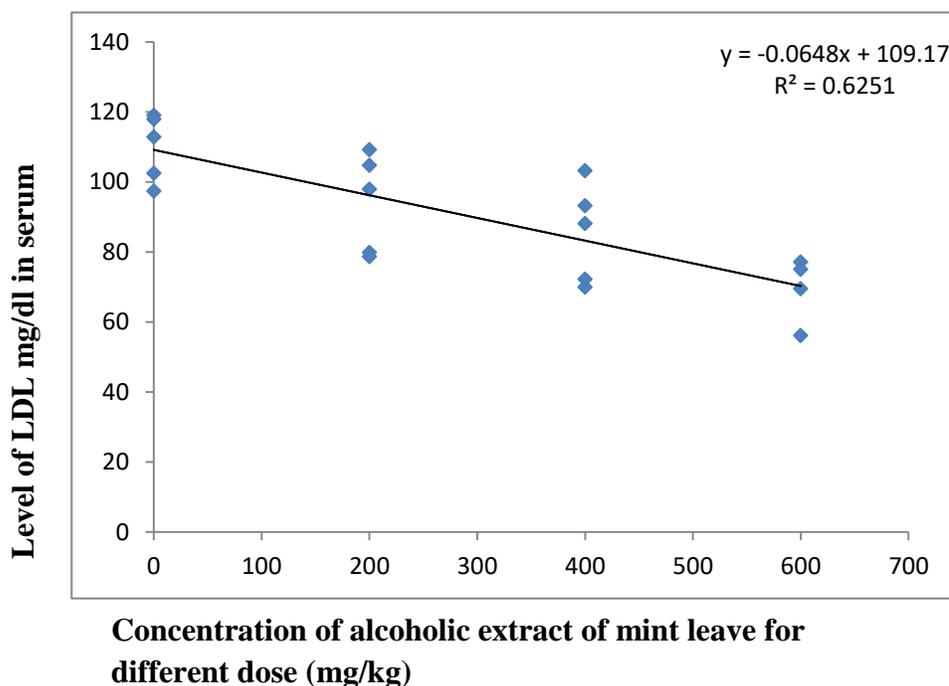


Figure (4.8) : The relationship between LDL concentrations and alcoholic extract of mint leaves (200,400,600 mg/kg).

4.1.5 Aggressive behavior study:

1. Visible signs

Each animal in the different groups are carefully examined on daily basis before and after experimental exposure for possible clinical signs because of testosterone injections and these signs:

- a. Hair loss in testosterone-injected animals compared to the control group and animals injected with testosterone propionate +alcoholic extract of mint leaves TP+ ALMP (600 mg/kg) show in figure (4.9).
- b. Little hair loss in the group of animals that are given testosterone propionate +alcoholic extract of mint leaves TP+ ALMP (600 mg/kg) show in figure (4.9).
- c. appearance of blood pimples in group II injected with testosterone propionate show in figure (4.9).



Figure (4.9) : visible signs noted in rats groups A- hair loss of rat in TP group, B- Slight loss of hair rats in TP+ALMP groups, C- appearance of blood

d. Aggressive behavior



Figure (4.10) :Aggressive behavior in rats injected with testosterone propionate.

4.1.6: Behavior study

4.1.6.1:Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the aggressive behavior of the rats.

The effect of alcoholic extract of mint leaves (*Mentha spicata*) on the aggressive behavior of the female rat were shown in table (4.5) . Injection of testosterone propionate (6 mg/kg) into rats in the II group for 60 days led to a significant decreased ($P<0.05$) in all mean of Latency to the first threat and thrust (sec.). When showed significant increased ($P<0.05$) in all mean of Latency to the first threat and thrust (sec.) in the group III treated with alcoholic extract of mint leave (100 mg/kg).

The result showed significant increased ($P<0.05$) in all mean the number of threat and thrust in the group II treated with testosterone propionate (6 mg/kg) compared to the group III as well as the rats in the group III treated with alcoholic extract of mint leave (100 mg/kg) showed significant decreased ($P<0.05$) in all mean the number of threat and thrust compared to

the group II. Whereas rats in the group I(control) have significant decreased ($P<0.05$) in the mean the number of threat and thrust compared to the V group. No significantly ($p>0.05$) difference was noted between control group I and III,IV, V groups of rats treated by TP + AML.

Changes in the Latency to the first attack (sec.) of rats treated with TP, AML are shown in table (4.5). TP alone caused, a significant decreased ($p<0.05$) in the Latency to the first attack (sec.) in the group II of rats that are treated with testosterone propionate (6 mg/kg) compared to the control and the group III affective dose (100mg/kg) treated with AML. When the rats in the group III treated with alcoholic extract of mint leave (100 mg/kg) showed significant increased ($p<0.05$) in the Latency to the first attack (sec.) compared to the group II.

The result showed significant increased ($P<0.05$) in all mean the number of attack in the group II treated with testosterone propionate (6 mg/kg) compared to the group III as well as the rats in the group III treated with alcoholic extract of mint leave (100 mg/kg) showed significant decreased ($P<0.05$) in all mean the number of attack compared to the group II. No significantly ($p>0.05$) difference is noted between control group I and III group of rats treated by AML.

The result in the table (4.5) showed significant increased ($P<0.05$) in the number of keep down in the group II treated with testosterone propionate (6 mg/kg) compared to the group III as well as the rats in the group III treated with alcoholic extract of mint leave (100 mg/kg) showed significant decreased ($P<0.05$) in the number of keep down compared to the group II.

No significantly ($p>0.05$) difference is noted between control group I and III, VI groups of rats treated by TP + AML.

Table (4.5) : Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the aggressive behavior of the rats treated with testosterone propionate for 60 days (n =5).

Parameters Groups	Latency to the first threat and thrust (sec.)	Number of threat and thrust	Latency to the first attack (sec.)	Number of attack	Number of keep down
G I (control)	120.82±13.12	11.00±1.87	176.07±8.99	3.40±1.14	2.40±1.14
G II TP(6mg/kg)	15.48±3.68 ^a	45.00±7.97 ^a	31.69±5.59 ^a	17.60±2.07 ^a	22.40±3.65 ^a
G III (AML 100mg/kg)	306.18±16.69 ^{ab}	8.40±1.52 ^b	359.79±60.45 ^{ab}	5.60±1.14 ^{bd}	1.20±0.45 ^b
G IV (TP+ AML 200mg/kg)	213.06±21.83 ^{abc}	14.00±3.39 ^{bc}	256.01±5.63 ^{abc}	9.40±1.82 ^{abc}	12.40±3.05 ^{abc}
G V (TP+ AML 400mg/kg)	238.21±14.76 ^{abcd}	7.00±1.87 ^{bd}	285.11±7.07 ^{abc}	8.00±1.58 ^{abc}	8.20±1.48 ^{abcd}
G VI (TP+ AML 600mg/kg)	278.16±27.20 ^{abcde}	4.20±1.64 ^{abd}	350.98±11.97 ^{abd}	6.60±2.61 ^{abd}	4.40±1.14 ^{bcde}

TP : testosterone propionate. AML: alcohol extract of mint leaves. TP + AML: testosterone propionate + alcohol extract of mint leaves.

a: The mean is significantly different at the $P\leq 0.05$ as compared to group I (control).

b: The mean is significantly different at the $P\leq 0.05$ as compared to group II.

c: The mean is significantly different at the $P\leq 0.05$ as compared to group III.

d: The mean is significantly different at the $P\leq 0.05$ as compared to group IV.

e: The mean is significantly different at the $P\leq 0.05$ as compared to group V.

4.1.6.2 Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the aggressive behavior of the rats treated with testosterone propionate.

Aggressive behavior is usually measured by the sum of the number of threats, thrusts, attacks and keep down. The pie chart demonstrates the effect of alcoholic extract of mint leaves (*Mentha spicata*) on the female rats' decreased aggressive behavior, as shown in (Fig 4.11) Injection of testosterone propionate (6 mg/kg) into rats in Group 2 for 60 days led to a significant rise ($P<0.05$) in aggressive behavior.

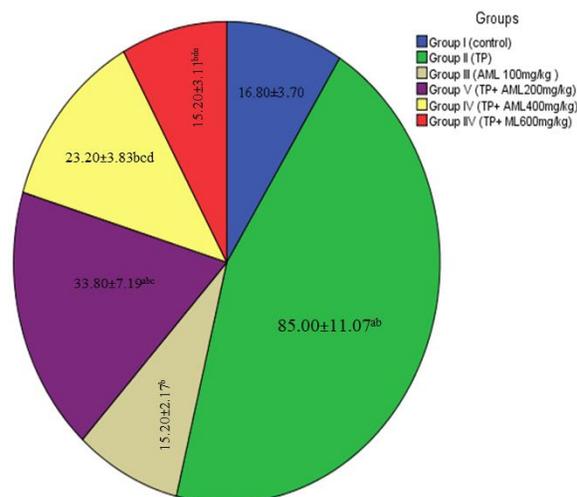


Figure (4.11) : aggressive behavior in female rats treated with testosterone propionate.

4.1.7: Phytochemical components of alcoholic extract of mint leaves.

The result in the table (4.6) showed Phenolic compounds and Tannins formation of a white precipitate. When the flavonoids, glycosides and coumarins formation of a yellow coloration. The saponins formation 1 cm layer of form and the terpenoids formation reddish brown color.

Table (4.6): Phytochemical components of alcoholic extract of mint leaves.

	Phytochemical constituents	Test	Result	Appearance
1.	Alkaloids	Wagner's test	-	Reddish precipitate
		Picric acid test	-	An orange color
		Mayer's reagent	-	Cream precipitate
		Iodine test	-	A blue color
2.	Phenolic compounds	Lead acetate test	+	White precipitate
3.	Tannins	FeCl ₃	+	White precipitate
4.	Flavonoids	Aluminium chloride	+	Yellow coloration
5.	Saponins	Froth	+	1 cm layer of foam
6.	Glycosides	NaOH	+	Yellow color
7.	Terpenoids	Salkowski	+	Reddish brown color
8.	Coumarins	NaOH test	+	yellow color
9.	phlobatannins	% HCl	-	Red precipitate
10.	Carbohydrates	α -naphthol + H ₂ SO ₄	-	violet color ring
11.	Chalcons	Ammonia	-	Reddish color
12.	Cardiac glycosides	Keller-Killani test	-	A blue color solution
13.	Proteins and amino acids	Xanthoproteic test	-	Yellow colored solution
14.	Phytosterols	Hess's response	-	Pink ring
15.	Quinons	Alcoholic KOH test	-	Red to blue color
16.	Anthocyanins	HCl test	-	Pink-red solution which turns blue-violet after addition of ammonia

Chapter Five

Discussion

5.1: Effect of alcoholic extract of mint leave (*Mentha spicata*) on body weight in female rats.

This study showed the efficacy of testosterone in increasing body weight of female rats that suffer from a high level of testosterone compared with the control group and other groups it may be because of insulin resistance and a change in the distribution of fat in the body and this study agrees with what is stated Huang *et al.* (2014) testosterone administration at supraphysiologic doses is associated with significant gains in lean body mass, chest press power, and loaded stair climbing power.

The results of the experiment support the effectiveness of the alcoholic extract of mint leaves in lowering the body weight in female rats that suffer from a high level of testosterone compared with the control group , as well as when comparing the groups with each other . It may be because it works to increase the secretion of salivary glands, which increases the activity of digestive enzymes and this study agrees with Ölmez *et al.* (2021) drinking spearmint tea contributes to stimulating the feeling of satiety and fullness for a long time, thus reducing the amount of calories consumed. It also agrees with Torki *et al.* (2021) spearmint tea helps improve bowel movement and thus improve the digestion process, as it works by activating fat-digesting enzymes so that fat is burned and used as an energy source in the body. Nemati *et al.* (2022) It has been proven that spicata oil has properties that make it contribute to suppressing appetite, thus eating less food. In a study conducted on 13 people who are given peppermint oil capsules, it is found that peppermint oil contributed to significantly reducing their appetite for food (Nemati *et al.*, 2022).

5.2 : Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the testosterone and dopamine levels in serum of female rats.

The results of the experiment support the effectiveness of the alcoholic extract of mint leaves in lowering the levels of testosterone and dopamine in female rats that suffer from a high level of testosterone compared with the control group , as well as when comparing the groups with each other. It may be because mint leaves contain menthol, which causes low testosterone levels (Ashkar *et al.*, 2020). This result is consistent with Alae *et al.* (2020) note that mint is beneficial in decreasing free testosterone level and hirsutism in women with mild hirsutism with PCOS. Fahimi *et al.* (2021) a study has shown that menthol in mint lowers testosterone levels in men, which ultimately leads to a decrease in libido. In addition, mint may reduce sperm production if consumed in excess, which causes impotence.

The results in the figure (4.9-B) showed that giving the alcoholic extract of mint leaves as a treatment to female rats that were dosed with testosterone resulted in mild hair loss compared to the II group that was dosed with testosterone alone. There was noticeable hair loss as shown in the figure (4.9-A), therefor the result of Alae *et al.*(2020).

This result is consistent also with what he found Alae *et al.*(2020) it is known that spearmint is beneficial in decreasing free testosterone levels and hirsutism in women with mild hirsutism with PCOS, and its adverse histopathological effects on kidney, liver and uterine tissue in animals were observed. Mohammed *et al.* (2021) *M. spicata* has a lethal effect on the testis by causing a significant in experimental animals. Past studies showed structural changes in testicular tissues, morphological deformities and

inhibition of spermatogenesis of different mammalian species treated with peppermint (Mohammed *et al.*, 2021)

The study showed that there is an inverse, linear, negative significant relationship between the concentration of the alcoholic extract of mint leaves and the levels of testosterone and dopamine in the serum of female rats. This study also agrees with Amoura *et al.*(2015) ; Ataabadi, *et al.* (2017) and sari *et al.* (2018) found that peppermint tea affects testicular function as it lowers testosterone and dopamine, and this may be because peppermint tea has anti-androgenic properties in both animals and women. Also agrees with Nozhat *et al.* (2014) and Akdoğan *et al.* (2007) Spearmint reduces free testosterone concentration of serum this is due to the anti-androgens present in it.

5.3 : Effect of alcoholic extract of mint leaves (*Mentha piperita*) on the levels of TNF- α and IL-2 in serum female rats.

This study showed the efficacy of testosterone in increasing TNF- α and IL-2 levels in the serum of female rats that suffer from a high level of testosterone compared with the control group. It may be because TNF- α stimulates the basal secretion of testosterone in whole testicular cells as well as in purified Leydig cells (Warren *et al.*, 1990). This result was consistent with what who found by Ganesan *et al.* (2012) where it was shown that high doses of testosterone lead to an increase in TNF- α levels.

While the alcoholic extract of mint leaves led to the reduction of TNF- α levels in the serum of female rats that suffer from a high level of testosterone compared with the control group and other groups It may be because the

alcoholic extract of mint leaves inhibits the production of TNF- α (Pelvan *et al.*, 2022)

The present results was consistent with Arumugam *et al.* (2008) that found the anti-inflammatory activity of hexane, chloroform, ethyl acetate and aqueous fractions of ethanol extract of *M. spicata* was evaluated on carrageenan (acute) and cotton pellet (chronic) induced inflammation in rats. Together ethyl acetate and aqueous fractions are found to be effective in chronic inflammation; whereas, only ethyl acetate fraction is effective in acute inflammation and also agreed with the results of Modarresi *et al.* (2019) as the cytokines are increased in the *M.Spicata* treatment groups. Mentha oil is known to have a significant antibacterial effect it may be because high levels of menthol, and according to several of studies, the alcoholic extract compounds of mint leaves showed significant anti-inflammatory activity (Boussouf *et al.*, 2017).

The results of the current study showed that the essential oils of the *M.Spicata* have the most effective inhibitory effects on tumor necrosis factor alpha TNF- α and IL-2 and this may be because mint contains menthol and tanning substances that have antispasmodic and anti-inflammatory effects (Orhan *et al.*, 2016). This result is also consistent with Kim *et al.* (2020), that observed inhibition of pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-5, and IL-8 cytokines treated with *M.Spicata* . This may be because testosterone affects the work of microglia in the brain, which leads to the release of high levels of TNF from these cells since mint lowered testosterone, TNF- α also decreased (Jayaraman *et al.*, 2021).

There was a significant negative linear relationship between the level of the TNF- α in the serum of female rats and the concentration of the alcoholic extract of mint leaves (*Mentha Spicata*) might suggest it's because the alcoholic extract of mint leaves inhibits the production of TNF- α .

5.4 : Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the levels of lipid profile in serum female rats.

This study showed that the efficacy of testosterone on lipid profile levels in serum of female rats that suffer from a high level of testosterone compared with the control group . This result was consistent with Uchida *et al.* (2021) that found the effect of testosterone on liver lipid content with castrated and testosterone-administered male rats and demonstrated that castration reinstated hepatic lipid accumulation through protein restriction in mature male rats. Castration enhanced hepatic steatosis is reported to be induced by a high-fat.

These results are also consistent with (Baik *et al.*, 2020) that found a low testosterone level or testosterone deficiency reportedly increases adiposity in the liver and several peripheral tissues. Yuefeng *et al.* (2022) indicate that the level of the hormone testosterone is sufficient to increase the risk of cardiovascular diseases related to cholesterol, as the increase in testosterone leads to a decrease in cholesterol and LDL. While this study does not agree with Choi *et al.* (2011) that showed the deficiency of testosterone leads to high cholesterol in the blood. This result was consistent with Baik *et al.* (2020) found that serum testosterone levels are inversely correlated with total cholesterol and LDL cholesterol levels and it has been demonstrated markedly increased serum cholesterol levels in testosterone-deficient male mice.

The results of the experiment support the effectiveness of the alcoholic extract of mint leaves in lowering the levels of lipid profile in female rats that suffer from a high level of testosterone compared with the control group, as well as when comparing the groups with each other. It may be because the rich fiber content in mint helps prevent indigestion and reduce high cholesterol levels, and reduce the risk of weight gain and obesity (Khursheed *et al.*, 2017).

The present results is consistent with Badal *et al.* (2011) that found treatment with an alcoholic extract of mint leaves in mice, lead to a significant decrease in blood cholesterol and triglycerides. This study demonstrated the efficacy of *M. Spicata* in improving the lipid profile of animals. A remarkable fact is that this plant is normally consumed in the form of tea, but some beneficial effects (significant reduction of triacylglycerols and increase in HDL-c) has been achieved with the juice from the leaves (Barbalho *et al.*,2009) .

This result was also consistent with Al-Fartosi *et al.* (2014) as it is shown that the treatment with mint causes a significant decrease in the levels of cholesterol, LDL, and triglycerides, and a significant increase in the levels of HDL because Flavonoids may augment the activity of lecithin acyl transferase (LCAT) which plays an important role in the incorporation of free cholesterol into HDL, causing an increase in the serum HDL concentration. This result does not agree with Nagarajan and Doss. (2023) that found *M. Spicata* was one of the main causes of the elevated myocardial cholesterol level in isoproterenol-induced myocardial damage is accelerated lipid production. The impact of testosterone on serum lipids showed an increase in the TG levels may be because enlargement of abdominal adipose

tissue and may be due to its influence on the functions of regulated hepatic enzymes which are associated with cholesterol uptake, synthesis, and clearance. Studies in rats have shown that testosterone engages in the cholesterol metabolism regulation of hepatic cells only in females (Zarei *et al.*, 2018).

There was a significant negative linear relationship between the level of the Trig, cholesterol and LDL in the serum of female rats and the concentration of the alcoholic extract of mint leaves (*Mentha Spicata*) might suggest it's because the rich fiber content in mint helps prevent indigestion, reduce high cholesterol levels, and reduce the risk of weight gain and obesity (Nagarajan and Doss, 2023).

5.5: Effect of alcoholic extract of mint leave (*Mentha Spicata*) on aggressive behaviour in female rats.

This study showed that the efficacy of testosterone in increasing aggressive behaviour in the serum of female rats that suffer from a high level of testosterone compared with the control group. It may be because testosterone has a profound effect on the brain circuits involved in threat and aggression (Johansen *et al.*, 2020). This study agrees with Njoroge *et al.* (2015) and Geniole *et al.* (2020) that found a single dose of testosterone increased aggressive behavior in female rats. This study agrees with Estumano *et al.*(2019) showed that a single injection of the hormone testosterone is sufficient to increase functional communication between and within the hemispheres, and it also modifies physiology, addiction and behavior, encouraging hostility and aggression. The study showed of the

short-term oral administration of testosterone to women showed a range of changes, including features associated with aggression (Carré and Archer, 2018).

This study also agrees with Aluja *et al.* (2015) studies in healthy men confirm the hypothesis that high testosterone levels impact risk-taking or impulsive behavior. In a later study, it has been exhibited that early-onset users under testosterone rage are more impulsive and show deficits in behavioral disinhibition, affective processing, and planning. Bennett *et al.* (2018) found that increases in aggression are due to increases in both testosterone and cortisol levels associated with stress levels. Gouveia *et al.* (2023) increased testosterone levels lead to a reduction in serotonergic availability in the limbic system, supporting that testosterone modulates serotonergic activity and further contributes to the maintenance of aggressiveness.

The results of the experiment support the effectiveness of the alcoholic extract of mint leaves in lowering the aggressive behaviour in female rats that suffer from a high level of testosterone compared with the control group , as well as when comparing the groups with each other . It may be because of its soothing mint scent and this study agrees with Moss *et al.* (2023) the ambient aroma of spearmint may be a simple and effective intervention to reduce aggressive behaviour. It was hypothesized that the presence of spearmint aroma would reduce aggressive behaviors and negative feelings such as aggression and stress, whilst increasing alertness and calmness. Abbasi-Maleki *et al.* (2017) *M. spicata* extract with typical antidepressants looks promising and its potential as a supplementary agent for the management of depression. Since the alcoholic extract of mint leaves

reduced testosterone, Conclude from our study in this research that the alcoholic extract of mint leaves also reduced aggressive behavior.

Bennett *et al.* (2018) found that increases in aggression are due to increases in both testosterone and cortisol levels associated with stress levels. Batalhão *et al.* (2019) showed results indicating that increases aggressiveness in Nile tilapia, perhaps due to a decrease in serotonin levels in the brain and changes in dopamine-genes and dopamine levels. Geniole *et al.* (2019) found that in vivo, shows that the correlation between testosterone and both threat-related amygdala functions and self-aggression is enhanced among humans and they suggested testosterone thus appears to promote human aggression through inducing dopaminergic pathways.

Wagels *et al.* (2020) showed Many neural networks have been associated with aggressive behavior, and studies have been conducted on many different species, including rats. High testosterone levels can decrease the activity of the medial region of the orbitofrontal cortex (OFC) within the prefrontal cortex (PFC) and stimulate aggressive behavior. One of the possible mechanisms by which testosterone can reduce the activity of the OFC is by regulating serotonin.

Conclusions and Recommendations

Conclusion and Recommendations

Conclusion:

The current study indicated the protective role of the alcoholic extract of mint leaves in reducing the:

1. Levels of testosterone, dopamine.
2. Proinflammatory cytokines(TNF- α , IL-2)
3. Aggressive behavior and total cholesterol, triglycerides, and low-density lipoprotein in the serum of female rats treated with testosterone propionate.
4. Spicata leave extract also led to improving the levels of high-density lipoprotein in the serum of female rats treated with testosterone propionate in female rats suffering from high testosterone.

Conclusion and Recommendations

Recommendations:

- 1- Studying the effect of alcoholic extract of mint leaves on other hormones such as serotonin, estrogen, progesterone, LH, and FSH.
- 2- Study of sexual and maternal behavior in female rats that suffer from high testosterone.
- 3- Studying the licorice plant because it may cause low testosterone.
- 4- Studying some medicinal plants rich in tryptophan and its role in raising testosterone because it is responsible for mood, sleep, and adaptation.

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اجريت الدراسة الحالية في أناث الجرذان البيض لمعرفة تأثيرات المستخلص الكحولي لأوراق نبات النعناع *Mentha spicata* على مستوى هرمون الشحمون الخصوي والسلوك العدواني الى أناث الجرذان التي تعاني من ارتفاع مستوى التستوستيرون ، بالإضافة الى ذلك تقدير مستوى بعض المعايير الفسيولوجية والمناعية التي لها علاقة مع السلوك العدواني . أجريت تلك الدراسة في البيت الحيواني لقسم علوم الحياة /كلية العلوم/جامعة بابل للمدة من تشرين الثاني (2022) الى آذار (2023) . أستخدم في هذه الدراسة 30 من أناث الجرذان (*Rattus rattus*) قسمت الى ستة مجاميع (خمسة جرذان لكل مجموعة) وعلى النحو التالي : **المجموعة الاولى G1** من الجرذان تلقت جرعة يومية 0,1 مل من زيت السمسم بالتجريب عن طريق الفم كمجموعة سيطرة ولمدة 60 يوماً". **المجموعة الثانية (GII)** فكانت الموجبة حيث حقنت الجرذان تحت الجلد بمادة بروبيونات التستوستيرون 6 ملغم / كغم ، أما **المجموعة الثالثة (GIII)** فقد اعطيت عبر الفم جرعة من 100ملغم / كغم من المستخلص الكحولي لأوراق نبات النعناع . **المجموعة الرابعة (GV)** من الجرذان فقد جرعت 6ملغم/كغم من بروبيونات التستوستيرون (تحت الجلد) و200 ملغم/كغم من المستخلص الكحولي لأوراق النبات (عبر الفم) ، أما **المجموعة الخامسة (GV)** فقد جرعت 6ملغم/كغم من بروبيونات التستوستيرون و400 ملغم/كغم من المستخلص الكحولي لأوراق النبات ، في حين جرعت **المجموعة السادسة (GVI)** ببروبيونات التستوستيرون (6ملغم/كغم) و600 ملغم / كغم من المستخلص الكحولي للنبات .كل تلك الحيوانات قد خدرت بواسطة الكلوروفورم وشرحت في اليوم الستين من التجربة ، وبعد ذلك تم اخذ عينات الدم من كل المجاميع الحيوانية وذلك لغرض تقدير مستويات الدهون ، بعض المعايير الفسيولوجية (مستوى التستوستيرون والدوبامين) والمناعية (الحركيين الخلويين عامل تنخر الورم – الفا و الانترليوكين -2).

بينت نتائج هذه التجربة ان الجرذان المعاملة (المجموعة الثانية) بروبيونات التستوستيرون (6 ملغم/كغم) قد أظهرت ارتفاعاً معنوياً ($P < 0.05$) في مستويات عامل تنخر الورم – الفا ($TNF\alpha$) والانترليوكين -2 (IL-2) مقارنة بمجموعة السيطرة (الاولى) والمجموعات الاخرى ، في حين اظهرت الجرذان في المجاميع الرابعة والخامسة والسادسة المعاملة ببروبيونات التستوستيرون (6ملغم/كغم) والمستخلص الكحولي لأوراق النعناع (200، 400، 600 ملغم/كغم) على التوالي لمدة 60 يوماً" انخفاضاً معنوياً ($P < 0.05$) في مستويات تلك الحركيات الخلوية عند المقارنة مع المجموعة الثانية. كذلك أظهرت النتائج ان الجرذان المعاملة (المجموعة الثانية) بروبيونات التستوستيرون (6 ملغم/كغم) أظهرت ارتفاعاً معنوياً في مستويات هرموني التستوستيرون والدوبامين مقارنة بمجموعة السيطرة (الاولى) والمجموعات الاخرى ، في حين اظهرت الجرذان في المجموعات الرابعة والخامسة والسادسة المعاملة ببروبيونات التستوستيرون والمستخلص الكحولي

لأوراق النعناع (200، 400، 600 ملغم/كغم) على التوالي انخفاضا معنويا في مستويات هرموني التستوستيرون والدوبامين عند المقارنة مع المجموعة الثانية.

بالإضافة الى ذلك بينت النتائج وجود ارتفاع معنوي ($P < 0.05$) في مستويات الكليسيريدات الثلاثية ، الكولسترول الكلي ، البروتين الدهني منخفض الكثافة والبروتين الدهني منخفض الكثافة جدا" في المجموعة الثانية المعاملة ببروبيونات التستوستيرون 6 ملغم\كغم مقارنة بالمجموعة الثالثة، بينما انخفض تركيز البروتينات الدهنية عالية الكثافة في المجموعة الثانية. لقد بينت النتائج (المجموعة الثالثة) وجود انخفاض معنوي ($P < 0.05$) في مستويات الكولسترول الكلي ، الكليسيريدات الثلاثية ، البروتينات الدهنية منخفضة والمنخفضة الكثافة جدا" مقارنة مع المجموعة الثانية ، بينما ارتفع تركيز البروتينات الدهنية عالية الكثافة في المجموعة الثانية . اظهرت النتائج وجود ارتفاع معنوي ($P < 0.05$) في السلوك العدواني لدى المجموعة الثانية من الجرذان مقارنة بمجموعة السيطرة والمجموعات الاخرى، في حين كان هناك انخفاض معنوي ($P < 0.05$) في السلوك العدواني في المجموعات الثالثة والرابعة والخامسة والسادسة مقارنة بالمجموعة الثانية .

نستنتج من هذه الدراسة ان التأثيرات الوقائية الى المستخلص الكحولي لأوراق نبات النعناع قد تمثلت بانخفاض مستويات الحركيات الخلوية الالتهابية و مستويات الدهون ، في حين ازداد مستويات الهرموني التستوستيرون والدوبامين ، كذلك بينت الدراسة وجود زيادة في السلوك العدواني للجرذان في المجموعة الثانية ، في حين لوحظ نقصان في السلوك العدواني الى المجاميع الرابعة والخامسة والسادسة على التوالي .



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قسم علوم الحياة

تأثير مستخلص أوراق النعناع على بعض المعايير الفسلجية والمناعية والسلوك العدواني
للإنات الجرذان البيض المعاملة بهرمون الشحمون الخصوي

رسالة

مقدمة الى مجلس كلية العلوم- جامعة بابل

كجزء من متطلبات نيل شهادة الماجستير في العلوم /علوم الحياة

من قبل

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