T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHYSIOLOGY

INVESTIGATION OF OBESOGENIC EFFECTS OF HEXACHLOROBENZENE, DDT AND P,P-DDE IN MALE RAT MODEL

THE MASTER OF SCIENCE THESIS

ZEYAD AL-OBEIDI, DVM.

İSTANBUL-2021

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 06.10.2021 and numbered 2021/10-05.

Prof. Dr. Bayram Yılmaz

Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

08/08/2021

Zeyad AL-OBEIDI

DEDICATION

To my family…

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Zeyad Al-Obeidi

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ABSTRACT

Al-Obeidi, Z. (2021). Investigation of Obesogenic Effects of Hexachlorobenzene, DDT and p,p-DDDE in Male Rat Model, Yeditepe University, Institute of Health Sciences, Department of Physiology, MSc thesis, İstanbul.

Obesity continues to be a global public health problem. Recent studies reported that some environmental pollutants may have obesogenic effects. Peroxisome proliferator activating receptor gamma (*Pparγ*) and uncoupling protein-1 (*UCP1*) genes play roles in adipogenesis. Their expression in white adipose tissue (WAT) and brown adipose tissue (BAT) is of importance. In the present study, we aimed to investigate obesogenic effects of dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB) and dichlorodiphenyldichloroethylene (DDE). Secondly, we aimed to investigate the serum sex hormones (luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone), as well as testicular histology. Thirty-two adult male Sprague-Dawley rats were randomly divided into four groups: control, HCB, DDT and DDE. Organochlorine pesticides (OCPs; 5mg/kg) dissolved in corn oil were administered to the animals by oral gavage every other day for five weeks. Controls received vehicle alone. The body weights of the animals were monitored throughout the experiments. At the end, the animals were decapitated, BAT and WAT samples were collected to analyse *Pparγ* and *UCP1* levels. Testes were dissected out for histological examination. Serum samples were collected to measure levels of LH, FSH, testosterone and serum cholesterol and triglyceride levels. Body weights of the animals did not significantly change among the groups. Serum cholesterol and triglyceride were similar. Serum LH and FSH levels were not significantly altered, but testosterone was significantly reduced in all groups ($p<0.05$). HCB administration significantly increased *Pparγ* expression only in WAT ($p<0.05$). DDT significantly decreased *Pparγ* and *UCP1* expressions in both WAT and BAT ($p<0.01$). DDE significantly decreased *Pparγ* expression only in WAT ($p<0.001$). Testicular histology was found to deteriorated by OCPs. In conclusion, HCB-induced expression of *Pparγ* in WAT obesogenic activity of HCB. In addition, DDT and DDE-induced decreases in expression of *Pparγ* and *UCP1* in WAT suggest that these two pesticides may reduce adipogenesis.

Keywords: Adipogenesis, DDT, DDE, Hexachlorobenzene, Obesogen, Testosterone.

ÖZET

Al-Obeidi, Z. (2021). Hekzaklorobenzen, DDT ve p,p-DDE'nin Obesogenik Etkilerinin Erkek Sıçan Modelinde Araştırılması, Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Fizyoloji Anabilim Dalı, Yüksek Lisans Tezi, İstanbul.

Obezite önemli bir global halk sağlığı problemidir. Bazı çevresel kimyasal kirleticilerin obezogenik etkiler gösterdiği konusunda çalışmalar yayınlanmıştır. Peroksizom proliferatör aktive edici reseptör gama (*PPARγ*) adipogenez sürecinde; uncoupling protein-1 (*UCP-1*) genleri de enerji metabolizmasında rol oynar. Hem *PPARγ* hem de *UCP-1*'in beyaz yağ doku (BYD) ve kahverengi yağ dokudaki (KYD) ifadeleri bu bakımdan önemlidir. Bu çalışmada, hekzaklorobenzen (HKB), diklorodifeniltrikloroetan (DDT) ve diklorodifenildikloroetilen (DDE)'nin obezogenik etkilerinin araştırılması amaçlandı. Ayrıca, serum luteinize edici hormon (LH), folikül stimülan hormon (FSH) ve testosterone değerleri ile testis histolojisi de incelendi. Otuz iki erişkin erkek Sprague-Dawley sıçan randomize olarak dört gruba ayrıldı: Kontrol, HKB, DDT ve DDE. Hayvanlara oral gavaj yoluyla iki günde bir olmak üzere, beş hafta boyunca mısırözü yağında çözülmüş organoklorlu pestisitler (OKP) 5mg/kg dozunda uygulandı. Kontrol grubundaki sıçanlara sadece taşıt solüsyonu uygulandı. Deney süresi boyunca hayvanların vücut ağırlıkları takip edildi. Deneylerin sonunda hayvanlar dekapite edildi ve BYD ve KYD örneklerinde *PPARγ* ve *UCP1* seviyeleri incelendi. Testis dokusu histolojik inceleme için diseke edildi. Serum örnekleri LH, FSH, testosterone, trigliserit ve total kolesterol seviyelerini analiz etmek için toplandı. Deney süresince gruplar arası vücüt ağırlıklarında anlamlı değişiklik gözlenmedi. Serum kolesterol ve trigliserit düzeyleri gruplar arasında farklılık göstermedi. Serum LH ve FSH seviyeleri pestisit uygulamasından etkilenmezken, pestisit uygulanan tüm gruplarda testosterone azaldı ($p<0,05$). HKB uygulaması BYD'de *PPARγ* ifadesini artırdı ($p<0,05$). DDT ise hem BYD hem de KYD'de *PPARγ* ve *UCP1* ifadelerini azalttı ($p<0,01$). DDE ise yalnızca BYD'de *PPARγ* ifadesini azalttı ($p<0,001$). Testis dokusunun pestisit uygulaması sonucu etkilendiği gözlendi. HKB'nin *PPARγ*'nın BYD'deki ifadesinin artışı, HKB'nin obezojenik aktiviteye sahip olabileceğini göstermektedir. Ayrıca, DDT ve DDE'nin BYD'de *PPARγ* ve *UCP1* ifadesini azaltması bu iki OKP'nin adipogenezi azaltabileceğini düşündürmektedir.

Anahtar kelimeler: Adipogenez, DDT, DDE, Hekzaklorobenzen, Obezogen, Testosteron.

LIST OF SYMBOLS and ABBREVIATIONS

AhR	Aryl hydrocarbon/dioxin receptor
AR	Androgen receptor
BMI	Body mass index
DDE	*p,p´*-dichlorodiphenyldichloroethylene
DHT	Dihydrotestosterone
EDCs	Endocrine disrupting chemicals
ER	Estrogen receptor
FNDC5	Fibronectin type III domain-containing protein 5
FSH	Follicle-stimulating hormone
HCB	Hexachlorobenzene
LH	Luteinizing hormone
NCDs	Noncommunicable diseases
OCP	Organochlorinated pesticide
PCBs	Polychlorinated biphenyls
PGC-1α	PPAR-γ coactivator 1-alpha
POPs	Persistent organochlorine pollutants
PPARs	Peroxisome proliferator-activated receptors
RXR	Retinoid X receptor
T2D	Type 2 diabetes
T4	Thyroxine
TBT	Tributyltin
TSH	Thyroid-stimulating hormone
WHO	World Health Organization

1. INTRODUCTION and PURPOSE

Fat accumulation in the body is defined as obesity which leads to risk factors for several chronic diseases including diabetes, cardiovascular diseases, stroke and cancer [1]. There has been a global increase in obesity as its prevalence has doubled through the last three decades, and hence it has become an important public health problem [2]. An imbalance between energy intake and expenditure is the main cause of obesity which consequently leads to disruption in metabolism [3]. Sedentary lifestyle and immobility are important in obesity and metabolic syndrome [4]. A study analyzing the obesity trend between 1988 and 2006 revealed that although the average caloric intake and physical activity remained same, mean body mass index (BMI) has increased over the years [5].

Many theories have associated the development in average BMI to obesogens. These obesogens constitute an element of the endocrine disrupting chemicals (EDCs) responsible for the changes in metabolism to favor lipid storage which leads to the tendency of obesity. Food and drink packaging, cleaning products and pesticides are all carriers of these substances [5]. Obsogens have been found to operate using various mechanisms. They have the ability to mimic natural hormones which can lead to undesired biological effects [5]. These chemicals' ability to mimic are dependent on their properties that are similar to that of the natural hormones, which includes lipophilicity and small molecular weight [5]. Three important characteristics that affect obesogens' ability to act the same as xenohormones are the partition constant, half-term, and molecular weight.

EDCs can present in two classes. First, natural as phytoestrogens placed in grains, fungi, grasses, herbs, legumes, and fruits are not stronger if compared with endogenous estrogens. And second, the chemicals that have made it by a human such as organic compound from carbon and many components such as hydrogen, nitrogen, and chlorine tend to rebellion and produce an adverse effect on human health [6].

Exposure to these substances can be directed through contacts with them, such as insecticides, herbicides, fumigants, fungicides, or indirectly by ingested food or water. Different sources have Endocrine disruptors that can enter the environment, especially when the plastic and many materials are burned because of manufacturing processes.

EDCs can work in many different mechanisms, firstly, by binding with a nuclear receptor as estrogen receptor (ER), secondly by nuclear receptor antagonists third,

convert testosterone to estradiol by altering the normal function which inhibiting aromatases as the P450 family members CYP19 and CYP3A1. Fourth, Stimulating the expression of the P450 enzymes can change the level of hormones by the effect on the endocrine system. Also, change neuronal synapse formation has been verified in endocrine-disrupting chemicals [6].

Hereditary and environmental agents such as the quantity of food, and lack of physical exercises are strongly believed to be the major causative agents for metabolic diseases [7]. In the last years, it has been documented that EDCs activate adipogenesis and prompts obesity. These EDCs were therefore termed “obesogens” [7].

The term endocrine disruptor returned to a workshop organized by Theo Colborn and colleagues in the early 1990s. They published a statement termed wingspread, which highlighted EDCs effects on the environment and on human health [7]. Endocrine disruptors were labelled by USA’s EPA in 1996 as an exogenous factor that impedes the hormone’s functions. The functions that are disrupted includes synthesis, secretion, transport, binding, action, or removal of normal hormones in the body, which perform the function of keeping homeostasis, reproduction, development, and manners [7]. Pharmaceuticals, dioxin, dioxide-like compounds, polychlorinated biphenyls, organochlorinated pesticides, and plasticizers are among a wide range of natural and artificial substances that are responsible for endocrine disruption [7].

It is easy to find products that contain endocrine disruptors daily. Examples of these products include plastic bottles, metal food cans, detergents, flame retardants, food, toys, cosmetics, and pesticides. Studies point out that endocrine disruptors may present a higher risk during fetal and early postnatal growth when organ and neural systems form. Endocrine disruption is considered one of the main issues in health and the environment. Many other environmental contaminants including polychlorinated biphenyls (PCBs) and organochlorinated pesticides (OCPs) have been described as having the "endocrine disruptor" effect [7]. Extensive environmental contamination problems, and several human health issues are triggered by these persistent organic pollutants. These persistent organic pollutants (POPs) produce extensive environmental contamination and indicate several human health problems. Carcinogenic, neurotoxic, hepatotoxic, nephrotoxic, immunotoxin effects and congenital disabilities are all caused by POPs [7]. These contaminants can instigate long-term effects on adipose tissue, metabolic activity,

hormones and eventually weight if people are exposed to them on a regular regularly. Additionally, people who are exposed in their prenatal stages risk becoming obese later in life. Understanding and identifying these obesogens is essential in reforming world-wide health since the issue of obesity is a concern to all and constitutes a billion-dollar industry worldwide [7].

In the present study, obesogenic effects of HCB, DDT and p,p-DDE were investigated in male rat model. Effects of these OCPs on adipogenesis, testicular histology and the levels of *uncoupling protein 1 (UCP1)* and *Pparγ* in white and brown adipose tissues were examined following five weeks exposure period.

2. LITERATURE REVIEW

2.1. Obesity

2.1.1. Definition and Epidemiology

Noncommunicable diseases (NCDs) such as cardiovascular disease, fatty liver disease, stroke, dementia, and various types of cancers are the major cause of both early disability and mortality[8, 9]. As a main risk factor for NCDs, obesity is not only associated with decreased life expectancy by about 5-20 years, but also with other issues including unemployment and social issues such as increased economic burden for the society[8]. Obesity is defined as the excessive fat accumulation leading to impaired health by the World Health Organization (WHO) and individuals having a body mass index (BMI) ≥30 kg/m^2 are considered obese[10].

There has been a dramatic increase in the incidence of obesity Worldwide over the last two decades[8, 11]. It has recently been reported that over one-third of the population is classified as overweight or obese[11]. If the present trends continue, it is estimated that 57.8% of whole population will be overweight or obese by 2030[12].

2.1.2. Pathogenesis

In general, the major cause of obesity is an imbalance between calorie intake (and hence fat accumulation) and energy expenditure[8]. However, obesity is a multifactorial and complex disease. Its etiology includes both individual factors such as genetics, internal signals (between the brain and adipose tissue) and environmental factors[8, 13]. Social factors such as eating culture and sedentary lifestyle are also among the causes of obesity [8, 13].

Genetic causes of obesity can be classified under three categories: (1) monogenic, (2) syndromic obesity and (3) polygenic reasons [14]. The monogenic cause of obesity includes the gene mutations occur largely in the leptin/melanocortin pathway centrally regulating food intake [15]. Monogenic obesity is a rare and severe early-onset form of the obesity which is related with several endocrine disorders[15]. In syndromic obesity, several other factors including neurodevelopmental problems, mental retardation and organ-associated problems appear to accompany with the severe obesity[15]. Polygenic obesity refers to not only the genetic factors underlying the obesity, but also the environmental

factors[14-16]. In the last decade, several endocrine disrupting chemicals have been reported to have obesogenic effects, and thus such chemicals are called "obesogens" [7, 17, 18] .

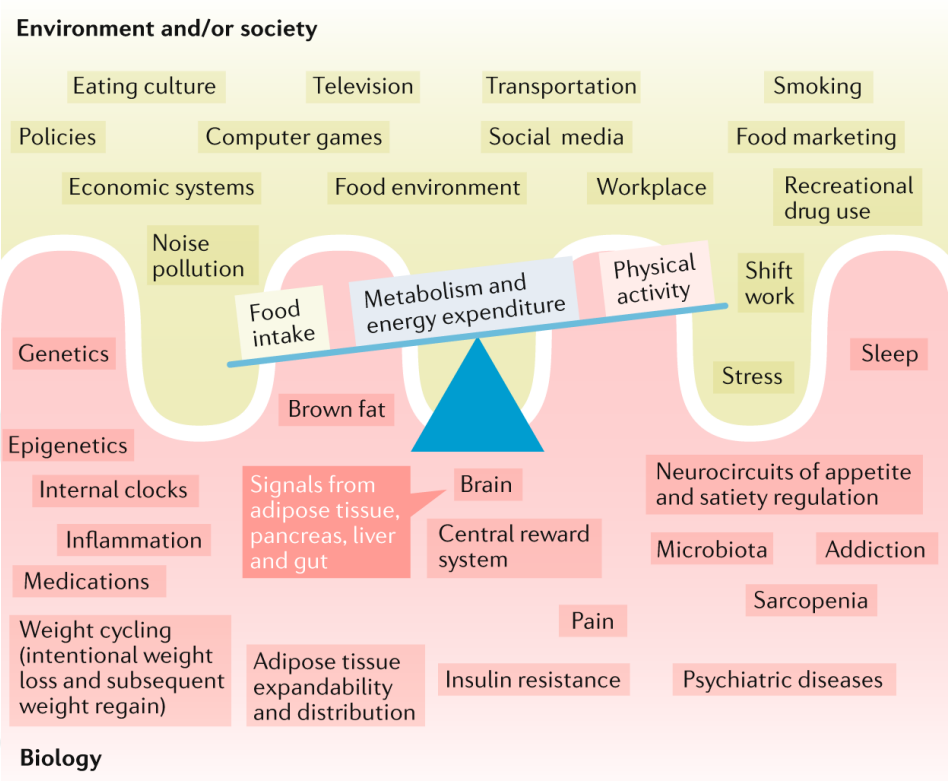

Figure 1. Multifactorial and complex nature of obesity (Adapted from Blüher, 2019)[8].

2.2. Endocrine Disrupting Chemicals and Obesogens

Endocrine disrupting chemicals (EDCs) were initially defined as the exogenous chemicals that interfere with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior [7]. Later, the Endocrine Society statement re-defined endocrine disruptors as "an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action" [19]

EDCs include a wide range of natural and synthetic substances including pharmaceuticals, phytoestrogens, industrial chemicals (such as polychlorinated biphenyls (PCBs), dioxin and dioxin-like compounds), pesticides, heavy metals and plasticizers [20, 21]. Residues of such industrial chemicals, agricultural pesticides, detergents, plasticizers, flame retardants, and cosmetics may be found in everyday products [17]. People are exposed

to a broad range of EDCs through different routes. Entrance of environmental pollutants to the organism generally happens in an unconscious manner during the course of life [22]. Dietary ingestion is the main entering route of most EDCs to the human body, and this causes more than 90% of the total chemical exposure [7]. In addition, human exposure to these environmental pollutants include by inhalation and dermal uptake to some extent [23]. It has been reported that EDCs may pose the greatest risk during fetal and neonatal development when formation and development of organ systems are very active [7].

There has been an enormous increase in the production of different chemicals during the last several decades globally. Even though these chemicals have proven to be useful for many aspects of modern life, it recently has been recognized that they may cause environment pollution and pose threat to human health. Today, there are thousands of manufactured chemicals. Of these substances, it is estimated that more than 1000 may have endocrine-acting properties [7].

It has been suggested that some EDCs may cause obesity by promoting adipogenesis [5, 24]. Obesogens were described as a subgroup of EDCs that may lead to disrupted adipogenesis and induce obesity[5]. Associations between the exposure to EDCs and elevated adiposity have been implicated by various studies [25-29]. Moreover, *in utero* exposure to obesogens may cause obesity as well as leading to other NCDs at later stages of life [30].

Lipid homeostasis may be disrupted by obesogens through various mechanisms [7, 17]. These mechanisms include (1) increasing the number of adipocytes, (2) increasing the size of adipocytes, (3) altering endocrine regulation of adipose tissue development, (4) changing brain regulation of appetite, satiety and food preference, (5) altering basal metabolic rate and (6) energy balance in favor of calorie storage and (7) changing insulin sensitivity in the liver, skeletal muscle, pancreas, gastrointestinal system and the brain [17].

2.2.1. Persistent Organic Pollutants (POPs)

Persistent organic pollutants (POPs) are substances that persist in the environment for a long period of time and may exert adverse effects on human health[7]. They are lipophilic substances, can contaminate the food chain and can bioaccumulate fat tissue. Thus, these chemicals may reach to high concentrations that may negatively affect various functions in the organism. POPs caused environmental pollution is not just local

but can be a global toxic threat since they can be transported over long distances and dispersed around the globe by air and ocean currents [22]. In 2001, it was decided at the Stockholm Convention that necessary measures should be taken in order to prevent the release of POPs into the environment and reduce their contamination to the food chain as far as is technically feasible and economically acceptable [31, 32]. Although regulations and implementations have been introduced, POPs can still be found in environmental and human biological samples [22].

About 20 years ago, 12 POPs were identified as "dirty dozen" at the Stockholm Convention, producing severe adverse effects on humans and the ecosystem [31, 32]. A guideline for storing and eliminating a list of chemicals was generated that was appended in 2008 and 2014 [7]. The dirty dozen included pesticides, industrial chemicals, and by-products, namely aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, PCBs, polychlorinated dibenzo-p-dioxins, and polychlorinated dibenzofurans [31, 32].

POPs have become an important global problem. There have been numerous experimental and epidemiological studies to investigate their biological and toxic effects, human exposure and risk assessment [7]. These pollutants have been produced intentionally and used in a wide variety of commercial (such as PCBs, chlorophenols and chlorobenzenes) and agricultural (such as DDT) applications because of their useful technological or pesticide properties [33, 34]. Other persistent and very toxic pollutants such as polychlorinated dibenzo-p-dioxins and dibenzofurans are formed as undesirable by-products, e.g., during the manufacture of the chemicals, waste combustion, the chlorine bleaching of pulp and paper, some metallurgical processes [35, 36]. Over the past three decades, environmental and epidemiological studies have shown that POPs have spread Worldwide [37]. Such a global level of contamination is a somewhat expected consequence in view of the physical and chemical properties of POPs. It has been reported that they are highly resistant to chemical and biological degradation. PCBs and other chlorinated pollutants, particularly the highly chlorinated ones, have been known for some time to persist in soils, water, sediments, and biota for long periods of time [22, 37, 38]. In addition, POPs are nonpolar molecules that can accumulate in fatty tissues [39, 40]. This results in their biomagnification in the higher levels of the food chain. Since humans are at the top of the food chain, high concentrations of these lipophilic and persistent compounds are

often found in human biological samples [37]. It has been estimated that 90% of organochlorine body burden in the general population occurs through dietary intake. Thus, it is envisaged that levels of these pollutants are proportional to the dietary intake (such as fish, animal fat, dairy products, cereals and vegetables consumption). Studies on POPs available from various countries are abundant, and inventories of sources and possible pollution sites are being developed. Cross-sectional epidemiological studies in adults, amount of POPs such as PCBs, *p,p´*-dichlorodiphenyldichloroethylene (DDE), and hexachlorobenzene (HCB) have been associated with disturbance of metabolism including obesity, insulin resistance, metabolic syndrome and type 2 diabetes (T2D) [41].

2.2.2. Polychlorinated biphenyls

These are different congeners having different numbers (1–10) and positions (ortho, meta, para) of chlorine atoms around biphenyl rings [22]. They were widely used as insulators in capacitors and transformers because of their inflammability and insulating properties such as hydraulic fluids and paints, and related products. Although these industrial chemicals were banned in most countries in the late 1970s, they remain global contaminants in both the environment and the human body because of lipophilic nature and persistent properties [22]. PCBs with biphenyl rings substituted with zero chlorine in the ortho position that assumes a coplanar biphenyl orientation in solution, and PCBs with one to four chlorines in the ortho position that assume noncoplanar biphenyl orientation [22]. Several PCB congeners and mixtures have been documented to have neurotoxic, carcinogenic, immunotoxin, hepatotoxic, nephrotoxic, and cytotoxic influence in different experimental models and also in human studies [7]. It has been reported that noncoplanar PCBs are structurally similar to 17β-estradiol (E2) and therefore can mimic estrogenic impact in the cell [42]. Conversely, coplanar PCBs mimic dioxin in that they bind with a relatively high affinity to the aryl hydrocarbon receptor (AHR) [43]. It regulates the transcription of a large group of dioxin-responsive genes and results in a reduction in cytosolic estrogen level (i.e. anti-estrogenic effect)[7].

2.2.3. Organochlorine Pesticides (OCPs)

Definition of pesticides include insecticides, herbicides, fungicides, and rodenticides. Based on their chemical structure, there are five major types of pesticides; organochlorines (such as DDT, endosulfan, lindane, mirex, dieldrin, chlordane), organophosphates (such as malathion, parathion), carbamates (such as carbaryl, carbofuran, aminocarb), pyrethroids (such as cypermethrin, permethrin) and triazines [44]. It has been noted that atrazine is the most studied triazine herbicide [45]. Most classes of pesticides may produce adverse influences on human health [46]. It has been documented that OCPs pose the greatest danger as EDCs due to their lipophilic trait and thus bioaccumulation potential [7]. OCPs are among the POPs. Occupational and residential exposure to pesticides is an important problem and subject of several epidemiological studies [7]. As mentioned above, primary route of exposure of populations to pesticides occurs through food consumption and ingestion of these chemicals. However, inhalation and dermal absorption of OCPs (such as DDT) may be the other important routes of exposure [7, 22]. Although use of these category of pesticides was limited and banned in most countries about thirty years ago, OCPs are still widely spread in the environment due to their persistence, bioaccumulation. They are hazardous to the environment and pose multiple endocrine-disrupting risks to human health [47]. Some OCPs structurally resemble E2 and can bind to ER and mimic the endogenous ligand's effects. Some xenohormones may exhibit anti-estrogenic actions by binding to AHR and starting transcription of cytochrome p450 enzymes that metabolize E2. Residues of OCPs have been detected in human tissues in epidemiological studies conducted in various countries [48, 49]. Such epidemiological studies have reported OCP levels in human biological samples (such as a serum, fat tissue, breast milk) and examined possible relationship(s) to various diseases and disorders in humans [7]. Residues of OCPs have even been detected in cord blood samples [50].

2.2.4. Hexachlorobenzene (HCB)

HCB is one of the most widespread environmental pollutants. Although use of HCB was terminated in most countries, it is still released into the environment as a byproduct of various manufacturing methods [51]. HCB exposure elicits toxic effects in humans and laboratory animals [52, 53]. Chronic exposure of humans to HCB produces

several effects, such as triggering of porphyria, increased synthesis of liver microsomal enzymes, neurological symptoms, immunological disorders, and thyroid dysfunctions [54]. The main exposure can occur in people exposed to a contaminated site[51]. When heated to decomposition, HCB emits highly toxic fumes of hydrochloric acid, other chlorinated compounds, carbon monoxide and carbon dioxide [55]. Dimethyl formamide and HCB react violently above 65°C [55]. There is no current commercial use of HCB in the United States and most countries. However, HCB was used as a fungicide on the seeds of onions, sorghum, wheat, and other grains until 1984 when the last registered use of the compound as a pesticide was voluntarily cancelled[55]. Previously HCB was also used in the production of pyrotechnic and ordinance materials for the military, the production of synthetic rubber, as a porosity controller in the manufacture of electrodes, a chemical intermediate in dye manufacturing, and a wood preservative[55].

There have been several studies about the adverse effects of HCB administration to *in vitro* and *in vivo* studies and human exposure. Disruptive effects of HCB on thyroid functions have been reported[28]. In an epidemiological study conducted over a period of 14 years, high levels of HCB in blood samples of several hundreds of residents nearby an electrochemical factory in Spain were associated with decreased serum levels of thyroxine, but thyroid-stimulating hormone (TSH) values were not affected[50]. In an earlier study from the same research group, no significant differences were observed in serum levels of TSH in 189 workers at the factory in comparison to 419 other residents living in Flix (Spain) despite five-fold higher blood HCB concentrations in the workers[56]. It has been reported that HCB is a weak agonist of the AHR, a ligand-dependent transcription factor the in cytosol and nucleus of mammalian cells[57]. These studies have suggested that HCB has endocrine disrupting potential. However, there are relatively few studies on possible obesogenic effects of HCB.

2.2.5. Dichlorodiphenyltrichloroethane (DDT)

In 1948, Paul Müller was awarded with the Nobel Prize in Physiology or Medicine for the discovery of DDT as an effective insecticide. Originally, it was used to control vector-borne diseases, such as malaria, and hence use of this substance as a pesticide rapidly increased. However, DDT was banned in 1972 in the United States due to inappropriate environmental effects. Later, several other countries adopted a similar

policy and banned the production and use of DDT following the 2001 Stockholm Convention. However, DDT is still recommended for indoor residual spraying to control malaria vectors[58]. The direct exposure toxic effects of DDT in humans have been examined and it was found that DDT is involved reproductive disease, neurological disease, developmental abnormalities, and cancer[59]. The US Center for Disease Control in 2010 published that 33% of adults in the US are obese, and 17% of children between ages 2 to 19 are obese. Obesity has not only increased in the United States, but also increased in almost every country[59]. DDT and its metabolite DDE are POPs due to their physicochemical characteristics, permitting biomagnification and their storage in the lipid-rich adipose tissue of mammalians[58]. In a new integrated systematic study and meta-analysis, *p,p´*-DDT and *p,p´*-DDE were named as "presumed" to be obesogenic for humans, based on prospective epidemiological observations integrated with experimental evidence of increased rodent adiposity and impaired energy expenditure[58]. It has been documented that DDT is a strong endocrine disruptor by playing as an androgen receptor antagonist and decreasing levels of testosterone in both serum and testis[60]. Such hydrophobic elements could accumulate in the environmental matrix and lead to bioaccumulation in the food chain; particularly in animal fat tissue. In general, these substances are very stable and have a long persistent time[60]. A growing body of literature exhibits relationships between exposure to insecticides, including DDT and DDE, and adverse health effects in humans, such as weight gain and glucose homeostasis change[61]. Moreover, *in vivo* studies have shown that rodents fed a high-fat diet containing POPs promote several metabolic abnormalities, such as increasing visceral adiposity and progressed macrophage infiltration to adipose tissue, and DDE induces fasting hyperglycemia[62].

2.2.6. Dichlorodiphenyldichloroethylene (DDE)

DDE is a common breakdown product of DDT produced by removal of hydrogen chloride. Both DDT and DDE are POPs that bioaccumulate in the lipid-rich fatty tissue of humans[7]. It has been described that DDT and DDE are endocrine disruptors by acting as androgen receptor antagonist [7]. Several cross-sectional epidemiological investigations have combined exposure to *p,p´*-DDE (DDE) with metabolic syndrome, T2D and obesity [62]. DDE, the primary bioaccumulate metabolite of the insecticide, *p,p´*-DDT is a persistent

lipophilic environmental contaminant that has been discovered in the serum and fatty tissues of humans and animals over the world. DDE is more widespread environmentally, more lipophilic, and less acutely toxic than DDT. Though DDT was banned in the United States in 1972, DDE can still be detected in most countries' food supplies and human populations. Recent human cohort studies have established an increased relative risk of developing T2D in individuals with the highest exposure levels [62]. In addition, various *in vivo* studies indicate that rodents fed a high-fat diet containing POPs develop several metabolic abnormalities, including increased visceral adiposity and increased macrophage infiltration to adipose tissue, and DDE induces fasting hyperglycemia [62]. It has been reported that hepatic induction of CYP2B by DDT and DDE through feeding of rats has been associated with liver carcinogenesis, tumor promotion and increment of the liver/body weight ratio [63]. As mentioned above, these persistent pollutants act as endocrine disruptors, mimicking or antagonizing endogenous estrogens and androgens, changing the pattern of synthesis and metabolism of natural hormones, and modifying receptor levels [63]. Several of these hormones contribute to the control body weight homeostasis. High plasma E2 levels in women have been positively correlated with weight control [63]. There are various studies to elucidate a potential correlation between several chronic diseases (such as insulin resistance, T2D, fatty liver disease, atherosclerosis, hypertension, stroke, cancer, asthma) and environmental pollution with OCPs [64-66]. In the recent years, possible implications of DDT and DDE in the development of obesity have gained more attention.

2.3. Peroxisome Proliferator-Activated Receptors (PPARs)

It has been reported that long term exposure to EDCs may lead to weight gain and consequently obesity. Tributyltin chloride and triphenyltin chloride can bind with retinoid X receptor (RXR) and peroxisome proliferator-activated receptor, nuclear receptors that are responsible for lipid homeostasis and adipogenesis [37, 67]. At the cellular level, obesogenic activity can arise due to disruptive endocrine effects by impeding with peroxisome proliferator-activated receptors (PPARs) and some steroid receptors[7].

PPARs are responsible for translating signals that control cellular bioenergetics, works as nutrition sensors, and controls the metabolism to adjust systemic metabolism [68]. It has three types as PPARα, γ, and δ (also known as PPARβ). They also have pro-

oxidative or storage functions [69]. PPARs are linked with the RXR and can create their heterodimers. These heterodimers alter the expression of target genes [5]. Therefore, heterodimers bind with PPAR responsive elements and modulate with coactivator and corepressor proteins, changing the metabolism rate of free fatty acids, eicosanoids, and xenobiotics [69]. PPARα and PPARγ are very important cellular components because they are considered to be pharmacological targets for treatment strategy / management of obesity. Understanding how the PPAR receptors regulate metabolism and energy homeostasis in the body is of importance as there is a global increase in obesity [69].

Thiazolidinedione drugs have been utilized as a cure to insulin T2D target that PPARγ works on increasing sensitivity to insulin with the side effect that promotes adipogenesis[5]. Tributyltin induces preadipocytes to differentiate into adipocytes in the 3T3-L1 cell line, increases adiposity, and reduces metabolic functions in rats by raising PPARγ [7]. It has been reported that various EDCs may alter adipogenesis by interfering with PPAR-γ function. They regulate lipid influx, adipocyte reproduction and differentiation. Some EDCs link to certain nuclear receptors, change gene expression that promotes obesity[7]. Various obesogens may act thorough different mechanisms for activating the PPARγ/RXR heterodimer. Research on obesogenic actions of EDCs is important to understand underlying molecular mechanisms. Investigation of the effects of obesogens on the PPARγ/RXR heterodimer may provide valuable insight for eliminating obesogenic influences [5].

2.4. Aryl Hydrocarbon/dioxin Receptor (AHR) Pathway and Adipogenesis

The aryl hydrocarbon/dioxin receptor (AHR) is a linked transcription factor and acts against environmental toxicants. In the last decades, AHR has been shown to play important roles in various cells and tissues[70]. It has been reported that inhibition of AHR pathway reduces obesity and fatty liver diseases in rats[71]. Low-density lipoproteins (LDLs) are known to activate AHR signaling[71]. LDLs are derived from fats and transported by the blood. Lipoprotein particle in LDLs contains an apolipoprotein B protein, 50– 60 ancillary proteins and ~5,000 fat molecules and having different types from cholesterol, phospholipids, and triglycerides[71].

Formation of adipogenesis has been widely studied by various laboratories by using an *in vitro* system, 3T3-L1 cells[72]. The associated hormonal activities of insulin (IDM), the glucocorticoid dexamethasone and the phosphodiesterase inhibitor isobutyl methylxanthine give further cell propagation in previously quiescent, confluent cells[72]. That is leading to growth an irreversible cessation, and finally by stimulation of the same genes in adipocytes *in vivo*[72]. The variations in gene expression include brief appearance of c-myc and c-jun, thus supported by sequential elevations of C/EBPβ, C/EBPα, and PPARγ [72]. Drugs that activate PPARγ, such as the thiazolidinedione BRL-49653, enhance the hormonal mix IDM effects. These drugs are essential for stimulation of adipogenesis in MEF cells such as 10T1/2 [72].

2,3,7,8-Tetrachlorodibenzo-p-dioxin can redirect triglycerides of adipose tissue to the liver and diabetic properties in animals, involving reduced glucose uptake in the adipose tissues. TCDD has been shown to reduce the activity of a series of adipogenic genes [72]. AHR may affect adipogenesis by altering PPAR-γ expression.

The aim of the present study was to investigate obesogenic effects of chronic HCB, DDT and *p,p´*-DDE exposure in male rat model by analyzing adipogenesis, testicular histology and the levels of *UCP1* and *Pparγ* in white and brown adipose tissues.

3. MATERIALS and METHODS

3.1. Animals

Thirty-two adult male Sprague-Dawley rats were included in this study. They were fed with standard rat chow and had access to water *ad libitum*. Animals were maintained in a controlled environment of 21±2 °C, 50±10% relative humidity, and a 12-12 h light/dark cycle at the Yeditepe University Medical School Experimental Research Centre (YÜDETAM). The rats were adapted to these conditions for five weeks. All the experimental procedures involving animals were approved by the Yeditepe University Ethics Committee on Animal Research.

3.2. Experimental Groups and Administration of the Drugs

HCB (#45522), DDT (#31041) and DDE (#35487) were obtained from Sigma-Aldrich and dissolved in corn oil. The animals were randomly divided into four groups (n=8 / group). All groups received 5 mg/kg of the OCPs under investigation via oral gavage administered every other day for five weeks. Animals in the control group received corn oil alone by oral gavage. Body weight and body temperature (rectal temperature) parameters were recorded weekly.

At the end of five weeks of HCB, DDT and DDE administration period, all animals were terminated by decapitation. Blood glucose levels of the animals were measured by using a glucometer. Blood samples were collected by cardiac puncture, serum was separated and kept at -80ºC until analyzed. White adipose tissue (WAT) and brown adipose tissue (BAT) samples were collected and kept -80ºC for quantitative real-time polymerase chain reaction (qRT-PCR) experiments. Testes tissues were dissected out and fixed in Bouin's solution for histological analyses.

3.3. Enzyme-Linked ImmunoSorbent Assay (ELISA) for Serum Hormone Levels

The serum samples were thawed on ice and serum luteinizing hormone (LH; E-EL-R0026, Elabscience) and follicle-stimulating hormone (FSH; E-EL-R0391, Elabscience) and testosterone (E-EL-0155, Elabscience) levels were determined by ELISA method according to the manufacturer's instructions.

3.4. RNA Isolation, cDNA Synthesis and Gene Expression Analyses

WAT and BAT samples were thawed on ice and were homogenized in TRI Reagent by using a Bullet Blender X24 (NextAdvance) and RNA was isolated according to the manufacturer's instructions (DirectZol™ RNA MiniPrep Plus, Zymo Research, R2072). Isolated RNA was quantified on a NanoDrop 2000 spectrophotometer (Thermo Scientific). cDNA was synthesized from equal amounts of RNA (50-150 ng) for each set of experiments according to manufacturer's instructions (iScript™ cDNA Synthesis Kit, Bio-Rad, 1708891).

Primers for *Pparγ*, *UCP1* and *β-Actin* were designed by using the online primer designing tool Primer3web version 4.1.0 (https://primer3.ut.ee/). The primer list for *Pparγ*, *UCP1* and *β-Actin* are indicated in Table 1.

Table 1. Primers for *Pparg*, *UCP1* and *β-Actin* used in qRT-PCR.

Primer ID	**Sequence (5´-3´)**
Pparg_F	TCAAACTCCCTCATGGCCAT
Pparg_R	GCATTGTGAGACATCCCCAC
UCP1_F	CAACACTGTGGAAAGGGACG
UCP1_R	CAGCTGGGTACACTTGGGTA
Actin_F	TCTTCCAGCCTTCCTTCCTG
Actin_R	CACACAGAGTACTTGCGCTC

Table 2. Thermal cycle conditions used in qRT-PCR.

Step	**Temperature**	**Duration**	**Number of Cycles**
Initial Denaturation	95 ºC	3 min	1
Denaturation	95 ºC	10 sec	
Annealing	48 ºC	20 sec	55
Extension	52 ºC	30 sec	

In order to analyze possible changes in *Pparγ* and *UCP1*, sequences were amplified by qRT-PCR by using SsoAdvanced™ Universal SYBR® Green Supermix (Bio Rad, 1725270) on a CFX96 Touch Real-Time PCR Detection System (Bio Rad) with the thermal cycle conditions indicated in Table 2. Relative gene expression was assessed by using $2^{-\Delta\Delta CT}$.

3.5. Tissue Processing and Morphological Analysis

At the end of the study, decapitation was performed for tissue isolation from the experimental and control group animals. Then, the abdominal region was dissected and testes were isolated. Testes were fixed in Bouin's solution (Histo Plus, #HCM-BOU-1000) for 36 hours. Testes were processed for tissue slicing and staining.

The fixative was thoroughly removed from the tissues. Afterwards, the testes were processed through graded alcohols, cleared in xylene, and embedded in liquid paraffin. Five μm sections were taken from the paraffin blocks by using a microtome (Leica, #RM2245). Sections were incubated for 1 hour at 60°C to remove paraffin and left in xylene for 2x20 min to completely remove paraffin. Then, the tissue sections were incubated in decreasing alcohol series for 10 minutes each (100%, 90%, 80% and 70%). The sections were washed with distilled water and stained with Hematoxylin staining (Bio Optica, Gill3 #05-06015), then washed with tap water and they were stained with Eosin (Sigma, #HT110132). After washing with distilled water, the sections were incubated in increasing alcohol series (70%, 80%, 90%). After incubating in 100% alcohol, sections were placed 30 min in xylene. The slides were covered with coverslip using xylene-based mounting medium (Bio Optica, #05-BMHM508). Imaging was performed by using light microscopy (Leica DM 6000).

3.6. Statistical Analysis

Data was analyzed by using GraphPad Prism 7 (GraphPad Software, USA). All data were presented as mean ± standard deviation (STD). The distribution of the data was analyzed by Shapiro-Wilk normality test. The outliers in the datasets were determined and removed by using ROUT method (Q=1%)[73]. Data were statistically analyzed by using One-Way Analysis of Variance (One-Way ANOVA) followed by Dunnett's multiple comparison test. $P<0.05$ was considered statistically significant.

4. RESULTS

4.1. Body Weight and Blood Glucose

Body weight and body temperature did not significantly differ among the groups throughout the experimental period (Figure 2a and b). We also determined that blood glucose levels at the end of the experimental period were similar (Figure 3).

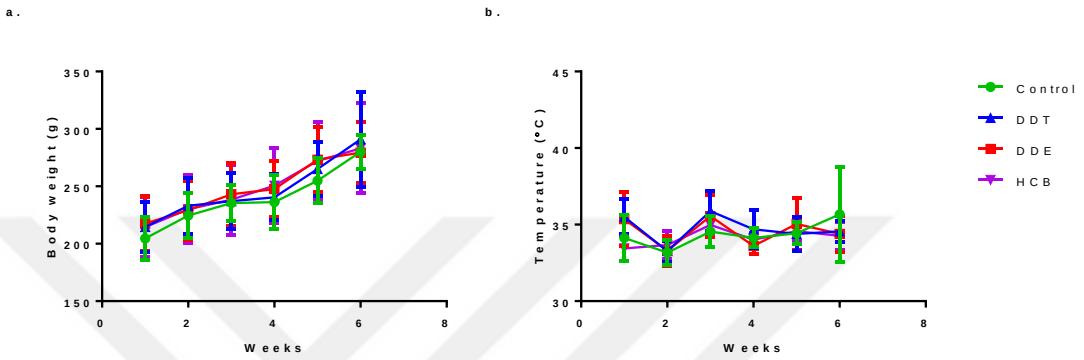

Figure 2. (a) Body weight and **(b)** body temperature (rectal temperature) measurements of the animals throughout the experimental period.

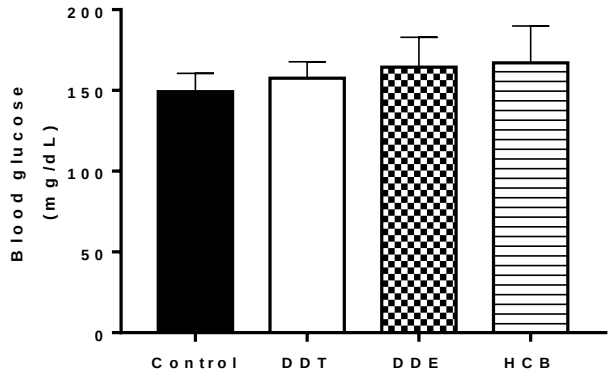

Figure 3. Blood glucose levels of the animals at the end of experimental period.

4.2. Serum Hormone Levels

No significant differences were observed in serum FSH (Figure 4a) and LH (Figure 4b) levels among the groups at the end of the experimental period. However, serum testosterone levels were found to be significantly lower in all DDT, DDE and HCB groups compared to control group (Figure 4c; $p<0.001$, $p<0.05$ and $p<0.001$, respectively).

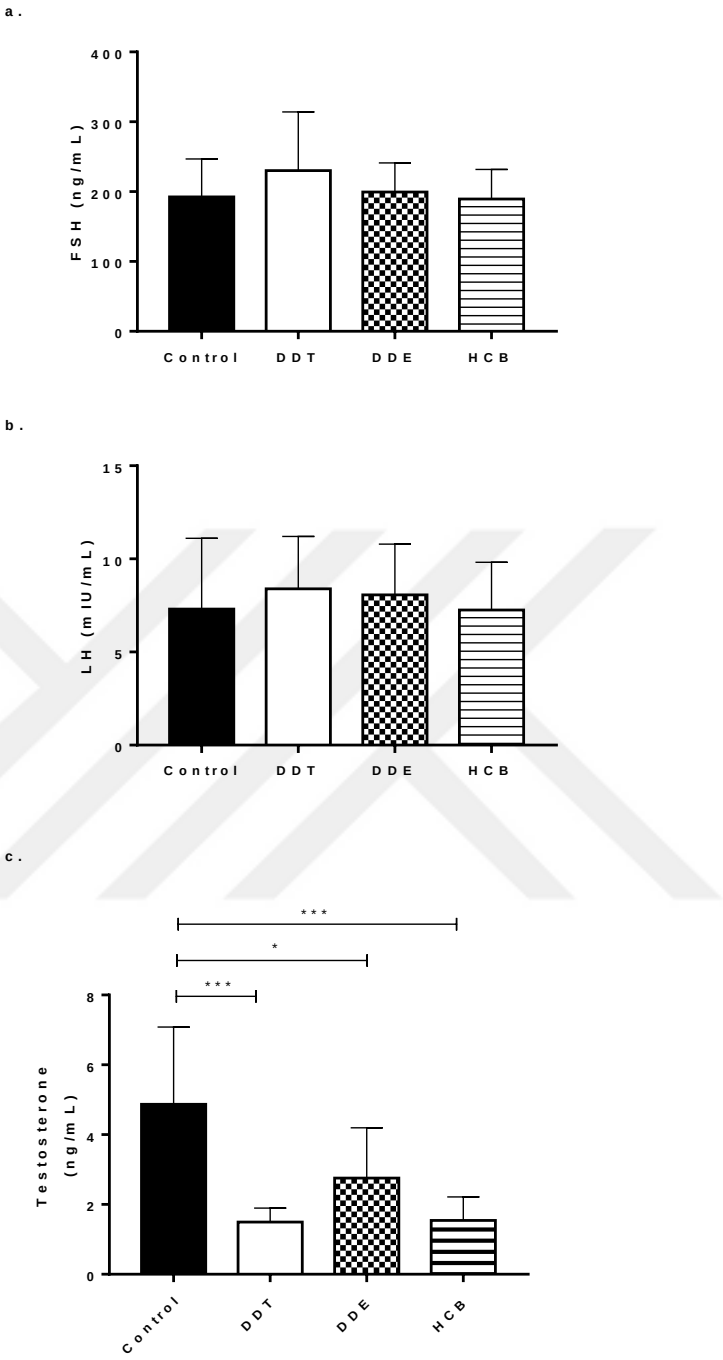

Figure 4. Serum **(a)** FSH **(b)** LH and **(c)** testosterone levels of the animals throughout the experimental period (*p<0.05 and ***p<0.001).

4.3. *Pparγ* and *UCP1* levels in WAT and BAT

HCB significantly increased the *Pparγ* levels (p<0.05), while DDT and DDE significantly reduced both *Pparγ* and *UCP1* levels (p<0.001) in WAT (Figure 5a). On the

other hand, DDT significantly reduced both *Pparγ* and *UCP1* levels ($p<0.01$), while HCB and DDE did not cause any significant alterations in the expression levels of *Pparγ* and *UCP1* in BAT (Figure 5b).

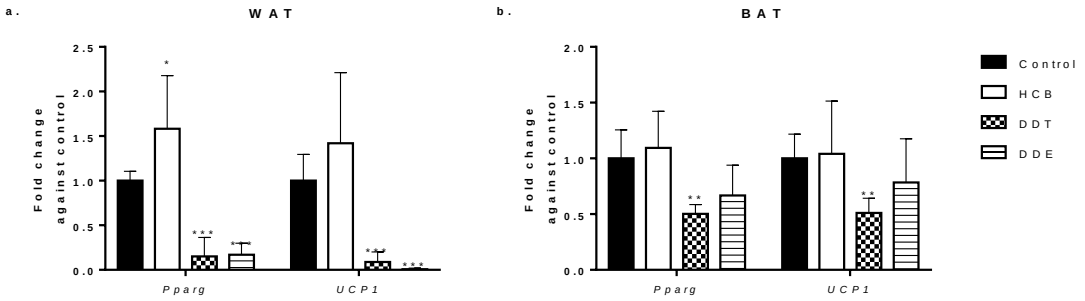

Figure 5. *Pparγ* and *UCP1* expression levels in **(a)** WAT and **(b)** BAT. (*$p<0.05$, **$p<0.01$ and ***$p<0.001$).

4.4. Morphological Analysis of Testes

Testicular morphology following chronic administration of DDT, DDE and HCB groups was examined under light microscope after processing with Hematoxylin-Eosin staining.

In the control group, testicular parenchyma had a normal appearance (

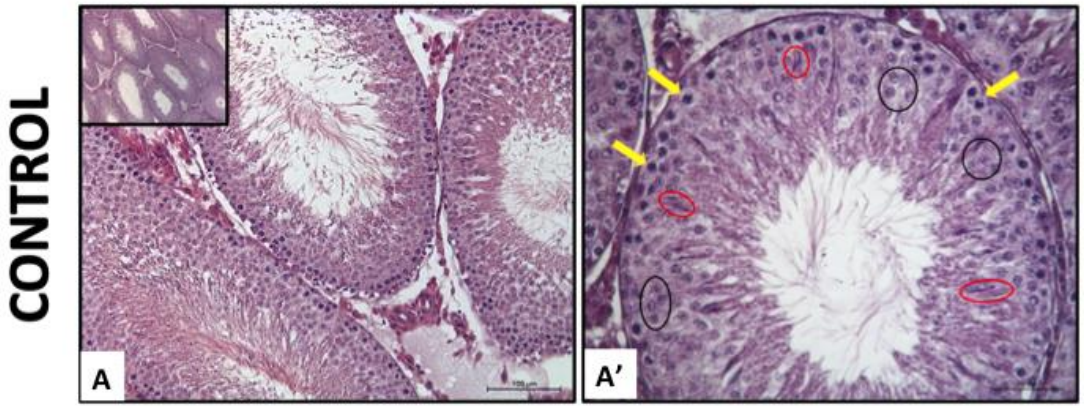

Figure **6**). Peritubular tissue surrounding the seminiferous tubule was normal. Spermatogenic cells and Sertoli Cells were seen in the seminiferous tubule epithelium. In the seminiferous tubules, 1-2 rows of gonocytes located on the basal lamina were seen. Primary spermatocytes with oval nuclei were identified in the seminiferous cord epithelium. Spermatids located close to the lumen and darker nuclei were seen.

Spermiogenesis was detected from spermatozoa cells located in the lumen and sperm cells were seen in the lumen with their tail. Sertoli cells were located along the seminiferous tubule epithelium. Leydig cells, connective tissue and blood vessels were distinguished in the interstitial area (

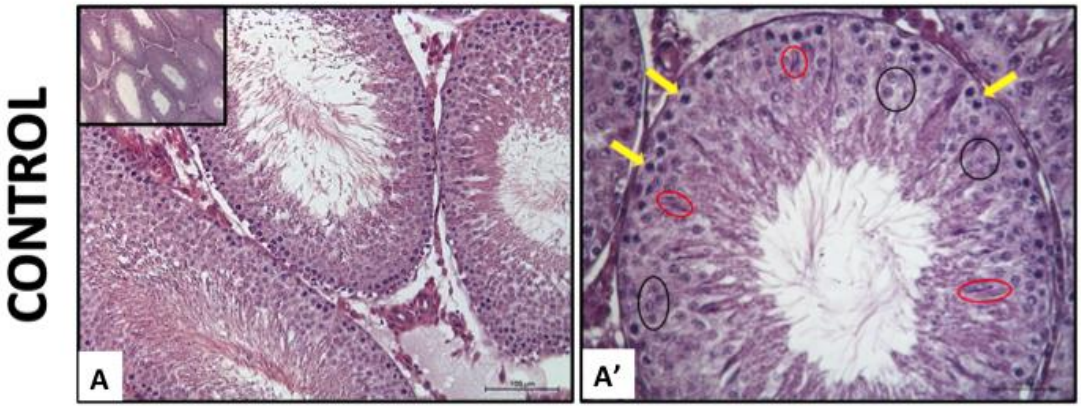

Figure **6**).

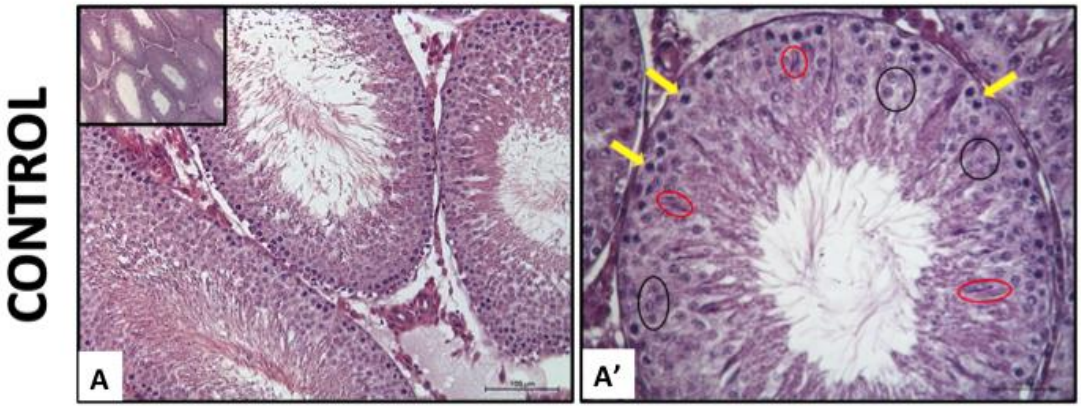

Figure 6. Testicular morphological examination of the control group. Figure showing seminiferous tubules containing spermatogonia (yellow arrows) resting on the basement membrane, primary spermatocyte (black circles) and sperm cells (red circles**). A.** Control group 20X magnification; **A'**, 40X magnification.

It was determined that there was a significantly thin connective tissue on the basal part of the seminiferous tubule in the DDT group compared to the control group. It was observed that some parts of the basal lamina generally curved towards the inner part of the tubule or towards the inter-tubular area. In the epithelium of seminiferous tubule,

fragmentation was observed in the spermatogenic cells and at the same time, we detected a significant decrease in the number of cells. It was observed that the primary spermatocytes were generally enlarged and hypertrophic. While head of sperm cells were detected embedded in the tubule, their tails were not seen as healthy in the lumen. It was determined that the diameter of the seminiferous tubule was smaller compared to the control group (Figure 7).

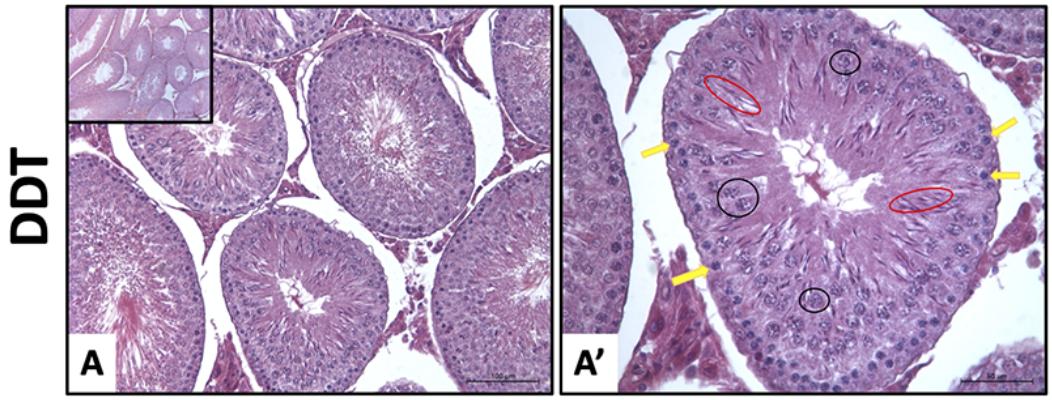

Figure 7. Testicular morphological examination of DDT group. Figure showing seminiferous tubules containing spermatogonia (yellow arrows) resting on the basement membrane, primary spermatocyte (black circles) and sperm cells (red circles). **A.** DDT group 20X magnification; **A'**, 40X magnification.

In the DDE group, thin connective tissue located at the basal part of the seminiferous tubules was detected. It is seen that spermatogenesis and spermiogenesis occur successfully in the DDE group, which is one of the sperm cells in the lumen. The nuclei of the gonocytes located at the base of the seminiferous cords were seen as pycnotic. Cells in the interstitial space exhibited morphology similar to the control group. It was determined that the diameter of the seminiferous tubule was smaller compared to the control group (Figure 8).

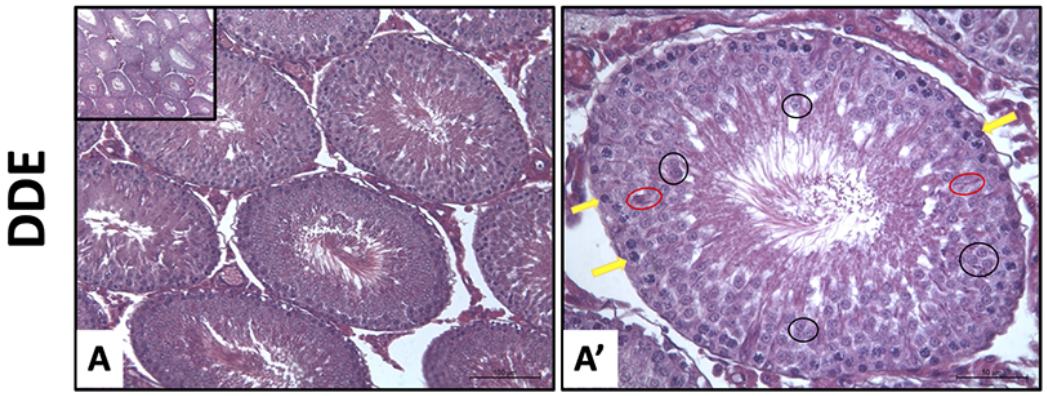

Figure 8. Testicular morphological examination of DDE group. Figure showing seminiferous tubules containing spermatogonia (yellow arrows) resting on the basement membrane, primary spermatocyte (black circles) and sperm cells (red circles). **A.** DDE group 20X magnification; **A'**, 40X magnification.

In the HCB group, thin and morphological deterioration were detected in the connective tissue located in the basal part of the seminiferous tubules. The decrease in the number of cells seen in the seminiferous tubule epithelium was remarkable. Hypertrophy was determined in primary spermatocytes. The head of the sperm cells seem to have reached almost to the seminiferous tubule basal membrane. This situation made us think that the blood-testicular barrier might be impaired. At the junction of the basal and luminal part, separations were observed between Sertoli cells. The nuclei of Sertoli cells have a pycnotic appearance. It was determined that the diameter of the seminiferous tubules was smaller than the control group (Figure 9).

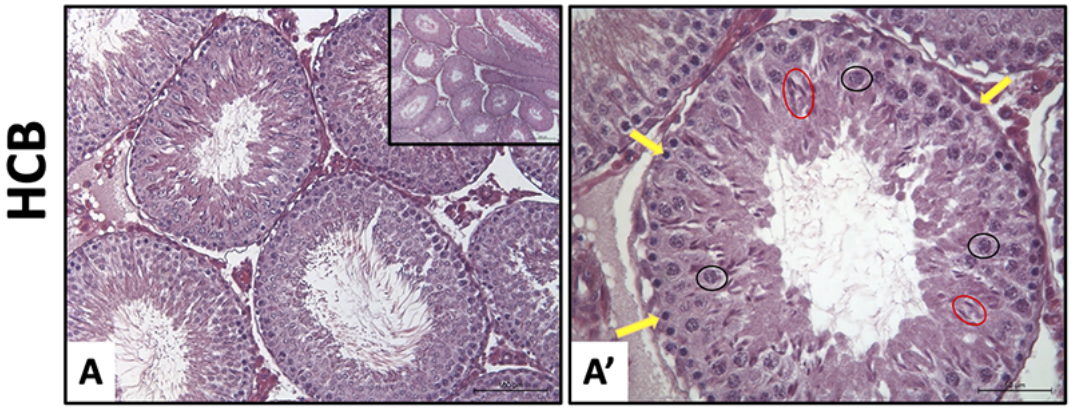

Figure 9. Testicular morphological examination of HCB group. Figure showing seminiferous tubules containing spermatogonia (yellow arrows) resting on the basement membrane, primary spermatocyte (black circles) and sperm cells (red circles). **A.** HCB group 20X magnification; **A'**, 40X magnification.

Morphological comparison of control, DDT, DDE, and HCB group testis tissue was shown in Figure 10.

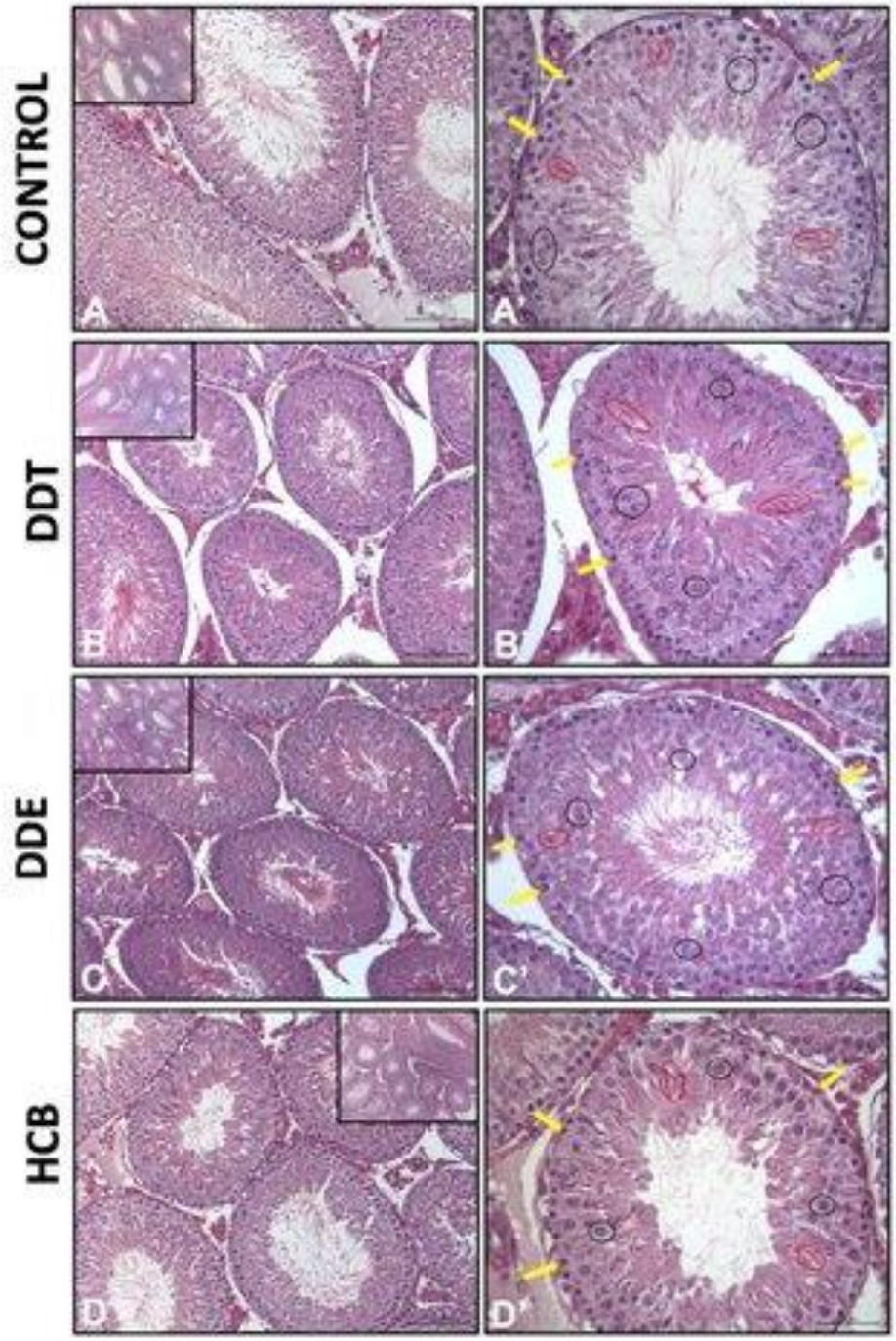

Figure 10. Morphological comparison of control, DDT, DDE and HCB group testis tissue. Figure showing seminiferous tubules containing spermatogonia (yellow arrows) resting on the basement membrane, primary spermatocyte (black circles) and sperm cells (red circles). **A.** Control group **(A-A')**, DDT group **(B-B')**, DDE group **(C-C')**, HCB group **(D-D')**.

4.5. Serum Cholesterol and Triglyceride levels

Serum cholesterol and triglyceride levels were not significantly altered following chronic administration of DDT, DDE or HCB.

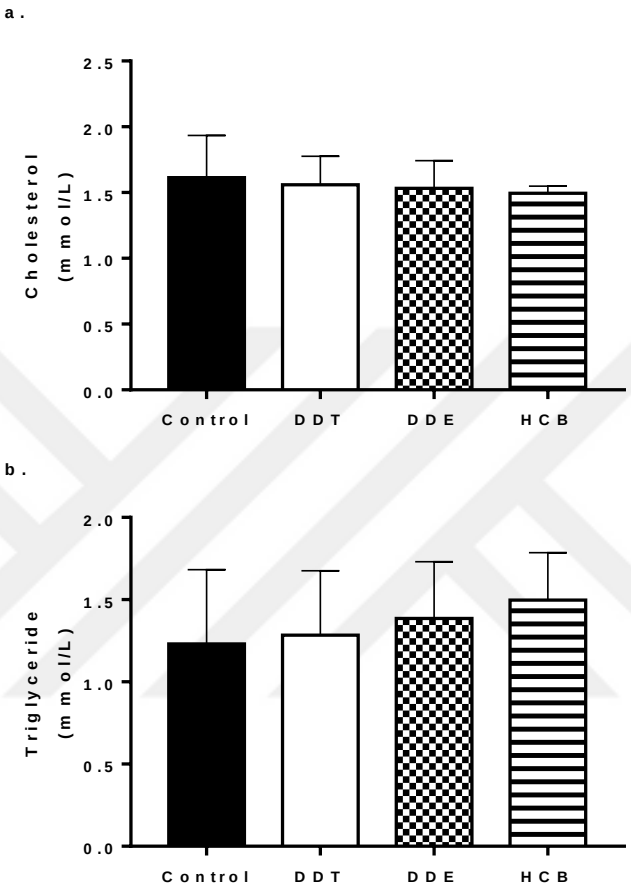

Figure 11. Effect of DDT, DDE, and HCB on serum **(a)** cholesterol and **(b)** triglyceride levels.

5. DISCUSSION and CONCLUSION

Results of the present study have shown that chronic oral exposure to low doses of DDT, DDE, and HCB for five weeks affected testicular function and morphology differently in the rat. Serum testosterone levels were significantly reduced by all three OCPs examined in parallel to altered morphology in the testicular tissue. However, there was no significant change in serum LH and FSH levels. Previous studies have reported that DDT-induced decrease in testosterone levels could be caused by reduced release of this androgen by the Leydig cells. High doses of DDT (30-120 mg/kg) have been reported to cause a decrease in the testosterone levels in flounders [23]. Similar findings have been documented in the literature in studies where another OCP, lindane decreased serum testosterone levels due to alterations in Leydig cells [74]. Physiologically, the anterior pituitary hormone, LH, regulates the release of testosterone from the Leydig cells in the testes. However, a local disruption in the steroidogenesis and/or metabolism has been reported to account for this decline. It has been reported that exposure to various doses of DDT for 24 h increased the activity of at least seven CYP dependent testosterone-hydroxylating enzymes, that results in reduced serum testosterone levels [75]. The size of the seminal vesicles of rats after exposure to DDT and DDE were significantly reduced. A previous report suggested this effect of DDT on the testes may partly mediated by the antiandrogenic activity of this compound [74]. Eventually, histological observations showed chemicals pesticides have the ability to effect on the tubules in the testis because they showed more severe alteration comparing with the normal group. Actually, several seminiferous tubules demonstrated exfoliation of germ cells in the lumina. This case can be observed upon treatment with different toxicants *in vivo* [76]. *p,p´*-DDE was shown to induce testicular apoptosis and oxidative stress in both prepubertal [77] and adult rats [78]. Moreover, elevated *p,p´*-DDE was shown to be associated with increased risk of testicular germ cell tumors [79].

A recent study from South Africa has reported indoor residual spraying of DDT and DDE to control malaria mosquitoes [80]. Serum samples were obtained from men (a total of 535 men, aged 18-40) living near the indoor residual spraying areas. High levels of DDT and DDE were detected in their blood that were associated significantly reduced LH and FSH concentrations compared to men living in non-indoor residual spraying

villages [80]. However, previous studies reported that exposure to DDT and DDE in rat Hershberger assay caused slight increase in serum LH and FSH levels, but these changes were not found to be statistically significant [81]. Previous epidemiological studies reported that high levels of DDT and DDE were not associated with significant alterations in gonadotropin and testosterone levels in men exposed to dietary contamination with these OCPs [82]. Similar findings were also observed in Swedish fisherman [83] and DDT spray-workers in Sardinia, Italy [84].

In our study, while DDT caused significant decreases in *PPARγ* and *UCP1* levels in both WAT and BAT, DDE caused significant decreases only in WAT. We determined that HCB significantly increased the *PPARγ* levels only in WAT but did not affect other parameters neither in WAT nor BAT. There are studies reporting that exposure to DDT and DDE is associated with increased prevalence of obesity and metabolic disease [85, 86]. The most recommended mechanism of action for obesogenic compounds involves disruption of PPARγ, which is considered a main regulator of adipogenesis and lipid homeostasis [87]. The expression and activation of PPARγ are sufficient for initiating the adipogenic differentiation program and maintaining adipocyte phenotype, integrity and function based on multiple different genetic mouse models [88]. It is known that PPARγ mainly regulates the expression of genes implicated in adipocyte differentiation and adipocyte maintenance. In addition, PPARγ also governs the expression genes involved in lipogenesis, fatty acid transport and gluconeogenesis [89]. *In vitro* studies have shown that PPARγ is expressed higher in *p,p′*-DDT-treated cells than the differentiated 3T3-L1 cells [90] and mesenchymal stem cells [91]. Tai et al. have also demonstrated that activation of PPARγ in brown adipocytes leads to increase in adipogenesis and lipid metabolism [92]. It has been suggested in another study that perinatal DDT exposure decreases *PPARγ* levels in BAT, impairs thermogenesis and the metabolism of carbohydrates and lipids which may increase susceptibility to the metabolic syndrome in adult female offspring [93]. It has been reported that in mammals, PPAR-γ coactivator 1-alpha (PGC-1α)/fibronectin type III domain-containing protein 5 (FNDC5) promotes the browning of beige fat cells in WAT resulting in enhanced thermogenesis and increased energy expenditure [94]. In a study on *PPARγ*-knockout mice have showed reduced wet weight of BAT when compared to wild type animals but, no difference found in terms of body weight was found [95]. UCP1 is expressed in BAT and plays a key role in the body's energy consumption and heat production [96, 97]. Moreover, UCP1 greatly increases the proton

conductivity of the mitochondrial inner membrane and catalyzes adaptive thermogenesis in mammalian BAT [98]. It has been shown that increased expression of UCP1 in white adipocytes leads to "browning" of WAT. In a study, in which mice were injected with adenovirus particles containing full-length FNDC5, it was shown that there was a decrease in WAT and an increase in the expression of *UCP1*, which provides browning in WAT, resulting in an increase in energy expenditure and the development of brown adipocyte-like cells [99].

Considering our study results, the fact that DDT and DDE decreased both *PPARy* and *UCP1* levels in WAT means that the browning of white adipocytes decreased [99]. This data supports the effect of DDT on *PPARy* in the study conducted by La Merrill et al. [93]. Likewise, the suppressive effect of DDT on *PPARy* and *UCP1* was similar to that of BAT, which proves that it reduces energy expenditure. However, despite these suppressive effects of DDT on *PPARy* and *UCP1*, the absence of any increase in body weights of rats was not an expected finding for us. At the same time, the lack of a significant difference in body temperature, which is accepted as an indicator of energy expenditure, is not in line with the recommendations of Hofmann et al. [94]. When we evaluate all the data of our study together, we can say that all three of these pesticides can accumulate in the adipose tissue due to their lipophilic nature [100] and may disrupt the functions of WAT and BAT, thus promoting the development of metabolic diseases, especially obesity.

The cholesterol and triglyceride levels were not significantly altered by neither DDT nor DDE. There were significant inconsistencies between this study`s findings between the report by Teeyapant et al. [101], where both DDT and DDE have strong effect on changing the serum of cholesterol and triglyceride levels which was the opposite of what this study found. In addition to HCB, there was no change or effect on both serum cholesterol and triglyceride levels in this present study which is contrary to previous research that exposed HCB to increased cholesterol and triglyceride levels in inhabitants of Eastern Slovakia [102].

In summary, exposure to DDT, DDE, and HCB at a dose of 5 mg/kg for a period of five weeks do not promote browning of WAT in male rats according to *Pparγ* and *UCP1* expression results. In addition, HCB-induced expression of *Pparγ* in WAT suggests it may have obesogenic activity, while DDT and DDE-related decreases in expression of *Pparγ* and *UCP1* in WAT suggest anti-adipogenic activity. On the other

hand, serum testosterone levels significantly reduced in all groups upon pesticide treatment, while there were no significant alterations in serum FSH and LH levels. Additionally, DDT, DDE and HCB had a strong influence on male rat testicular morphology and spermatogenesis. In conclusion, HCB-induced expression of Pparγ in WAT obesogenic activity of HCB. In addition, DDT and DDE-induced decreases in expression of Pparγ and UCP1 in WAT suggest that these two pesticides may reduce adipogenesis. Our findings also implicate that OCPs may affect testosterone levels through direct effects on testes, rather than influencing gonadotropin release.

6. REFERENCES

1. Tremmel M, Gerdtham UG, Nilsson PM, Saha S. Economic Burden of Obesity: A Systematic Literature Review. *Int J Environ Res Public Health*. 2017;14(4)

2. Cano-Sancho G, Salmon AG, La Merrill MA. Association between Exposure to p,p'-DDT and Its Metabolite p,p'-DDE with Obesity: Integrated Systematic Review and Meta-Analysis. *Environ Health Perspect*. 2017;125(9):096002.

3. Romieu I, Dossus L, Barquera S, et al. Energy balance and obesity: what are the main drivers? *Cancer Causes Control*. 2017;28(3):247-258.

4. Lavie CJ, Ozemek C, Carbone S, Katzmarzyk PT, Blair SN. Sedentary Behavior, Exercise, and Cardiovascular Health. *Circ Res*. 2019;124(5):799-815.

5. Griffin MD, Pereira SR, DeBari MK, Abbott RD. Mechanisms of action, chemical characteristics, and model systems of obesogens. *BMC Biomed Eng*. 2020;2:6.

6. Elobeid MA, Allison DB. Putative environmental-endocrine disruptors and obesity: a review. *Curr Opin Endocrinol Diabetes Obes*. 2008;15(5):403-8.

7. Yilmaz B, Terekeci H, Sandal S, Kelestimur F. Endocrine disrupting chemicals: exposure, effects on human health, mechanism of action, models for testing and strategies for prevention. *Rev Endocr Metab Disord*. 2020;21(1):127-147.

8. Blüher M. Obesity: global epidemiology and pathogenesis. *Nat Rev Endocrinol*. 2019;15(5):288-298.

9. Organization WH. *Tackling NCDs:'best buys' and other recommended interventions for the prevention and control of noncommunicable diseases*. 2017.

10. Whitlock G, Lewington S, Sherliker P, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet*. 2009;373(9669):1083-96.

11. Chooi YC, Ding C, Magkos F. The epidemiology of obesity. *Metabolism*. 2019;92:6-10.

12. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. *International Journal of Obesity*. 2008;32(9):1431-1437.

13. González Jiménez E. Obesity: etiologic and pathophysiological analysis. *Endocrinol Nutr*. 2013;60(1):17-24.

14. Thaker VV. Genetic and Epigenetic Causes of Obesity. *Adolesc Med State Art Rev*. 2017;28(2):379-405.

15. Huvenne H, Dubern B, Clément K, Poitou C. Rare Genetic Forms of Obesity: Clinical Approach and Current Treatments in 2016. *Obes Facts*. 2016;9(3):158-73.

16. Hinney A, Vogel CI, Hebebrand J. From monogenic to polygenic obesity: recent advances. *Eur Child Adolesc Psychiatry*. 2010;19(3):297-310.

17. Darbre PD. Endocrine Disruptors and Obesity. *Curr Obes Rep*. 2017;6(1):18-27.

18. Heindel JJ, Newbold R, Schug TT. Endocrine disruptors and obesity. *Nat Rev Endocrinol*. 2015;11(11):653-61.

19. Zoeller RT, Brown TR, Doan LL, et al. Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society. *Endocrinology*. 2012;153(9):4097-110.

20. Caserta D, Maranghi L, Mantovani A, Marci R, Maranghi F, Moscarini M. Impact of endocrine disruptor chemicals in gynaecology. *Hum Reprod Update*. 2008;14(1):59-72.

21. Wang C, Yang L, Wang S, et al. The classic EDCs, phthalate esters and organochlorines, in relation to abnormal sperm quality: a systematic review with meta-analysis. *Sci Rep*. 2016;6:19982.

22. M.; N, JL. D. Sources of Human Exposure. In: Carpenter DO, ed. *Effects of persistent and bioactive organic pollutants on human health*. John Wiley & Sons; 2013:8-25.

23. Mills LJ, Gutjahr-Gobell RE, Haebler RA, et al. Effects of estrogenic (o,p'-DDT; octylphenol) and anti-androgenic (p,p'-DDE) chemicals on indicators of endocrine status

in juvenile male summer flounder (Paralichthys dentatus). *Aquat Toxicol*. 2001;52(2):157-76.

24. Grün F, Blumberg B. Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. *Endocrinology*. 2006;147(6 Suppl):S50-5.

25. Erkin-Cakmak A, Harley KG, Chevrier J, et al. In utero and childhood polybrominated diphenyl ether exposures and body mass at age 7 years: the CHAMACOS study. *Environ Health Perspect*. 2015;123(6):636-42.

26. Valvi D, Mendez MA, Martinez D, et al. Prenatal concentrations of polychlorinated biphenyls, DDE, and DDT and overweight in children: a prospective birth cohort study. *Environ Health Perspect*. 2012;120(3):451-7.

27. Valvi D, Mendez MA, Garcia-Esteban R, et al. Prenatal exposure to persistent organic pollutants and rapid weight gain and overweight in infancy. *Obesity (Silver Spring)*. 2014;22(2):488-96.

28. Mendez MA, Garcia-Esteban R, Guxens M, et al. Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. *Environ Health Perspect*. 2011;119(2):272-8.

29. Liu G, Dhana K, Furtado JD, et al. Perfluoroalkyl substances and changes in body weight and resting metabolic rate in response to weight-loss diets: A prospective study. *PLoS Med*. 2018;15(2):e1002502.

30. Chamorro-Garcia R, Blumberg B. Current Research Approaches and Challenges in the Obesogen Field. *Front Endocrinol (Lausanne)*. 2019;10:167.

31. Convention S. Stockholm convention on persistent organic pollutants. Accessed August 19, 2020. http://chm.pops.int/Portals/0/Repository/convention_text/UNEP-POPS-COP-CONVTEXT-FULL.English.PDF

32. Convention S. The new POPs under the Stockholm Convention. Secretariat of the Stockholm Convention 2014. Accessed August 19, 2020. http://chm.pops.int/TheConvention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspx

33. Tsai WT. Organochlorine Insecticides. In: Wexler P, ed. *Encyclopedia of Toxicology (Third Edition)*. Academic Press; 2014:711-713.

34. Stringer R, Johnston P. Chlorine and the environment: an overview of the chlorine industry. 2001;

35. Holt E, Weber R, Stevenson G, Gaus C. Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) impurities in pesticides: a neglected source of contemporary relevance. *Environ Sci Technol*. 2010;44(14):5409-15.

36. Srogi K. Levels and congener distributions of PCDDs, PCDFs and dioxin-like PCBs in environmental and human samples: a review. *Environmental Chemistry Letters*. 2008;6(1):1-28.

37. Covaci A, Hura C, Schepens P. Selected persistent organochlorine pollutants in Romania. *Sci Total Environ*. 2001;280(1-3):143-52.

38. Safe S. Endocrine disruptors and falling sperm counts: lessons learned or not! *Asian J Androl*. 2013;15(2):191-4.

39. Atkinson S, Crocker DE, Ortiz RM. Endocrine Systems. In: Würsig B, Thewissen JGM, Kovacs KM, eds. *Encyclopedia of Marine Mammals (Third Edition)*. Academic Press; 2018:318-328.

40. Reaves DK, Ginsburg E, Bang JJ, Fleming JM. Persistent organic pollutants and obesity: are they potential mechanisms for breast cancer promotion? *Endocr Relat Cancer*. 2015;22(2):R69-86.

41. Tang-Péronard JL, Heitmann BL, Jensen TK, et al. Prenatal exposure to persistent organochlorine pollutants is associated with high insulin levels in 5-year-old girls. *Environ Res*. 2015;142:407-13.

42. Calaf GM, Ponce-Cusi R, Aguayo F, Muñoz JP, Bleak TC. Endocrine disruptors from the environment affecting breast cancer. *Oncol Lett*. 2020;20(1):19-32.

43. Pessah IN, Lein PJ, Seegal RF, Sagiv SK. Neurotoxicity of polychlorinated biphenyls and related organohalogens. *Acta Neuropathol*. 2019;138(3):363-387.

44. Carpenter DO. *Effects of Persistent and Bioactive Organic Pollutants on Human Health*. Wiley; 2013.

45. Sathiakumar N, MacLennan PA, Mandel J, Delzell E. A review of epidemiologic studies of triazine herbicides and cancer. *Crit Rev Toxicol*. 2011;41 Suppl 1:1-34.

46. Kalyabina VP, Esimbekova EN, Kopylova KV, Kratasyuk VA. Pesticides: formulants, distribution pathways and effects on human health - a review. *Toxicol Rep*. 2021;8:1179-1192.

47. Sun J, Pan L, Zhan Y, et al. Contamination of phthalate esters, organochlorine pesticides and polybrominated diphenyl ethers in agricultural soils from the Yangtze River Delta of China. *Sci Total Environ*. 2016;544:670-6.

48. Nakata H, Kawazoe M, Arizono K, et al. Organochlorine pesticides and polychlorinated biphenyl residues in foodstuffs and human tissues from China: status of contamination, historical trend, and human dietary exposure. *Arch Environ Contam Toxicol*. 2002;43(4):473-80.

49. Chen MW, Santos HM, Que DE, et al. Association between Organochlorine Pesticide Levels in Breast Milk and Their Effects on Female Reproduction in a Taiwanese Population. *Int J Environ Res Public Health*. 2018;15(5)

50. Sala M, Ribas-Fitó N, Cardo E, et al. Levels of hexachlorobenzene and other organochlorine compounds in cord blood: exposure across placenta. *Chemosphere*. 2001;43(4-7):895-901.

51. García MA, Peña D, Alvarez L, et al. Hexachlorobenzene induces cell proliferation and IGF-I signaling pathway in an estrogen receptor alpha-dependent manner in MCF-7 breast cancer cell line. *Toxicol Lett*. 2010;192(2):195-205.

52. Ezendam J, Vos JG, Pieters R. Research articles mechanisms of hexachlorobenzene-induced adverse immune effects in brown norway rats. *J Immunotoxicol*. 2005;1(3):167-75.

53. Castilla R, Asuaje A, Rivière S, et al. Environmental pollutant hexachlorobenzene induces hypertension in a rat model. *Chemosphere*. 2018;195:576-584.

54. Chiappini F, Alvarez L, Lux-Lantos V, Randi AS, Kleiman de Pisarev DL. Hexachlorobenzene triggers apoptosis in rat thyroid follicular cells. *Toxicol Sci*. 2009;108(2):301-10.

55. Toxicological profile for hexachlorobenzene (Public Health Service, Agency for Toxic Substances and Disease Registry) (2015).

56. Sala M, Sunyer J, Otero R, et al. Health effects of chronic high exposure to hexachlorobenzene in a general population sample. *Arch Environ Health*. 1999;54(2):102-9.

57. Randi AS, Sanchez MS, Alvarez L, Cardozo J, Pontillo C, Kleiman de Pisarev DL. Hexachlorobenzene triggers AhR translocation to the nucleus, c-Src activation and EGFR transactivation in rat liver. *Toxicol Lett*. 2008;177(2):116-22.

58. Elmore SE, La Merrill MA. Oxidative Phosphorylation Impairment by DDT and DDE. *Front Endocrinol (Lausanne)*. 2019;10:122.

59. Skinner MK, Manikkam M, Tracey R, Guerrero-Bosagna C, Haque M, Nilsson EE. Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. *BMC Med*. 2013;11:228.

60. Migliaccio V, Sica R, Scudiero R, Simoniello P, Putti R, Lionetti L. Physiological Adaptation to Simultaneous Chronic Exposure to High-Fat Diet and Dichlorodipheniletylhene (DDE) in Wistar Rat Testis. *Cells*. 2019;8(5)

61. Kim J, Sun Q, Yue Y, et al. 4,4'-Dichlorodiphenyltrichloroethane (DDT) and 4,4'-dichlorodiphenyldichloroethylene (DDE) promote adipogenesis in 3T3-L1 adipocyte cell culture. *Pestic Biochem Physiol*. 2016;131:40-5.

62. Mangum LH, Crow JA, Stokes JV, et al. Exposure to p,p'-DDE Alters Macrophage Reactivity and Increases Macrophage Numbers in Adipose Stromal Vascular Fraction. *Toxicol Sci*. 2016;150(1):169-77.

63. Rodríguez-Alcalá LM, Sá C, Pimentel LL, et al. Endocrine Disruptor DDE Associated with a High-Fat Diet Enhances the Impairment of Liver Fatty Acid Composition in Rats. *J Agric Food Chem*. 2015;63(42):9341-8.

64. Kaur N, Swain SK, Banerjee BD, Sharma T, Krishnalata T. Organochlorine pesticide exposure as a risk factor for breast cancer in young Indian women: A case-control study. *South Asian J Cancer*. 2019;8(4):212-214.

65. Hoppin JA, Umbach DM, London SJ, et al. Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study. *Eur Respir J*. 2009;34(6):1296-303.

66. Son HK, Kim SA, Kang JH, et al. Strong associations between low-dose organochlorine pesticides and type 2 diabetes in Korea. *Environ Int*. 2010;36(5):410-4.

67. Kanayama T, Kobayashi N, Mamiya S, Nakanishi T, Nishikawa J. Organotin compounds promote adipocyte differentiation as agonists of the peroxisome proliferator-activated receptor gamma/retinoid X receptor pathway. *Mol Pharmacol*. 2005;67(3):766-74.

68. Moreno M, Lombardi A, Silvestri E, et al. PPARs: Nuclear Receptors Controlled by, and Controlling, Nutrient Handling through Nuclear and Cytosolic Signaling. *PPAR Res*. 2010;2010

69. Ament Z, Masoodi M, Griffin JL. Applications of metabolomics for understanding the action of peroxisome proliferator-activated receptors (PPARs) in diabetes, obesity and cancer. *Genome Med*. 2012;4(4):32.

70. Roman Á C, Carvajal-Gonzalez JM, Merino JM, Mulero-Navarro S, Fernández-Salguero PM. The aryl hydrocarbon receptor in the crossroad of signalling networks with therapeutic value. *Pharmacol Ther*. 2018;185:50-63.

71. Moyer BJ, Rojas IY, Kerley-Hamilton JS, et al. Obesity and fatty liver are prevented by inhibition of the aryl hydrocarbon receptor in both female and male mice. *Nutr Res*. 2017;44:38-50.

72. Alexander DL, Ganem LG, Fernandez-Salguero P, Gonzalez F, Jefcoate CR. Aryl-hydrocarbon receptor is an inhibitory regulator of lipid synthesis and of commitment to adipogenesis. *J Cell Sci*. 1998;111 (Pt 22):3311-22.

73. Motulsky HJ, Brown RE. Detecting outliers when fitting data with nonlinear regression - a new method based on robust nonlinear regression and the false discovery rate. *BMC Bioinformatics*. 2006;7:123.

74. Ben Rhouma K, Tébourbi O, Krichah R, Sakly M. Reproductive toxicity of DDT in adult male rats. *Hum Exp Toxicol*. 2001;20(8):393-7.

75. Sierra-Santoyo A, Hernández M, Albores A, Cebrián ME. DDT increases hepatic testosterone metabolism in rats. *Arch Toxicol*. 2005;79(1):7-12.

76. Breslin WJ, Paulman A, Sun-Lin D, Goldstein KM, Derr A. The inhibin B (InhB) response to the testicular toxicants mono-2-ethylhexyl phthalate (MEHP), 1,3 dinitrobenzene (DNB), or carbendazim (CBZ) following short-term repeat dosing in the male rat. *Birth Defects Res B Dev Reprod Toxicol*. 2013;98(1):72-81.

77. Shi YQ, Wang YP, Song Y, et al. p,p'-DDE induces testicular apoptosis in prepubertal rats via the Fas/FasL pathway. *Toxicol Lett*. 2010;193(1):79-85.

78. Marouani N, Hallegue D, Sakly M, Benkhalifa M, Ben Rhouma K, Tebourbi O. p,p'-DDT induces testicular oxidative stress-induced apoptosis in adult rats. *Reprod Biol Endocrinol*. 2017;15(1):40.

79. McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, Erickson RL. Persistent organochlorine pesticides and risk of testicular germ cell tumors. *J Natl Cancer Inst*. 2008;100(9):663-71.

80. Bornman M, Delport R, Farías P, et al. Alterations in male reproductive hormones in relation to environmental DDT exposure. *Environ Int*. 2018;113:281-289.

81. O'Connor JC, Frame SR, Davis LG, Cook JC. Detection of the environmental antiandrogen p,p-DDE in CD and long-evans rats using a tier I screening battery and a Hershberger assay. *Toxicol Sci*. 1999;51(1):44-53.

82. Hagmar L, Björk J, Sjödin A, Bergman A, Erfurth EM. Plasma levels of persistent organohalogens and hormone levels in adult male humans. *Arch Environ Health*. 2001;56(2):138-43.

83. Rignell-Hydbom A, Rylander L, Giwercman A, Jönsson BA, Nilsson-Ehle P, Hagmar L. Exposure to CB-153 and p,p'-DDE and male reproductive function. *Hum Reprod*. 2004;19(9):2066-75.

84. Cocco P, Loviselli A, Fadda D, et al. Serum sex hormones in men occupationally exposed to dichloro-diphenyl-trichloro ethane (DDT) as young adults. *J Endocrinol*. 2004;182(3):391-7.

85. Lee DH, Steffes MW, Sjödin A, Jones RS, Needham LL, Jacobs DR, Jr. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. *PLoS One*. 2011;6(1):e15977.

86. Taylor KW, Novak RF, Anderson HA, et al. Evaluation of the association between persistent organic pollutants (POPs) and diabetes in epidemiological studies: a national toxicology program workshop review. *Environ Health Perspect*. 2013;121(7):774-83.

87. Gore AC, Chappell VA, Fenton SE, et al. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocr Rev*. 2015;36(6):E1-e150.

88. Rosen ED, Hsu CH, Wang X, et al. C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. *Genes Dev*. 2002;16(1):22-6.

89. Hernandez-Quiles M, Broekema MF, Kalkhoven E. PPARgamma in Metabolism, Immunity, and Cancer: Unified and Diverse Mechanisms of Action. *Front Endocrinol (Lausanne)*. 2021;12:624112.

90. Moreno-Aliaga MJ, Matsumura F. Effects of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (p,p'-DDT) on 3T3-L1 and 3T3-F442A adipocyte differentiation. *Biochem Pharmacol*. 2002;63(5):997-1007.

91. Strong AL, Shi Z, Strong MJ, et al. Effects of the endocrine-disrupting chemical DDT on self-renewal and differentiation of human mesenchymal stem cells. *Environ Health Perspect*. 2015;123(1):42-8.

92. Tai TA, Jennermann C, Brown KK, et al. Activation of the nuclear receptor peroxisome proliferator-activated receptor gamma promotes brown adipocyte differentiation. *J Biol Chem*. 1996;271(47):29909-14.

93. La Merrill M, Karey E, Moshier E, et al. Perinatal exposure of mice to the pesticide DDT impairs energy expenditure and metabolism in adult female offspring. *PLoS One*. 2014;9(7):e103337.

94. Hofmann T, Elbelt U, Stengel A. Irisin as a muscle-derived hormone stimulating thermogenesis--a critical update. *Peptides*. 2014;54:89-100.

95. Lasar D, Rosenwald M, Kiehlmann E, et al. Peroxisome Proliferator Activated Receptor Gamma Controls Mature Brown Adipocyte Inducibility through Glycerol Kinase. *Cell Rep*. 2018;22(3):760-773.

96. Boss O, Muzzin P, Giacobino JP. The uncoupling proteins, a review. *Eur J Endocrinol*. 1998;139(1):1-9.

97. Kim-Han JS, Reichert SA, Quick KL, Dugan LL. BMCP1: a mitochondrial uncoupling protein in neurons which regulates mitochondrial function and oxidant production. *J Neurochem*. 2001;79(3):658-68.

98. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev*. 2004;84(1):277-359.

99. Boström P, Wu J, Jedrychowski MP, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*. 2012;481(7382):463-8.

100. Gutgesell RM, Tsakiridis EE, Jamshed S, Steinberg GR, Holloway AC. Impact of pesticide exposure on adipose tissue development and function. *Biochem J*. 2020;477(14):2639-2653.

101. Teeyapant P, Sikaphan S, Parnmen S. Bioaccumulation of DDT residues in human serum: An historical use of DDT indoor residual spraying in malaria endemic regions of Thailand. *EnvironmentAsia*. 2014;7(2):1-6.

102. Langer P, Ukropec J, Kocan A, et al. Obesogenic and diabetogenic impact of high organochlorine levels (HCB, p,p'-DDE, PCBs) on inhabitants in the highly polluted Eastern Slovakia. *Endocr Regul*. 2014;48(1):17-24.

7. APPENDICES

7.1. Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu (HADYEK)

ETİK KURUL KARARI

Protokol No	Toplantı Tarihi	Toplantı Sayısı	Karar No	Proje Yürütücüsü
2021-025	02.03.2021	2021/03	2021/03-11	Prof. Dr. Bayram Yılmaz
'Investigation of Obesogenic Effects of Hexachlorobenzene, DDT and p,p-DDE in Male Rat Model' isimli proje oy birliğiyle etik açıdan uygun görülmüştür.				

Hayvan Türü / Irkı	Toplam Hayvan Sayısı	Hayvanın Cinsiyeti
Sıçan / Spraque Dawley	32	Erkek

Görevi	Adı Soyadı	Katılım Durumu
Başkan	Prof. Dr. Bayram YILMAZ	
Başkan Vekili	Prof. Dr. Erdem YEŞİLADA	
Üye	Dr. Veteriner Hekim Engin SÜMER	
Üye	Prof. Dr. M. Ece GENÇ	
Üye	Prof. Dr. Rukset ATTAR	
Üye	Prof. Dr. Gamze TORUN KÖSE	
Üye	Doç. Dr. Ediz DENİZ	
Üye	Doç. Dr. Aylin YABA UÇAR	
Üye	Doç. Dr. Burcu GEMİCİ BAŞOL	
Üye	Hakan GÖKSEL	
Üye	Ahmet ŞENKARDEŞLER	

7.2. Curriculum Vitae

Kişisel Bilgiler

Adı	Zeyad	**Soyadı**	Al-Obeidi
Doğum Yeri		**Doğum Tarihi**	

Öğrenim Durumu

Derece	Alan	Mezun Olduğu Kurumun Adı	Mezuniyet Yılı
Doktora			
Yüksek Lisans			
Lisans	Veterinerlik	Tripoli University, Libya	2014
Lise			

Başarılmış birden fazla sınav varsa (KPDS, ÜDS, TOEFL; EELTS vs), tüm sonuçlar yazılmalıdır

Bildiği Yabancı Dilleri	Yabancı Dil Sınav Notu (**#**)
İngilizce	
Arapça	

İş Deneyimi (Sondan geçmişe doğru sıralayın)

Görevi	Kurum	Süre (Yıl - Yıl)
Medical Representative	Middle East Medical Company	2016-2017
Pharmacist	Vet Pharmacy	2015-2016
Veterinary doctor	Vet Clinic	2014-2015

Bilgisayar Bilgisi

Program	Kullanma becerisi
Microsoft Office	Orta

*****Çok iyi, iyi, orta, zayıf olarak değerlendirin

Bilimsel Çalışmaları

SCI, SSCI, AHCI indekslerine giren dergilerde yayınlanan makaleler

Diğer dergilerde yayınlanan makaleler

Uluslararası bilimsel toplantılarda sunulan ve bildiri kitabında (*Proceedings*) basılan bildiriler

Hakemli konferans/sempozyumların bildiri kitaplarında yer alan yayınlar

Diğer (Görev Aldığı Projeler/Sertifikaları/Ödülleri)