Ministry of Higher Education & Scientific Research University of Kerbala
College of Veterinary Medicine Physiology, Pharmacology and Biochemistry Department

The Role of *Nigella sativa* and Curcumin vs Oxidative Stress Toxicity induced by Bisphenol A in male rats' reproductive system

A Thesis

Submitted to the Council of the College of Veterinary Medicine/ University of Kerbala, as a Partial Fulfillment of the Requirements for the Degree of Master in Science of Veterinary Medicine/ Physiology

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1445A.H **2024A.D**

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Dedication

For those who strive to console me and make me happy.... My father

For whom is that paradise under her feet...My mother

To my wife who supported me and encouraged me in every step (Abrar)

My dear son (Al- Hassan)

To those whom gave me the strength and support… "My sister and my brothers"

I am greatly thankful for your tolerance and your kind support to successful Complete my research.

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Summary

Bisphenol A (BPA) could be considered as one of the most studied endocrine disruptors. BPA is an endocrine disruptor and an environmental pollutant; it can produce free radicals, which cause damage in various tissues including the tissues of reproductive system. The male reproductive system plays a crucial role in influencing the overall health as well as well-being of males, in recent years, there has been a growing prevalence of male reproductive health issues, leading to heightened concerns, various potential substances and factors are being scrutinized for their possible impact on male reproductive health at all.

Nigella sativa oil (NS) oil, had been demonstrated to help as s scavenger of free radicals and function as an antioxidant with a variety of male reproductive diseases. Besides, Curcumin (Cur.) has also been demonstrated to treat several sorts of male reproductive problems in experimental animals, hence increasing fertility; as well as, to scavenge free radicals and function as an antioxidant.

This study aimed to investigate the protective role of NS oil and Cur. against the toxic effect of BPA in male rats' reproductive system by estimation the levels of the antioxidant enzyme Superoxide Dismutase (SOD) and Malondialdehyde(MDA), the level of sexual hormones such as (follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone), and lastly, the level of cytokines as (Interleukin6(IL-6) and tumor necrosis factor Alpha (TNF-α)). Furthermore, detect if there are any histological changes before and after treatment with this herb.

48 adult male rats, with weights of about (200-350 grams) and ages between (eight -10 weeks). These male rats were divided into eight groups six rats in each group. This study was conducted from November/2023 – March/2024.

The effect of NS oil and Cur. as protective agents against BPA on the oxidative stress parameters, showed that The effect of NS oil and Cur. as protective medicinal plants against BPA, on the oxidative stress parameters showed that, NS oil and Cur.

had no significant values in MDA compared with the control group but in BPA group there was a significant increase in MDA (P value=0.0001), in the same time there were increase in the concentration of both SOD (P value=0.0003), and catalase (P value<0.0001) significantly, with decrease in BPA group.

Moreover, the results revealed that, there were significant increase in FSH (P value=0.0001), LH (P value=0.0003) and testosterone (P value<0.0001) in the groups of BPA+NS oil, BPA+CUR. and BPA+ NS oil+ CUR , as well as, decrease in the levels of these hormones in BPA group comparing with the control.

The effect of NS oil, Cur. and the combination of NS oil+ Cur. on the cytokines found that, there were a significant decrease of IL-6 and TNF-α, (P value= 0.0001) and (P value=0.02) respectively in groups NS oil+ BPA, Cur.+ BPA and the combination of NS oil+ Cur+ BPA comparing with BPA group and control group. Besides, histopathological study of cross sections of testis and epididymis showed that the BPA-treated group showed detrimental effect of this chemicals on the testes and epididymis, while administration of NS oil and Cur. keeping the normal features of the testicular and epididymal section as comparing to the BPAtreated group. The group treated with combination of NS oil and Cur. had no synergistic effect when compared with groups treated with NS oil or Cur. alone. The study concluded that, NS oil and Cur. had enhanced protective effects against BPAinduced toxicity male reproductive system in rats. While the administration of NS oil and Cur. had no synergistic or antagonistic effect on healing the adverse effect of BPA.

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List of Abbreviations

Abbreviation	Meaning
B.W.	Bady weight
BPA	Bisphenol A
CAT	Catalase
Cur	Curcumin
D.W.	Distilled water
EDCs	Endocrine disrupting compounds
ER	estrogen receptor
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
gm	Gram
H_2O_2	hydrogen peroxide
HPG	hypothalamic-pituitary-gonadal
IL-6	Interleukin-6
LPO	lipid peroxidation
LH	luteinizing hormone
MDA	Malondialdehyde
MIS	Mullerian inhibiting substance
Ns	Nigella sativa
NO	nitric oxide
PGE2	prostaglandin E2

ROS	Reactive Oxygen Species
ERK	regulated protein kinase
SOD	Superoxide Dismutase
TQ	thymoquinone
TNF-α	tumor necrosis factor Alpha

Chapter One

Introduction

1. Introduction

Bisphenol A is an industrial chemical known for its role as an environmental endocrine disruptor, exhibiting estrogenic properties; it finds extensive application in the manufacturing of various consumer goods such as specific plastics like polycarbonate and epoxy resin, as well as thermal paper; additionally, BPA has the potential to migrate from dental sealants (Ramakrishna *et al.,* 2022). Endocrine disrupting compounds (EDCs) are external substances capable of either triggering, or inhibiting natural hormone reactions within organisms; thus, disrupting reproductive and developmental processes (CzarnyKrzymińska *et al.,* 2023). BPA's interaction with hormonal receptors, rather than hormones themselves; results in increased expression of these cells; additionally, BPA exhibits similar effects on sexual maturation as di-ethyl-stilbestrol (Kang *et al.,* 2023).

The male reproductive system of rats consists of the testes, epididymis, vas deferens, seminal vesicles, prostate gland, and penis; these structures work together to produce, store, and deliver sperm for reproduction (Suckow *et al.,* 2005). Moreover, the male reproductive system plays a crucial role in influencing the overall health as well as well-being of males; in recent years, there has been a growing prevalence of male reproductive health issues, leading to heightened concerns; so, various potential substances and factors are being scrutinized for their possible impact on male reproductive health at all; Bisphenol A (BPA) could be considered as one of the most studied endocrine disruptors (Jambor *et al.,* 2021).

The use and effectiveness of medicinal herbs in treatment of various diseases has been received enormous attention and research efforts were made on extraction and examination of the properties of the herbal compounds in the treatment of different types of diseases and providing detailed mechanisms of

drug performance of these compounds (Ahmadi *et al*., 2016). Among the medical herbs, flavonoids are a large subgroup of the family of natural polyphenolic compounds that are the result of secondary metabolism in plants (Mansouri *et al*., 2020). Such a growing interest in medicinal plants has inspired scientists to clarify their effects on male fertility based on the available literature, some of these plants increase sperm count and motility while a number of others alter the secretion of hormones by the testicles (Khaki *et al.,*2009).

Nigella sativa (Ns) seeds have been used extensively in human history for culinary and medicinal purposes dating back over 1400 years; besides, the seeds are described as aromatic and bitter in taste (Leisegang *et al.,* 2021). As a result, Ns has a potential therapeutic agent for the treatment of several disorders, such as fertility (Ates *et al.,*2022).

The flavonoids have been very effective in the prevention and control of common diseases complex (Chen and Chen ,2013). Amongst the wide range of the medical herbs, curcumin is an effective ingredient of turmeric plant with the scientific name of "longa Curcuma" (Modaresi *et al.,* 2017).

Curcumin (Cur.) is a yellow phenolic pigment obtained from the rhizome of turmeric which has indicated that have a broad range of biological and pharmacological functions (Alizadeh *et al.,* 2018). Curcumin has also been demonstrated to treat several sorts of male reproductive problems in experimental animals, hence increasing fertility (Khorsandi *et al.,*2022). Curcumin's ability to scavenge free radicals and act as an antioxidant agent. as well as an increase in serum testosterone levels, may be responsible for its beneficial effects (Lu *et al.,* 2022).

2. Aim of this study:

The study aimed to investigate the protective role of *Nigella sativa* and Curcumin against the toxic effect of bisphenol A in male rats' reproductive system by estimation the levels of sexual hormones, the level of cytokines, histological changes before and after treatment with these herbs. This can be conducted by the following objectives:

1- Physiological Parameters:

a) Measuring the level of antioxidant such as catalase (CAT), superoxide dismutase (SOD) and oxidant malondialdehyde (MDA).

b) Measuring the level of sexual hormones Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone).

2- Serological Parameters:

- Measurement the level of Interleukins (IL-6 and TNF-α).

3- Histological study: Cross section of the testes and epididymis (H&E).

Chapter Two

Review of The Related Literature

2. Review of the Related Literature

2.1. Bisphenol A (BPA):

Bisphenol A (BPA), chemically known as 2,2-bis(-hydroxyphenyl) propane, was initially synthesized in 1891 and subsequently discovered in the 1930s to possess estrogenic properties; presently, with an annual production exceeding 2 million tons, BPA serves as the primary monomer utilized in the manufacturing of polycarbonate plastics and epoxy resins (Gul *et al*., 2021). BPA is a crystalline chemical compound with the molecular formula $C_{15}H_{16}O_2$, polycarbonate plastics derived from BPA exhibit exceptional strength and stability, capable of withstanding high temperatures and resisting high-impact collisions; while BPA in its polymeric form is not considered hazardous, it becomes unstable in acidic or basic solutions and when exposed to UV light; under such conditions, polymeric BPA may convert into its monomeric form, potentially releasing BPA into food, beverages, or the environment; the LD50 of BPA in rats via oral administration is reported to be 3.25 g/kg (Mileva *et al.,*2014).

Bisphenol A (BPA) is recognized as a significant industrial component with notable implications as an endocrine disruptor and environmental contaminant due to its estrogenic properties, it is widely utilized in the production of various consumer goods, including specific plastics like polycarbonate, thermal paper, and epoxy resin; furthermore, BPA has the capacity to migrate from dental sealants, adding to its potential exposure pathways in humans and the environment (Ramakrishna *et al.,* 2022). Endocrine disrupting compounds (EDCs) are external substances capable of either triggering, or inhibiting natural hormone reactions within organisms; thus, disrupting reproductive and developmental processes (Czarny-Krzymińska *et al.*, 2023).

BPA's interaction with hormonal receptors, rather than hormones themselves; results in increased expression of these cells; additionally, BPA exhibits similar effects on sexual maturation as di-ethyl-stilbestrol (Kang *et al.,* 2023).

Bisphenol A (BPA) exerts some of its effects by binding to nuclear steroid receptors such as ERα and Erβ; this interaction triggers estrogen receptor (ER)nuclear signaling pathways, which subsequently modulate the expression of estrogen-responsive genes (Gámez *et al.,*2014). Exposures to environmental toxicants, including BPA, are recognized as significant contributors to male infertility, as highlighted by Manfo *et al.* (2014). BPA stands out as one of the most prevalent environmental contaminants globally, owing to its widespread production and utilization as a common ingredient in plastic manufacturing; given the extensive integration of plastics into various aspects of modern life, BPA's presence makes a substantial contribution to nearly all product categories, underscoring its potential impact on reproductive health (Shi *et al.,*2018; Dumitrascu *et al*.,2020).

Exposure to BPA in food materials occurs through various routes, including heating, prolonged use, contact with acidic or alkaline substances, and microwave exposure; these conditions can facilitate the transfer of BPA from packaging or containers into the food itself, thereby leading to BPA intake by consumers; though, such contact with food can result in the presence of non-conjugated BPA in the serum of adults and even in fetuses, highlighting the potential for BPA exposure during critical stages of development (Ramakrishna *et al.*,2021).

Liu *et al.* (2022) told that, BPA has the potential to migrate into water and food, particularly under conditions of high temperature, acidity, and alkalinity. Moreover, BPA leaching from food packaging, especially in warm environments, constitutes a primary route of BPA exposure through ingestion, Additionally, inhalation and dermal contact with BPA, which is omnipresent in environmental matrices such as indoor dust, soil, and aquatic systems, also represent significant exposure pathways (Ramakrishna *et al.,*2021).

2.2. Male Reproductive System:

The male reproductive system comprises both internal and external components; internally, it includes the testes, epididymis, vas deferens, prostate, while externally it involves the scrotum and penis; these structures are richly supplied with blood vessels, glands, and ducts to facilitate sperm production, storage, and release for fertilization, as well as to generate crucial androgens for male growth and development (Tiwana and Leslie, 2017). The male reproductive system serves to generate androgens, notably testosterone, which sustain male reproductive capabilities, and to facilitate spermatogenesis and its conveyance into the female reproductive system for fertilization; the testes function dually as both endocrine and exocrine organs, as they play a role in androgen secretion and in the production and transportation of sperm (Gurung *et al.,* 2022). The cornerstone of the male reproductive system is the testis, functioning as the central organ; its importance lies in its dual role, encompassing the synthesis of steroid hormones and the production of spermatozoa (Carreau *et al.,* 2002). The male reproductive system comprises the epididymis, a vital tubular coiled structure that links the testis to the vas deferens, characterized by its caput (head), corpus (body), and cauda (tail) segments as noted by Cornwall (2009). Within the epididymis, the process of spermatozoa maturation and storage takes place, as emphasized by Beu *et al.* (2009).

Maintaining the structural integrity of the epididymis is crucial for preserving the optimal environment necessary for male fertility and the viability of sperm, as underscored by (Favor and Cagnon, 2006). Furthermore, in mammalian reproductive function, the accessory sex glands, such as the seminal vesicles and the prostate gland, play pivotal roles (Tolba and Mandour, 2018).

2.3. Hormones of Reproductive System:

The primary male androgen, testosterone, originates from Leydig cells within the testes; it can undergo peripheral conversion into dihydrotestosterone, a more potent form, via 5-alpha-reductase, or into estradiol via aromatase; additionally, Sertoli cells in the testes produce inhibin B and Mullerian inhibiting substance (MIS) hormone; key regulators of these processes include follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are secreted by the anterior pituitary gland and controlled by gonadotropin-releasing hormone (GnRH) from the hypothalamus; together, these hormones orchestrate the hypothalamicpituitary-gonadal axis, crucial for male sexual development and function (Mawhinney and Mariotti, 2013; Gurung *et al.,* 2022).

Luteinizing hormone (LH) stands as a pivotal gonadotropin within the regulatory framework of the reproductive system; originating from the anterior pituitary gland, its receptor is situated within the gonads, where it executes essential reproductive functions (Oduwole *et al.,* 2021).

Follicle stimulating hormone (FSH), a vital gonadotropin, is secreted by the basophilic cells of the anterior pituitary gland, contributing significantly to gonadal hormone synthesis and the regulation of reproductive processes. Gonadotropin-releasing hormone, predominantly produced by hypothalamic neurons, travels to the anterior pituitary, where it binds to gonadotropin-releasing hormone receptors, thereby regulating FSH secretion in the hypothalamicpituitary-gonadal (HPG) axis (Wang *et al.,* 2021).

The hormones LH, FSH, and testosterone play indispensable roles in spermatogenesis; LH, emanating from the pituitary gland, stimulates testosterone production, while testosterone is essential for the initiation and maintenance of sperm production (Mantovani, 2002). Furthermore, both FSH and testosterone collaborate in fostering spermatid growth and facilitating sperm release.

(Alboghobeish *et al.,* 2019). The pivotal regulatory pathway in male reproductive physiology revolves around the HPG axis, orchestrating vital functions through the interplay of FSH and LH, alongside the maintenance of elevated intratesticular testosterone levels; LH governs testosterone production by Leydig cells, specialized endocrine cells situated within the testicular interstitium, as elucidated by Mruk and Cheng (2015). Testosterone stands as a cornerstone for male virilization and, in conjunction with FSH, initiates and sustains spermatogenesis; this synergistic effect of testosterone and FSH primarily targets Sertoli cells, lining the seminiferous tubules, providing crucial support for germ cells as they progress through the developmental stages towards mature sperm, as discussed by Oduwole *et al.* (2021) as shown in Figure (2-1).

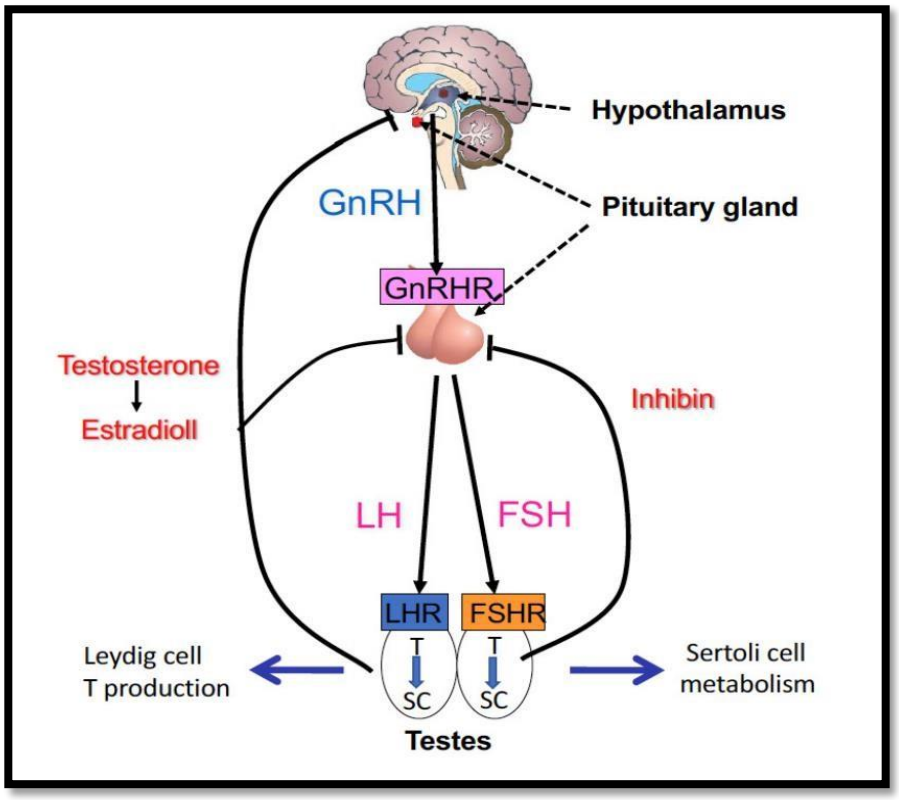


Figure (2-1): Hormonal regulation in the reproductive system hinges on (GnRH), generated by the hypothalamus. Upon binding to its receptors (GnRHR), GnRH initiates the synthesis and secretion of gonadotropins (Oduwole *et al.*, 2021).

2.4. The Effect of BPA on:

2.4.1. The Males Reproductive System:

In recent decades, the surge in industrialization has coincided with increased leaching of industrial pollutants into the environment, consequently elevating human and animal exposure to toxic chemicals; among these compounds, Endocrine Disrupting Compounds (EDCs) are notable for their capacity to interfere with cellular pathways, particularly hormonal pathways governing reproductive processes; the disruption of these pathways by EDCs has been linked to a spectrum of adverse health effects (Adegoke *et al.,*2020). As shown in Figure (2-2)

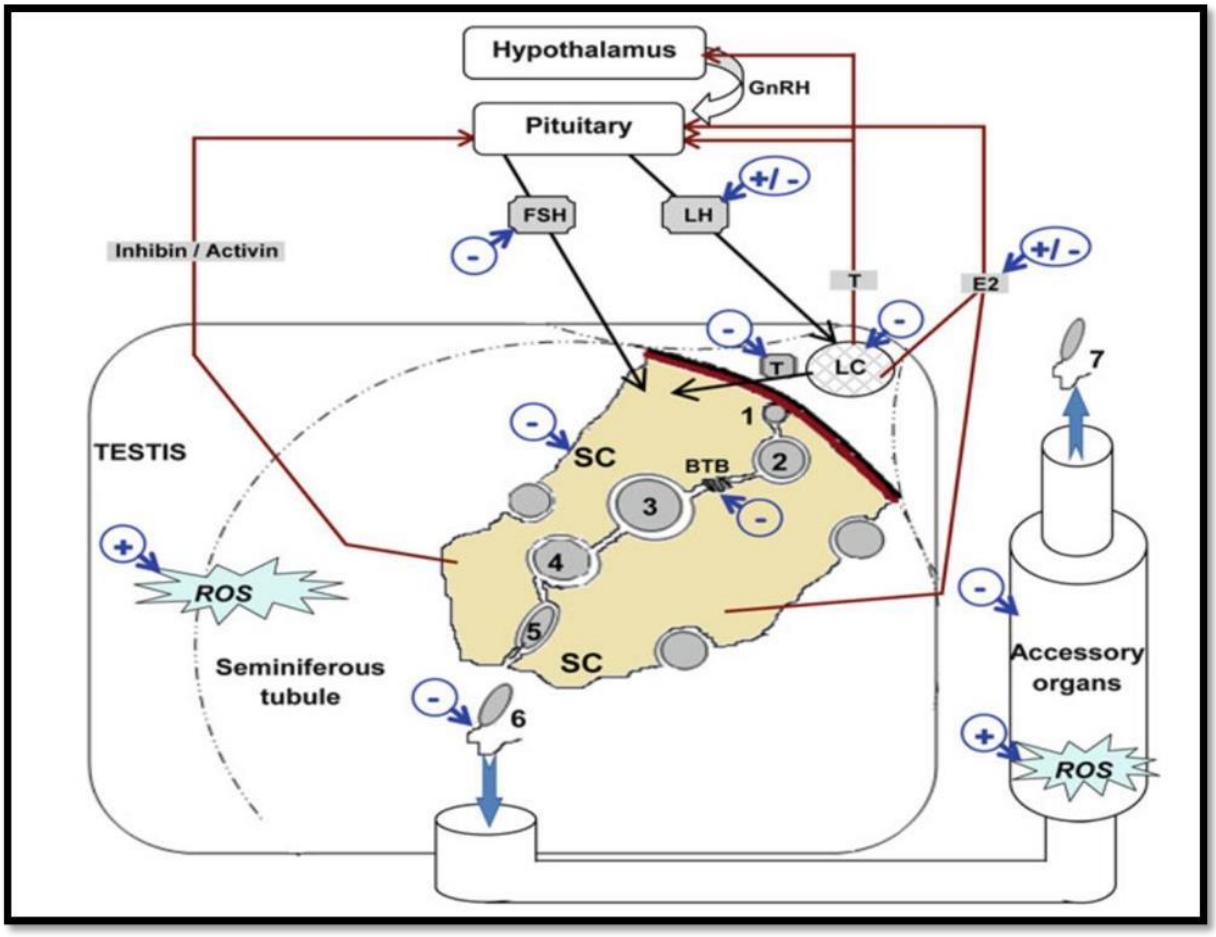


Figure (2 -2): A schematic diagram showing the main effects and action sites of BPA on male reproductive function (Manfo *et al*.,2014).

BPA stands out as one of the most recognized EDCs, as highlighted by Sabry *et al.* (2021). It serves as a synthetic estrogen agonist extensively used in the manufacturing of everyday products; these EDCs, including BPA, are external substances capable of either stimulating or inhibiting natural hormone reactions within organisms, thereby disrupting reproductive and developmental processes, as discussed by Czarny-Krzymińska *et al.* (2023). BPA's interaction with hormonal receptors, rather than the hormones themselves, leads to increased expression of these receptors; additionally, BPA has been observed to exert similar effects on sexual maturation as diethylstilbestrol (Kang *et al.,*2023).

Bisphenol A (BPA) possesses the capability to interact with and bind to androgen receptors, potentially exerting adverse effects on reproductive function; such interactions may contribute to diminished male fertility, characterized by conditions like cryptorchidism, testicular dysgenesis, and cancer (Cimmino *et al.* 2020). Furthermore, animal studies have indicated that BPA can negatively impact sperm quality and disrupt the integration of germ cell nests, as demonstrated by Ramakrishna *et al.* (2022).

Moreover, estrogen receptors are widely distributed throughout various organs, encompassing the thyroid glands, reproductive organs, and other tissues; consequently, the interaction between BPA and these receptors elicits heightened cellular expression, thereby impacting sexual maturity, as noted by Raheem *et al.* (2020). For instance, numerous laboratory rodent studies propose that the plasticizer BPA may function as a reproductive toxicant, although significant uncertainties persist regarding the actual risk for humans Kazemi *et al.* (2016).

Exposure to EDCs has been associated with several detrimental effects on male fertility, as outlined by Gámez *et al.* (2014); these effects include a reduction in sperm quality, a higher incidence of conditions like cryptorchidism and

hypospadias, as well as an increased risk of testicular and prostate cancer, among others.

Research conducted by Mínguez-Alarcón *et al.* (2016) on male rats exposed to a dose of 2.4 µg/kg/day of BPA between postnatal days 21 and 35 revealed notable impacts on reproductive parameters; this exposure led to reduced serum levels of luteinizing hormone (LH) and testosterone, accompanied by an increase in testicular size, as well as reductions in epididymal weight and daily sperm production. Furthermore, recent findings suggest that low doses of BPA can impair spermatogenesis in adult male rats by suppressing reproductive hormone production and promoting germ cell apoptosis; these studies collectively underscore the significant adverse effects of BPA exposure on male reproductive health (Hass *et al.,*2016). As shown in Figure (2-3).

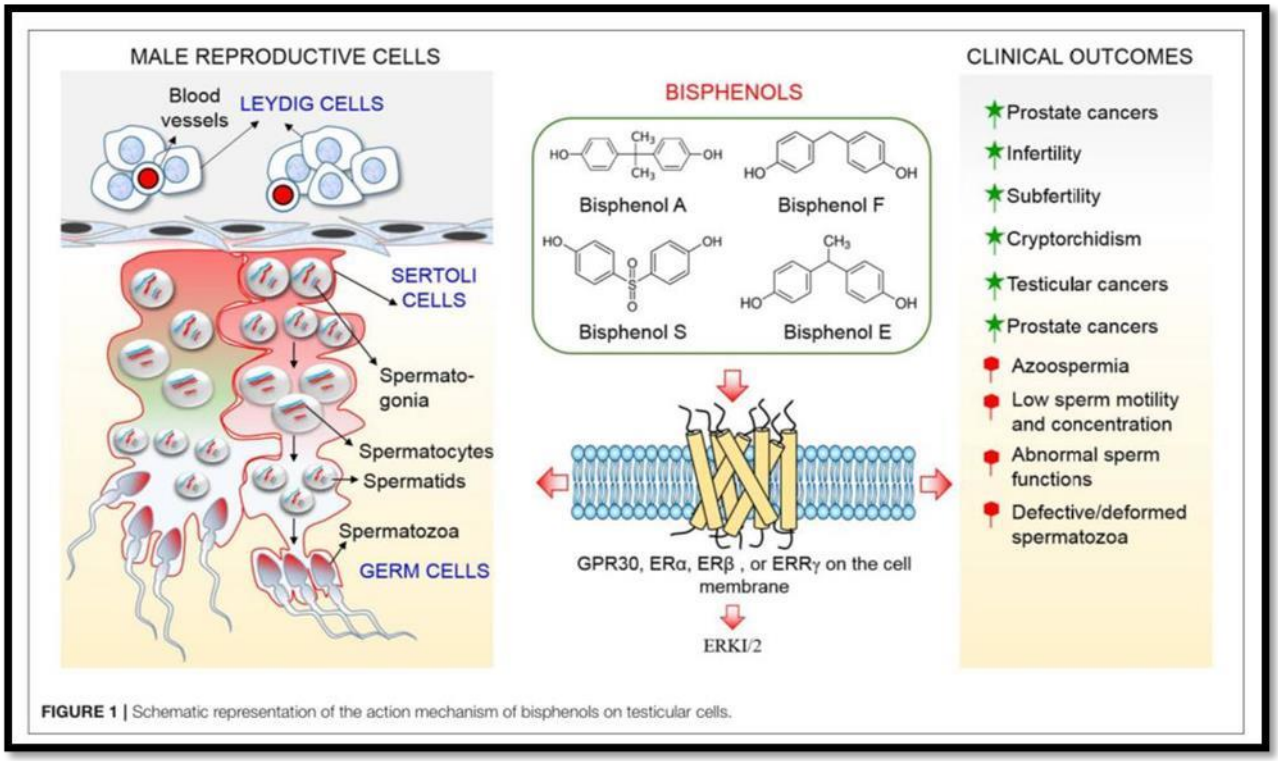


Figure (2-3): The action mechanism of BPA on testicular cells (Adegoke *et al.*, 2020).

Bisphenol A (BPA) exerts detrimental effects on spermatogenesis and semen quality through various mechanisms, as observed by Zang *et al.* (2016); these include impairing meiosis, inducing apoptosis of spermatids, and depressing the

function of the HPG axis. Moreover, BPA has been shown to influence Leydig cell steroidogenesis as mentioned by Matuszczak *et al.* (2019) and De Toni *et al.* (2020); this includes affecting the expression of enzymes such as 17αhydroxylase/17,20 lyase and aromatase, as well as interfering with the binding of LH to its receptor.

2.4.2. The Oxidative Stress:

Environmental toxicants like BPA can negatively impact male reproductive function by disrupting the pro-oxidant–antioxidant balance within the testes, consequently impairing testicular function, according to Wong and Cheng (2011); these findings collectively underscore the multifaceted ways in which BPA can disrupt male reproductive health.

Indeed, oxidative stress induced by BPA in the testes can instigate various physiological alterations; this oxidative stress often arises from heightened levels of reactive oxygen species (ROS), molecules characterized by oxygen atoms with unpaired electrons; it's worth noting that while radicals are a common form of ROS, certain non-radical molecules categorized as ROS can also generate free oxygen ions during regular metabolic processes, this imbalance between ROS production and the body's antioxidant defences can lead to cellular damage and dysfunction in the testicular environment (Wu *et al.,* 2020; Hussein *et al.,* 2023). Exactly; BPA disrupts redox homeostasis by increasing oxidative mediators and reducing the activity of antioxidant enzymes; this imbalance leads to mitochondrial dysfunction, alterations in cellular signalling pathways, and ultimately triggers apoptosis, or programmed cell death; the cumulative effect of these disruptions contributes to the overall detrimental impact of BPA on cellular health and function (Othman *et al.,* 2016).

2.4.3. The Immune Response:

BPA's potential to disrupt the immune system is concerning; by interfering with various cytokine signals, BPA can lead to dysregulation of cytokine signalling pathways; this disruption may indeed play a significant role in the development of allergies, autoimmune disorders, inflammation, cancer, and various other diseases; the intricate interplay between BPA exposure and immune system dysregulation (Xiang *et al.,* 2023). Karunarathne's findings shed further light on the inflammatory effects of BPA; at low concentrations, approximately ≤1 μM, BPA can trigger the release of proinflammatory mediators such as prostaglandin E2 (PGE2) and nitric oxide (NO), as well as proinflammatory cytokines like tumor necrosis factor alpha (TNF-α) and IL-6; these findings highlight the potential of BPA to exacerbate inflammation, contributing to the pathogenesis of various inflammatory conditions (Karunarathne *et al.,* 2021). Indeed, while IL-6 plays a crucial role in the body's immune response and host defence mechanisms, dysregulated and prolonged IL-6 production can lead to significant inflammatory issues; abnormal and prolonged IL-6 expression during immune events can result in systemic inflammatory response syndrome and cytokine release syndrome, these conditions are characterized by excessive inflammation throughout the body, which can lead to severe tissue damage and organ dysfunction; therefore, maintaining the balance of IL-6 production is essential for proper immune function and prevention of inflammatory complications. (Jordan *et al.,* 2017).

Absolutely, IL-6 is indeed classified as an inflammatory cytokine and plays a multifaceted role in both innate and adaptive immune responses, it is produced by various cell types, including immune cells such as macrophages, T cells, and B cells, as well as non-immune cells like fibroblasts and endothelial cells; IL-6 is involved in a wide range of physiological processes, including the regulation of immune cell differentiation, activation, and proliferation, it also contributes to the acute phase response, inflammation, and tissue repair; furthermore, IL-6 can

modulate the function of other immune cells and is implicated in the pathogenesis of various autoimmune diseases, inflammatory disorders, and cancer; its pleiotropic effects highlight its importance in orchestrating immune responses and maintaining immune homeostasis (Abbasifard and Khorramdelazad, 2020).

TNF-α is a cytokine with diverse effects on various cell types; it serves as a major regulator of inflammatory responses and has been implicated in the pathogenesis of numerous inflammatory and autoimmune diseases, as noted by Jang *et al.* (2021). Typically, TNF-α binds to its receptors, primarily TNFR1 and TNFR2, and initiates molecular signaling pathways that regulate biological functions such as inflammation and cell death, as discussed by Pobezinskaya and Liu (2012), this interaction between TNF-α and its receptors plays a crucial role in orchestrating immune responses and maintaining tissue homeostasis.

2.5. Medicinal Plants and Fertility:

Herbalism, an alternative or folk medicine practice, involves utilizing various parts of plants, as well as their extracts and oils, for medicinal purposes; this approach to healthcare has gained significant traction in recent years and has captured the attention of researchers worldwide; in certain Asian and African countries, as reported by the World Health Organization (WHO) in 2008, up to 80% of the population relies on traditional medicine for primary healthcare needs; this reliance underscores the enduring importance of herbalism and traditional healing practices in many cultures around the globe (Mosbah *et al.*, 2018). Indeed, a vast array of medicinal plants harbor pharmacologically active compounds, making them valuable for treating various ailments (D'Cruz *et al.,* 2010). Plants and their derivatives have historically played a pivotal role in global healthcare, owing to their diverse biological activities, it's noteworthy that approximately thirty percent of all modern drugs have origins in plants, furthermore, plants have a rich folklore of use in promoting fertility, with traditional knowledge highlighting their fertility-enhancing properties and aphrodisiacal qualities, this

longstanding cultural association underscores the multifaceted importance of plants in both traditional and modern healthcare practices, especially concerning reproductive health and wellness (Chaachouay and Zidane, 2024).

Complementary therapies for infertility have garnered increased attention in recent years; various antioxidants, nutritional approaches, and medicinal plants have been proposed as potential treatments for fertility issues in both infertile and sub-fertile couples, these complementary therapies often aim to address underlying factors such as oxidative stress, hormonal imbalances, and nutritional deficiencies that may contribute to infertility, while more research is needed to fully understand the efficacy and safety of these approaches, they offer promising avenues for individuals seeking alternative or adjunctive treatments for fertility problems (Abdi *et al*.,2017).

Certainly, various medicinal plants have been traditionally utilized worldwide for their antifertility or fertility-boosting effects, reflecting the rich heritage of herbal medicine across cultures; these properties have also become the subject of modern scientific research, indicating the enduring relevance and potential therapeutic value of these plants, as highlighted by Jain *et al.* (2015), WHO advocates for the exploration of medicinal plants and encourages researchers to delineate the rational use of these plants as potential sources for developing new treatments. This emphasis underscores the importance of integrating traditional knowledge with contemporary scientific approaches to unlock the therapeutic potential of medicinal plants in addressing fertility-related issues (Roozbeh *et al.,*2021).

Flavonoids and phenolic compounds found in certain plants are known for their potent antioxidant properties; these compounds play a crucial role in neutralizing oxygenated free radicals, thereby protecting sperm cells from oxidative damage by scavenging free radicals, flavonoids and phenolic compounds help to maintain sperm quality and improve various fertility

parameters; their antioxidant activity contributes to preserving sperm viability, motility, and DNA integrity, ultimately enhancing male fertility, this protective effect highlights the potential of plant-derived antioxidants as complementary therapies for addressing infertility issues (Hadree *et al.,* 2022). Among the most promising medicinal plants, which are believed to have healing and medicinal properties, *Nigella sativa L.,* is determined as one of the most famous plants that has been used for both the seeds and oil to promote health and fight diseases overwhelming thousands of years (Mosbah *et al.,*2018).

2.5.1. Nigella sativa:

Black cumin (*Nigella sativa L.*), belonging to the Ranunculaceae family, as classified in diagram below.

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Order: Ranunculales

Family: Ranunculaceae

Genus: Nigella

Species: *N. sativa* **) Ahmad *et al*.,2021).**

It is an herbaceous plant celebrated for its medicinal and aromatic properties; this dicotyledonous plant typically reaches heights of about 20-30 cm as illustrated in figure (2-4), its small yet potent seeds have been prized for their various health benefits and have been used for centuries in traditional medicine systems across different cultures (Demirci *et al.,* 2019), according to different climatic conditions and in various geographies (Sevim *et al*.,2021). Indeed, NS commonly referred to as black cumin or black seed in English and Habbat-albarakah in Arabic, is an aromatic, short-lived annual plant; it holds a significant

place in traditional medicine systems across various cultures due to its therapeutic properties (Mosbah *et al.,* 2018).

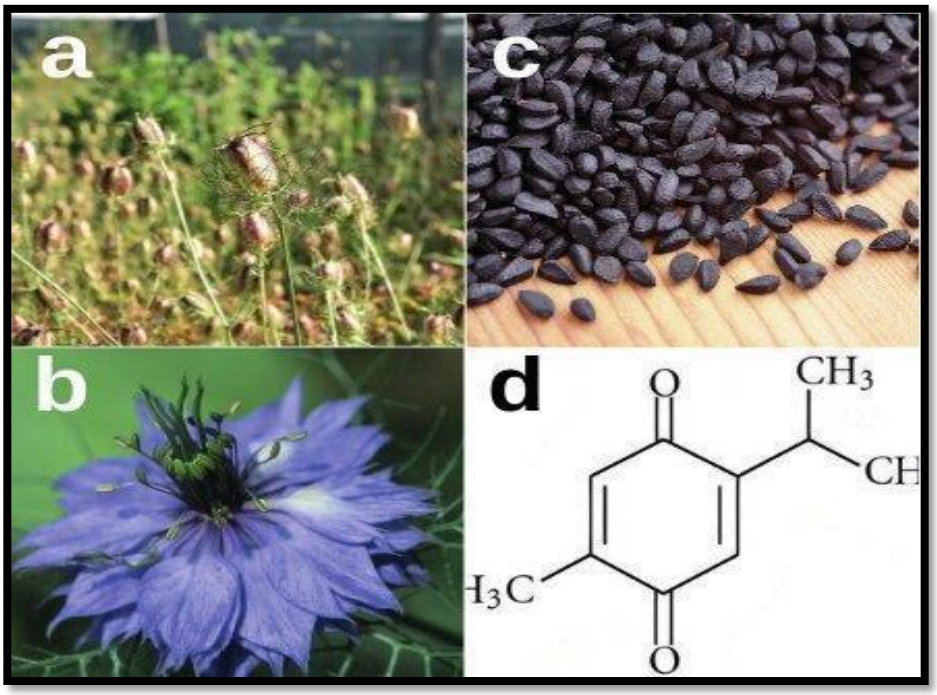


Figure (2-4): The Nigella sativa plant (a), its flower (b) and seeds (c); and the chemical structure of bioactive component of seeds, thymoquinone (TQ). (Dhaybi *et al.,* 2023).

Indeed, the seeds of NS have a long history of being revered for their healing properties, in Islamic religion, as referred by Prophet Muhammad to black cumin seeds as having potent healing powers, similarly, the seeds are mentioned in the Holy Bible as the "curative black cumin" additionally, in ancient medical texts, such as those attributed to Hippocrates, Dioscorides, and Pliny the Elder, NS is described as Melanthion, Discroides, and Gith, respectively, these historical references underscore the longstanding recognition of NS as a valuable medicinal plant across different cultures and civilizations (Mohammad *et al.*,2009). Black seeds contain 91.50-94.48% dry matter, 16.00-26.70% protein, 34.49-41.60% fat, 23.50-33.20% total carbohydrate, 24.90% nitrogen-free extract

matter, 7.94-8.40% cellulose, 3.77-4.86% ash, amino acids (arginine, glutamic acid, aspartic acid), minerals (Ca, K, P, Na, Fe, Se, Mg, Cu, Zn, Mn and carotene calcium), and vitamins (thiamin, riboflavin, niacin, pyridoxine, and folic acid, A and C), it is also contains 0.5-1.6% essential oils (nigellon, thymoquinone, dithymoquinone, thymohydroquinone, carvacrol, thymol, α and β pinene, dlimonene, p-cymen), alkaloids (nigellicine, nigellimine, and nigellidine), sterols (betasosterol, sykloartenol, sychloeikolenol, sterol esters, sterol glucosides), saponins, and quinones (Tufan *et al.,* 2015).

2.5.1.1. The Curative Effect of *Nigella sativa* (NS):

Nigella sativa (NS) is among the plants that are believed to fight diseases and promote health (Pakdel *et al.,* 2017). It is extensively employed as an ingredient in nutraceuticals, the concept of nutraceuticals emerged from the significant link between ideal nutrition and longevity, and it has garnered favor among dietitians, nutritionists, food scientists, physicians, and professionals in the food and pharmaceutical industries (Hannan *et al.,*2021; Hatipoglu *et al.,* 2023).

Işık *et al.* (2019), reported that the seeds of NS are rich in some fatty acids such as oleic acid, linolenic acid and palmitoleic acid. It is found that, the seeds are good source of protein, crude fat, crude cellulose, and macro minerals, and it contained 0.5-1.6% yellowish volatile fatty acid (Kumar *et al.,* 2017). Numerous studies have indicated that black seed possesses antimicrobial, antiviral, diureticreducing, antidiabetic, antitumoral, and antioxidant attributes attributed to its phenolic compound content (Usta *et al.,* 2016; Sevim *et al.,*2021).

2.5.1.2. The Effect of *Nigella sativa* L. on Fertility and Hormones:

Infertility presents as a multifaceted condition, encompassing both substantial medical and economic dimensions; approximately 30% of cases of infertility stem from male factors (Isidori *et al.,*2006), chemotherapy, drug

treatment, toxins, and environmental factors (AL Chalabi *et al.,* 2020). These factors can influence the function of natural hormones, impede spermatogenesis, and hinder the transformation of young spermatids into mature ones, consequently, the utilization of medicinal herbs has been widely documented for various purposes due to their accessibility, affordability, and the comparatively lower incidence of side effects associated with natural compounds compared to synthetic drugs (Adib-Hajbaghery and Rafiee ,2018).

Numerous herbal plants exert an influence on fertility, with one of the most significant benefits being their antioxidant properties, which promote spermatogenesis; these antioxidant compounds enhance hormone production, sperm count, and motility, thereby augmenting fertility (Khan and Khan,2014). Plant seed oil serves as a natural source of antioxidants and finds various medicinal applications (AL Chalabi *et al.,* 2020).

Multiple studies suggest the testicular protective effects of NS seeds; these seeds possess an impressive phytochemical profile, which can counteract toxicities induced by feed additives; they achieve this by mitigating excessive free radical production and enhancing the redox circuitry (Karimi *et al.,*2019). For example, thymoquinone, a prominent phytochemical found in Nigella sativa seeds, serves as a potent testicular protectant, exhibiting antioxidant, antiapoptotic, and endocrine modulatory properties (Hassan *et al.,* 2019; AbdElkareem *et al.,*2021).

Research has demonstrated that NS oil can mitigate the deterioration in epididymal sperm characteristics, furthermore, both NS oil and seeds have been found to enhance sperm production, semen quality, sperm count, and volume of semen, they also increase seminiferous tubular diameter, sperm motility, and percentage fertility, while decreasing the sperm abnormality index; additionally, they lead to increased hormonal levels (Mahdavi *et al.,*2015). Both the seed and

oil of NS have been reported to be safe for oral intake, exhibiting a high safety margin (Assi *et al.,* 2016). Related studies have shown that both the administration of NS seed and oil improve sperm counts, sperm viability, and motility, they also lead to increases in testicular weights, while decreasing sperm abnormalities and concentrations of testosterone (Hala,2011; Mahdavi *et al.,*2015). Likewise, elevated levels of reproductive hormones were observed following the administration of NS oil in male rats (Juma and Hayfaa, 2011). Moreover Assi *et al.,* (2016) told, NS oil used as a therapeutic rather than a prophylactic regimen.

2.5.2. The Curcumin:

Curcumin (Cur.) is a potent ingredient found in the turmeric plant, scientifically known as *Curcuma longa*; Its chemical name is "diferuloylmethane," and its chemical formula is $C_{21}H_{20}O_6$ (Eghbali *et al.,* 2023). As illustrated in Figure (2-4).

Curcumin, chemically known as 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6heptadiene-3,5-dione, is the primary curcuminoid found in *Curcuma longa*; it constitutes approximately 77% of the curcuminoids present, followed by desmethoxycurcumin at 17% and bisdemethoxycurcumin at 3%, widely recognized as a popular nutraceutical, Cur. is utilized worldwide for both medicinal and food purposes (Liu and Chen, 2013; Wang *et al*., 2017).

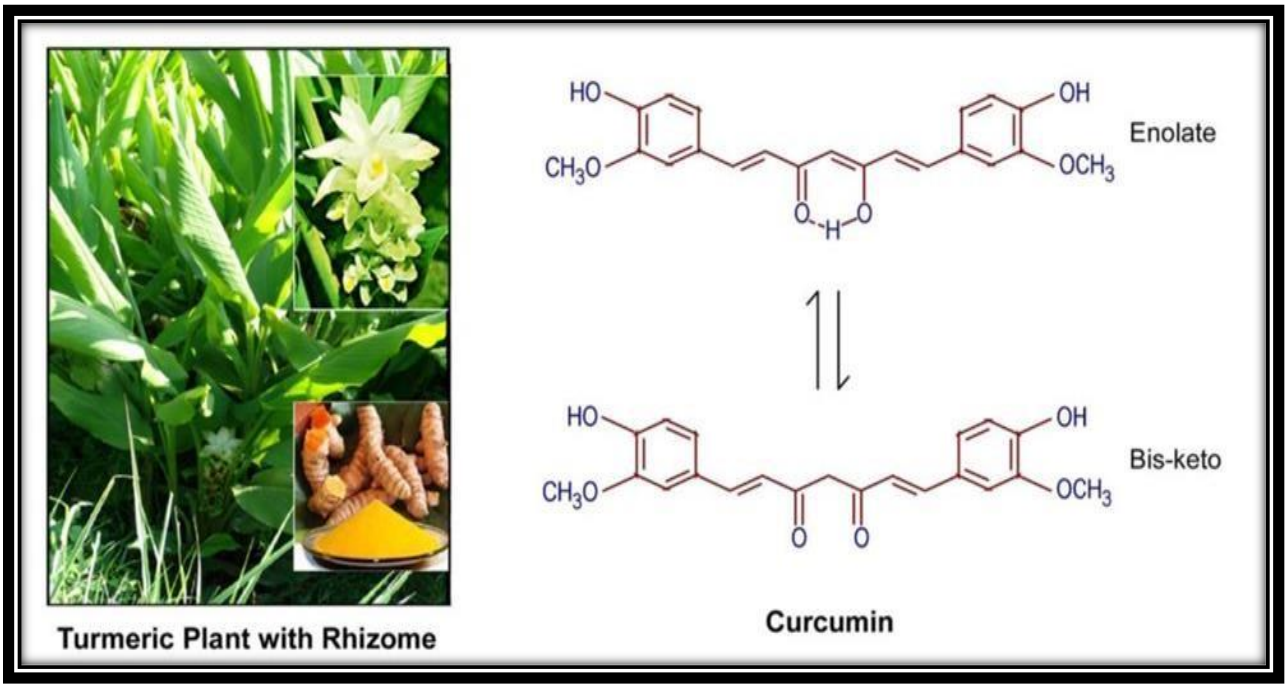


Figure (2-5): Curcuma longa Plant and chemical structure of curcumin, the active ingredient of rhizome turmeric (Sa and Das,2008).

Curcumin, the active component found in ground rhizome, or turmeric, is a well-known Indian spice; with a molecular weight of 368.4, it serves various purposes, including as a food preservative, food coloring agent, and herbal medicine (Mohebbati *et al.,*2017). The color yellow of turmeric is attributed to its polyphenolic pigments, specifically the curcuminoids (Farid *et al.,* 2017; Zahra *et al*., 2021).

Curcumin comprises approximately 2 to 8% of the compounds found in turmeric and is primarily responsible for its characteristic yellow/golden color; additionally, Cur. has been identified as the key component responsible for many of the beneficial properties associated with turmeric (Koroth *et al.,* 2019). Despite its low inherent toxicity, Cur. possesses a multitude of properties that have significant impact and applications in various pharmacological developments; these include its roles as an antioxidant, anti-inflammatory, antimicrobial, and anti-cancer agent, making it a valuable compound for drug development (Sun *et al.,* 2019; Mansouri *et al.,* 2020).

The median lethal dose of Cur. is reported to be 2 g/kg, clinical trials have demonstrated that continuous use of curcumin for up to 4 months showed no apparent side effects (Chandra *et al.,* 2007; El-Sherbiny *et al.,* 2022). Anecdotal reports indicate that dietary consumption of curcumin up to 150 mg per day is not associated with any adverse effects in humans (Sa and Das, 2008).

Curcumin is generally considered safe, and even at high doses, it has been found to be non-toxic to both animals and humans; the safety of curcumin has been reported by the United States Food and Drug Administration (FDA) (Liu *et al.,* 2016; Alizadeh *et al.,* 2018). However, some studies have reported adverse effects associated with it, including nausea, diarrhea, ulcers, rash, and yellow stool (Zahra *et al.,* 2021). Hence, various nano-preparations of Cur. can be utilized in cancer therapy due to its non-toxic nature. (Rahimi *et al.,* 2016; Alizadeh *et al.,* 2018).

Because of its low absorption in the intestines, Cur. is quickly cleared from circulation, as noted by Anand *et al.* (2007). Subsequently, in the liver, curcumin undergoes metabolism via glucuronidation and sulfation before being excreted in the feces and urine, as highlighted by Mirzaei *et al.* (2017).

2.5.2.1. Curcumin and Male Reproductive System:

The male reproductive system comprises the gonads and androgen hormones, the epididymites, the ductus or vasa deferens, the seminal vesicles, the ejaculatory ducts, penis, and various accessory structures like the prostate and bulbourethral glands, Cur. has been shown to affect this system and is known to target these structures (Noorafshan and Esfahani, 2013). The testis is a known target organ for injury resulting from exposure to both chemotherapeutic and toxic environmental agents, Cur. has demonstrated its ability to alleviate various forms of male reproductive disorders in experimental animals, thereby enhancing fertility (Khorsandi *et al*.,2013). The ameliorative effect of curcumin may be

attributed to its ability to scavenge free radicals and act as an antioxidant agent; additionally, it might enhance serum levels of testosterone, contributing to its beneficial effects on male reproductive health (Noorafshan *et al.,* 2011).

Study was carried out by Farombi *et al*., (2007) found the therapeutic effects of curcumin on the di-n-butyl phthalate (DBP)-induced testicular damage in rats. Mathuria and Verma (2008), investigated the effects of Cur. on toxicity induced in mice spermatozoa, the toxicity notably reduced sperm count, viability, and motility, leading to various morphological defects; however, treatment with curcumin improved the toxin-induced decrease in sperm count, immobilization, and viability, and enhanced the morphological characteristics of the sperm. Curcumin has been demonstrated to be a potent scavenger of various reactive oxygen species, including hydroxyl radicals, nitrogen dioxide radicals, and superoxide radicals (Tousson *et al*., 2014).

2.5.2.2. The Effect of Curcumin on Sperm and Hormones:

The antioxidant properties of Cur. play essential protective roles, particularly in the reproductive system; an appropriate balance between oxidants and antioxidants is crucial for the efficient performance of the reproductive system; studies have indicated that curcumin can safeguard the structural integrity and functional activity of spermatozoa, especially when oxidative damage to germ cells is elevated, this protective effect may be attributed to curcumin's phenol group, which helps prevent oxidative cellular damage (Azza *et al*.,2011).

Curcumin has shown potential for ameliorating both the antioxidant status and activity of reproductive cells and tissues in mice (Doust and Noorafshan, 2011). The balance between oxidant and antioxidant levels is often disrupted during the cryopreservation of semen, though, Cur. has been demonstrated to help preserve this balance through its antioxidant capacity (Soleimanzadeh and Saberivand,2013; Boroumand *et al .,*2018).

Alcoholic extract of turmeric has been found to enhance in vitro sperm activation in infertile men, however, further research is needed to investigate the specific influence of curcumin on sperm function, such as motility, capacitation, acrosome reaction, and fertilization in vitro, as well as its impact on fertility in vivo (Fakhrildin, 2011; Soni *et al*.,2015).

The inhibitory effects of Cur. on lipid peroxidation by scavenging the superoxide anion and hydroxyl radicals have been well documented (Daneshyar *et al.,* 2011). Several studies have indicated the beneficial effects of dietary supplementation with Cur. or turmeric on meat quality and stability, liver enzyme activity, and immune response (Zhang *et al.,* 2015). Moreover, Cur. has been shown to enhance semen quality when added to semen extenders during the freeze/thawing process (Kazemizadeh *et al.,* 2019).

In addition, there is growing evidence suggesting that, Cur. may exert potential gonad protective effects (Azza *et al.,* 2011) due to its antioxidant (Sahoo *et al.,* 2008), anti-inflammatory (Farombi *et al.,* 2007), anticancer (Cort *et al.,* 2012), and antiapoptotic activities, as well as its ability to modulate estrogen and androgen activities (Mohamadpour *et al.,* 2017). Evidence also shows that Cur. and several of its analogs may exert chemo-preventive and antitumor effects against reproductive system cancers in both females and males by modulating estrogen- and/or androgen-related pathways (Banik *et al.,* 2017; Mohajeri *et al.,* 2020).

Curcumin also exhibits a protective effect on DNA damage repair, prevents spermatogenic cell apoptosis, and aids in the development of testicular tissue (Bulku *et al.,* 2012). In livestock, curcumin has been utilized to enhance enzyme activity, performance, egg quality, and antioxidative state (Ahmadi, 2010). It has been reported that nursing lambs fed diets containing Cur. exhibit significantly

improved daily weight gain, production performance, metabolism, and immune performance (Jaguezeski *et al.,* 2018; Jiang et al., 2019; Molosse *et al.,* 2019).

With its wide array of health benefits and therapeutic roles, it seems unlikely that a single pathway can account for all these activities; studies have thus moved forward to investigate the underlying mechanisms contributing to the healthpromoting properties of this compound; according to conducted research, it can be hypothesized that a complex interaction between three main events inflammation, oxidative stress, and immunity likely contributes to the various therapeutic roles of Cur. (Menon and Sudheer, 2007; Jurenka, 2009; Boroumand *et al.,* 2018). Considering the wide range of pharmacological properties that curcumin offers, numerous studies have investigated its potency as a therapeutic agent in various diseases, fundamental research conducted on cell cultures, animal models, as well as pilot and clinical trials, has provided strong evidence for the health-promoting activities of this compound; Cur. is recognized as a powerful antioxidant, which has been acclaimed as a potent healing herbal component, reported to be 300 times more influential than Vitamin E according to scientific reports (Sahebkar *et al.,* 2015; Alizadeh *et al.,* 2018).

Chapter Three:
Methodology

3.1. Materials:

3.1.1. Instruments and Equipment:

All the devices utilized as a part of this study are summarized in table (3-1).

Table (3-1) Apparatus and equipment with their manufactures

NO.	Apparatus & Equipment	Company	Manufactures
1.	Anatomical set (Scissors, Forceps, Scalpel)	Chemo lab	India
2.	Balance	Denver	Germany
3.	Beakers)1000 500 ,250 ,100)	Chemo lab	India
4.	Centrifuge	Hettich	Germany
5.	Colony flask	Chemo lab	India
6.	Cotton	Entrepreneur	India
7.	Digital balance	Denver	Germany
8.	Disposable Syringes	Medical ject	.S.A.R
9.	ELISA - Reader and washer	BioTek	USA
10.	Eppendorf's tubes	Chemo lab	India
11.	Filter paper	Chemo lab	India
12.	Freezer	Hitachi	Japan
13.	Gel tube	Chemo lab	India
14.	Hot plate	Alssco	India
15.	Incubator	Biotelk	
16.	Insulin syringe	eldawlia	Egypt
17.	light microscope with camera	MEIJI	Japan
18.	Mince machine	Hitachi	Japan
19.	Oven	Daihan- Lab. Tech	Korea
20.	Pipette tips (10 – 1000) µl volume	Chemo lab	India
21.	Refrigerator	Kelon	Japan
22.	Rotary Microtome	Histo-Line Lab. Mod. MRS 3500	Italy
23.	Sensitive balance	Sartorius	Germany
24.	Slides and cover slides	China MHECO	China
25.	Spectrophotometer	Labomed	UK
26.	Staining Gar	Harshman	Germany
27.	Surgical gloves	Chemo lab	India
28.	Syringe (1 ml, 5 ml)	Chemo lab	India

29.	Test tubes	Chemo lab	India
30.	Water bath	FALC BI	Italy

3.1.2. Chemicals and Kits:

All the chemicals and the standard kits used in this study are shown in table (3-2)

Table (3-2): Chemicals and Kits with their suppliers

No.	Chemicals & Kits	Company	Suppliers
1	Aluminum and potassium sulphate	BDH	England
2	Bisphenol A (97% purity)	High media	India
3	Catalase (CAT)Activity Assay Kit	Solarbio	China
4	Chloroform	Scharlau	Spain
5	D.P.X	Thomas Baker	India
6	Eosin Stain	Himedia Lab Put. Ltd Company	India
7	Ethanol	,Merk	Germany
8	Follicle Stimulating Hormone (FSH) ELISA Kit	Solarbio	China
9	Formalin10%	TEDIA company	USA
10	Glacial acetic acid	SdfcL	India
11	Hematoxylin Stain	Himedia Lab Put. Ltd Company	India
12	Luteinizing Hormone (LH) ELISA Kit	Solarbio	China
13	Malondialdehyde (MDA) kit	Solarbio	China
14	Normal saline	Labort	India
15	Paraffin wax	,Merck	Germany
16	Perchloric acid	SdfcL	India
17	Rat Interleukin 6(IL-6) ELISA Kit	Solarbio	China
18	Rat Tumor necrosis factorα (TNF-α) ELISA Kit	Solarbio	China
19	Red Mercuric oxide	bdh	England
20	Superoxide Dismutase (SOD) Activity Assay Kit	Solarbio	China
21	Testosterone Hormone Kit	Solarbio	China
22	Xylene	Scharlau	Spain

3.2. Examination Methods:

3.2.1. Experimental Protocol:

Forty-eight adult male rats, with weights of about (200-350 grams) and ages between (eight-10 weeks) and were placed in the animal house of the College of Veterinary Medicine/ University of Kerbala, from November/2023 – March/2024, in special cages and these animals were provided with the appropriate conditions, in

terms of temperature round (25 ±5 C°), as well as ventilation and light system was 14/10 hours light/dark cycle with a relative humidity of 50±5%. These animals were left for two weeks for adaptation with standard experimental condition.

3.2.2. Ethical Approval:

The study was conducted in adherence to the ethical principles out-lined in the Helsinki Declaration. The study protocol, subject information, and per-mission form were examined and authorized by the local ethics committee under the reference number UOK. VET. PH.2023.082.

3.2.3. Experimental Design:

48 white male rats were divided into eight groups, figure (3-1). Each group consists of six male rats, which used as the following:

1- Group 1(Control Negative Group): Animals received orally normal saline, daily for 5 weeks.

2- Group 2: Animals received Bisphenol A(BPA) (100 mg/kg) of body weight daily, oral gavage for five weeks (Atabay and Kalender,2023).

3- Group 3: Animals received BPA (100 mg/kg) + Nigella sativa oil (85 µl/kg), orally gavage daily for five weeks (Fadishei *et al*.,2021).

4- **Group 4:** Animals received BPA (100 mg/kg) + Curcumin (100 mg/kg), orally gavage daily for five weeks (Atabay and Kalender,2023).

5- **Group 5**: Animals received BPA (100 mg/kg) + Curcumin (100 mg/kg) + Nigella sativa oil (85 µl/kg). Orally gavage daily for five weeks.

6- **Group 6**: Animals received Nigella sativa oil (85 µl/kg), orally gavage daily for five weeks.

7- **Group 7:** Animals received Curcumin (100 mg/kg), orally gavage daily for five weeks.

8- **Group 8**: Animals received Curcumin (100 mg/kg) + Nigella sativa oil (85 µl/kg). orally gavage daily for five weeks.

Chapter Three: Methodology

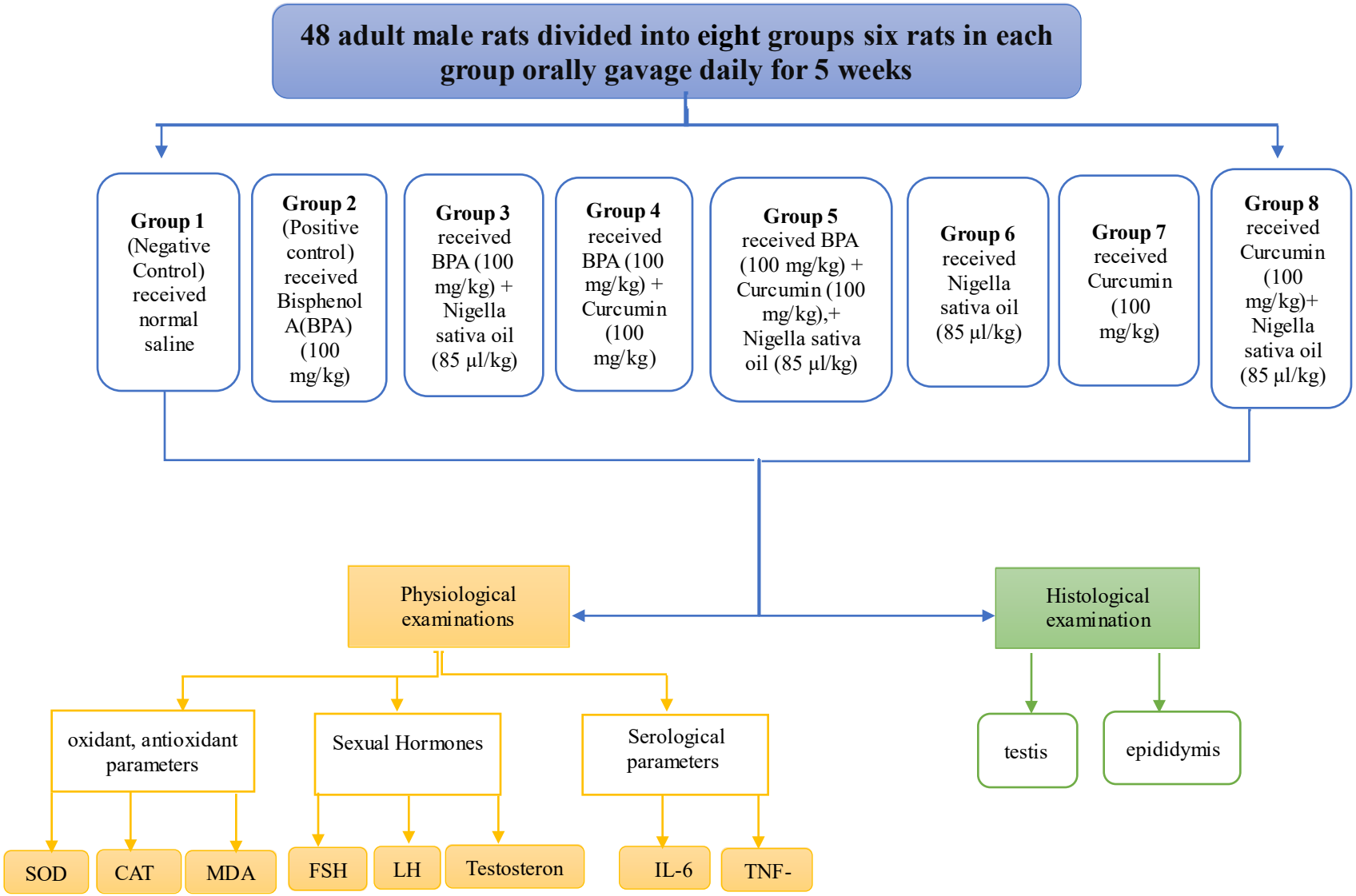

48 adult male rats divided into eight groups six rats in each group orally gavage daily for 5 weeks
Group 1 (Negative Control) received normal saline
Group 2 (Positive control) received Bisphenol A(BPA) (100 mg/kg)
Group 3 received BPA (100 mg/kg) + Nigella sativa oil (85 µl/kg)
Group 4 received BPA (100 mg/kg) + Curcumin (100 mg/kg)
Group 5 received BPA (100 mg/kg) + Curcumin (100 mg/kg),+ Nigella sativa oil (85 µl/kg)
Group 6 received Nigella sativa oil (85 µl/kg)
Group 7 received Curcumin (100 mg/kg)
Group 8 received Curcumin (100 mg/kg)+ Nigella sativa oil (85 µl/kg)
Physiological examinations
Histological examination
oxidant, antioxidant parameters
Sexual Hormones
Serological parameters
testis
epididymis
SOD
CAT
MDA
FSH
LH
Testosteron
IL-6
TNF-

Figure (3-1): Experimental Design

3.3. Samples Collection:

3.3.1. Collection of Blood Samples:

After ending the five weeks of the trial period, blood samples were taken, and before that, the animals were controlled and made comfortable by inhaling chloroform. Sterile medical syringes of 5 ml were accustomed to extract 5 ml of cardiac blood using the heart puncture method, and the blood was then centrifugated in a special gel tube that did not contain anticoagulant, at a speed of 4000 revolutions per minute for 5 minutes. In order to obtain the serum. Once the serum had been separated, it was put in Eppendorf tubes for storage and kept in the fridge at (-30°C) while the assays were working.

3.3.2. Collection of The Organs for Histological Section:

Animals were killed at the conclusion of the experiment after being put under chloroform anesthesia. The animals were then dissected to remove sections (testis and epididymis), and each of them was isolated separately. The organs were then preserved in formalin at a concentration of 10% in clean plastic containers after being numbered for use in the histological section. The two stains, Eosin and Hematoxylin, were made by adhering to the researcher's suggestions and the staining method's instructions. (Overmyer *et al*., 2015).

3.4. Physiological Parameters Measurement:

3.4.1. Estimation of Antioxidants Concentrations:

3.4.1.1. Estimation of Superoxide Dismutase (SOD) Activity:

Concentration of SOD activity was measured using specific kit for it depending on method of according to manufacturer instructions of the producing company, as shown **(appendix** I).

3.4.1.2. Estimation of Catalase activity:

Level of Catalase (CAT) activity was measured using specific kit to it. Depending on the method of manufacturer instructions of the producing company, as shown **(appendix** II).

3.4.1.3. Estimation of Malondialdehyde (MDA) activity:

The level of Malondialdehyde (MDA) activity was measured by ELISA specific kit Specific to measure MDA depending on method provided as instructions of the producing company, as shown **(appendix III**).

3.4.2. Estimation of Sexual Hormones:

3.4.2.1. Estimation of Testosterone Hormone:

Estimation of concentration of Testosterone hormone (TH) was measured using a specific Kit to measure hormone depend on method according to instructions of the producing company, as shown **(appendix** IV).

3.4.2.2. Estimation of Follicle-stimulating Hormone (FSH):

level of Follicle-stimulating hormone (FSH) was measured using a Kit to measure hormone depend on method of manufacturer instructions according the producing company, as shown **(appendix** V).

3.4.2.3. Estimation of Luteinizing Hormone (LH):

level of Luteinizing hormone (LH) was measured by a specific Kit used to measure hormone depend on method according to the instructions of manufacture that produced company, as shown **(appendix VI**).

3.4.3. Determination of Cytokines Levels:

3.4.3.1. Interleukin-6 (IL-6):

The level of IL-6 was measured using a specific kit of ELISA to it, depending on method of manufacturer instructions of the producing company, as shown **(appendix VII**).

3.4.3.2. Tumer Necrosis Factor-α (TNF-α):

Concentration of TNF-α was measured by Kit specific to it, according to method instructions manufacturer of the producing company, as shown **(appendix VII**I).

3.5. Histological Section Method:

3.5.1. Dehydration and Clearing:

Water was removed from the tissue by putting it through rising concentration of alcohol for two hours for each level of alcohol (70%, 80%, 90%, 95%, and 100%). The tissues were then submerged in xylene for five minutes (Bancroft *et al*., 2013).

3.5.2. Infiltration:

Following the lamination procedure, the samples were transferred to glass bottles containing (1:1) mixture of xylene and filter, which have a melting point of 57–60°C, and melted paraffin wax, which has a melting point of 57–60°C, in order to maintain the wax's melted state and ensure the complete impregnation process. For wax models, they were moved to other paraffin wax bottles inside the oven for two hours, then moved them again to other paraffin wax bottles for another two hours (Bancroft *et al.,* 2013).

3.5.3. Embedding:

In order to create wax molds that could hold the samples, special iron molds into which the models had been inserted were filled with wax. The models were then allowed to set at laboratory temperature before being removed from the mold and kept until it was time to cut them (Bancroft *et al.,* 2013).

3.5.4. Sectioning:

The models were cut to a thickness of 5 micrometers using a rotary manual microtome (Bancroft *et al.,* 2013).

3.5.5. Staining:

The following special dye were used to coloring the tissue sections of testis and epididymis (Bancroft *et al.,* 2013).

3.6. Statistical Analysis:

One-way ANOVA followed by Dunnett's multiple comparisons test was performed using GraphPad Prism version 10.0.0 for Windows, GraphPad Software.

Chapter Four

Results and Discussion

4. Results and Discussion:

4.1. Excluded Criteria:

As mentioned earlier, the last group (6th, 7th and 8th groups) used to detect that, the NS oil and Curcumin had no toxic effect and the toxic effect, if present, was due to BPA only. Group 6: Animals received Nigella sativa oil (85 µl/kg), orally gavage daily for 5 weeks. Group 7: Animals received Curcumin (100 mg/kg), orally gavage daily for 5 weeks. Group 8: Animals received Curcumin (100 mg/kg) + Nigella sativa oil (85 µl/kg). orally gavage daily for 5 weeks. Therefore, the effect of these compounds estimated on oxidative stress parameters (MDA, SOD and catalase), hormones of reproductive system (FSH, LH and testosterone) and cytokines (IL-6 and TNF-α). The results obtained, showed in the table (1) and figure (4-1: A, B and C).

The results confirmed that, there were no significant values (P value ≥ 0.05) between the control group and NS oil group, Curcumin group as well as NS oil group+ Curcumin group to exclude the effect of NS oil according to parameters used in this study.

Table (4-1): The effect of NS oil and Cur. on the oxidative stress parameters, reproductive hormones and cytokines.

Parameter	Mean of Control	Mean of NS oil	Mean of Cur.	Mean of NS oil+ Cur.	*P* value
MDA (µl)	16.12	20.46	20.10	18.76	0.6770
SOD (U/ml)	260.2	227.7	261.1	298.7	0.7587
Catalase (µl)	6.510	7.872	7.437	7.044	0.7398
FSH (ng/ml)	28.19	26.94	26.15	26.23	0.3965
LH (IU/L)	4.214	4.727	5.136	4.533	0.6306

Testosterone (pg/ml)	246.9	256.3	250.1	213.8	0.9817
IL-6	25.49	25.82	24.06	24.44	0.4938
TNF-α	125.4	154.3	149.9	145.2	0.7836

P value Significant ≤ 0.05

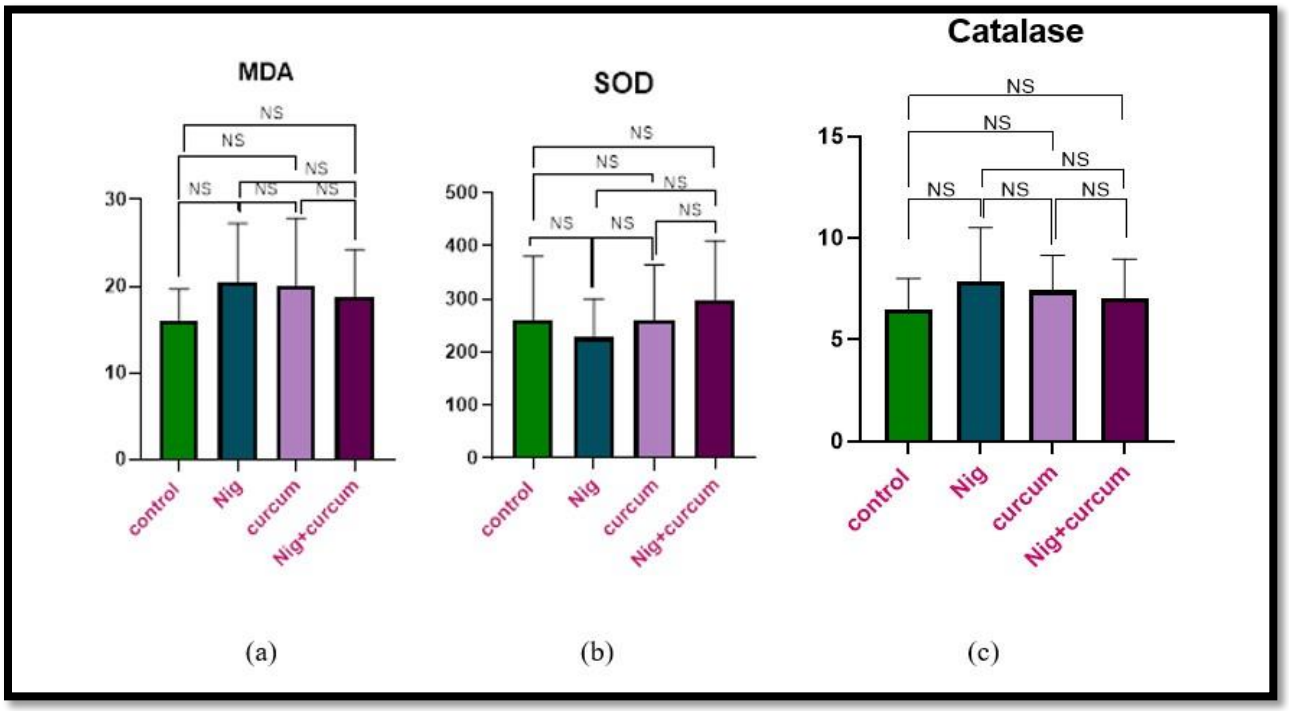


Figure (4-1 A): (a) the effect of NS oil, Cur. and Nig oil+ Cur. on the MDA, (b) The effect of NS oil, Cur. and Nig oil+ Cur. on the SOD, (c) the effect of NS oil, Cur. and Nig oil+ Cur. on the catalase.

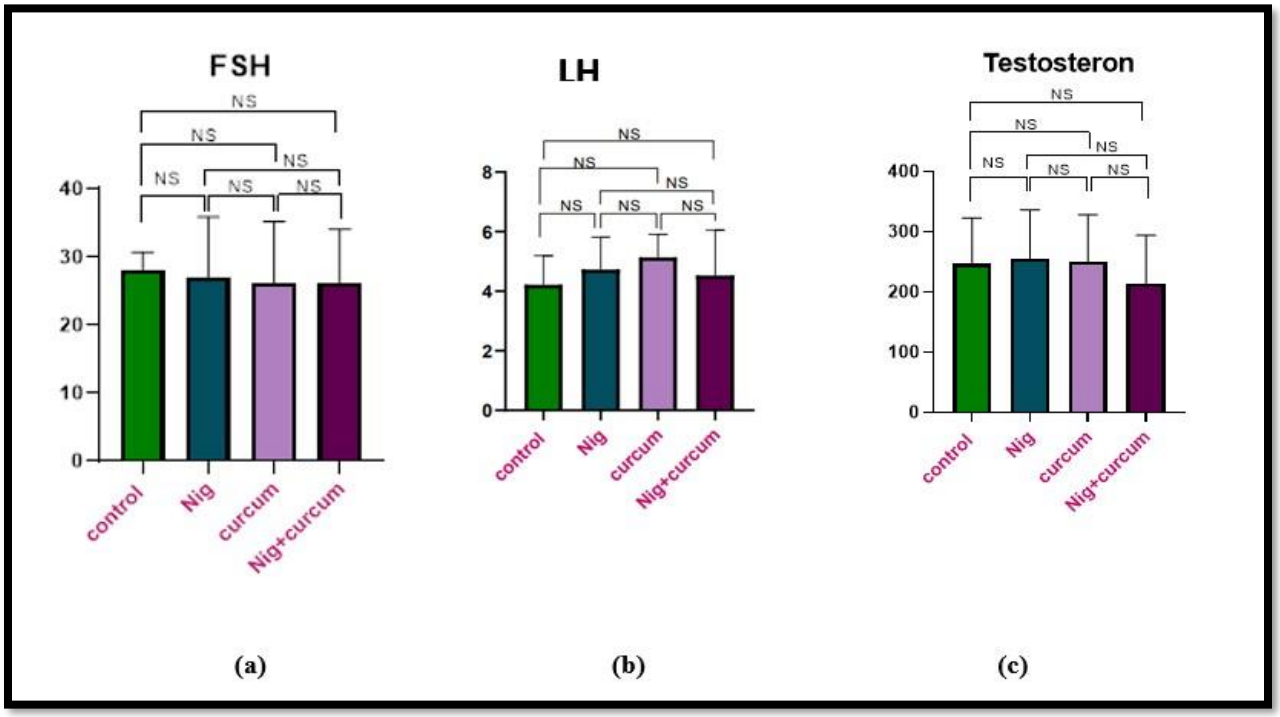


Figure (4-1 B): (a) the effect of NS oil, Cur. and Nig oil+ Cur. on the FSH, (b) The effect of NS oil, Cur. and Nig oil+ Cur. on the LH, (c) the effect of NS oil, Cur. and Nig oil+ Cur. on the testosterone.

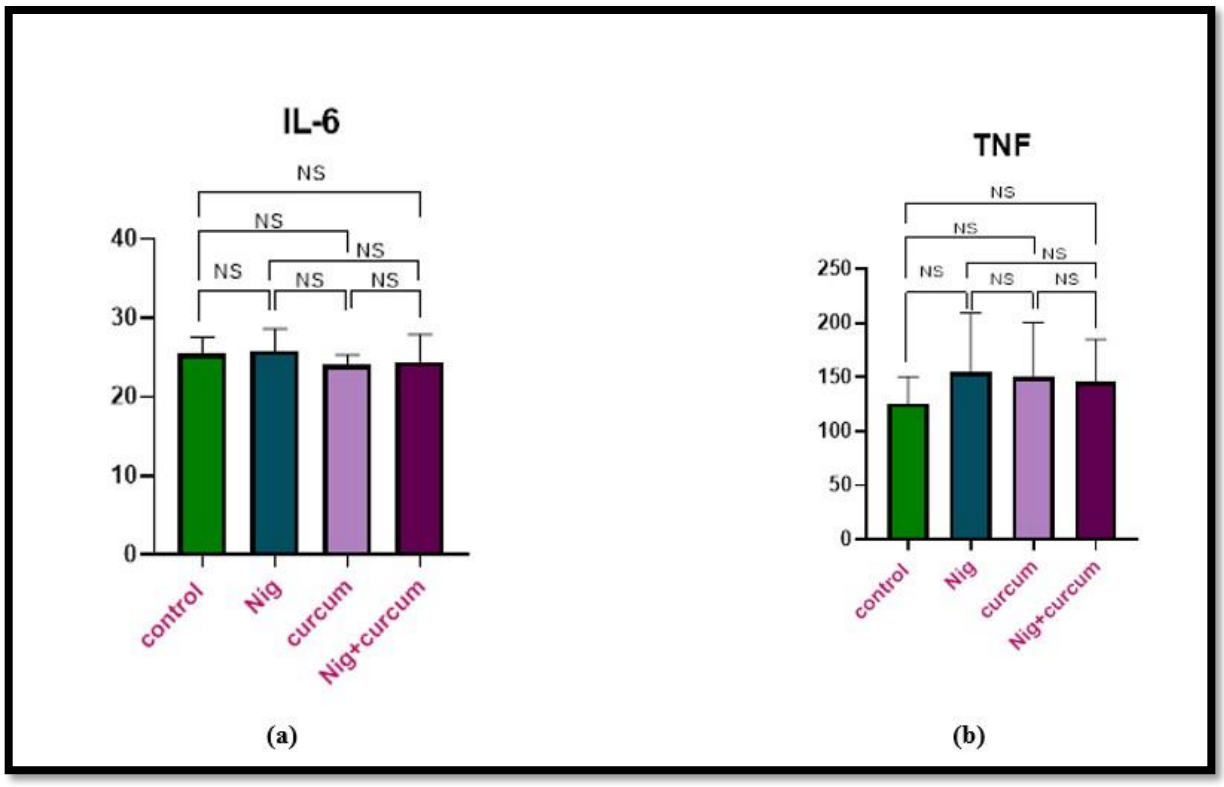
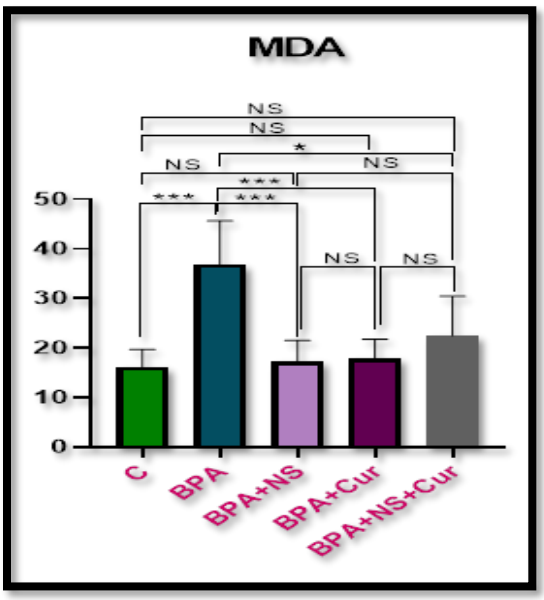


Figure (4-1 C): (a) the effect of NS oil, Cur. and Nig oil+ Cur. on the IL-6, (b) The effect of NS oil, Cur. and Nig oil+ Cur. on the TNF.

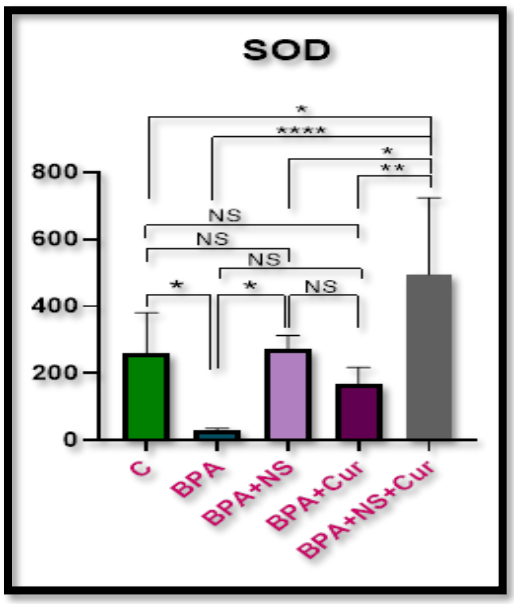
4.2. The Effect of NS oil and Curcumin Vs BPA on The Physiological Parameters:

4.2.1. The Effect on Oxidative Stress Parameters:

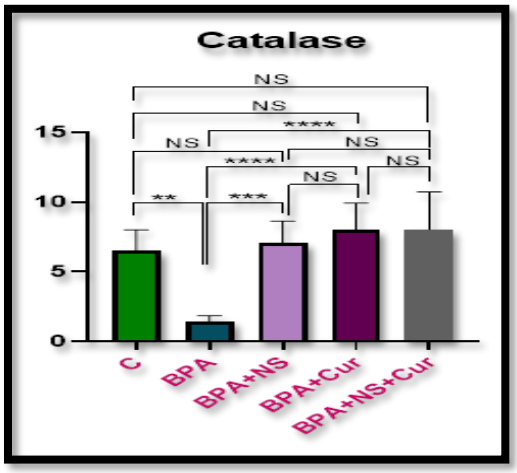
The effect of NS oil and Cur. as protective medicinal plants against BPA, on the oxidative stress parameters showed that, NS oil and Cur. had no significant values in MDA compared with the control group but in BPA group there was a significant increase in MDA (P value=0.0001), as shown in figure (4-2A) and, in the same time there were increase in the concentration of both SOD (P value=0.0003), and catalase (P value<0.0001) significantly, with decrease in BPA group, figure (4-2 B) and catalase figure (4-2 C) comparing with the control group.



(A) *P* value=0.0001



(B) *P* value=0.0003



(C) *P value*<0.0001

Figure (4-2): The effect of BPA as well as BPA+NS oil, BPA+ Cur. and BPA+NS oil+ Cur. on the MDA (A), SOD (B) and catalase (C).

Exposure to environmental toxins like BPA exacerbates the generation of ROS, causing oxidative stress in the testicles; furthermore, these toxins disrupt the balance between free radicals and antioxidants in the testis, ultimately impairing its function and negatively impacting male reproductive health (Meli *et al.,*2020). The utilization of medicinal plants had shown promise in enhancing semen volume and improving sperm quality among infertile males; investigations into the mechanisms of action of these herbs against infertility have revealed that their use significantly regulates not only the levels of male sex hormones but also enhances sperm motility and concentration (Roozbeh *et al.,* 2021; Ali *et al.,* 2022).

Exposure to BPA has been shown to impact sexual differentiation, brain development, and social behaviour; consequently, there was growing evidence suggesting that BPA's stimulation of ROS could significantly contribute to its toxicity and carcinogenicity (Attia *et al.,* 2021). In groups exposed to BPA intoxication, there was a notable reduction in the activity of SOD and catalase, accompanied by a considerable increase in MDA levels; this observation aligns with the findings of Geetharathan, who also reported similar results while investigating the effects of BPA on the tissues of pregnant rats (Geetharathan, 2016). In groups treated with BPA, the activity of MDA was increased, leading to BPA-induced cognitive dysfunctions and oxidative stress in those rats (Jain *et al.,* 2011). Bisphenol A (BPA) exhibited a markedly higher increase in MDA levels compared to the control rats ($p \leq 0.05$) (Abo-Zaid *et al*., 2022).

Various studies have documented the positive impacts of NS oil on sperm and reproductive parameters. So, El-Beshbishy *et al.* (2011) conducted an experiment, where male rats were orally administered BPA for 14 days. They observed a decrease in testicular antioxidant enzymes such as SOD and catalase; moreover, there was an

increase in the levels of hydrogen peroxide (H_2O_2) and lipid peroxidation in the testes and spermatozoa of BPA-treated animals.

The explanation for the improvement in reproductive functions was the decrease in levels of MDA and the restoration of antioxidant enzymes in reproductive organs; Lipid peroxidation not only leads to the failure of antioxidant mechanisms in preventing excessive of ROS formation, including oxygen radicals and their reaction products, but also causes damage to biological molecules, resulting in cell and tissue damage; besides, the increase in antioxidant status and the decrease in MDA concentration in the reproductive system in NS oil-treated group suggest a protective effect against oxidative stress and its detrimental effects on reproductive health totally (Ghlissi *et al.,* 2012).

Salem (2005) found that, the black seed oil and its active component, thymoquinone (TQ), exhibit antioxidant properties by bolstering the scavenger system, resulting in a protective effect against various insults. Equally, CiesielskaFiglon *et al.,* (2023) had reported that, the use of NS oil elevated the activity of the antioxidant defence system; additionally, NS oil treatment reduced tissue MDA levels and prevented the inhibition of SOD and catalase enzyme activities.

Atabay and Kalender, (2023) demonstrated that Cur. mitigated the impact of BPA, suppressing the antioxidant enzyme system in host tissues; this resulted in a notable decrease in MDA levels and a significant increase in catalase and SOD compared to rats treated with BPA alone; therefore, Cur. partially prevented the adverse effects induced by BPA. The inclusion of phenolic compounds within the structure of Cur. was crucial for elucidating its capability to counteract oxygenderived free radicals, which were accountable for lipid peroxidation within cells (Apaydin *et al.,* 2019). Curcumin exerted a protective influence on SOD and CAT, whose levels decrease due to BPA induction; additionally, Cur. diminished lipid peroxidation, as evidenced by reduced MDA levels (Elsayed *et al.,* 2016).

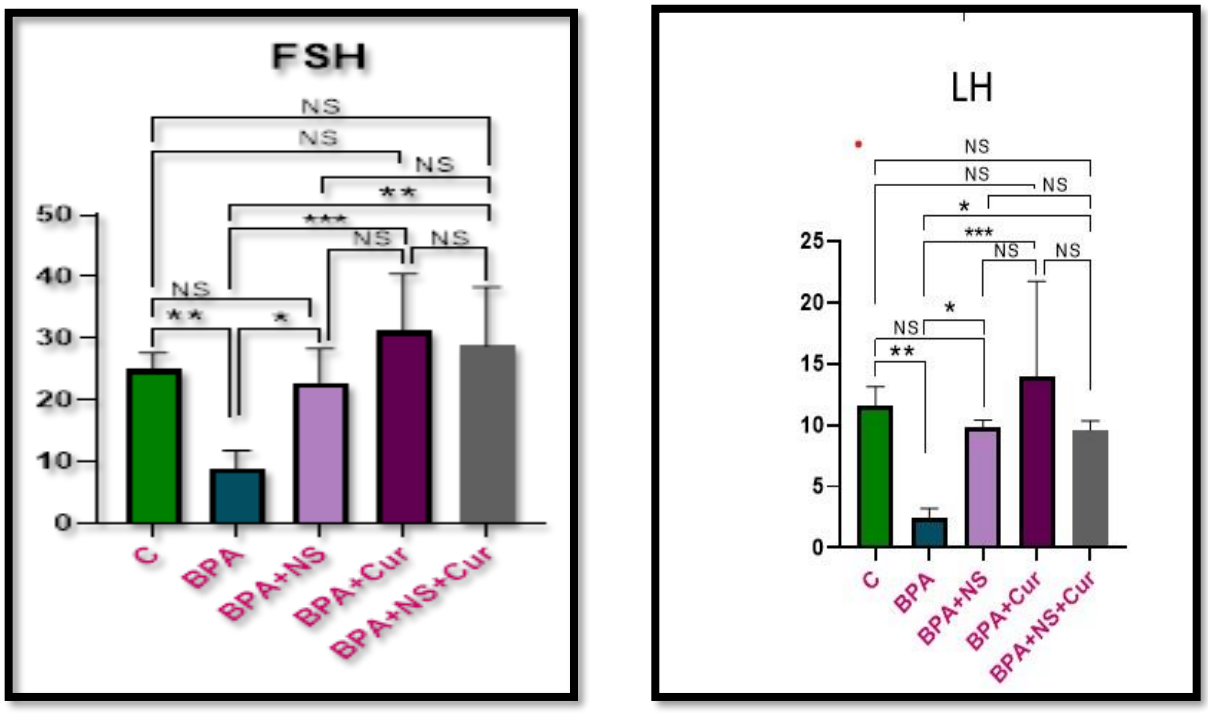
Curcumin had the potential to halt peroxidative changes in sperm and testicular membranes, resulting in improved sperm motility and a reduction in sperm defects; furthermore, the administration of Cur. might alleviate the suppression of antioxidant enzymes or even stimulate their synthesis, thereby mitigating the oxidative harm induced by BPA (Noorafshan and Ashkani-Esfahani, 2013). So, it maintained the structural and functional integrity of seminiferous tubules to a degree comparable to that of the control group, indicating its protective effect on the testes.

As a result, in the current investigation, co-administration of curcumin leads to significant improvements in antioxidant enzymes and lowered oxidative stress.

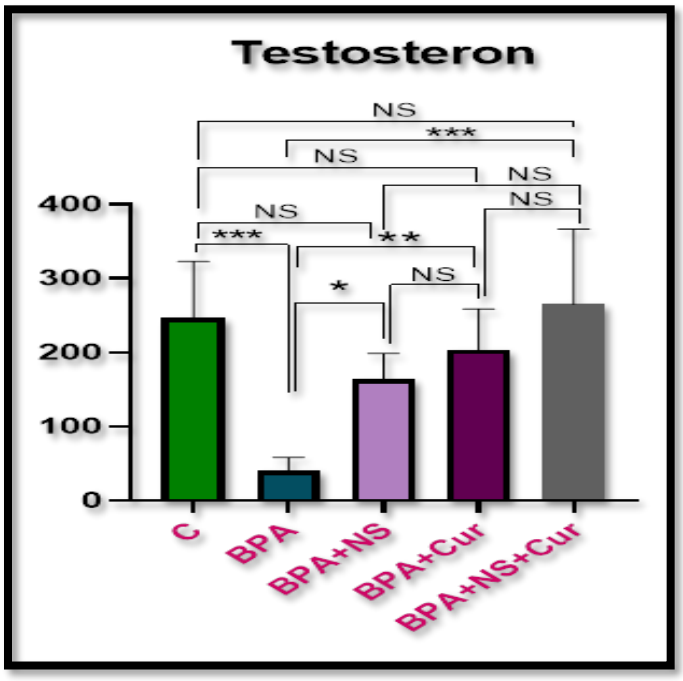
The results found that there were no significant differences between the NS oil+ Cur. group together and NS oil group or Cur. group alone on the oxidative stress parameters except SOD. Suggested that, there was no synergistic effect between two herbs in case of MDA and catalase groups. In spite of that, Abd El-Kader *et al.,* (2018) told that, administration treatment of combination of NS extract and Cur. extract together had synergistic effects in renal damage improvement.

4.2.2. The Effect on The Reproductive Hormones:

The effect of BPA, NS oil, Cur. and NS oil+ Cur. on the reproductive hormones comparing with the control group showed in figure (4-3), and the results revealed that, there were significant increase in FSH (P value=0.0001), LH (P value=0.0003) and testosterone (P value<0.0001) in the groups of BPA+NS oil, BPA+CUR. and BPA+ NS oil+ CUR , as well as, decrease in the levels of these hormones in BPA group comparing with the control.



(A) *P* value=0.0001 **(B)** *P* value=0.0003



(C) *P value*<0.0001

Figure (4-3): The effect of BPA as well as BPA+NS oil, BPA+ Cur. and BPA+NS oil+ Cur. on the FSH (A), on the LH (B) and on the testosterone (C).

Certain concentrations of BPA and its analogues have been associated with decreased levels of LH, FSH, and testosterone as mentioned in some studies. This effect was particularly noticeable when exposure happened during adulthood, affecting the HPG axis , the findings suggested that, BPA and its analogues competitively bind to androgen receptors (AR) and ER , while also stimulating KiSS1 expression in the hypothalamus, this disturbance in the HPG axis feedback mechanism led to alterations in GnRH secretion, consequently resulting in fluctuations in pituitary secretion of LH and FSH, most studies had reported lower levels of these hormones as a result of these effects (Shamhari *et al.,* 2021).

Moreover, BPA had been observed to decrease testosterone concentration in both serum and testicular tissue, with more pronounced effects seen in higher dose groups compared to lower dose groups; it was hypothesized that, BPA could potentially impair Leydig cells, leading to a decline in intratesticular testosterone levels and subsequently suppressing spermatogenesis; however, this hypothesis requires validation through further experiments (Zang *et al.,* 2016). Moreover, Wisniewski *et al.* (2015) uncovered that, all hormones were assessed and found to be altered in groups exposed to BPA; specifically, blood concentrations of FSH were decreased by 38%, LH concentrations were reduced by 65%, and testosterone levels were diminished by 98% in these BPA-treated group.

Oral administration of NS oil had been shown to enhance fertility and improve the reproductive system in adult male rats; this improvement in reproductive function is likely attributed to its constituents, including proteins, vitamins such as A, B, and C, as well as essential minerals like zinc, copper, and magnesium, which contribute to increased weight of reproductive organs; furthermore, black seeds oil contain alkaloids and phenols, which are believed to stimulate the secretion of testosterone, further enhancing reproductive health in male rats (Juma, 2011). Administration of NS oil alone, notably elevated plasma testosterone, LH, and FSH levels; leading to a considerable correction of hormone level imbalances and

subsequent enhancement of semen quality, Khamis *et al.* (2023) observed a significant increase in reproductive organ weight and circulating levels of testosterone, FSH, and LH in rats treated with NS, suggesting activation of the hypothalamic-pituitary-testicular axis, which stimulated steroidogenesis and spermatogenesis processes.

Our findings revealed an elevation in testosterone, FSH, and LH levels in the group treated with Cur. +PBA agreed with studies by Alizadeh *et al.* (2018). Karimi *et al.* (2019), suggested that Curcumin could enhance reproductive hormone levels in male rats, possibly by safeguarding Leydig cells, this aligned with prior research demonstrating Curcumin's protective effects through its anti-apoptotic, antioxidant, and antigenotoxic properties (Akinyemi *et al.,* 2015). Furthermore, Cur. inhibited cortisol secretion by suppressing adrenocorticotropic hormone and increasing mRNAs encoding steroid-controlling proteins (Mohamadpour *et al.,* 2018). The report had also indicated a significant increasing in serum testosterone levels following treatment with Curcumin (Alizadeh *et al.,* 2018).

In a study conducted by Noorafshan and Ashkani-Esfahani (2013), they were showed that, Cur. protected Leydig cells of mice from the damage caused by consumption of harmful materials. It was seen that, consumption of these materials inhibited testosterone production and caused testicular atrophy and enhanced mitochondrial diameter by more than three folds; while in Cur-treated mice the necrosis of Leydig cells was decreased (Giannessi *et al.,* 2008).

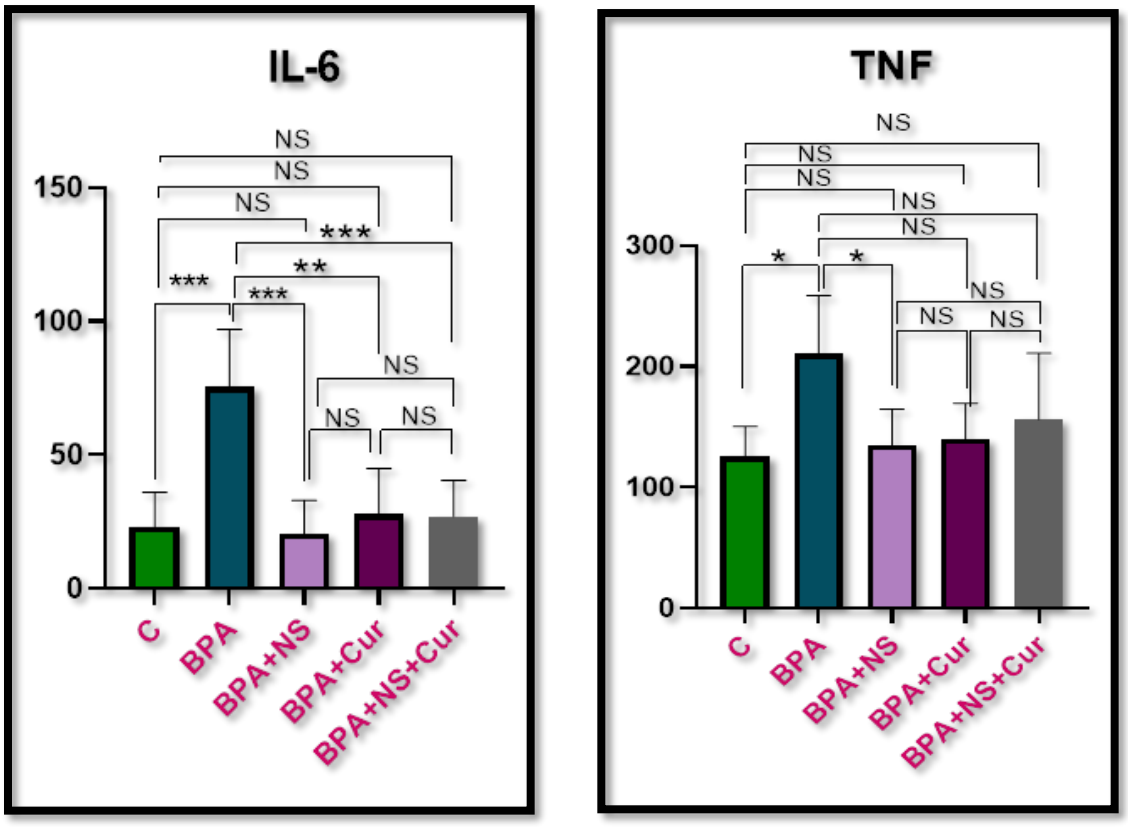
According to the National Cancer Institute, Curcumin is deemed nontoxic, nonmutagenic, and nonteratogenic; it could inhibit lysosomal enzymes by stabilizing the membrane; and positive effects of Cur. on prostate cancer had been noted (Dorai and Katz, 2001). This indirectly suggested that, Cur. notably improved sperm parameter from toxicity; indeed, treatment with Cur. alone showed no adverse effects on sperm count, viability, motility, or morphological structures (Mathuria and Verma, 2008). Multiple studies had investigated the effects of Cur. on various sexual

glands, including ovaries, breasts, testes, and endometria, these effects were attributed to its anti-inflammatory (Farombi *et al.,* 2007), anticancer (Cort *et al.,* 2012), antioxidant (Sahoo *et al.,* 2008), and anti-apoptotic properties (Zahra *et al.,* 2021). Additionally, Cur. had been reported to possess biological properties such as being an antineoplastic and anti-mutagenic agent by several investigations (Andrew and Izzo, 2017).

Our results found that, there were no significant differences between the combination group of NS oil+ Cur. and NS oil group or Cur. group alone on the reproductive hormones. Suggested that, there was no synergistic effect between two herbs.

4.3. The Effect of NS oil and Curcumin Vs BPA on The Cytokines:

The effect of NS oil, Cur. and the combination of NS oil+ Cur. on the cytokines showed in the figure (4-4), and the results were obtained found that, there were a significant decrease of IL-6 and TNF-α, (P value= 0.0001) and (P value=0.02) respectively in groups NS oil, Cur. and the combination of NS oil+ Cur comparing with BPA group and control group.



(A)

***P value*= 0.0001**

(B)

***P* value=0.02**

Figure (4-4): The effect of BPA as well as BPA+NS oil, BPA+ Cur. and BPA+NS oil+ Cur. on the IL-6 (A) and on the TNF-α (B).

The impact of BPA on immune system cells had been extensively documented, albeit with variations depending on the specific model used; it was worth noting that fluorescence quantification revealed a 2.9-fold increase in BPA-induced levels of IL-6 compared to the control group; similarly, when comparing the expression levels of TNF-α between the control or vehicle groups with those exposed to BPA, there was a 3-fold increase in TNF-α expression (Palacios-Arreola *et al.,* 2022). Also, BPA triggered a pro-inflammatory response within the cells, characterized by an increase in the expression of IL-6 and TNF-α (Chen *et al.,* 2018). According to a study done by Hassani *et al.* (2017), exposure to BPA was associated with elevated serum levels of inflammatory biomarkers IL-6 and TNF-α.

The levels of IL-6 serving as a cellular safety marker, and TNF-α, a cytokine, hold significance during the acute phase of inflammation (Mijwel *et al.,* 2023; AlMayah *et al.,* 2023). Elevated levels of these factors are observed in response to chemical substances like acetaminophen, carbon tetrachloride and BPA, contributing to liver inflammation (You *et al.,* 2008). Treatment with NS oil at various doses led to a notable reduction in serum IL-6 levels compared to the BPAgroup; additionally, administration of NS oil at doses of 21, 42, and 84 µL/kg significantly mitigated TNF-α levels ($p<0.05$) compared to the BPA-group (Fadishei *et al.,* 2021). Given that, heightened levels of IL-6 and TNF-α were indicative of insulin resistance and inflammation disorders resulting from exposure to BPA, and considering the evidence from the current study on emerging factors, it can be inferred that TQ and NSO may be utilized to alleviate symptoms associated with insulin resistance disorders (Zelová and Hošek, 2013).

Various mechanisms underlying the modulation of cytokine/chemokine formation/release by BPA had been suggested; one such mechanism proposes that, these effects were linked to the impact of the agents on ER in immune cells; because BPA exhibited estrogenic activities for both ER-alpha and ER-beta; furthermore, BPA selectively recruits co-regulators to the bisphenol-ER-alpha complex, potentially accounting for the divergent biological actions induced by these agents; besides, Qiu et al. demonstrated that BPA and BPS up-regulated TNF-α and IL-6 (Qiu *et al.,* 2018).

Cho *et al.,* (2007) found that, Curcumin suppressed the expression of TNF-α and IL-6, demonstrating its anti-inflammatory characteristics and its capacity to hinder the progression of inflammatory pathways; furthermore, it mitigated the expression of TNF-α and IL-6 in neutrophils under inflammatory conditions, thereby reinstating the delayed apoptosis of neutrophils (Cho *et al.,*2020)

Curcumin additionally restrained the release of inflammatory cytokines from macrophages; it achieved this by inhibiting MCP-1 production through the

suppression of NF-κB pathways; consequently, there was a notable reduction in the production of inflammatory cytokines like IL-6 and TNF-α; moreover, curcumin displayed the ability to impede macrophage inflammation and apoptosis by suppressing the extracellular regulated protein kinase (ERK) signalling pathway (Allegra *et al.,* 2022).

Considering their activation patterns and functions, macrophages can be classified into two subtypes: classically activated M1 macrophages and alternatively activated M2 macrophages; M1 macrophages are known for their proinflammatory actions and tissue-damaging effects, while M2 macrophages contribute to antiinflammatory responses and tissue remodelling Cur. directed macrophages towards the M2 phenotype by activating peroxisome proliferator-activated receptor γ ; furthermore, in another investigation, curcumin facilitated the conversion of M1 macrophages into the M2 phenotype by inhibiting the TLR4/MAPK/NF-κB pathway (Yang *et al.,* 2024).

4.4. Histopathological Study:

Histopathological examination of testes and epididymis for each group under study was done, and the results revealed in the following figures, figure (4-5) had shown the testicle of a rat from the control group (injected with the normal saline) with the normal structure of the testicular tissue. The middle cavity is filled with sperm, with Leydig cells in the interstitial tissue, the germinal epithelial cell layer and also, sperm progenitors. Figure (4-6) had shown the epididymis of a rat in the BPA-treated group (100 mg/kg B.W.), noted that, the lack of sperm in the lumen of the tubule, the decrease in the diameter of the lumen of the tubules and also, the destruction of the epithelial cells lining the tubules.

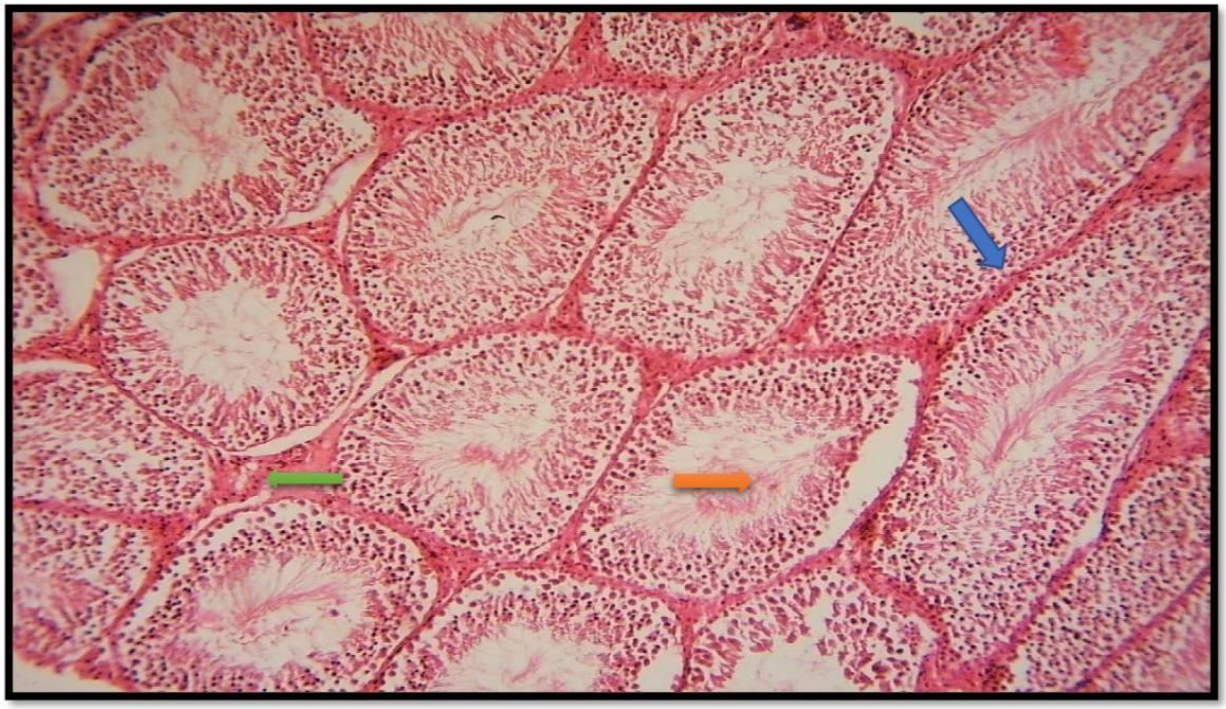


Figure (4-5): A cross-section of the testicle of a rat from the control group showing the normal structure of the testicular tissue. The middle cavity is filled with sperm ( ) with Leydig cells in the interstitial tissue ( ) and the germinal epithelial cell layer ( ) (H & E 40X)

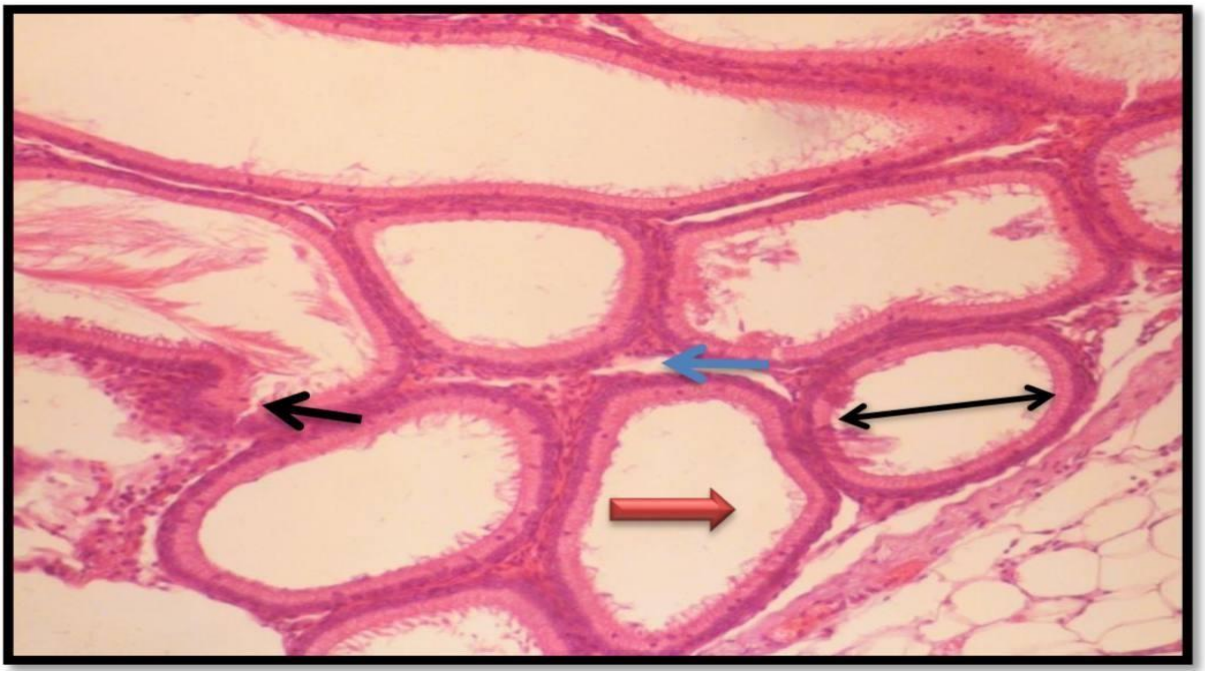


Figure (4-6): a cross-section of the epididymis of a rat in the group treated with Bisphenol (100 mg/kg B.w.), showing the lack of sperm in the lumen of the tubule ( ), the decrease in the diameter of the lumen of the tubules ( ), and the destruction of the epithelial cells lining the tubules) and the lack of Peritubular smooth muscle ( ) (H&E 40X).

The figure (4-7) shown the testicle of a rat in the group treated with BPA (100 mg/kg) of body weight, noted that there were interstitial spaces between the seminiferous tubules, few sperm in the cavities of the tubules, reduction and disintegration of the interstitial tissue, lack of Leydig cells and a decrease in the size of the germinal epithelial cell layer with cell degeneration necrosis of cells lining the tubules.

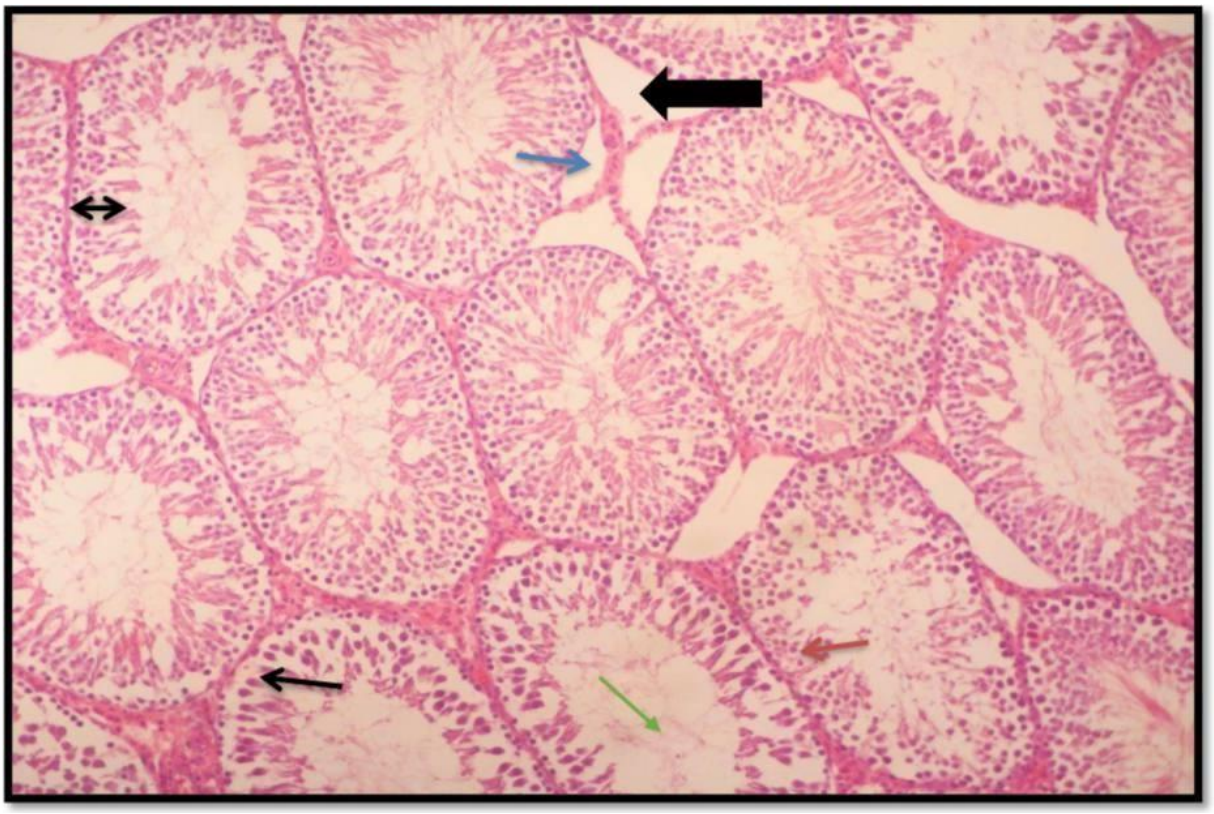


Figure (4-7): A cross-section of the testicle of a rat in the group treated with Bisphenol A (100 mg/kg) of body weight, in which it is noted that there are interstitial spaces between the seminiferous tubules ( ) and few sperm in the cavities of the tubules ( ) with reduction and disintegration of the interstitial tissue and a lack of Leydig cells ( ) and a decrease in the size of the germinal epithelial cell layer ( ) with cell degeneration ( ) necrosis of cells lining the tubules ( ) (H & E 40X).

A notable decrease in seminiferous tubule diameter was showed in animals receiving the highest BPA dosage (100 mg/kg BW), with even those subjected to the lowest dose exhibiting seminiferous tubules lacking spermatozoa (Mendiola *et al.*, 2010). Moreover, there were indications of seminiferous epithelium disintegration,

including loosening intercellular bridges between germ cells; additionally, immature and degenerating germ cells were observed in the tubular lumen (Kato *et al.,* 2006). The reduced diameter of the seminiferous tubules could potentially be linked to the thinning of the seminiferous germinal epithelium, particularly at the highest BPA dosage (Gurmeet *et al.,* 2014).

Exposure of male mice to BPA through oral ingestion suggested potential concerns related to infertility, genital tract malformations, and increased cancer rates in estrogen-sensitive target tissues (De Toni *et al.,* 2020). Certainly, BPA has been identified as a potential testicular toxicant in animal models, as indicated by Ullah *et al.* (2019). Additionally, adverse effects of BPA exposure on the developing testis and prostate stem cells of rodents have been reported (Maamar *et al.,* 2015).

In mice, BPA triggered the development of morphologically multinucleated giant cells in testicular seminiferous tubules, characterized by possessing more than three nuclei each (Kamel *et al.,* 2018). Likewise, a reduction in sperm counts and motility was noted, along with an increase in sperm morphological abnormalities, after 2 weeks of BPA administration at doses ranging from 10 to 40 mg/kg body weight (Cariati *et al.,* 2019). In humans, BPA has also been associated with adverse effects; a study conducted in China revealed that male factory workers exposed to high levels of BPA at work experienced sexual dysfunction, manifested by reduced sexual desire and increased difficulties with erectile function and ejaculation (Hart, 2020).

Besides, Mendiola *et al.,* (2010), were observed that, there was a significant alteration in the histology of the reproductive tissues, characterized by a reduction in the number of sperm in the lumen of the epididymis and a decrease in the height of epithelial tissues of seminiferous tubules. This outcome was not unexpected concerning estrogen; while it was crucial for normal epididymis function at specific concentrations, it exerted inhibitory effects on the brain, pituitary, and gonadal axis

in males; It was well-documented that, elevated levels of E2 inhibit spermatogenesis and the secretion of testosterone in the testes (Ullah *et al.,* 2018).

The results of cross-section of the testicle of a rat from the NS oil treated group revealed in the figure (4-8). The figure, shown normal tissue of the testicle with seminal tubules, and its cavities filled with sperm with Leydig cells in the interstitial tissue and layer germinal epithelial cells, and also, sperm progenitors.

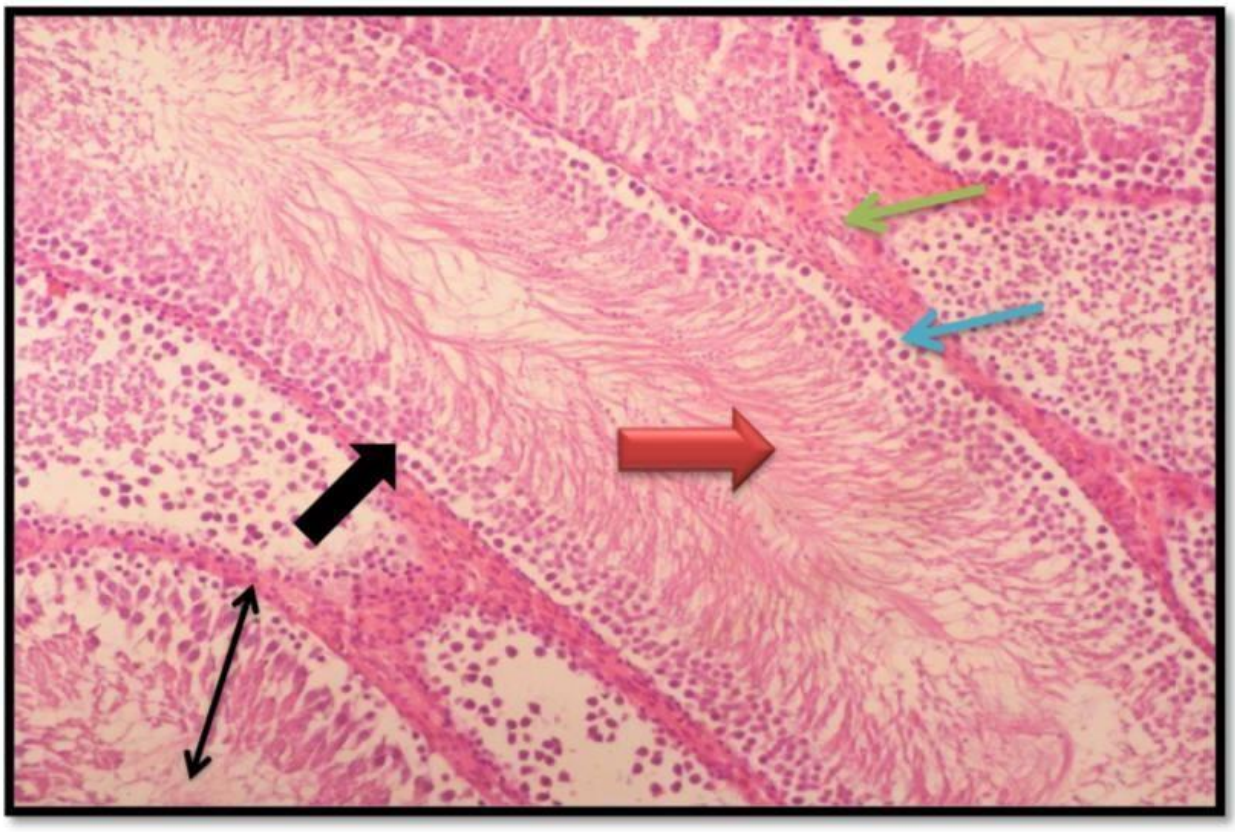


Figure (4-8): A cross-section of the testicle of a rat from the treat ed group: NS oil (85 µl/kg) of body weight, showing normal tissue of the testicle with seminal tubules ( ) and its cavities filled with sperm ( ) with Leydig cells in the interstitial tissue ( ) and layer Germinal epithelial cells ( ) and sperm progenitors ( )(H&E 40X).

The figure (4-9) of the cross section of the epididymal duct of a rat in the group introduced BPA (100 mg/kg) and NS oil (85 µl/kg) shown the normal structure of the epididymis with regular epididymal tubules and increased sperm numbers in

Epididymal lumen. As well as, peritubular smooth muscle cell pleocytosis, and increased diameter of the epididymal tubules.

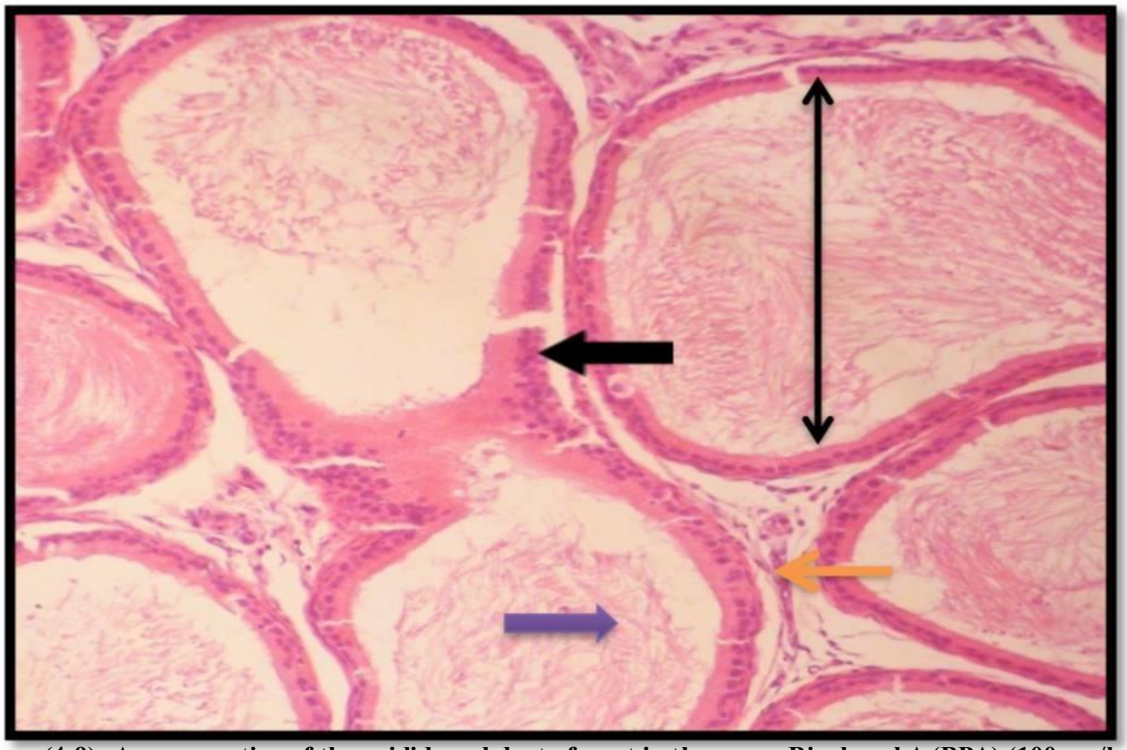


Figure (4-9): A cross-section of the epididymal duct of a rat in the group Bisphenol A (BPA) (100 mg/kg) with Nigella sativa oil (85 µl/kg) showing the normal structure of the epididymis with regular epididymal tubules ( ) and increased sperm numbers in Epididymal lumen ( ) and peritubular smooth muscle cell pleocytosis ( ) and increased diameter of the epididymal tubules ( ) (H & E 40X).

Figure (4-10) showed cross-section of the epididymal duct of a rat in the group introduced BPA (100 mg/kg) and NS oil (85 µl/kg), the results improvement the normal structure of the epididymis with regular epididymal tubules. Also, the presence of sperm in the lumen of the epididymis, and the presence of stationary cilia.

The function of accessory reproductive organs and the spermatogenesis process are reliant on androgens. Consequently, a notable enhancement in cauda epididymis sperm motility was noted in the experimental group. This augmentation might be attributed to the functional impact of NS on oxidative phosphorylation enzyme

activity (Mohammad *et al.,* 2009). The presence of antioxidant compounds in black seeds suggests a potential for enhancing tissue quality and function (Pakdel *et al*., 2017).

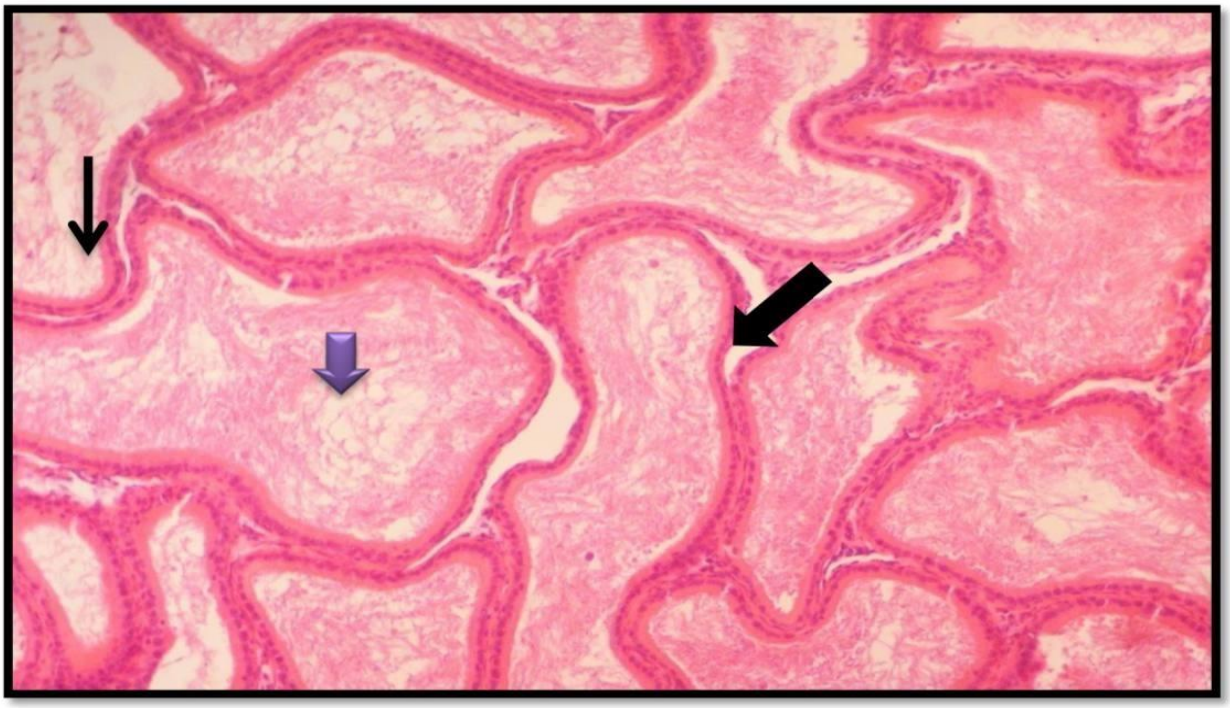


Figure (4-10): A cross-section of the epididymal duct of a rat in the BPA group (100 mg/kg) with Nigella sativa oil (85 µl/kg) showing the normal structure of the epididymis with regular epididymal tubules ( ) and the presence of sperm in the lumen of the epididymis ( ) and The presence of stationary cilia ( ) (H & E 40X).

It became evident that NS served as a protective agent against testicular tissue damage, with evidence indicating a boost in sperm production (Juma, 2011). These findings suggest that NS seed powder exerted therapeutic effects on testicular tissue and Leydig cells, thereby ensuring the maintenance of testosterone production at consistent levels throughout the experimental duration (Assi *et al.,*2016).

The active phytochemical compounds found in NS seeds and oil, such as thymoquinone (TQ), thymol and α-hederin, serve as crucial guardians of testicular health against harmful agents; they achieve this by inhibiting iron-related lipid peroxidation, nuclear factor kappa B, cyclooxygenase, and lipoxygenase, while

simultaneously enhancing both enzymatic and non-enzymatic components of the antioxidant network; additionally, the hormonal rebalancing within the reproductive axis, coupled with the antioxidant and anti-apoptotic properties of TQ, along with NS's vasodilatory effects on microvasculature, play pivotal roles in restoring testicular histological patterns and shielding it from degenerative and necrotizing chemo-toxicants; moreover, the stimulatory effects of NS on spermatogenesis contribute to the preservation of spermatogenic cells across various developmental stages, ensuring their progression to mature sperm (Abd-Elkareem *et al.,* 2021).

Several studies had consistently demonstrated the protective effects of NS oil against testicular damage induced by toxic substances; additionally, investigations utilizing the alcoholic extract of NS oil had revealed significant enhancements in the production of active and motile sperm cells, as well as improvements in epididymal sperm reserves, blood testosterone levels, and fertility indexes; these favorable outcomes have been associated with an increase in embryo numbers; furthermore, NS had been shown to stimulate sperm motility and activity by elevating spermatogenesis hormones in the pituitary gland and modulating oxidative phosphorylation enzymes; consequently, the extract held promise as a potential natural remedy for infertility, suggesting its potential as a candidate for the development of novel therapeutic agents (AL-Chalabi *et al.,* 2020).

The fatty acid methyl esters found in NS oil exhibit potent free radical scavenging properties, act as antioxidants and demonstrate anti-apoptotic activity (Abd-Elkareem *et al.,*2021). Hence, these oilseeds served various therapeutic purposes owing to their composition of active compounds, including alkaloids such as, nigellicine, nigelline, and nigellone, along with chemical constituents like thymoquinone, quinine, and thymohydroquinone; these substances were recognized for their anti-inflammatory and anti-apoptotic properties, as well as their ability to provide protection against toxicity (Assi *et al.,* 2016).

The characterization of NS oil had unveiled the presence of specific bioactive constituents, shedding light on the mechanisms underlying its observed antioxidant and anti-apoptotic effects in this study.

Figure (4-11), cleared the effect the Cur. (100 mg/kg) on the testicle of a rat which showing the normal structure of the testicular tissue, and also the middle cavity is filled with sperm with Leydig cells in the interstitial tissue and sperm progenitors.

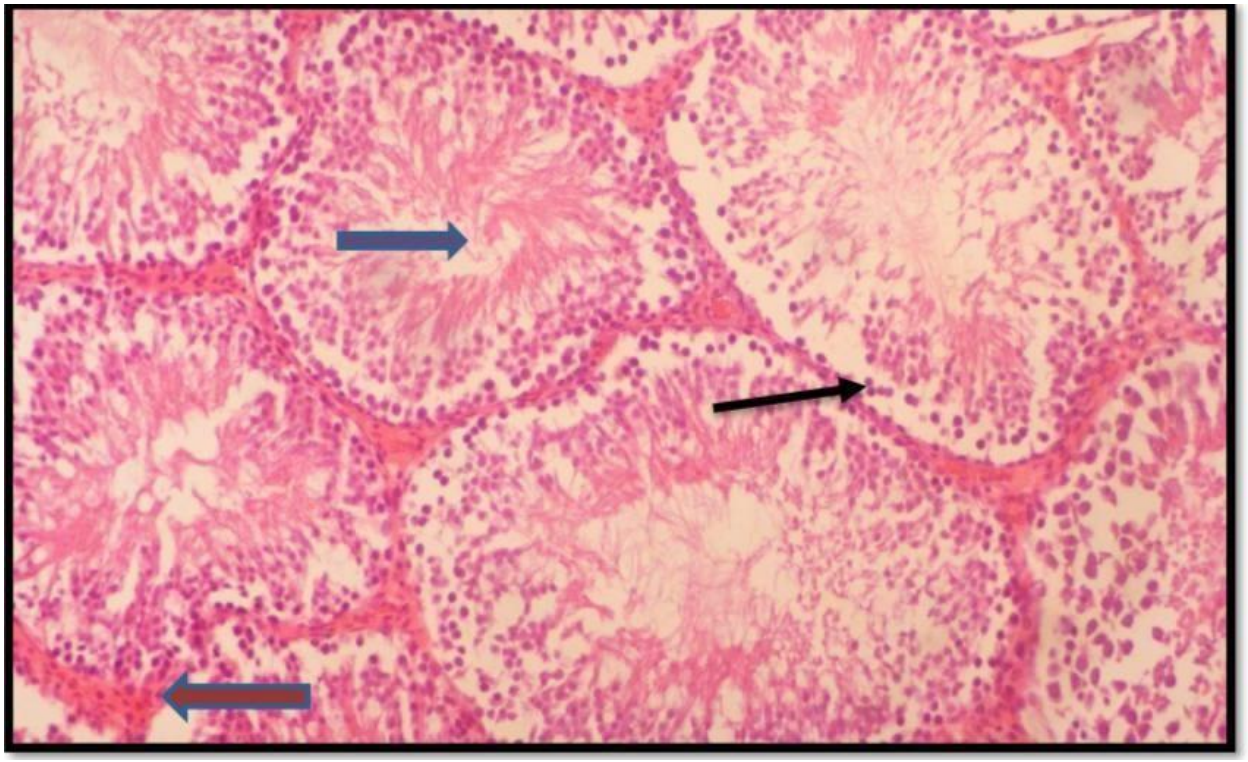


Figure (4-11): A cross-section of the testicle of a rat from the Curcumin (100 mg/kg) group showing the normal structure of the testicular tissue. The middle cavity is filled with sperm ( ) with Leydig cells in the interstitial tissue ( ) and sperm progenitors ( ) (H & E 40X).

Cross-section in figure (4-12) of the epididymal duct of a rat in the treated group with BPA (100 mg/kg) and Cur. (100 mg/kg, shown the normal structure of the epididymis with regular epididymal tubules, increased sperm numbers in epididymal

lumen, peritubular smooth muscle cell pleocytosis, and increased diameter of the epididymal tubules.

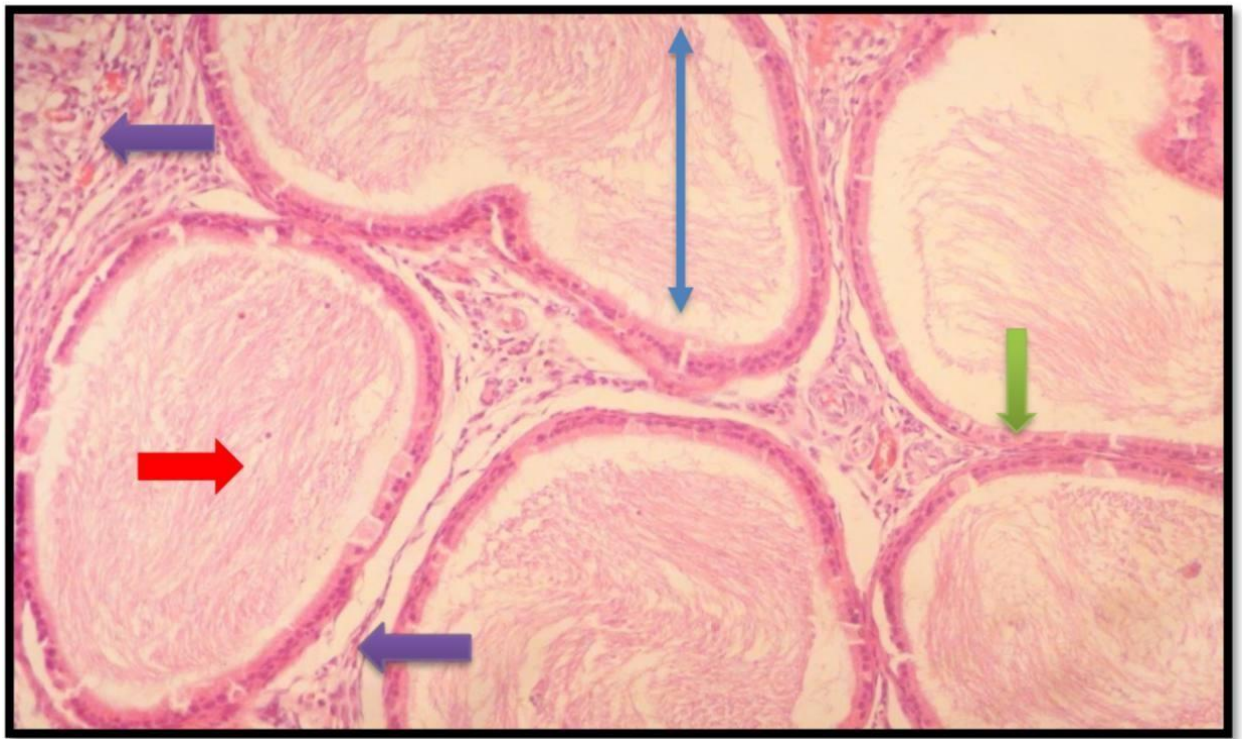


Figure (4-12): A cross-section of the epididymal duct of a rat in the group Bisphenol A (BPA) (100 mg/kg) with Curcumin (100 mg/kg) showing the normal structure of the epididymis with regular epididymal tubules ( ) and increased sperm numbers in epididymal lumen ( ) and peritubular smooth muscle cell pleocytosis ( ) and increased diameter of the epididymal tubules ( ) (H & E 40X).

The effect of BPA (100 mg/kg) and Cur. (100 mg/kg) of body weight, on the testicle of a rat from the treatment group appear in a cross-section in the figure (4-13), and the results found very close to normal of the testicle with seminal tubules and its cavities filled with sperm with Leydig cells in the interstitial tissue and layer germinal epithelial cells and sperm progenitors.

The protective efficacy of Cur. on the testicular tissue could be attributed to its ability to impede cellular damage and apoptosis, induced by oxidative stress within the spermatogenic cells of seminiferous tubules and Leydig cells (Mohamadpour *et*

al., 2014). The protective mechanisms of Cur. had been linked to its regulation of LPO and enhancement of antioxidant activity, resulting in the reduction of free radical levels, through its free radical scavenging activity, Cur. exerted protection against degenerative diseases (Aktas *et al.,* 2012). Moreover, Cur treatment fortifies the cellular integrity of the testes by stimulating the production of antioxidant products while simultaneously mitigating lipid peroxidation (Cheraghi *et al.,* 2017).

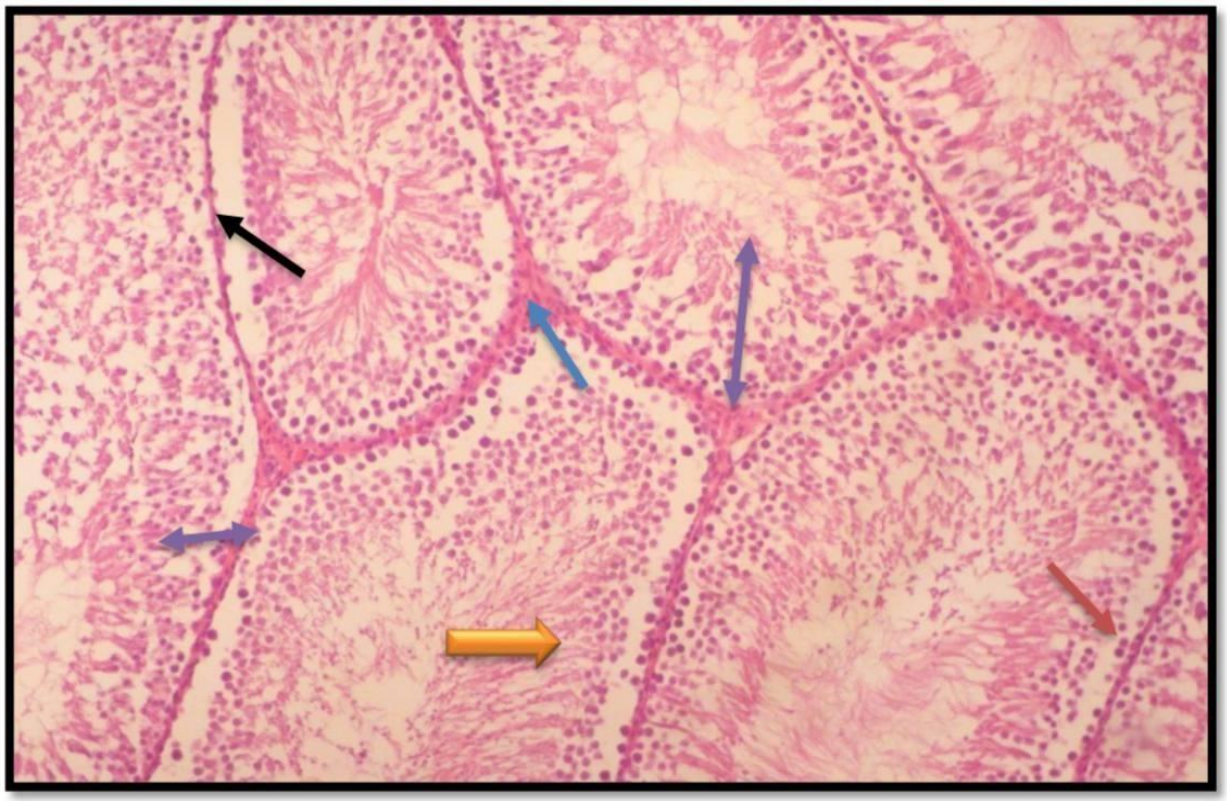


Figure (4-13): A cross-section of the testicle of a rat from the treatment group: BPA (100 mg/kg) and Curcumin (100 mg/kg) of body weight, showing improvement to normal of the testicle with seminal tubules ( ) and its cavities filled with sperm ( ) with Leydig cells in the interstitial tissue ( ) and layer Germinal epithelial cells ( ) and sperm progenitors ( )(H&E 40X).

The advantageous impacts of consuming Cur. on testicular tissue, evident in both acute and chronic models of testicular injury, are compellingly validated at both histological and biochemical levels, these findings suggest the potential utility of curcumin as a therapeutic intervention for stress-induced testicular dysfunction; treatment with Cur. notably attenuated apoptosis in rat testes, leading to increases in mean seminiferous tubule diameter and mean testicular biopsy score values;

furthermore, a significant reduction in testicular tissue oxidative stress, accompanied by a decrease in sperm abnormalities, an increase in total sperm count, and elevation in testosterone levels, were demonstrated (Santonastaso *et al.,* 2021).

Histopathological examination revealed normal spermatogenesis with notable regenerative features observed in the seminiferous tubules, characterized by the absence of atrophy or interstitial edema (Karimi *et al.,*2019). The steroidogenic influence of Cur. might stem from its indirect elevation of testosterone levels by impeding the metabolism of testosterone; Cur. had been contemplated as a testosterone-increasing agent primarily by acting as an aromatase inhibitor, a key enzyme responsible for converting testosterone into estrogen (Ahmed-Farid *et al*., 2017). Various studies had underscored that Cur., akin to other dietary polyphenols, mitigated the effects of toxic damage across various tissues (Scazzocchio *et al.,* 2020).

Curcumin had also demonstrated potential in inhibiting oxidative damage induced by chronic stress in crucial organs such as the brain, liver, and kidneys; so, indicated that Cur. fulfilled that role by preserving the activity of SOD and glutathione peroxidase, while reversing the stress-induced inhibition of catalase; these actions of Cur. ultimately led to a decrease in lipid peroxidation, thereby mitigating the detrimental effects of chronic stress on tissues (Boroumand *et al*.,2018). Furthermore, curcumin had been shown to alleviate testicular damage, cellular apoptosis, and spermatogenic cell death induced by various toxic compounds (Jiang *et al*.,2019).

A possible explanation for the decrease in testosterone output could be the heightened apoptotic cell death observed in Leydig cells, as demonstrated by histopathological examination and in a recent study. Although apoptosis is crucial for regulating the population of Leydig cells and maintaining testosterone levels; an excessive increase in apoptosis can lead to a decline in testosterone production. This

decline may subsequently increase germ cell apoptosis, potentially resulting in infertility in male rats.

The epididymal duct of a rat in the BPA (100 mg/kg) with Cur. (100 mg/kg) and NS oil (85 µl/kg) in the treated group showing that, the tissue is very improvement to normal tissue with regular epididymal tubules, the presence of sperm in the lumen of the epididymis, the increases in the diameter of the lumen of the tubules figure (4-14).

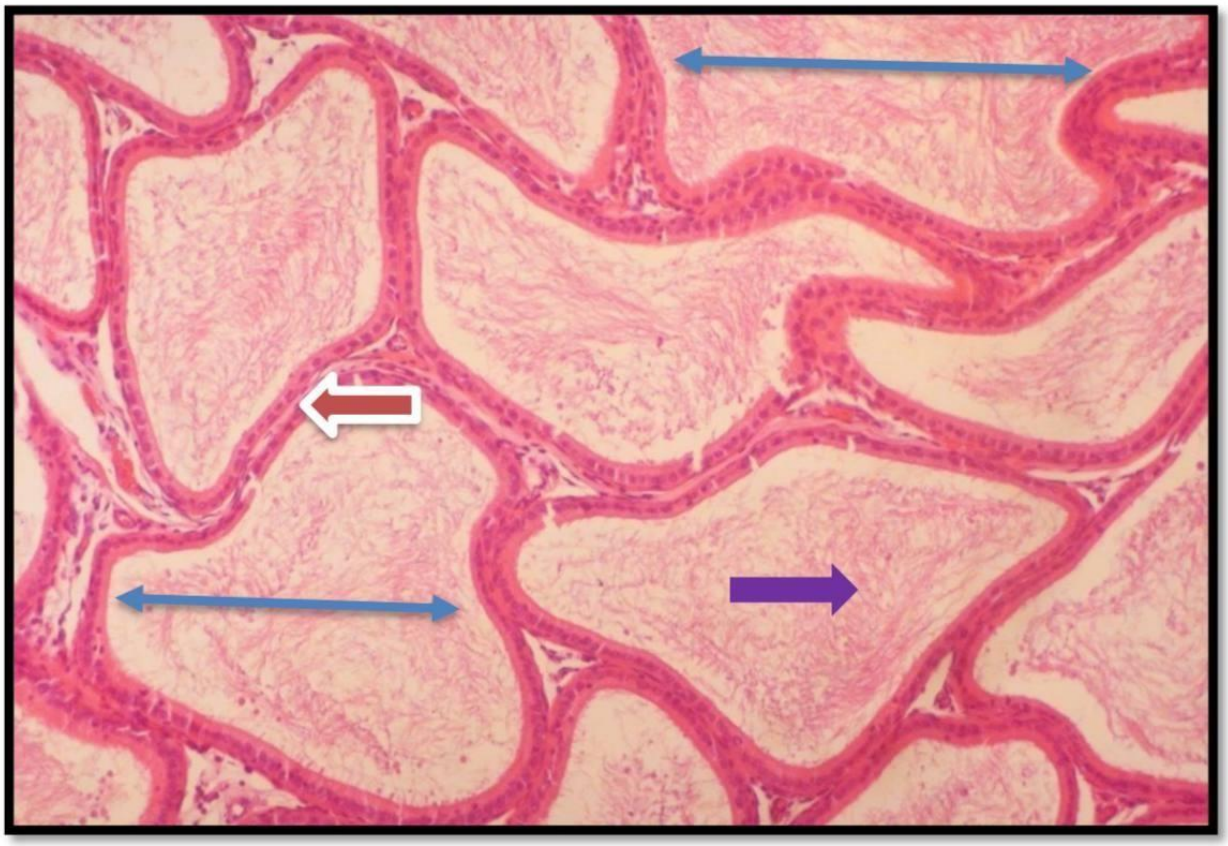


Figure (4-14): A cross-section of the epididymal duct of a rat in the BPA (100 mg/kg) with Curcumin (100 mg/kg) and Nigella sativa oil (85 µl/kg) group showing the tissue is improvement to normal tissue with regular epididymal tubules ( ) and the presence of sperm in the lumen of the epididymis ( ) and the increases in the diameter of the lumen of the tubules ( ) (H & E 40X).

While figure (4-15), shown the testicle of the treated group rats with BPA (100 mg/kg) and Cur. (100 mg/kg) and NS oil (85 µl/kg) of body weight, and appear clear improvement in the seminiferous tubules and their cavities filled with sperm, the

presence of Leydig cells in the interstitial tissue, and an increase in the number of layers of germinal epithelial cells that make up sperm and sperm progenitors.

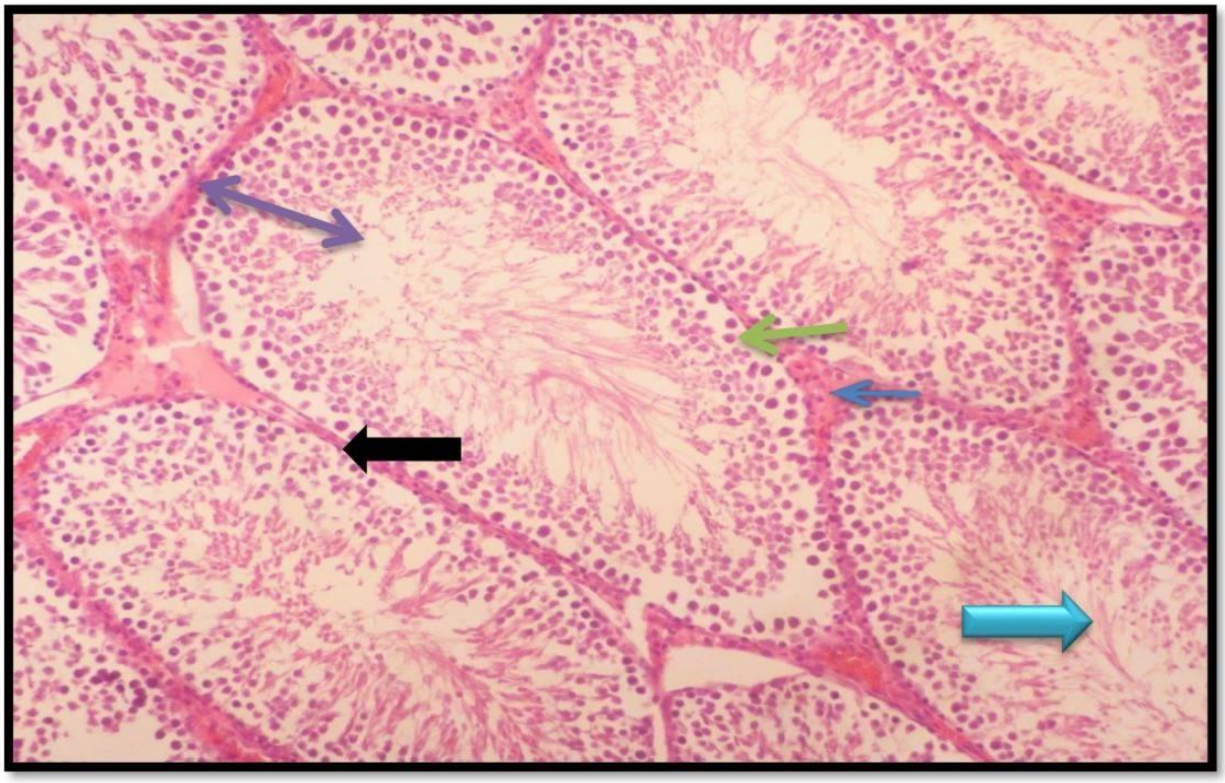


Figure (4-15): A cross-section of the histological section of the testicle of a rat from the group treated with BPA (100 mg/kg) and Curcumin (100 mg/kg) and Nigella sativa oil (85 µl/kg) of body weight, showing a clear improvement in the seminiferous tubules ( ) and their cavities filled with sperm ( )with the presence of Leydig cells in the interstitial tissue( ) and an increase in the number of layers of germinal epithelial cells that make up sperm ( ) and sperm progenitors ( ) (H & E 40X).

The study, conducted by Ezz *et al.,* (2011), demonstrated the promising anticonvulsant and potent antioxidant effects of combining Cur. and NS oil; this combination showed efficacy in reducing oxidative stress and enhancing excitability, as well as ameliorating certain adverse effects of medications. The potential antagonistic interaction between Cur and NS prompts inquiries regarding the clinical viability of their combination for inducing cell cycle arrest and DNA damage repair; as, similarity in target mechanisms might contribute to this antagonism (Saleh *et al.,* 2012).

Chapter Five:Conclusions and Recommendations

5. Conclusions and Recommendations:

5.1. Conclusions:

1- Bisphenol A is artificial chemical compounds, which had an adverse effect on the male reproductive system.

2- BPA increased MDA concentration, and in the same time decreased the concentration of both catalase and SOD, and Nigella sativa oil and Curcumin contain protective role against the effect of BPA, NS oil and Cur. played a crucial role as antioxidants by decreasing MDA concentration and increasing the concentration of catalase and SOD.

3- BPA decrease the concentration of the reproductive hormones (LH, FSH and testosterone) and both NS oil and Cur. could increase the concentration of the reproductive hormones (LH, FSH and testosterone).

4- BPA increased the concentration of inflammatory cytokines (IL-6 and TNF-α) and NS oil and Cur. decreased the concentration of inflammatory cytokines (IL6 and TNF-α).

5- The histopathological investigation of both testes and epididymis detected the presence of abnormal features of these tissues due to the adverse effect of BPA, the two compounds' NS oil and Cur. maintain the normal structure of the testes and epididymis.

5.2. Recommendations:

1- Using other medicinal plants and knowing their effect on the damage caused by BPA.

2- Study the effect NS oil and Cur. in various concentrations and detect the most appropriate protective concentrations.

3- Study the effect of NS oil and Cur. on the genes expression of reproductive hormones *lh*, *fsh* and *dht* besides, the genes responsible of the enzymes of oxidative stress.

4- Investigation the residues of BPA in tissues of various organs in different types of living organisms and found the relation with different types of diseases.

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Appendixes

Appendix I

Determination of Serum Concentration of (SOD). A)

Principal:

Assay Principle This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Rat SOD2 antibody. SOD2 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Rat SOD2 Antibody is added and binds to SOD2 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated SOD2 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Rat SOD2. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

B) Procedure:

1. All reagents, standard solutions, and samples were prepared as instructed. All reagents were brought to room temperature before use. The assay was performed at room temperature.

2. The number of strips required for the assay was determined. The strips were inserted into the frames for use. The unused strips were stored at 2-8°C.

3. 50 µl of the standard was added to the standard well. Note: Antibody was not added to the standard well because the standard solution contained biotinylated antibody.

4. 40 µl of the sample was added to the sample wells, and then 10 µl of Rat SOD2 antibody was added to the sample wells. Next, 50 µl of streptavidin-HRP was added to both the sample wells and standard wells (Not blank control well). The plate was mixed well and covered with a sealer. Incubation was done for 60 minutes at 37°C.

5. The sealer was removed, and the plate was washed 5 times with wash buffer. Each well was soaked with 300 µl of wash buffer for 30 seconds to 1 minute for each wash. For automated washing, each well was aspirated or decanted, and the plate was washed 5 times with wash buffer. The plate was then blotted onto paper towels or other absorbent material.

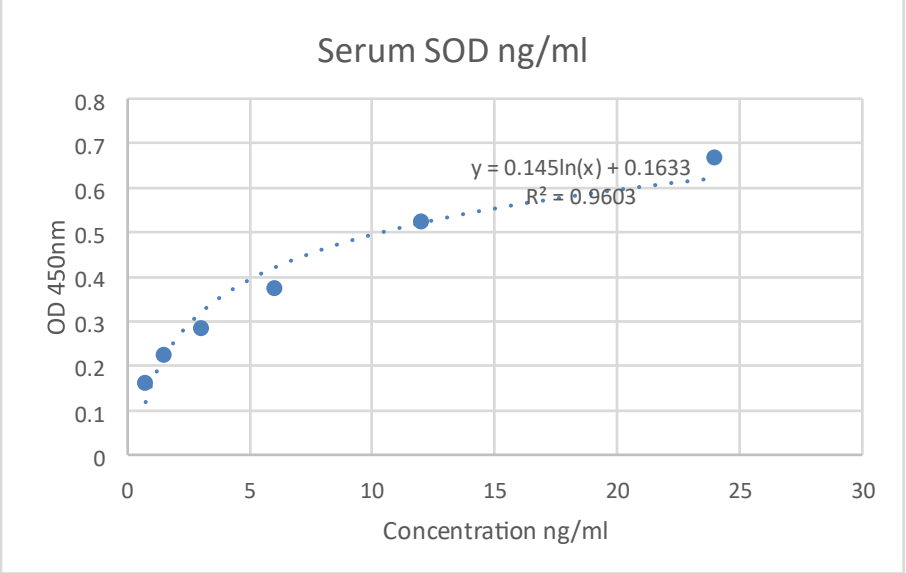
6. 50 µl of substrate solution A was added to each well, followed by adding 50 µl of substrate solution B to each well. The plate was incubated, covered with a new sealer, for 10 minutes at 37°C in the dark.

7. 50 µl of Stop Solution was added to each well, and the blue color changed into yellow immediately.

8. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

C) Calculation:

Known concentrations of Rates Super oxide dismutase (SOD) Standard and its corresponding reading OD were plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of (SOD) in sample was determined by plotting the sample's O.D. on the Y-axis. The original concentration calculated by multiplying the dilution factor.



Appendix II

Determination of Serum Concentration of Catalase (CAT): A)

Principle:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Rat CAT antibody. CAT present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Rat CAT Antibody is added and binds to CAT in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated CAT antibody. After incubation unbound StreptavidinHRP is washed away during a washing step. Substrate solution is then added and colour develops in proportion to the amount of Rat CAT. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450

nm

B) Procedure:

1. All reagents, standard solutions, and samples were prepared as instructed. All reagents were brought to room temperature before use. The assay was performed at room temperature.

2. The number of strips required for the assay was determined. The strips were inserted into the frames for use. The unused strips were stored at 2-8℃.

3. 50 µl of standard was added to the standard well. Note: Antibody was not added to the standard well because the standard solution contained biotinylated antibody.

4. 40 µl of the sample was added to the sample wells, and then 10 µl of Rat CAT antibody was added to the sample wells. Next, 50 µl of streptavidin-HRP was added to both the sample wells and standard wells (Not blank control well). The plate was mixed well and covered with a sealer. Incubation was done for 60 minutes at 37℃.

5. The sealer was removed, and the plate was washed 5 times with wash buffer. Each well was soaked with 300 µl of wash buffer for 30 seconds to 1 minute for each wash. For automated washing, each well was aspirated or decanted, and the plate was washed 5 times with wash buffer. The plate was then blotted onto paper towels or other absorbent material.

6. 50 µl of substrate solution A was added to each well, followed by adding 50 µl of substrate solution B to each well. The plate was incubated, covered with a new sealer, for 10 minutes at 37℃ in the dark.

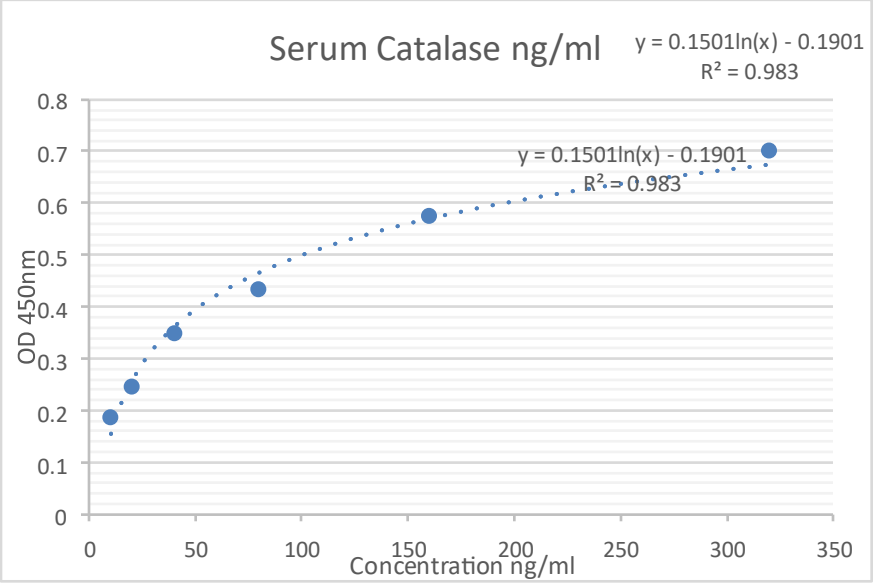
7. 50 µl of Stop Solution was added to each well, and the blue color changed into yellow immediately.

8. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

C) Calculation:

Known concentrations of Rates Catalase Standard and its corresponding reading OD were plotted on the log scale (x-axis) and the log scale (y-axis)

respectively. The concentration of Catalase in sample was determined by plotting the sample's O.D. on the Y-axis. The original concentration calculated by multiplying the dilution factor.



Appendix III

Determination of Serum Concentration of Malondialdehyde (MDA): A)

Principle:

This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of Thiobarbituric acid to form a red MDA-TBA complex which can be measure at 535 nm.

Stock TCA – TBA – HCl

B) Procedure:

To 0.4 ml of serum, 0.6 ml TCA-TBA-HCl reagents were added. It was mixed well and kept in boiling water bath for 10 minutes. After cooling 1.0 ml freshly prepared 1N NaOH solution was added to eliminate centrifugation. This

absorbance of pink color was measured at 535 nm against blank which contained distilled water in place of serum. In blank 0.4 ml distilled water and 0.6 ml TCATBA-HCl reagent was mixed and boiled. Blank was always taken.

C) Calculation:

Extinction coefficient of MDA at 535 nm is = 1.56 × 105

MDA concentration = χ / 0.0624 nmol / ml.

Appendix IV

Measuring Serum Testosterone Levels: A)

Principle of a test:

Testosterone assay is a sandwich ELISA immunoassay. The sample (or calibrator/control, if applicable), magnetic microbeads coated with antitestosterone monoclonal antibody, ABEI labelled with another monoclonal antibody are mixed thoroughly and incubated at 37° C, forming sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then perform a wash cycle. Subsequently, the starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier within 3 seconds as relative light units (RLUs), which is proportional to the concentration of testosterone present in the sample (or calibrator/control, if applicable).

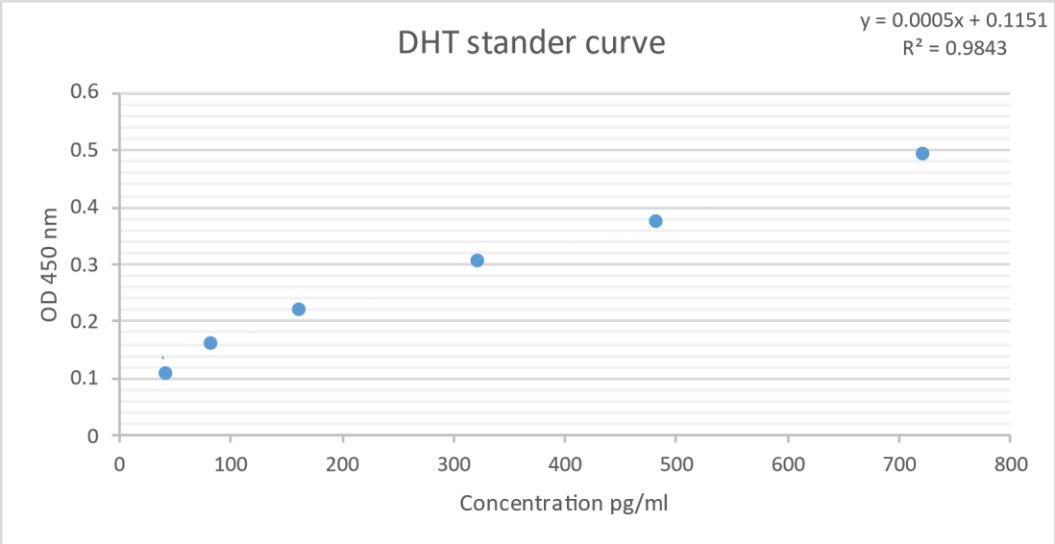
B) Procedure:

1. The micro plate wells were formatted for each serum reference, control and patient specimen to be assayed in duplicate.
2. A volume of 50 µl of appropriate serum reference, control or specimen was pipetted into the assigned well.
3. A volume of 100 µl of testosterone-enzyme reagent was added to all wells.
4. The micro plate was gently swirled for 20-30 seconds to mix and cover.

5. The plate was incubated for 60 minutes at room temperature.
6. The contents of the micro plate were discarded by decantation or aspiration, if decanting, blot the plate dry with absorbent paper.
7. The plate was washed three times with 300 µl of the wash buffer.
8. A volume of 100 µl of working substrate solution was added to all wells, reagents were always added in the same order to minimize reaction time differences between wells, the plate wasn´t shacked after substrate addition.
9. The plate was incubated at room temperature for 15 minutes. A volume of 1050 µl of stop solution was added to each well and was gently mixed for 15-20 seconds.
10. The absorbance was read in each well at 450 nm in a micro plate reader. The results should be read within 30 minutes of adding the stop solution.

C) Calculations:

The standard curve of Testosterone determination was plotted in figure below and the testosterone level in each sample was determined.



Appendix V

Estimation of FSH levels: A)

Principle of a test:

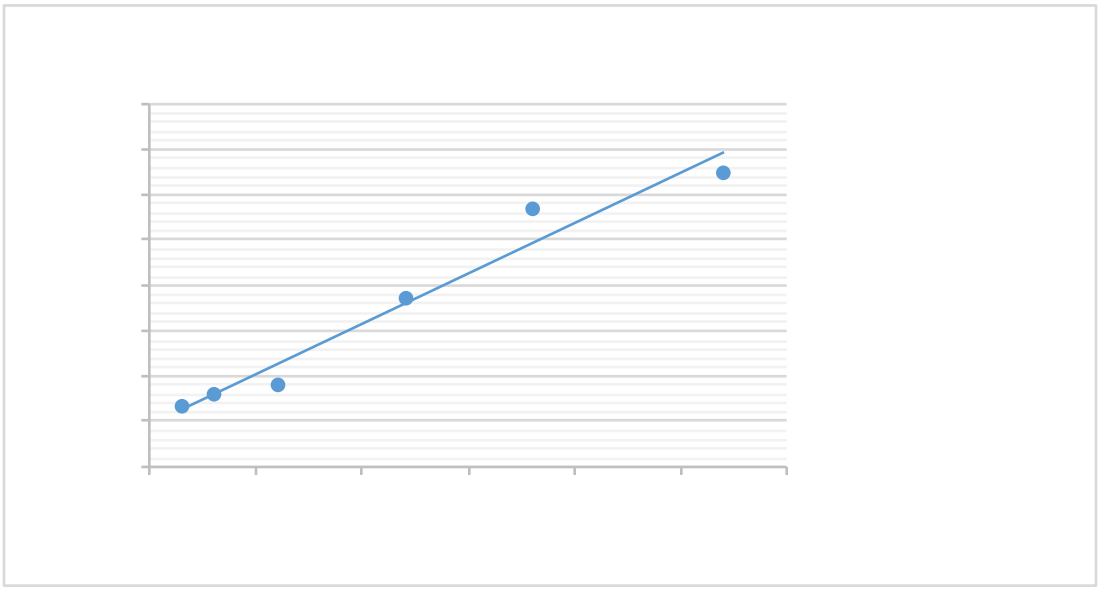
FSH assay is a measured using ELISA technique. The sample (or calibrator/control, if applicable), magnetic microbeads coated with anti-FSH monoclonal antibody, ABEI labelled with another monoclonal antibody are mixed thoroughly and incubated at 37° C, forming sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then perform a wash cycle. Subsequently, the starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier within 3 seconds as relative light units (RLUs), which is proportional to the concentration of FSH present in the sample (or calibrator/control, if applicable).

B) Procedure:

1. The micro plate wells were formatted for each serum reference, control and patient specimen to be assayed in duplicate.
2. A volume of 50 µl of appropriate serum reference, control or specimen was pipetted into the assigned well.
3. A volume of 100 µl of FSH-enzyme reagent was added to all wells.
4. The micro plate was gently swirled for 20-30 seconds to mix and cover.
5. The plate was incubated for 60 minutes at room temperature.
6. The contents of the micro plate were discarded by decantation or aspiration, if decanting, blot the plate dry with absorbent paper.
7. The plate was washed three times with 300 µl of the wash buffer.
8. A volume of 100 µl of working substrate solution was added to all wells, reagents were always added in the same order to minimize reaction time differences between wells, the plate wasn´t shacked after substrate addition. 9. The plate was incubated at room temperature for 15 minutes. A volume of 1050 µl of stop solution was added to each well and was gently mixed for 15-20 seconds.
10. The absorbance was read in each well at 450 nm in a micro plate reader. The results should be read within 30 minutes of adding the stop solution.

C) Calculations:

The standard curve of FSH determination was plotted in figure below, and the FSH level in each sample was determined.



Appendix VI

Measuring serum LH levels: **A)**

Principle of a test:

LH assay is a sandwich chemiluminescence immunoassay. The sample (or calibrator/control, if applicable), magnetic microbeads coated with anti-LH monoclonal antibody, ABEI labelled with another monoclonal antibody are mixed thoroughly and incubated at 37° C, forming sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then perform a wash cycle. Subsequently, the starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier within 3 seconds as relative light units (RLUs), which is proportional to the concentration of LH present in the sample (or calibrator/control, if applicable).

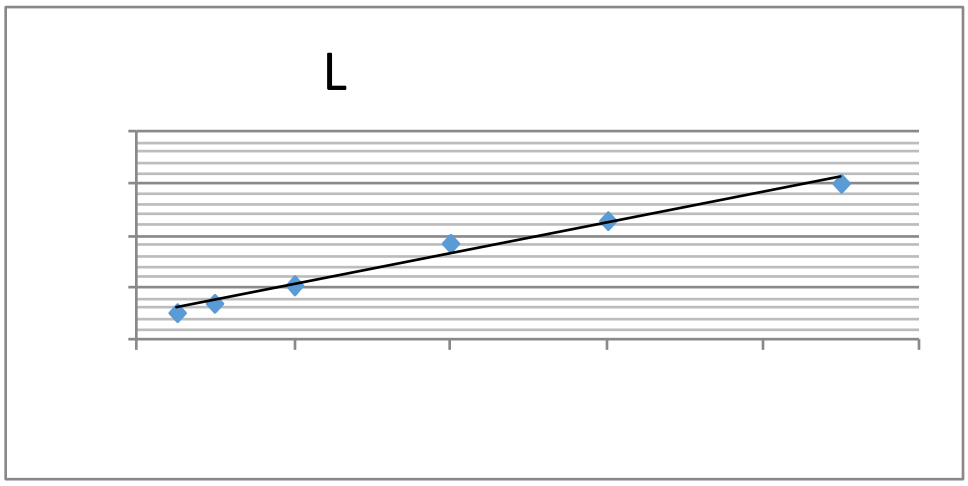
B) Procedure:

1. The micro plate wells were formatted for each serum reference, control and patient specimen to be assayed in duplicate.
2. A volume of 50 µl of appropriate serum reference, control or specimen was pipetted into the assigned well.
3. A volume of 100 µl of LH-enzyme reagent was added to all wells.
4. The micro plate was gently swirled for 20-30 seconds to mix and cover.
5. The plate was incubated for 60 minutes at room temperature.
6. The contents of the micro plate were discarded by decantation or aspiration, if decanting, blot the plate dry with absorbent paper.
7. The plate was washed three times with 300 µl of the wash buffer.
8. A volume of 100 µl of working substrate solution was added to all wells, reagents were always added in the same order to minimize reaction time differences between wells, the plate wasn´t shacked after substrate addition. 9. The plate was incubated at room temperature for 15 minutes. A volume of 1050 µl of stop solution was added to each well and was gently mixed for 15-20 seconds.
10. The absorbance was read in each well at 450 nm in a micro plate reader.

The results should be read within 30 minutes of adding the stop solution.

C) Calculations:

The standard curve of LH determination was plotted in figure below and the LH level in each sample was determined.



Appendix VII

Estimation of Interleukin 6 (IL-6)

A) Principle

A one - step immunosorbent, double-antibody enzyme-linked sandwich cycle to assay Interleukin 6 (IL-6) in human serum was used. The norm, test samples, and IL-6 antibodies labelled with HRP have been applied to wells pre-coated with IL-6 antibodies. A Chromogen solution A and B have been added after incubation and washed to eliminate the uncombine antibody. The liquid colour turned brown. The colour eventually becomes yellow at the effect of acid. The colour transition was measured by a spectrometer at a wavelength of 450 nm. The concentration of IL-6 in the samples was determined by comparing the O.D. of the samples to the standard curve.

B) Kit components:

Item	96 determinations
Micro Elisa strip plate	12*8strips
Standards (1 set)	0.3ml X6
Sample diluent	6.0ml X1
HRP-Conjugate reagent	10.0ml X1
20X Wash solution	25ml X1
Chromogen Solution A	6.0ml X1
Chromogen Solution B	6.0ml X1
Stop Solution	6.0ml X1

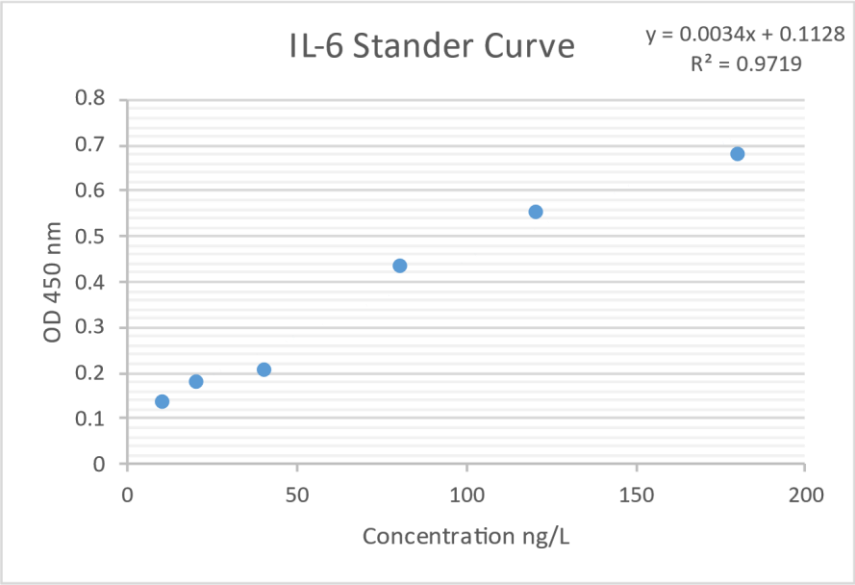
C) Procedure:

1. Standard addition: 50 µl of standards has been added to standard wells.
2. 10 µl of the testing sample has been added. Then, sample diluent 40µl was added to the testing sample well; nothing added to the Blank well.
3. 100 µl of HRP-conjugate reagent were added to each well, then covered and incubated for 60 minutes at 37℃.
4. Each well was washed five washes with a diluted wash solution.
5. Chromogen solution A (50 µl) and chromogen solution B (50µl) have been added to each well, then mixed and incubated for 15 minutes at 37℃.
6. Fifty microliters of stop solution have been added to each well. The colour in the wells were changed from blue to yellow.
7. The optical density (O.D.) was recorded at 450 nm by using a microtiter plate reader within 15 minutes.

D) Calculation of results:

The amount of IL-6 in unknown samples was determined by plotting the standard curve. The standard curve was generated by plotting the O.D. (450nm) obtained for six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis, then, the plot transformed into log-log

plot figure below to obtain a straight-line equation that was used in the transformation of absorbance into concentration.



Appendix VIII

Rat Tumor necrosis factor α (TNF-α) ELISA Kit

Purpose

Our Rat Tumor necrosis factor α (TNF-α) ELISA kit is to assay TNF-α levels in Rat serum, plasma, culture media or any biological fluid.

Principle

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to TNF-α. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for TNF-α is added to each Microelisa stripplate

well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain TNF-α and HRP conjugated TNF-α antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of TNF-α. You can calculate the concentration of TNF-α in the samples by comparing the OD of the samples to the standard curve.

Materials provided with the kit

	Materials provided with the kit	96 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	Microelisa stripplate	1	2-8°C
5	Standard: 360ng/L	0.5ml×1 bottle	2-8°C
6	Standard diluent	1.5ml×1 bottle	2-8°C
7	HRP-Conjugate reagent	6ml×1 bottle	2-8°C
8	Sample diluent	6ml×1 bottle	2-8°C
9	Chromogen Solution A	6ml×1 bottle	2-8°C
10	Chromogen Solution B	6ml×1 bottle	2-8°C
11	Stop Solution	6ml×1 bottle	2-8°C
12	wash solution	20ml (30X)×1bottle	2-8°C

Sample preparation

Serum preparation

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again

Procedure

1. Dilution of Standards

Dilute the standard by small tubes first,then pipette the volume of 50ul from each tube to microplate well,each tube use two wells ,total ten wells

240ng/L	Standard No.1	300µl Original Standard + 150µl Standard diluents
160ng/L	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
80ng/L	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
40ng/L	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
20ng/L	Standard No.5	150µl Standard No.4 + 150µl Standard diluent

2. In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.

3. Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.

4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).

5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.

6. Add 50 µl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3. 8. Washing as described in Step 5.

9. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.

10. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.

11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

المستخلص

يمكن اعتبار البيسفينول أ، أحد أكثر العوامل المسببة لاختلالات الغدد الصماء حيث يعتبر البيسفينول أ ملوثاً بيئيًا له تأثير مشابه لهرمون الاستروجين؛ ويمكن أن تنتج عنه الجذور الحرة التي تسبب ضررا في الأنسجة المختلفة بما في ذلك أنسجة الجهاز التناسلي. حيث يلعب الجهاز التناسلي الذكري دورًا اساسيا في التأثير على الصحة العامة للذكور؛ وفي السنوات الأخيرة، كان هناك االهتماما متزايد فيما يخص قضايا الصحة الإنجابية للذكور بسبب انتشار المواد والعوامل المختلفة والمحتمل بانها تؤثر تأثيرا سلبيا على الصحة الإنجابية بشكل عام .

تبين الدراسات أن زيت حبة البركة يساعد في التخلص من الجذور الحرة ويعمل كمضاد للأكسدة في مجموعة متنوعة من الأمراض التناسلية الذكرية. إلى جانب ذلك، ثبت أيضًا أن الكركم يعالج كذلك عدة أنواع من مشاكل الإنجاب لدى الذكور في حيوانات التجارب، وبالتالي يزيد الخصوبة؛ بالإضافة إلى التخلص من الجذور الحرة والعمل كمضاد للأكسدة.

هدفت هذه الدراسة إلى دراسة الدور الوقائي لزيت الحبة السوداء و الكركم . ضد التأثير السام لمرك ب البسفيول أ في الجهاز التناسلي لذكور الجرذان من خلال تقدير مستويات العوامل المضادة للأكسدة ومستوى الهرمونات الجنسية مثل) FSH) و)LH(والتستوستيرون وكذلك تحديد مستوى السيتوكينا ت المناعي ة مثل) IL-6) و (α- (TNF(علاوة على ذلك، ادراسة ما إذا كانت هناك أي تغيرات نسيجية قبل وبعد المعالجة بهذه المواد .

تم وضع ثمانية وأربعون ذكراً من الجرذان البالغة بأوزان حوالي)200 -350 جرام(وأعمارها ما بين) 8 -10 أسابيع(. وتم تقسيم ذكور الجرذان هذه إلى ثماني مجموعات بواقع 6 فئران في كل واحدة منها .

امتدت هذه الدراسة للفترة من تشرين الثاني / 2023- اذار/2024 .

ومن خلال دراسة تأثير زي ت الحبة السوداء و الكركم كعوامل وقائية ضد مادة البسفيول أ على معايير الجهد التأكسدي، أظهرت النتائج بأن هذه الأعشاب يمكن أن تقلل من تركيز MDA عند قيمة معنوي ة (P value=0.0001) وفي نفس الوقت تزيد من تركيز كل من الcatalase (P value<0.0001) والـ SOD. (P value=0.0003) كذلك بين ت النتائج بان استخدام الزيت و الكركم يمكن أن يزيد من تركيز (P value=0.0001) كذلك LH (P value=0.0003) والتستوستيرون (P value<0.0001) في

مجموعات BPA+CUR، BPA+NS. وBPA+ NS Oil+ CUR، بالإضافة إلى انخفاض مستويات هذه الهرمونات في مجموعة BPA مقارنة مع مجموعة السيطرة.

أ

كذلك توصلت الدراسة الى ان زي ت حبة البركة والكركم يمكن أن يقلل من السيتوكينات المضادة للالتهابا ت IL-6 وTNF-α عند مستويات معنوية (P value= 0.0001) و(P value=0.02) على التوالي. في كل من مجموعة المعالجة بزيت الحبة السو داء+ البسفينول أ ومجموعة الكركم+ البسفينول أ والمجموعة المركبة من زيت حبة البركة والكركم + البسفينول أ ،مقارنة مع مجموعة BPA ومجموعة السيطرة .

علاوة على ذلك، أظهرت الدراسة النسيجية المرضية لمقاطع من الخصية والبربخ أن المجموعة المعالجة بمادة البسفيول أ أظهرت تأثيراً ضاراً على انسجة الجهاز التناسلي على الإطلاق، في حين أن إعطاء زيت حبة البركة والكركم جعل من الممكن الحفاظ على السمات الطبيعية لنسيج الخصية والبربخ مقارنة بالمجموعة المعالجة بالبسفيول أظهرت النتائج انه لم يكن هناك أي تأثير تآزري من اعطاء مزيج من الحبة السوداء والكركم في المجموعة نفسه ا بالمقارنة مع المجموعات المعالجة بزيت حبة البركة أو الكركم بمفردهم. وخلصت الدراسة إلى أن لزيت الحبة السوداء والكركم كان له تأثيرات علاجية معززة ضد السمية في الجهاز التناسلي الذكري الناجمة عن مادة البسفيول أ في الجرذان في حين اعطاء زيت الحبة السودة والكركم لم يكن له أي تأثير تآزري أو معادٍ على شفاء التأثير الضار للبسفيول أ.

J·

وزارة التعليم العالي و البحث العلمي

جامعة كربلاء

كلية الطب البيطري فرع

الفسلجة و الادوية و الكيمياء الحياتية

دور حبة البركة والكركمين في مواجهة سمية الإجهاد التأكسدي الناجم عن البيسفينول أ في الجهاز التناسلي لذكور الجرذان

رسالة

مقدمة إلى مجلس كلية الطب البيطري جامعة كربلاء في استيفاء جزء من متطلبات درجة ماجستير في

الطب البيطري / فرع الفسلجة

بواسطة

ماجد حامد رسول

اشرا ف

أ.م.د. علي جاسم جعفر

أ.م.د. جمان خليل ابراهيم

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