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The Impact of Anti-obesity Potential of Orlistat and Iron Oxide Nanoparticles on some Hormonal and Biochemical Parameters in Male Mice

A Thesis

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بسم الله الرحمن الرحيم

﴿ وَأَنْ لَيْسَ لِلْإِنْسَانِ إِلَّا مَا سَعَى (39) وَأَنَّ سَعْيَهُ سَوْفَ يُرَى (40) ثُمَّ يُجْزَاهُ الْجَزَاءَ الْأَوْفَى (41) ﴾

صدق الله العلي العظيم

(سورة النجم: الايات **39- 41**)

Supervisor's Certificate

We certify that this thesis entitled **((Evaluation the Levels of Some Hormones and Biochemical Parameters to Study the Anti –Obesity Potential of Iron Oxide Nanoparticles))**

"has been prepared under our supervision at the College of Science, University of Misan; as a partial fulfillment of the requirements for the degree of Master of Biology

Signature

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Recommendation of Head of Biology Department

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Dedication

- To my lady, my refuge and my medicine, my teacher and role model in life, my beloved and my friend, my mother.

- To the pure heart, to tolerance and humility, to kindness and affection, to my beloved father

- To my lifelong companions, my support, my strength, and the treasure of my life, to my dear sisters

I dedicate this research

Safa

Acknowledgment

Praise be to God always and forever

And after thanking God

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safa

Summary

Summary

This study was conducted in the animal house of the Biology Department, College of Science, from 2023/11/15 to 2025/5/25. University of Misan, to investigate the effects of oral administration of orlistat, iron oxide nanoparticles (IONPs), and their combination on various metabolic, hepatic, hormonal, and antioxidant parameters in obese male albino mice. A total of 70 mice were utilized, with 14 mice serving as a control group and the remaining 56 obese mice randomly assigned to four experimental groups: treatment with Orlistat, treatment with IONPs, treatment with Orlistat combined with IONPs, and an obese group without treatment.

The results of the current study were as follows:

A cording to the groups:

Obesity induction in male mice caused a significant ($P<0.05$) increase in blood glucose, lipid profile (TG, TC, LDL), ghrelin, glutathione, BMI, and body weight (final weight), along with a significant ($P<0.05$) decrease in HDL, ALT, and ALP.

Orlistat treatment led to a significant ($P<0.05$) decrease in glutathione but only slightly improved several metabolic markers without significant weight loss. IONPs administration led to a significant ($P<0.05$) decrease in blood glucose, TG, leptin, and final weight, although it led to a significant increase ($P<0.05$) in liver enzyme levels. The combined treatment of Orlistat and IONPs produced a significant ($P<0.05$) decrease in blood glucose, ghrelin, glutathione, and body weight, suggesting potential synergistic effects despite significant ($P<0.05$) increases in AST and ALP.

A cording to the periods:

In the first week:

Obesity induction caused significant (P<0.05) increases in blood glucose, lipid profile (TG, TC, LDL, VLDL), leptin, ghrelin, BMI, and final weight, along with significant (P<0.05) decreases in HDL and ALP, compared to the control group.

Treatment with orlistat showed improvements mainly through a significant (P<0.05) decrease in TG but significant (P<0.05) increases in liver enzymes (ALT, AST). while IONPs treatment caused a significant (P< 0.05) decrease in leptin levels but significant (P<0.05) increases in liver enzymes (ALT, AST). The combination therapy (Orlistat + IONPs) significant (P<0.05) decreases in leptin, ghrelin, and glutathione but was also associated with significant (P<0.05) increases in liver enzymes (AST and ALP).

In the third week:

Obesity induction caused significant (P<0.05) increases in blood glucose, TG, LDL, glutathione, BMI, and final weight, along with significant (P<0.05) decreases in HDL, ALT, and ALP, compared to the control group.

Orlistat treatment mainly lead to a significant (P<0.05) decrease in glutathione but significant (P<0.05) increase in ALP, IONPs treatment resulted in significant (P<0.05) decreases in blood glucose, TG, and final weight, yet caused significant (P<0.05) increases in ALT, AST, ALP, and LDL. The combination therapy (Orlistat + IONPs) demonstrated significant (P<0.05) decreases in blood glucose, TG, and final weight although it also led to significant (P<0.05) increases in LDL, ALP, and leptin levels.

The induction of obesity caused a clear disturbance in the study parameters. Treatment with Orlistat improved TG levels during the first week, whereas treatment with nanoparticles alone or in combination with Orlistat enhanced TG levels in the third week, leptin levels in the first week, and body weight in the third week. These findings indicate a potential positive role of nanoparticles in combating obesity and improving metabolic and hormonal parameters.

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List of Abbreviations

Abbreviation	Meaning
ACTH	Adreno-Corticotropic Hormone
AgRP	Agouti-related peptide
AITD	Autoimmune thyroid dysfunction
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMP	2-amino-2-methyl-1-propanol
Apo A-1	Apolipoprotein A-I
Apo B	Apolipoprotein B
AST	Aspartate aminotransferase
AT	Adipose tissues
BAT	Brown adipose tissues
BBS	Bardet-Biedl syndrome
β cells	Beta cells
BMI	Body mass index
CEPT	Cholesterol ester protein transfer
COVID-19	Coronavirus disease-2019
CRH	Corticoropin- Releasing Hormone
CRP	C-reactive protein
CVD	Cardiovascular disease
DMSO	Dimethyl sulfoxide
FDA	Food and Drug Administration

FFA	Free fatty acids
FT3	Free triiodothyronine
FT4	Free thyroxine
GORD	Gastro-oesophageal reflux disease
GGT	γ-glutamyl transferase
GH	Growth hormone
GHD	Growth hormone deficiency
GHRL	Ghrelin
GHSR	Growth Hormone Secretagogue Receptor
GHSR1-alpha	Ghrelin/growth hormone secretagogue receptor
GnRH	Gonadotropin releasing hormone
GSH	Glutathione
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
HPA	Hypothalamus-pituitary-adrenal
IGF-1	Insulin-like growth factor-1
INS	Insulin
IR	Insulin resistance
IONPs	Iron oxide nanoparticles
LDH	Lactate dehydrogenase
LDL	Low-density lipoproteins
LDL-c	Low density lipoprotein cholesterol
LEAP-2	Liver-expressed antimicrobial peptide 2

LEP	Leptin
IL-6	Interleukin-6
LPL	Lipoprotein lipase
LR	Leptin resistance
LSD	Least Significant Difference
MC4R	Melanocortin-4 receptor
MDH	Malate dehydrogenase
MHO	Metabolically healthy obesity
MS	Metabolic syndrome
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NADH	Nicotinamide adenine dinucleotide+hydrogen
NASH	Non-alcoholic steatohepatitis
Non-HDL-c	Non high density lipoprotein cholesterol
NPs	Nanoparticles
OHS	Obesity hypovenlitation syndrome
OSA	Obstructive sleep apnoea
PCOS	Polycystic ovary syndrome
PEG	Polyethylene glycol
PKC	protein kinase C
PWS	Prader-Willi syndrome
PYY	Peptide YY
RNS	Reactive nitrogen species
ROS	Reactive oxygen species

SAT	Subcutaneous adipose tissue
SCFA	Short chain fatty acids
SCN	Suprachiasmatic nucleus
SD	Standard deviation
SPIONs	Superparamagnetic iron oxide nanoparticles
SPSS	Statistical Package for the social sciences
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
T3	3,5,3′-triiodothyronine
T4	Thyroxine
TC	T otal Cholesterol
TG	Triglycerides
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
VAT	Visceral adipose tissue
VLDL	Very low density lipoprotein
WAT	White adipose tissues
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-to-hip ratio
WSR	Waist-to stature ratio

Chapter One

Introduction

1.1Introduction

Obesity is the abnormal or excessive accumulation of adipose tissue that threatens public health (Dragano *et al*., 2020). Can be assessed using body mass index (BMI) measures (Nimptsch *et al*., 2019). A BMI is equal or above 30 kg/m² is considered obese, whereas 25–29.9 kg/m² is considered overweight (Lauby-Secretan *et al*., 2016), the criteria for obesity and overweight differ in some populations, for example, in China, the criteria differ from the WHO criteria (Jin *et al*., 2023).

Obesity is commonly classified as simple (lifestyle-related) or morbid (severe) (Li *et al*., 2019). Simple obesity is associated with poor dietary habits and physical inactivity. Morbid obesity is primarily linked to endocrine, congenital, or metabolic disorders (Fjalldal *et al*., 2019), and Some drugs may contribute to obesity (Zhuo *et al*., 2018). Even mild obesity can worsen and cause pathological changes if left untreated (Li *et al*., 2022).

Obesity can cause various short-term adverse effects on physiological functions, such as elevated blood cholesterol, triglycerides, development of insulin resistance, and increased peripheral vascular resistance. More seriously, it can lead to various complications (Kumar and Kelly, 2017), particularly type 2 diabetes (T2DM), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cardiovascular disease (CVD), and some types of cancer (Aleksandrova *et al*., 2020).

Traditional approaches to obesity management have focused primarily on lifestyle modifications (Olateju *et al*., 2021), such as diet and exercise (Vettor *et al*., 2020). Lifestyle changes alone often require drugs for effective weight loss (Wharton *et al*., 2020).

Currently, several anti-obesity medications are approved and available, but these drugs vary in their efficacy and side effect profiles (Srivastava and Apovian, 2018). For example, orlistat is a weight-loss drug 2 approved by the US Food and Drug Administration (FDA) in 1999. As an intestinal lipase inhibitor, orlistat inhibits lipid-metabolizing enzymes, preventing the breakdown of triacylglycerols into absorbable fatty acids and mono-acylglycerols, leading to reduced intestinal absorption (subramaniyan and hanim, 2025). However, treatments used to combat obesity, including orlistat, are associated with unpleasant side effects comorbidities (Almeanazel *et al*., 2020).

Regarding bariatric surgery, it is considered the most effective treatment to date (Chacon *et al*., 2022). However, despite its great effectiveness, it is associated with several negative intraoperative outcomes and postoperative complications (anemia, depression, fractures, malabsorption, and more).

All these reasons have led to the search for new strategies to safely reduce the prevalence of obesity. Among these strategies is the development and improvement of anti-obesity nanomedicine (Sibuyi *et al*., 2019). Among the nanomaterials studied for biomedical applications, iron oxide nanoparticles (IONPs) have become a cornerstone over the years due to their high surface-to-volume ratio, biocompatibility, and magnetic 3 properties (Vangijzegem *et al*., 2023). Most importantly, clearance from the bloodstream and biodegradation of IONPs occur mainly through filtration in the kidneys or through capture in the liver and spleen (Feng *et al*., 2018)

Superparamagnetic iron oxide nanoparticles (SPIONs), a subtype IONPs consist of a magnetic iron oxide core, which is typically surface-modified by coating it with a hydrophilic and biocompatible polymer (Dulinska-Litewka *et al*., 2019). SPIONs can be administered orally (Gobbo,2024) SPIONs have been shown to be involved in regulating genes involved in lipid and glucose metabolism, suggesting they could be used as treatments for diabetes and obesity (Sharifi *et al*., 2013).

1.2 Aims of the study:

The aim of this study is to explore the potential of therapeutic agents in reducing body weight among individuals with obesity, with the intention of contributing to the development of more effective and targeted strategies for obesity management. In addition, the study seeks to examine the physiological changes associated with obesity which include the following parameters:

1- Biochemical Parameters

A- Glucose

B- Lipid profiles (Triglycerides, Total Cholesterol, High-density lipoprotein, low-density lipoprotein, and Very Low-Density Lipoprotein)

C- Liver enzymes (Alanine Aminotransferase, Aspartate Aminotransferase, and Alkaline Phosphatase)

2- Hormones (Insulin, Leptin, and Ghrelin)

3- Antioxidant (Glutathione)

4- Body weights (Initial weight, Weight gain, and Final weight)

5- Organs weights (Heart, Liver, and Kidneys)

6- Indicators (Body mass index and Lee index)

Chapter Two

Literature Reviews

2. Literature Review

2.1 Obesity

Obesity is defined as the excessive accumulation of fat, known as ectopic fat, in various parts of the body, organs or throughout the body. It is a chronic, progressive, relapsing condition with multiple factors that lead to adverse metabolic and psychosocial health consequences (Lin and Li, 2021).

By 2030 it is expected that, an estimated 14% of men and 20% of women in the world's total population will develop clinical obesity, additionally it is estimated that 18% of individuals will have a body mass index (BMI) greater than 30 kg/m^2, 6% will have a BMI greater than 35 kg/m^2, and 2% will have a BMI greater than 40 kg/m^2. Countries with high socioeconomic status and per capita income are also at a greater risk of experiencing an increased prevalence of obesity (Chandrasekaran and Weiskirchen, 2024).

A body mass index greater than 30 kg/m^2, being weight in kilograms divided by height in meters squared is used to identify obesity. For adults, a BMI of 25.0 to 29.9 kg/m^2 is defined as overweight, and a BMI of 30 kg/m^2 or higher is defined as obese (Jensen *et al*., 2014). In humans, the BMI measure is not used for children and adolescents aged 2 to 18 years; instead, it is recommended that a percentile scale based on the child's sex and age be used (Fitch *et al*., 2013). Anthropometric methods are the most convenient and most popular for estimating the extent of adiposity. Besides BMI, these include waist and hip circumferences, waist-to-hip ratio (WHR), skin fold thickness, and waist-to stature ratio (WSR). Since shorter individuals usually weigh less, weight alone cannot be used as a criterion to determine the amount of fat stores in an

individual, WSR and waist circumference are easy and relatively accurate techniques to estimate visceral fat (Parente *et al*., 2020).

Obesity can be classified into central or peripheral obesity. In central obesity, otherwise called "android" obesity, the distribution of fat is commonly on the upper part of the trunk (the chest and abdomen) and is more common in males, In the peripheral or "gynecoid" type of obesity, however, the distribution of fat is mainly on the hip and thighs and is more common in females (Liu *et al*.,2024). Adipose tissues (AT) represent a special connective tissue that contains adipocytes (which comprise 35–70% of AT mass), although lipid storage and both thermal and mechanical insulations are all classical functions of AT, the traditional view of AT as a passive reservoir for energy storage has been progressively replaced by the identification of AT as a multifunctional, essential, complex, and highly active metabolic, immune, and endocrine organ (Guerreiro *et al*., 2022). Which directly modulates many processes, including energy balance, metabolism, inflammation, and bone metabolism (Barchetta *et al*., 2019).

In mammals, AT is normally classified according to its localization and morphophysiological properties into white adipose tissues (WAT), brown adipose tissues (BAT), beige, and pink AT. WAT is the most abundant type of AT found in adult humans and is the main tissue involved in energy storage (Cinti, 2007). Both brown and beige adipocytes have gained increased interest as potential targets for the treatment of obesity and associated metabolic disorders. Finally, pink adipocytes are formed in females during pregnancy, lactation, and post lactation, where subcutaneous white adipocytes are converted to pink adipocytes, which then serve as milk-producing glands formed by lipid-rich elements (Cinti, 2018).

2.2 Factors influencing and causing obesity:

There are many factors influence and cause obesity, they can be divided into the following:

2.2.1 Endogenous causes of obesity including: Genetic and epigenetic disorders, maternal and birth-related factors, hormonal imbalances, microbiome, and infections.

2.2.1.1 Genetic and epigenetic disorders:

According to a study on family and twins showed that around 40-70% of the obesity variation in human are resulted from genetic factors (Wu *et al*., 2018).

Genetic causes of obesity can be broadly classified as:

1. Monogenic causes that result from a single gene mutation, primarily located in the leptin- melanocortin pathway. Many of the genes, such as AgRP (Agouti-related peptide), PYY (Peptide YY), or MC4R (the melanocortin-4 receptor), were identified for monogenic obesity disrupt the regulatory system of appetite and weight, hormonal signals (ghrelin, leptin and insulin) are sensed by the receptors located in the arcuate nucleus of the hypothalamus (Thaker, 2017).
2. Syndromic Obesity more than 80 distinct syndromic obesities have been described to date. Most common syndromes including Prader-Willi syndrome (PWS), Bardet-Biedl syndrome (BBS), and Alstrome syndrome share pathophysiology related to hypothalamic impairment (Hinney et al., 2022).

Epigenetics is a subdiscipline of genetics that studies heritable changes in gene expression in the absence of changes in the nucleotide sequence of genes (Regan and Shah, 2020), and it is that which connects the study of environmental factors to patterns of genetic change, such as between rapid changes in dietary habits and the observed obesity phenotype (Thaker, 2017).

Lifestyle, dietary pattern, gut microbiota, and other environmental factors, can affect epigenetic programming through the various periods of life, especially parental gametes, fetus and early postnatal development (Lopomo *et al*., 2016). According to the study, a healthy diet can positively influence the individual epigenetic profile; several data, indeed, indicate that normal-weight and non-diabetic people have epigenetic profiles different from those of obese and diabetic subjects (Ling and Ronn, 2019).

Non-nutritional risk factors associated with obesity such as hyperglycemia, inflammation, endocrine disruptors, hypoxia, and oxidative stress, as well as nutritional factors, appear to be involved in epigenetic modifications that influence adipogenesis and insulin sensitivity (Martinez *et al*., 2014).

2.2.1.2 Maternal and birth-related factors:

Epidemiological, clinical, and basic sciences research suggest that the foundations of an individual's lifelong health, including predisposition towards obesity and type 2 diabetes are largely established during the 'first 1000 days of life' from day of conception to completion of the second year of life. This is a highly sensitive period of growth and development in humans, where biological systems are formed and developed (Kinshella *et al*., 2021).

Both epidemiological and animal study have clearly established a link between maternal obesity and poor metabolic health in offspring. For example, in humans, maternal obesity in pregnancy increases the risk of offspring developing characteristics associated with metabolic syndrome such as obesity, type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD) (Glastras *et al*., 2018).

Increased offspring body weight and fat mass can be due to an increase in mass of the WAT, which was observed in both sexes. Sex differences also occur, as shown in male rat offspring having a larger adipocyte size (Litzenburger *et al*., 2020).

2.2.1.3 Hormonal disorders: Many types of hormones that play a role in the occurrence and development of obesity, such as:

A- Sexual hormones:

Sexual hormones are well known to play an important role in body composition glucose and lipid metabolism, of which especially androgens are characterized by a sexual dimorphism (Bianchi *et al*., 2018).

In men with hypogonadism, reduced testosterone concentrations are associated with obesity and insulin resistance (IR) (Molina-vega *et al*.,2018). Evidence indicates that testosterone deficiency in men induces adiposity and, at the same time, increased adiposity induces hypogonadism (Fernandez *et al*., 2019).

IR and compensatory hyperinsulinemia are pathophysiological hallmark features of low testosterone in obesity, resulting in impaired gonadotropin secretion and subsequent decreased secretion of testosterone from the testes (Kelly and Jones, 2015).

On the other hand, true hypogonadism in men can promote increased fat mass, which in turn may worsen the hypogonadal state, Low testosterone levels lead to a reduction in muscle mass and an increase in adipose tissue within abdominal depots, especially visceral adipose tissue (VAT) that can be reversed with testosterone therapy (Kapoor *et al*., 2006).

As adiposity increases, there is a further rise in aromatase activity that is associated with an even greater conversion of testosterone to estradiol (often

termed the 'testosterone-estradiol shunt'), which is thought to decreased Gonadotropin releasing hormone (GnRH) secretion, this further decreases testosterone levels that in turn further increases the preferential deposition of fat within abdominal depots: a 'hypogonadal-obesity cycle (Ylli *et al*., 2022).

In contrast to the favorable impact of testosterone in men, women with hyperandrogenemia are often obese and more likely to have metabolic syndrome. The most common cause of androgen excess in women is polycystic ovary syndrome (PCOS), which is closely linked to IR and obesity and has an unfavorable influence on women's quality of life (Wang *et al*., 2019).

Hyperandrogenism of ovarian origin is mainly due to defective intrinsic steroidogenesis in theca cells (Di Lorenzo *et al*., 2023).

A decline in estrogen may preferentially lead to central fat accumulation and promote visceral adiposity. Animal studies in mice and rats, as well as human ovarian suppression using GnRH agonists, resulted in visceral but not overall fat mass gain; when estrogen was added back, the visceral fat gain was reversed (Farahmand *et al*., 2021; Marlatt *et al*., 2022).

The physiologic and metabolic changes associated with menopause are a direct effect of estrogen deficiency, which has been shown to affect lipid metabolism, energy consumption, IR, and body fat composition, with a transition from a gynecoid to an android body shape and increased abdominal and visceral fat accumulation associated with increased cardiovascular disease (CVDs) and metabolic risks (Banack *et al*., 2023).

Ovarian estrogens promote peripheral fat storage in the gluteal and femoral subcutaneous regions and have a role in maintaining glucose homeostasis through their effects on insulin secretion and clearance. It is well established that estrogen promotes peripheral (vs. central) fat distribution and improves insulin sensitivity in women (Marlatt *et al*., 2022).

Menopausal weight gain has also been explained by the effect of estrogen deficiency on energy intake and expenditure. estrogen has been shown to have an inhibitory effect on appetite, and postmenopausal estrogen depletion is thought to centrally control energy intake (Zhu *et al*., 2023).

B- Adrenal gland:

The release of cortisol is under the control of the hypothalamus-pituitary-adrenal (HPA) axis. In a negative feedback loop, sufficient cortisol inhibits the release of both Adreno-Corticotropic Hormone (ACTH) and Corticoropin-Releasing Hormone (CRH). The HPA axis follows a circadian rhythm. Thus, cortisol levels will be high in the morning and low at night (Ramamoorthy and Cidlowski, 2016).

Cortisol is the primary hormone involved in biological responses to chronic stress. It is a member of the glucocorticoid family and a marker of (HPA) activity, also known to have the following effects: (i) redistribution of adipose tissue to the abdominal region, (ii) increase of appetite, and (iii) increased preference for more palatable foods (Van der Valk *et al*., 2018).

Higher cortisol levels have been detected in obese patients, and weight and cortisol levels are much higher in individuals who gain weight due to stress (Hewagalamulage *et al*., 2016). In addition, Herhaus *et al*. (2020) showed that cortisol reactivity under stress strongly predicts stress-related eating behavior, which in turn influences BMI. Although studies have indicated a correlation between cortisol levels and obesity, not all obese patients have elevated cortisol levels (Basu *et al*., 2005).

Chronically elevated cortisol levels increase appetite, lead to hypersecretion of insulin, and visceral fat accumulation in the long term (Garcia-Eguren *et al*., 2019). Moreover, chronic stress induces hypertrophy of adipocytes, promotes

the conversion of preadipocytes to mature adipocytes, and activates stromal fat immune cells (Rodriguez *et al*., 2015).

C- Growth hormone (GH): Growth hormone deficiency (GHD) is the most common hormonal deficit complicated by pituitary tumors (Seki *et al*., 2023), and it is known to cause obesity, dyslipidemia, and premature atherosclerosis (Yuen *et al*., 2019).

GH can enhance insulin sensitivity by regulating the synthesis and secretion of insulin-like growth factor-1 (IGF-1), insulin, and Free fatty acids (FFA) (Hjelholt *et al*., 2020). Adult obese patients with GHD suffer from metabolic disturbances and insulin sensitivity (Loftus *et al*., 2019).

Obesity is associated with reduced GH secretion since GH may be suppressed as a consequence of increased energy supply (Gar *et al*.,2020).

D- Thyroid hormones: The hypothalamic-pituitary-thyroid axis is a classic negative feedback loop. Hypothalamic thyrotropin-releasing hormone (TRH) stimulates the release of thyroid stimulating hormone (TSH) from the pituitary gland. TSH supports the synthetic machinery within the thyroid gland and stimulates the resorption of thyroglobulin from within the lumen of thyroid follicles. Thyroxine (T4) and 3,5,3′-triiodothyronine (T3) are both then released from the thyroid gland into the circulation in the proportion of approximately 14:1 (Jonklaas *et al*., 2014). Thyroid hormones are closely related to both obesity and metabolic disorders (van den Berg *et al*., 2017).

Free triiodothyronine (FT3) and thyroid-stimulating hormone (TSH) were found to be increased in morbid obesity (Michalaki *et al*., 2006). Another study indicated that obesity had a causal role in increasing FT3 levels (Taylor *et al*., 2016).

The change of thyroid hormones was associated with multiple metabolic risks. A prospective study found that decreased free thyroxine (FT4) within the subclinical and euthyroid range contributed to an increased risk of metabolic syndrome (MS) (Mehran *et al*., 2017).

Recent epidemiological and experimental data provided evidence of a bidirectional interaction between obesity and thyroid autoimmunity (Weetman, 2021).

The relationship between obesity and thyroid autoimmunity is not only based on epidemiological aspects but also on common pathogenetic mechanisms. A major player of this association is IR, that is present in 15.5 - 46% of the obese patients and represents the first mechanism in the relationship (Fahed *et al*., 2020).

A variable degree of leptin resistance (LR) is usually associated with IR and obesity and can be considered a second mechanism in the interplay between obesity and thyroid disorders (Duntas and Biondi, 2013).

The third mechanism is the increased oxidation of low-density lipoproteins (LDL) occurring in obese patients with IR.

The link between obesity and the risk of autoimmune thyroid dysfunction (AITD), which is the main cause of hypothyroidism in adults (Rotondi *et al*., 2009).

Hypothyroidism is associated with decreased thermogenesis, decreased metabolic rate, and has also been shown to correlate with a higher BMI and a higher prevalence of obesity. There is clinical evidence suggesting that even mild thyroid dysfunction in the form of subclinical hypothyroidism is linked to significant changes in body weight and represents a risk factor for overweight and obesity (Qiu *et al*., 2024).

2.2.1.4 The gut microbiome composition:

In recent years, an increasing amount of evidence has shown that an imbalance in the gut microbiota may be a factor leading to obesity (Gomes *et al*., 2018). Diet is the main factor influencing the imbalance of the gut microbiota (Stanislawski *et al*., 2019).

Up to 100 trillion symbiotic microbes live in the gut, called the gut microbiota, which comprises 10 times the number of cells in the body itself (Liu *et al*., 2021).

The gut microbiota relies on food residues that the human body does not digest, mucus secreted by the gut, and dead cells that are shed as nutrients to maintain its high population levels (Gentile and Weir, 2018). The active gut microbiota will produce a large number of physiologically active substances, including short-chain fatty acids, vitamins, and health-beneficial products such as anti-inflammatory, analgesic, and antioxidant products, along with potentially harmful products such as neurotoxins, carcinogens, and immunotoxins (Canfora *et al*., 2019).

The gut microbiota is responsible for metabolizing energy from the diet, e.g., indigestible dietary fibers, which are chemically polysaccharides and oligosaccharides. They are converted into short chain fatty acids (SCFA) that are either absorbed by the gut or excreted in feces, such as acetate, propionate, and butyrate. After absorption, SCFAs can induce lipogenesis and increase triglyceride stores through molecular pathways (Rosenbaum *et al*., 2015).

Higher concentrations of fecal SCFAs are associated with gut permeability, metabolic disorder markers, obesity, and hypertension. Although they can protect the host from diet-induced obesity, excessive SCFAs can provide extra energy for the host, thus promoting obesity (Liu *et al*., 2021).

2.2.1.5 Inflammation:

The WAT is the major fat-storing depot and also serves as the largest endocrine organ to secrete adipokines and cytokines systemically. Adipokines are involved in various metabolic and physiological signaling cascades and regulate insulin signaling, glucose uptake, fatty acid oxidation, and other energy-producing and metabolic processes, cytokines regulate inflammation and resolution of inflammation along with adaptive and reparative angiogenesis. Weight gain and obesity cause a phenotypic switch of WAT, which is characterized by the appearance of inflamed, dysfunctional adipocytes along with infiltration of immune cells in the stromal vascular fraction. Inflamed adipocytes secrete, both locally and systemically, proinflammatory cytokines, which in turn disrupt the normal function of AT itself as well as that of remote organs, (Kawai *et al*., 2020).

Demonstrated the essential role of inflammation in the establishment of insulin resistance following long-term consumption of an obesogenic Western-type diet. A unique feature of the inflammatory responses of expanding WAT is its duration and intensity, i.e., a persistent, low-grade inflammation that fails to resolve. Such obesity-related chronic low-grade inflammation and subsequent altered metabolism have been termed "metaflammation" (Hotamisligil, 2006).

It is currently well established that obesity instigates a more complex and intense inflammatory reaction in visceral WAT (VAT) than in subcutaneous WAT (SAT). Indeed, VAT contains more macrophages compared with SAT in obese mice and obese humans (Savulscu-Fiedler *et al*., 2024). With obesity and insulin resistance, VAT adipocytes experience a much higher degree of hypertrophy than SAT both in humans and in animal models (Hardy *et al*., 2011) Furthermore, VAT inflammation in obese humans is associated with decreased expression of lipogenic markers, probably due to the fact that more cells are

switched to an inflammatory rather than a lipid storage phenotype, which leads to the development of metabolic complications, such as ectopic lipid deposition in skeletal muscle and liver (Poulain-Godefroy et al., 2008). Since ectopic lipid deposition dampens peripheral insulin signaling, VAT inflammation is considered to have a major impact on obesity-related metabolic disorders such as systemic insulin resistance and development of T2DM (Hardy *et al*., 2011).

2.2.2 External causes of obesity: They include diet and physical activity, circadian misalignment, stress, and some medication

2.2.2.1 Diet and physical activity:

Obesity has reached epidemic proportions in many developed countries as western dietary patterns have been widely adopted. These are characterized by excess energy intake as well as regular consumption of processed or "fast" foods and limited consumption of fruits, vegetables, and whole grains (Agjei *et al*., 2025). Due to the rapid growth in technology and more scope of social media, physical inactivity has turned into a universal pandemic. Adults mostly prefer to remain sedentary, which makes them more vulnerable to disease or ill health (WHO, 2015).

2.2.2.2 Circadian misalignment:

The suprachiasmatic nucleus (SCN) and the peripheral clocks in peripheral tissues coordinate with each other in response to environmental cues, such as light, food, and sleep, to maintain circadian rhythms in almost all cells/tissues (Van den Berg *et al*., 2018). A number of biological processes, such as glucose and lipid homeostasis, energy expenditure, and hormone secretion, are regulated by the circadian clock, the SCN is mainly entrained by light signals, and the peripheral clocks can be modulated by temperature, food, and sleep as well as

hormonal cues (Peng and Chen, 2023). The abnormal expression of circadian genes, environmental misalignment like abnormal light/dark cycles, and behavioral misalignment, including feeding, sleep–wake cycles, and activity, can promote circadian disruption, which could contribute to obesity and obesity-related metabolic disorders (Li *et al*., 2020).

2.2.2.3 Stress:

Stress eating or emotional eating is a maladaptive behavioral response to stress. While some people may undereat and/or lose weight under stressful conditions, approximately 70% of individuals tend to overeat and/or gain weight in response to stress, the traditional, physiological model of stress includes the "fight or flight" mode in response to acute (i.e., life threatening) stress. In this model, acute stress triggers a cascade of sympathetic hormones that redirects bodily and organ functioning away from metabolic and appetitive mechanisms, such as food intake and digestion (Goens *et al*., 2023).

Instead of the acute stress most often faced by our premodern ancestors, in the modern world individuals are more likely to face stressors that are long-term, cumulative, and psychosocial in nature – that is, chronic stress. Chronic stress instigates an HPA-axis-regulated endocrine response, in particular the release of cortisol, which induces overeating of energy-dense foods (Dallman *et al*., 2003). Eating these "comfort foods"—those that are high in fat and carbohydrate content—may reduce the chronic-stress activation of the HPA-axis (Dallman, 2010).

2.3 Risks and complication of obesity:

The diseases and conditions associated with obesity and the possible effect are summarized in the table (2.1).

Table (2.1): The adverse consequences and impact of obesity (Lam *et al*., 2023).

Diseases and conditions associated with obesity	
Metabolic	**Pregnancy**
Diabetes mellitus (T2DM, T1DM) Dyslipidaemia Hypertension Polycystic ovary syndrome (PCOS)	Gestational hypertension Gestational diabetes mellitus Pre-term delivery Caesarian section
Cardiovascular	**Musculoskeletal**
Coronary heart disease Heart failure Atrial fibrillation Penipheral artery disease Venous thromboembolism Deep vein thrombosis Aortic valve stenosis	Osteoarthritis Intervertebral degeneration Back pain Sciatica
Respiratory	**Skin**
Obesity hypovenlitation syndrome (OHS) Obstructive sleep apnoea (OSA) Asthma	Atopic dermatilitis Psoriasis
Neurological	**Mental**
Stroke Cognitive impairment Dementia	Depression Anxity
Renal	**Infection**
Chronic kidney disease	Severe coronavirus disease-2019 (COVID-19)
Gastrointestinal	**Cancer**
Nonalcoholic fatty liver disease (NAFLD) Gastro-oesophageal reflux disease (GERD) Diverticular disease Crohn's disease Gallstone disease	Oesophageal adenocarcinoma Renal cell carcinoma Breast cancer Endometrial cancer Ovarian cancer Colorectal cancer Gastric cancer Liver cancer Gallbladder cancer Pancreatic cancer Thyroid cancer Meningioma
Rheumatological	
Gout Rheumatoid arthritis	

	Multiple myeloma

possible effects			
Overall clinical impact	**Psychological impact**	**Economic impact**	**Generational impact**
Poor health Disabilities Premature death	Reduced guality of life Poor self-esteem Stigmal/discrimination Social withdrawal and isolation	Increased healthcare expenditure Absenteeism /presenteeism	Death (stillbirth) Intrauterine growth retardation Large for gestational age /childhood obesity

2.4 Relationship of obesity and biochemical parameters:

2.4.1 Blood glucose:

Obesity causes an increase in insulin resistance and beta cell dysfunction through the induction of inflammation, endocrinopathies, and increased circulating FFA, multiple risk factors contribute to the development of obesity. Environmental factors, aging, diet, gut microbiota, and genetics are different factors that positively regulate obesity and adipose tissue dysfunction. Dysfunctional obese adipose tissue releases FFA, reactive oxygen species, and pro-inflammatory agents. Consequently, excess free fatty acids and other lipids, are deposited in cells of various organs, such as liver, muscle, and pancreas, creating lipotoxicity, leading to dysregulation of mitochondria, lysosomes, and endoplasmic reticulum. Dysfunctional organelles then further release excess FFA, pro-inflammatory agents, and reactive oxygen species (Ahmed *et al*., 2021). This increase in FFA mediates mitochondrial dysfunction and increases reactive oxygen species, decreasing the phosphorylation of insulin receptor substrates and impairing insulin receptor activity as shown in the figure (2.1)

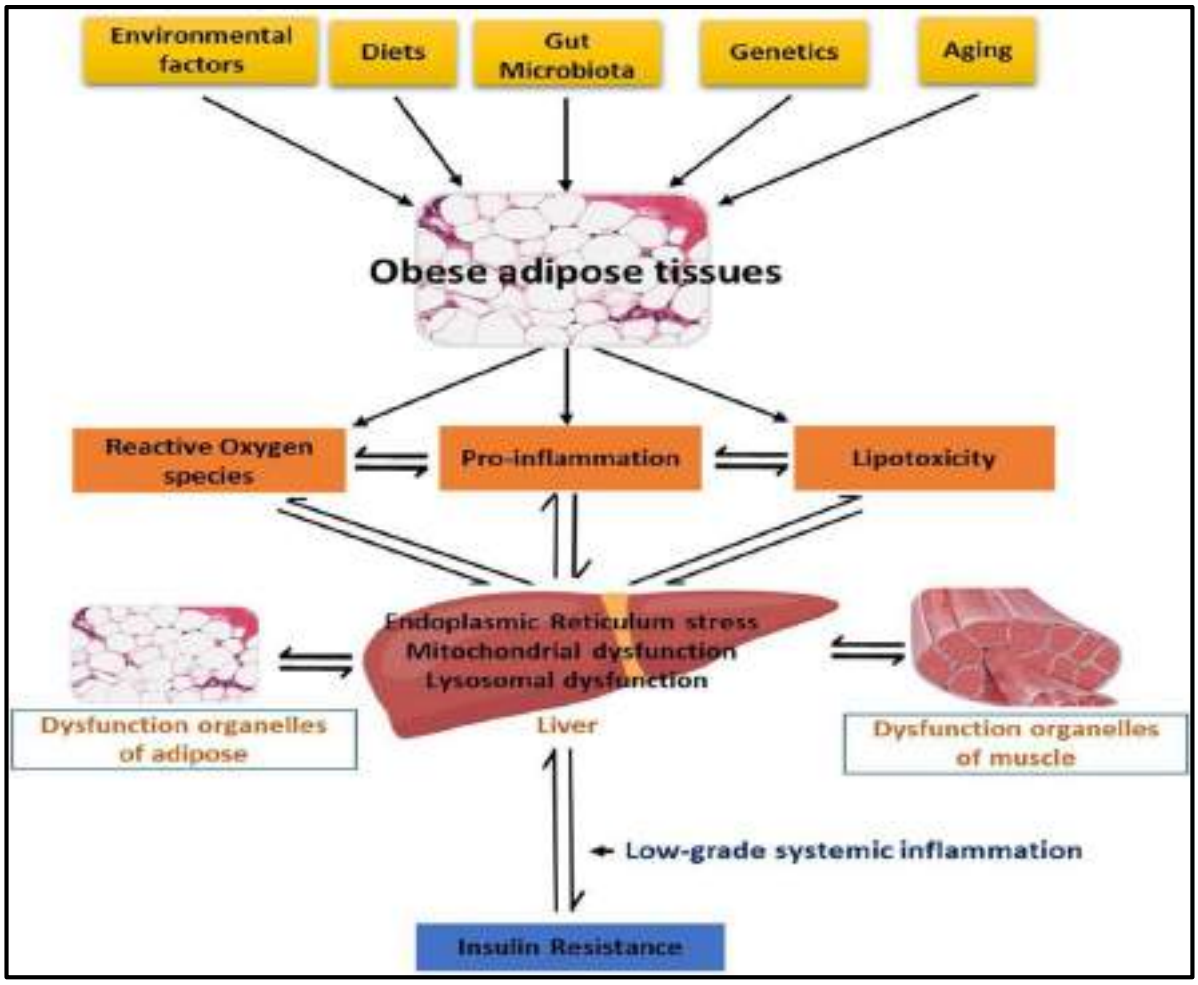

Figure (2.1): The lipodystrophic process is responsible for excess pro-inflammation to develop insulin resistance circumstances (Ahmed *et al*., 2021).

As we gain excess weight, our body's ability to regulate blood glucose becomes impaired, leading to higher blood glucose levels, usually associated with individuals with BMI >35 kg/m² and diabetes (Torres *et al*.,2023). Lipotoxicity from increased FFA can also cause hyperglycemia and eventually diabetes (Martyn *et al*., 2008).

2.4.2 Lipids profile:

The lipid abnormalities seen in patients who are obese which include elevated triglycerides (TG), very low density lipoprotein (VLDL), apolipoprotein B (Apo B), and non-high density lipoprotein cholesterol (non-HDL-c) levels, which are all commonly observed, HDL-C and apolipoprotein A-I (Apo A-I) levels are typically low, low density lipoprotein cholesterol (LDL-C) levels are frequently in the normal to slightly elevated range, but an increase in small dense LDL is often seen resulting in an increased number of LDL particles (Xiao *et al*., 2016). There are a number of different abnormalities that contribute to the dyslipidemia seen in patients with obesity. These abnormalities are driven by the combination of the greater delivery of FFA to the liver from increased total and visceral adiposity, IR, and a pro-inflammatory state, induced by macrophages infiltrating fat tissue. A key abnormality is the overproduction of VLDL particles by the liver, which is an important contributor to the elevation in serum TG levels. The rate of secretion of VLDL particles is highly dependent on TG availability, which is determined by the levels of fatty acids available for the synthesis of TG in the liver. An abundance of TG prevents the intrahepatic degradation of Apo B-100 allowing for increased VLDL formation and secretion (Bjornson *et al*., 2017).

The elevation in TG rich lipoproteins in turn has effects on other lipoproteins. Specifically, cholesterol ester transfers protein (CETP) mediates the equimolar exchange of TG from TG rich VLDL and chylomicrons for cholesterol from LDL and HDL, the increase in TG rich lipoproteins per se leads to an increase in CETP mediated exchange, increasing the TG content and decreasing the cholesterol content of both LDL and HDL. Additionally, obesity also increases the activity and mass of CETP (Kenneth and Feingold, 2023). This CETP-mediated exchange underlies the commonly observed reciprocal relationship of

low HDL-C levels when TG levels are high and the increase in HDL-C when TG levels decrease. The TG on LDL and HDL is then hydrolyzed by hepatic lipase and lipoprotein lipase leading to the production of small dense LDL and small HDL particles. Notably hepatic lipase activity is increased in patients who are obese with increased visceral adiposity, which will facilitate the removal of TG from LDL and HDL resulting in small lipoprotein particles. The affinity of Apo A-I for small HDL particles is reduced leading to the disassociation of Apo A-I and the breakdown of Apo A-I by the kidneys (Kenneth and Feingold, 2023). as shown in the figure (2.2).

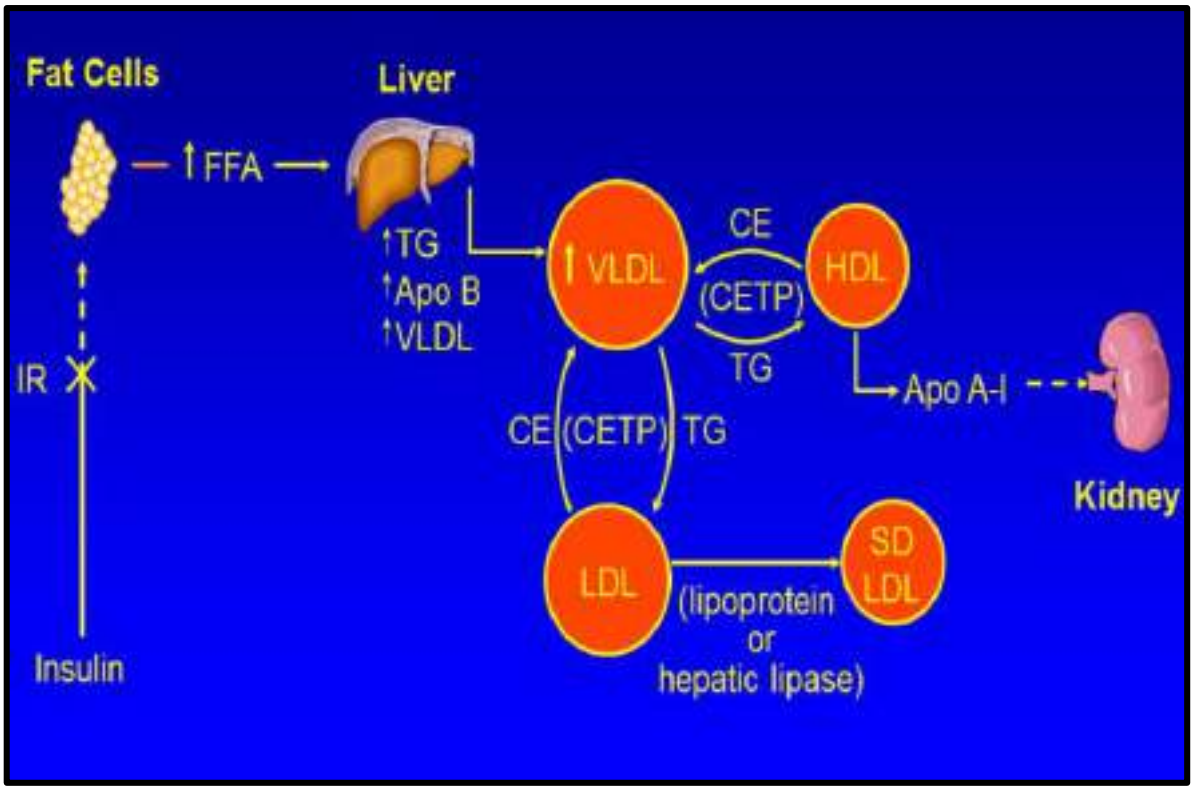

Figure (2.2): Changes in Lipid/Lipoprotein Metabolism Leading to the Dyslipidemia of Obesity (Kenneth and Feingold, 2023).

2.4.3 Liver functions:

A recent study reported that obesity may be associated with liver disease and progression of hepatic dysfunction, and obesity may impair liver function by a variety of mechanisms. In individuals with obesity, high levels of cytokines including interleukin-6 (IL-6) and C-reactive protein (CRP) may disrupt liver functions such as production of hepcidin which can lead to hepcidin-related iron deficiency anemia, and may lead to some types of liver diseases such as non-alcoholic fatty liver disease (NAFLD) and liver cancer (Doaei et al., 2019).

The adverse effects of obesity and metabolic dysfunction on the liver have only been partially understood. A retrospective study including 767 participants suggested that obesity severity was positively associated with liver disease severity (Seth *et al*., 2020). Furthermore, a large-population study showed that the two major subtypes of obesity, general and abdominal obesity, which can be measured by BMI and waist circumference (WC), respectively, were causal factors for NAFLD and chronic liver disease in 228,466 women and 195,041 men (Censin *et al*., 2019).

In addition to obesity, metabolic disorders including IR, dyslipidemia, and hypertension are also risk factors for liver disease (Aberg *et al*., 2022).

Obesity could induce metabolic dysfunctions, most likely by inflammatory mechanisms (Saltiel and Olefsky, 2017). But individuals with obesity may have limited or no features of poor metabolic health, which is referred to as metabolically healthy obesity (MHO) (Bluher, 2020).

The serum levels of four enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) are generally used in assessing liver functions, ALT and AST are found mostly in the liver, and serum levels of AST and ALT are considered

as specific markers for hepatic dysfunction, ALP is an enzyme that is primarily present in the liver, bones, intestine and kidneys,the higher serum levels of ALT, AST, ALP and GGT are reported in several diseases and increased levels of these enzymes are frequently reported in people with obesity (Jalili *et al*., 2022).

2.5 Relationship of obesity and hormones

2.5.1 Insulin:

Insulin is a hormone secreted by beta cells β cells of Langerhans islets located in the endocrine pancreas. It is responsible for regulating glucose metabolism as well as promoting actions such as lipogenesis, increase the transport of amino acids into the cell or decrease lipolysis. Also, it participates in multiple signaling transduction pathways (Rahman *et al*., 2021). Pancreatic β cells secrete insulin directly into the portal vein for delivery to the liver, which is the major site for insulin clearance. Plasma insulin concentration is determined by the balance between the rate of insulin secretion and the rate of insulin removal by the liver and extrahepatic tissues. A large portion (~50%) of the insulin secreted from β cells and delivered to the liver is cleared during first pass transit, and an additional 20% is cleared through subsequent passes; the remaining 30% of secreted insulin is primarily removed by the kidneys (about 20%) and skeletal muscle (about 10%) (Duckworth *et al*., 1998). The increase in both basal and postprandial plasma insulin concentrations observed in people with obesity is caused by both increased pancreatic insulin secretion and decreased fractional extraction and clearance of portal and peripheral plasma insulin (Gastaldelli *et al*., 2021).

Insulin resistance has long been associated with obesity (Wild *et al*., 2004). It was firmly believed that insulin resistance was the initial event and preceded hyperinsulinemia. From this perspective, hyperinsulinemia was considered to be a compensatory response to counteract insulin resistance in the body and insulin resistance the main factor in the development of obesity, type 2 diabetes, cardiovascular disease and cancer. Nowadays, this has been much debated and it appears that hyperinsulinemia may precede insulin resistance in obesity in some cases (Abdul-Ghani and DeFronzo, 2023). It is even shown to be a causal factor in that insulin hypersecretion from beta cells is the major deficiency and will subsequently lead to insulin resistance. Additionally, research indicates that hyperinsulinemia leads to a decrease in receptor affinity and number, favoring the development of insulin resistance (van Vliet *et al*., 2020). Beta-cell mass in subjects with obesity is assumed to increase, since plasma insulin levels in obese subjects increase to compensate for insulin resistance, a process known as hyperinsulinemia, in a rodent study, the beta-cell mass increased threefold in the animals with obesity induced by a high-fat diet, in adult humans, a study have reported that beta-cell mass increases by approximately 20% to 90% in obese individuals (Inaishi and Saisho, 2020).

When cells do not respond adequately to insulin, they cause a rise in blood glucose, which increases insulin production and secretion by pancreatic beta cells in an attempt to compensate normal glucose levels in the blood (Ahmed et al., 2021).

2.5.2 Leptin:

The last decade has witnessed an increase in the number of discovered adipokines, with more than 600 adipokines being secreted by adipose tissue (Flehmig *et al*., 2014).

Adipokines include leptin, adiponectin, resistin, and many others. The type and amount of adipokines produced by adipose tissue depend on the type of adipocytes (white or brown), their size, number, location, and interaction with other cells, discovered in 1994, leptin is an adipokine, a protein that functions as a hormone (Mancuso, 2016).

Leptin is produced and secreted primarily from adipose tissue into circulation to have effects in the CNS and peripheral organs. Relatively low levels of leptin are produced by other tissues, such as skeletal muscle, brain, stomach, pituitary gland, mammary epithelium or placenta, but this appears to result in local, as opposed to systemic, actions (Margetic *et al*., 2002).

Circulating leptin concentration is directly related to adipose tissue size; this informs the brain about available energy storage, leptin expression and circulating levels change with nutritional state, but also display a circadian oscillation with higher values in the evening and early morning (Martinez-Sanchez, 2020). Severe early obesity develops from rare genetic mutations that affect leptin signaling (Yazdi *et al*., 2015). Such mutations often lead to congenital leptin deficiency or high but ineffective leptin and leptin resistance (Dubern and Clement, 2012).

A growing body of evidence has demonstrated that increased adipose tissue mass contributes directly to an increase in circulating levels of leptin; thus, most common forms of obesity are characterised by hyperleptinaemia and by leptin resistance (Genchi *et al*., 2021).

Leptin resistance refers to the condition in which the brain or peripheral tissues are less sensitive (or do not respond) to leptin, and therefore leptin fails to promote its anticipated effects (Maffei and Giordano, 2022). This state could result in a vicious cycle, as it leads to a further increase in circulating leptin levels, and therefore to a worsening of leptin resistance. Therefore, leptin itself plays an important role in the development of its resistance (Gruzdeva *et al*., 2019).

Leptin resistance in obesity is believed to be caused by several factors, including chronic inflammation, insulin resistance, and changes in the hypothalamus and other brain regions that regulate appetite and energy balance, These factors can lead to a disruption in leptin signaling (Bjorbaek and Kahn, 2004).

2.5.3 Ghrelin:

First described by Kojima (1999) as an endogenous ligand for the Growth Hormone Secretagogue Receptor (GHSR). It is an acylated peptide hormone produced in the stomach, consisting of amino acids and a ligand for GHSR (Mihalache *et al*., 2019). Intriguingly, ghrelin had been known as the only endogenous ligand of GHS-R1a for nearly 20 years until 2018 when Ge and colleagues (2018) first reported liver-expressed antimicrobial peptide 2 (LEAP-2) as an endogenous blocker of GHS-R1a by screening this peptide against a panel of 168 engineered stable GPCR-expressing cell lines.

Ghrelin's main biological activities include stimulating growth hormone release, stimulating appetite, and carbohydrate metabolism. Increased circulating of ghrelin levels occurs during fasting and decreased ghrelin levels occur after food consumption in healthy people (Mihalache *et al*., 2019).

Ghrelin's notable effects of appetite stimulation, increased food intake, and increased fat storage have dubbed it the "hunger hormone". It acts on the hypothalamus, and is thought to be part of a neural network that is involved in feeding regulation, modulating the appetitive response to food cues as well as increasing brain response in areas responsible for visual processing, attention, and memory associated with images of food (Lv *et al*., 2018). Ghrelin has been shown to increase food intake by up to 30% when administered to humans (Young and Jialal, 2023).

Once secreted, ghrelin exerts its orexigenic effects by binding to the ghrelin/growth hormone secretagogue receptor (GHS-R1-alpha) (Koutouratsas *et al*., 2019). GHS-R1-alpha receptors appear on the arcuate and the ventromedial nuclei of the hypothalamus; these cells also host the receptors for leptin, a satiety hormone that opposes the effects of ghrelin (Makris *et al*., 2017).

The involvement of ghrelin in obesity is multifaceted, as it promotes adiposity, activates hypothalamic orexigenic neurons, and influences lipid metabolism (Tahir *et al*., 2023). Ghrelin, stimulates appetite and facilitates energy storage in the form of fat through various mechanisms. These mechanisms include the activation of hypothalamic orexigenic neurons, stimulating fat storage-related expression, the enhancement of lipogenesis, and the promotion of triglyceride uptake in adipocytes, primarily in WAT Additionally, ghrelin affects glucose homeostasis by reducing insulin sensitivity (Sovetkina *et al*., 2020).

Plasma ghrelin levels are lower in individuals with obesity than in lean individuals. With some exceptions, plasma ghrelin is consistently reported as being inversely correlated with body weight. This correlation has been found in both adult humans with obesity or metabolic syndrome and in mouse models of diet-induced obesity (Mani *et al*., 2019).

In diet-induced obese mice, fasting fails to increase plasma ghrelin, whereas in humans with obesity, plasma ghrelin levels are not suppressed after consumption of a meal (Gupta *et al*., 2021). Furthermore, plasma levels of the growth hormone secretagogue receptor (GHSR) antagonist and inverse agonist LEAP2 are higher in diet-induced obese mice and adult humans with obesity as compared to lean controls (Mani *et al*.,2019).

2.6 Relationship of obesity and antioxidants:

Glutathione (GSH) is a tripeptide synthesized from the amino acids glycine, cysteine, and glutamate and is the most abundant intracellular antioxidant and detoxicant in the human body (Uzun *et al*., 2007), especially abundant in the liver and kidney (Commandeur *et al*., 1995), while it is present at lower levels in the brain (Aoyama, 2021). The active thiol group is present as part of the cysteine residue and participates in antioxidant functioning either directly by detoxifying reactive oxygen species (ROS) and reactive nitrogen species (RNS) or indirectly via GSH-dependent peroxidase-catalyzed reactions, thus, GSH participates in many important detoxification reactions and has a high capacity for the prevention of oxidative stress (Teskey *et al*., 2018).

Increased intake of fats, carbohydrates, and saturated fatty acids, as well, especially trans-fatty acids, lead to increased oxidative stress via several biochemical processes such as the synthesis of superoxide anion via oxidative phosphorylation, glyceraldehyde auto-oxidation, protein kinase C (PKC) activation, and polyol and hexosamine pathways (Dandona *et al*., 2010).

Oxidative stress also plays a significant role in the development of obesity, by stimulating the deposition of adipose tissue, including preadipocyte proliferation, adipocyte differentiation and growth (Colak and Pap, 2021).

A decrease in glutathione availability is linked to various cardiometabolic disorders, and erythrocyte glutathione levels are decreased in obesity, the lower levels of GSH in individuals with obesity may result from the greater irreversible consumption of GSH relative to its de novo synthesis rate to counter the obesity-induced increased production of oxidants and the toxic byproducts associated with the higher rate of lipid metabolism. Alternatively, GSH biosynthesis may be compromised because of decreased availability of its amino acid precursors (Tan *et al*., 2024).

Amino acid metabolism is deranged in obesity, and studies have consistently reported lower plasma concentrations of glycine but higher concentrations of glutamate and cysteine (Rangel-Huerta *et al*., 2019).

2.7 The drug orlistat used to treat obesity:

Orlistat, a pancreatic lipase inhibitor, suppresses approximately 30% of the absorption of ingested fat. Orlistat has been approved as a prescription drug (120 mg × 3 times/day) or as an over-the-counter drug (60 mg × 3 times/day) for weight loss in more than 120 countries. Clinical trials performed in Japanese subjects have demonstrated that orlistat at a dose of 60 mg three times a day over 24 weeks (Shirai *et al*., 2019).

Orlistat is the only lipase inhibitor diet drug currently in clinical use and is the only anti-obesity medicine that does not act on the central nervous system or enter the bloodstream. Lipstatin, which is found from St. streptococci by Ballinger, is a potent irreversible inhibitor of pancreatic lipase. Roche Corp. succeeded in hydrogenating lipstatin to a more stable inhibitor called orlistat, which is a tetrahydro derivative of lipstatin and was approved by the Food and Drug Administration (FDA) as an anti-obesity drug in 1997, the chemical structure of orlistat (Liu *et al*.,2020). Is shown in Figure (2.3).

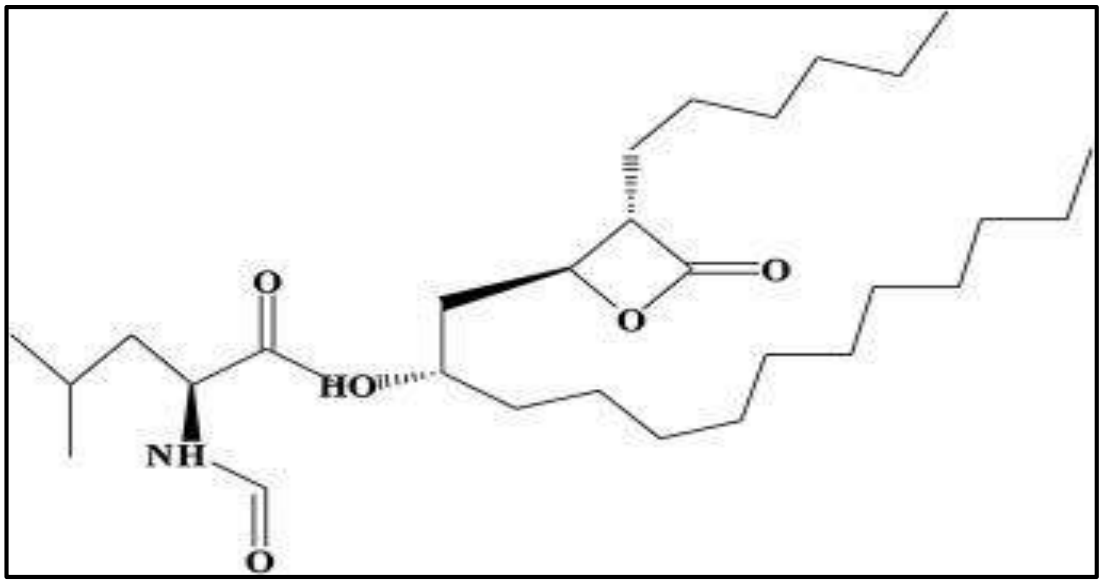

Figure (2.3): Chemical composition of orlistat (Liu *et al*.,2020).

Orlistat induces weight reduction via the inhibition of lipases in the mucous membranes of the stomach, small intestine, and pancreas, thereby preventing the breakdown of triglycerides into fatty acids and their absorption in the intestines (Drew *et al*., 2007).

The use of orlistat also results in the improvement of various cardiometabolic parameters, such as decreased insulin resistance, fasting plasma glucose level, low-density lipoprotein cholesterol level, and systolic and diastolic blood pressure. A meta-analysis of 30 studies reported that 21% more participants who use orlistat for 1 year achieve at least 5% or greater weight loss, and 12% more participants achieve a weight loss of 10% or more, than those who use a placebo (Rucker *et al*., 2007).

2.8 Iron oxide nanoparticles

Iron oxide is the most studied material in FDA approved nanomedicines (Bobo et al., 2016), at specific diameters (from 15 nm and no more than 100 nm). Magnetic iron oxide NPs (MNPs), which are made up of magnetite (Fe3O4) or maghemite (Fe_2O_3), have proved to be effective as contrast agents, drug delivery vehicles, and thermal-based therapeutics (Arias *et al*., 2018). Iron-oxide magnetic nanoparticles principally magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) have become a focal point of research because their properties make them highly promising for biomedical tasks such asusing Magnetic Resonance Imaging (MRI) (Bao *et al*., 2018). Radiolabeling and internal radiotherapy (Russell et al., 2021). Additional treatments include hyperthermia (Palzer et al., 2021), gene therapy (Mu *et al*., 2018), biomolecules separation (Eivazzadeh-Keihan *et al*., 2021), and drug delivery (Uskoković *et al*., 2019).

Magnetic nanoparticles (MNPs) furnish an ample surface for conjugating therapeutic agents, and an external magnetic field can steer these nano-carriers to precise anatomical sites (Estelrich *et al.*, 2015). Because permeability rises as particle size falls, many nanoparticles can traverse most biological barriers. Immobilising drugs on MNPs frequently improves their water solubility (Nowak-Jary *et al.*, 2020) and heightens cytotoxic potency against cancer cells (Nowak-Jary *et al.*, 2021). Moreover, iron-oxide MNPs are largely biodegradable, following a degradation route akin to ferritin breakdown in lysosomes that releases free iron ions (Skotland *et al*., 2010).

Toxicological studies are generally reassuring: routine doses of magnetic nanoparticles do not cause lasting organ injury (Couto *et al.*, 2016), whereas pathological changes in the liver and spleen have appeared only at very high exposures of around 500 mg Fe kg^{-1} (Katsnelson *et al.*, 2011).

The body clears these particles either through renal excretion (Du *et al.*, 2018) or via uptake by the mononuclear phagocyte system including phagocytes in blood, tissues, and lymph nodes (Feng *et al*., 2018). Integration of magnetic NPs with polymers can expand the scope of their application in a variety of ways. Polymers have been widely used to improve the stability, aqueous dispersion, biocompatibility, and bioavailability of magnetic NPs for in vivo applications (Ruggiero *et al*., 2016).

2.9 Obesity and the Role of Nanotechnology

Therapeutic control depends on pharmaceutical drugs to maintain a healthy body weight and prevent its progression to related chronic diseases. These pharmaceutical drugs are only recommended for overweight and obese patients who are non-responsive to lifestyle modification within the first 6 months and suffer from at least one of the obesity-induced diseases (Fasipe, 2018). There are various anti-obesity drugs available for short-term and long-term use, these drugs work mainly in the central nervous system, gut, and intestines to either suppress appetite, inhibit fat absorption, or increase energy expenditure (Kennett *et al*., 2010). The usefulness of anti-obesity drugs is mostly limited by non-specificity, poor efficacy, and bystander effects. In cases where the side effects surpassed the efficacy of the drugs, this led to the withdrawal of the most potent drugs (Giordano *et al*., 2016). Current pharmacological options for dyslipidaemia and obesity such as orlistat, lorcaserin, liraglutide, the phentermine topiramate combination, fibrates, statins, niacin, bile-acid resins, ezetimibe, sibutramine, bupropion, and naltrexone often bring an array of adverse reactions, including abdominal discomfort, nausea, insomnia, constipation, headache, and vomiting (Vekic *et al.,* 2019).

For patients with severe obesity, bariatric-metabolic surgery remains the gold-standard intervention, delivering durable weight reduction that translates into lower overall mortality (particularly from cardiovascular disease, diabetes, and cancer) and marked improvements in quality of life (Sbraccia and Finer, 2019).

Over the past decade, nanotechnology has emerged as a promising avenue to mitigate the shortcomings of conventional therapies, offering enhanced biocompatibility and refined biological targeting (Dhilip Kumar and Abrahamse *et al.*, 2022). Sibuyi and colleagues (2019) highlight three nanotech-driven strategies to combat obesity: (1) suppressing angiogenesis within white adipose tissue (WAT); (2) converting WAT into thermogenically active brown adipose tissue (BAT); and (3) inducing photothermal lipolysis of WAT. Particles such as hydrogel implants and MNs enable direct accumulation at the treatment site, resulting in high local drug concentrations and minimal systemic toxicity. Additionally, particles can serve as reservoirs for slow drug release, allowing for a more systematic effect. Moreover, particles can be administered systemically and be targeted to specific locations through active or passive targeting approaches, making their use a versatile drug delivery method (Kohane, 2007).

Microparticles are unlikely to cross most biological membranes because of their larger size. They can also cause acute and chronic inflammatory responses due to the slow degradation of particulate materials. Nanoparticles (NPs) address some of the limitations of microparticles and offer additional benefits, such as a high surface-to-volume ratio, customizable surface chemistry, and intracellular drug release. These advantages make them a hopeful delivery system for treating diseases, such as obesity (Tsou *et al*., 2019). The pharmacological therapy for obesity because their clinical benefit is modest and they provoke systemic toxicity manifesting as neuropsychiatric and cardiovascular events such as non-fatal myocardial infarction, stroke, depression, and anxiety the practical use of

these drugs is sharply curtailed (Wyatt, 2013). To counter these shortcomings, scientists have devised a suite of targeted delivery platforms: gold, phosphatidylcholine, and cholesterol nanoparticles; polymer systems built from PLGA-b-PEG, PLGA, dextran, or dextran-PEG; peptide-based liposomes; nano-emulsions formulated with Capryol PGMC and Cremophor RH40; and lipase-responsive nanocarriers, all designed to heighten therapeutic potency while minimising off-target effects. (Thovhogi *et al*., 2015). Sharifi and his team (2013) showed the involvement of SPIONs in the regulation of genes involved in lipid and glucose metabolism, suggesting that they could be used as therapeutics for diabetes and obesity.

Chapter Three

Materials and Methods

3. Material and methods

3.1 Apparatus: The apparatus and equipment that were used in the current study are listed in Table (3-1).

Table (3-1): The apparatus and equipment used with their producing companies and countries.

Apparatus	Company	Country
Balance	Kerm	Germany
Biosystem A15	Biosystems	Spain
Centrifuge	Hettich	Germany
Enzyme-Linked Immunosorbent Assay (Elisa)	Bio-Rad	America
Electrical balance	Kerm	Germany
Magnetic stirrer	Heidolph	Germany
Refrigerator	Kenwood	Japan
Vortex	Medilab	Korea

3.2 Kits: The kits that were used in this study are summarized in Table (3-2).

Table (3-2): The kits that are used with their producing companies and countries

Kits	Company	Country
Alanine Aminotransferase (ALT)	Biosystems	Spain
Alkaline Phosphatase (ALP)	Biosystems	Spain
Aspartate Aminotransferase (AST)	Biosystems	Spain
Cholesterol	Biosystems	Spain
Ghrelin	Shanghai YL Biont	China
Blood glucose	Biosystems	Spain
Glutathione	Shanghai YL Biont	China
High-density lipoprotein (HDL)	Biosystems	Spain
Insulin	Shanghai YL Biont	China
Iron Oxid	US nanomaterials Inc	USA
Leptin	Shanghai YL Biont	China
Orlistat	Pharma International	Jordan
Low-density lipoprotein (LDL)	Biosystems	Spain
Triglyceride	Biosystems	Spain
Very low-density lipoprotein (VLDL)	Biosystems	Spain

3.3 Instruments: The instruments that were employed in this study are summarized in **Table (3-3)**.

Table (3-3): The instruments used with their producing companies and countries

Instrument	Company	Country
Beaker	ISOLAB	Germany
Biofilm	Trust Labs	China
Chloroform	SD Fine-Chem Limited	Mumbi
Cotton	Citioglas	China
Cup 120 ml	Ningbo Trustlab instruments	China
Cylinder	ISOLAB	Germany
Dimethyl sulfoxide (DMSO)	Alpha Chemika	India
Distilled Water	Pioneer	Iraq
Ethanol 99%	Khair AL-joud	Iraq
Filter Paper	Trust Labs	China
Formalin	BDH	England
Gel Tubes	Trust Labs	China
Gloves	Higeen	Malaysia
Insulin Syringe	DMK	China
Iron oxide	US Nanomaterials Inc	USA
Masks	Xian Tao Diya	China
Micropipettes	Dragon	Germany
Micro Centerfuge Tubes	Ningbo Trustlab instruments	China
Normal saline	Cisen Pharmaceutical	China
Oral Gavage	Hebson	India
Plastic cages	Kajeen	Iran
Polyethylene glycol (PEG)	Thomas baker	India
Surgical Set	Hebson	India
Syringe	DMK	China
Tip	Ningbo Trustlab instruments	China

3.4 Laboratory Animals

This study was conducted between the period of 2023/11/15 to 2024/12/16. White laboratory male mice of the BALB/C strain, aged two months, were used in the current research. The mice had weights ranging from (15-18 g), obtained from the Ministry of Industry and Minerals / Industrial Research and Development Authority / Al-Razi Center (Baghdad/ Iraq) and raised in the animal facility of the Department of Biology/College of Sciences/ University of Misan, under controlled conditions in terms of temperature 20-25ºC and lighting cycle 12 hours light / 12 hours dark. For the duration of the study, the mice were placed in plastic cages of standard sizes (30 * 12 * 11) cm, and the cages were spread with sawdust bedding, which was changed weekly. The mice were acclimatized for two weeks before entering the experiment using the ration consisting of a group of substances shown in **Table (3-4)**.

Table (3-4): The feed ingredients according to Jawad (1996)

Substances	Percentage
Flour	75%
Animal protein	15%
Vegetable protein	6%
Milk	2%
Minerals and vitamins	1gm/kg

3.4.1 Animal fattening

After two weeks on normal feeding, mice were randomly divided into two groups. The first group remained on the normal diet as a control group, and the second group received a high-fat diet to increase weight. The level of dietary fat is directly related to increased body weight and body fat (Lu *et al*., 2013). Body weight was monitored and calculated weekly as shown in Table (3-5).

Table (3-5): **The components of the high-fat diet used to fatten mice; these components are for 195kg.**

Cow fat	25kg
Dried milk	5kg
Corn	50.189g
Soy	51kg
Flour	47kg
Bran	7kg
Protein premix	50g
Lime	5g
Garlic	1g
Antitoxin	50g
Mold inhibitor	25g
Oil	4,575g
D-calcium	5g
Hardening enzyme	50g
Barley	20kg
Wheat	20kg
Ground fish	20kg
Salt	50g

3.5 Evaluation of obesity in mice (Calculate BMI and Lee index)

In humans, BMI is calculated from weight in kilograms divided by height in square meters (kg/m^2). This concept has been adapted for rodent models of obesity using body weight and nose-to-anus length in (g/cm^2) (Vitarius *et al*.,2006). The Lee index is defined as the cube root of body weight (g) divided by the naso–anal length (mm). Correlations were also found between the Lee index and body fat content (Jagot *et al*.,1980).

3.6 Drug

The drug that was chosen to conduct the current experiments was Orlistat (120mg/ kg capsules; Pharma International Company, Jordan).

3.6.1 Doses

A stock solution/suspension was prepared daily (Tanenbaum and Tuffanelli,1980) by dissolving/suspending the required amount of Orlistat (weighed using a sensitive balance) in distilled water to achieve a final dose of 120 mg/ kg body weight.

3.7 Nanomaterial

The nanoparticles that were employed in this study were Iron Oxide nanoparticles Fe_3O_4 at a nano size ranging between 50-100nm. The nanoparticles were purchased readily from the particles produced by US Research Nano Materials, Inc. (Houston, TX, USA).

3.7.1 Nanoparticles Coating

Iron oxide nanoparticles were coated with a polyethylene glycol (PEG) polymer using the following procedure that included 0.43 g of iron oxide with 0.165 g of PEG, and 9.7 ml of ethanol added to a beaker. The mixture was then stirred on a magnetic stirrer at 250 rpm for five hours. Following stirring, the resulting suspension was washed several times with ethanol until the solution appeared clear, and the supernatant was discarded using an external magnet. The PEG-coated nanoparticles were subsequently collected, coated with a biofilm, and allowed to dry at room temperature (Chen *et al*.,2013).

3.7.2 Toxicity Assay

To assess cytotoxicity of iron oxide Fe_3O_4 nanoparticles for biomedical applications, an MTT assay was conducted using COS-7 cells seeded at 2×10^4 cells/well in 96-well plates with complete RPMI medium. After overnight incubation at 37°C with 5% CO_2, cells were exposed to 0.1, 1, and 10 mg/mL of bare and coated nanoparticles for 24 hours. Controls contained no particles. Following incubation, 5 µL of 5 mg/mL MTT solution was added per well and incubated for 4 hours. Formed formazan crystals were dissolved using 100 µL DMSO, and absorbance was measured at 570 nm and 690 nm. The absorbance of each well was read on a microplate reader (Magellan, Tecan, Australia) at wavelengths of 570nm and 690nm. The net absorbance was calculated by (A_{570}-A_{690}) (A refers to the area means absorbance). The relative cell viability (%) was calculated as ([A_{sample}] / [$A_{control}$]*100%, with the resultant value representing the number of living cells (Powles *et al*., 2020).

3.8 Experience design

A total of 70 adult male mice were used in this experiment, divided into five groups: a control group consisting of normal-weight mice and four groups of obese mice, with 14 mice assigned to each group, as follows:

1. Group A /Control: This group was given an oral dose of 0.2 ml of saline solution daily.
2. Group B /Obese mice: This is a group of obese mice only.
3. Group C /Treatment with anti-obesity drug /orlistat: This group was given orlistat, at a dose proportionate to body weight, at a volume of 0.2 ml per day.
4. Group D /Treatment only nanoparticles /iron oxide: This group was given iron oxide nanoparticles at a dose proportional to body weight, at a volume of 0.2 ml daily.
5. Group E /Orlistat + iron oxide: was given Orlistat with iron oxide nanoparticles at a dose proportional to body weight and a volume of 0.2 ml.

Within each group, seven mice were euthanized after 1 week of treatment, and the remaining seven mice were euthanized after 3 weeks of treatment.

The current experiment design is presented as the following **Figure (3-1)**

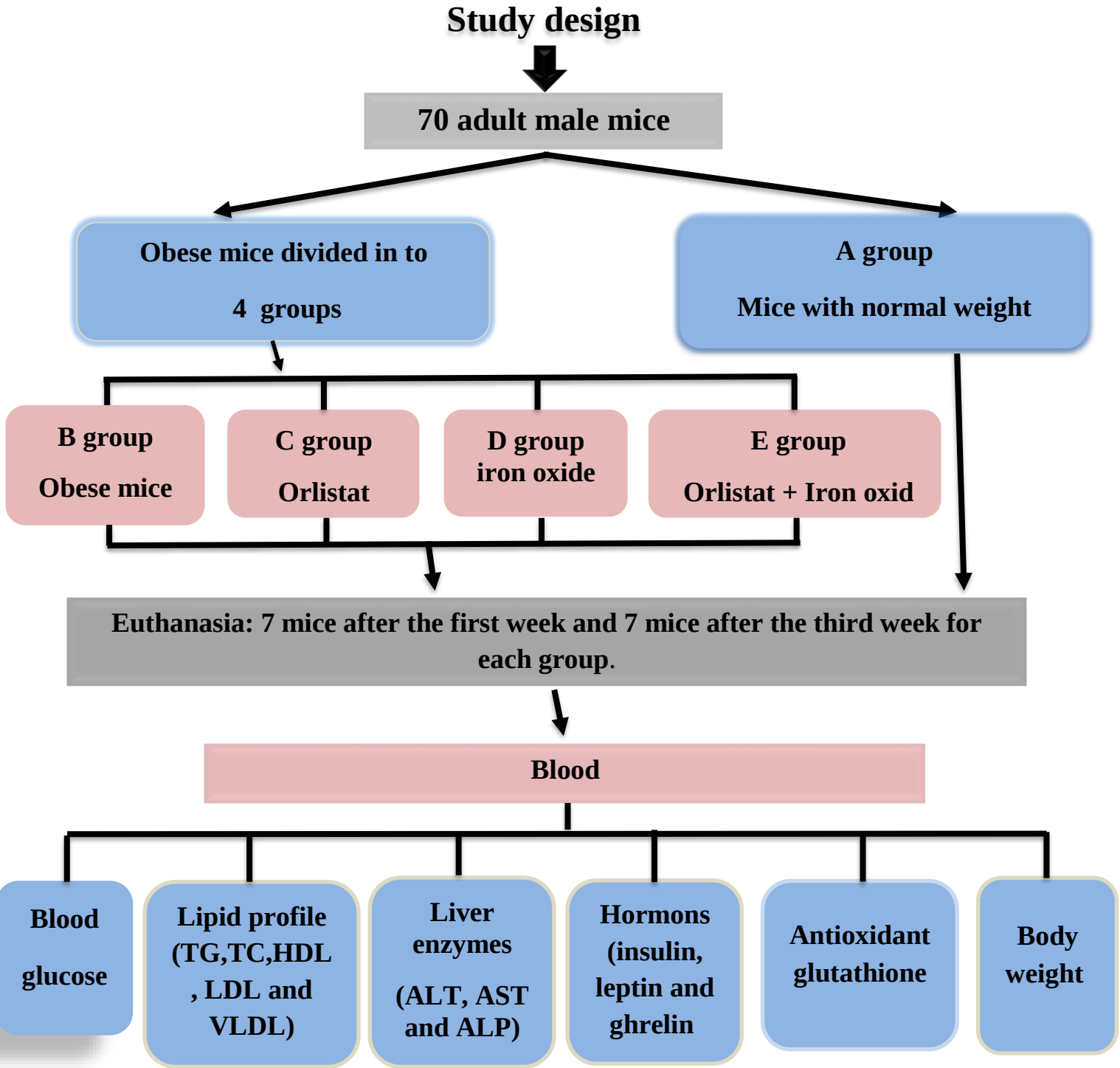

Figure (3.1): The experimental design

3.9 Blood Samples Collection

Mice were euthanized by asphyxiation with chloroform, blood was collected via cardiac puncture using a 3 mL syringe. After collection, the blood was transferred to a gel tube and then centrifuged at 3000 rpm for 15 min. The resulting serum was collected from the clear top layer, aliquoted into Eppendorf tubes, and stored in the freezer until analysis (Iriadam et al., 2006).

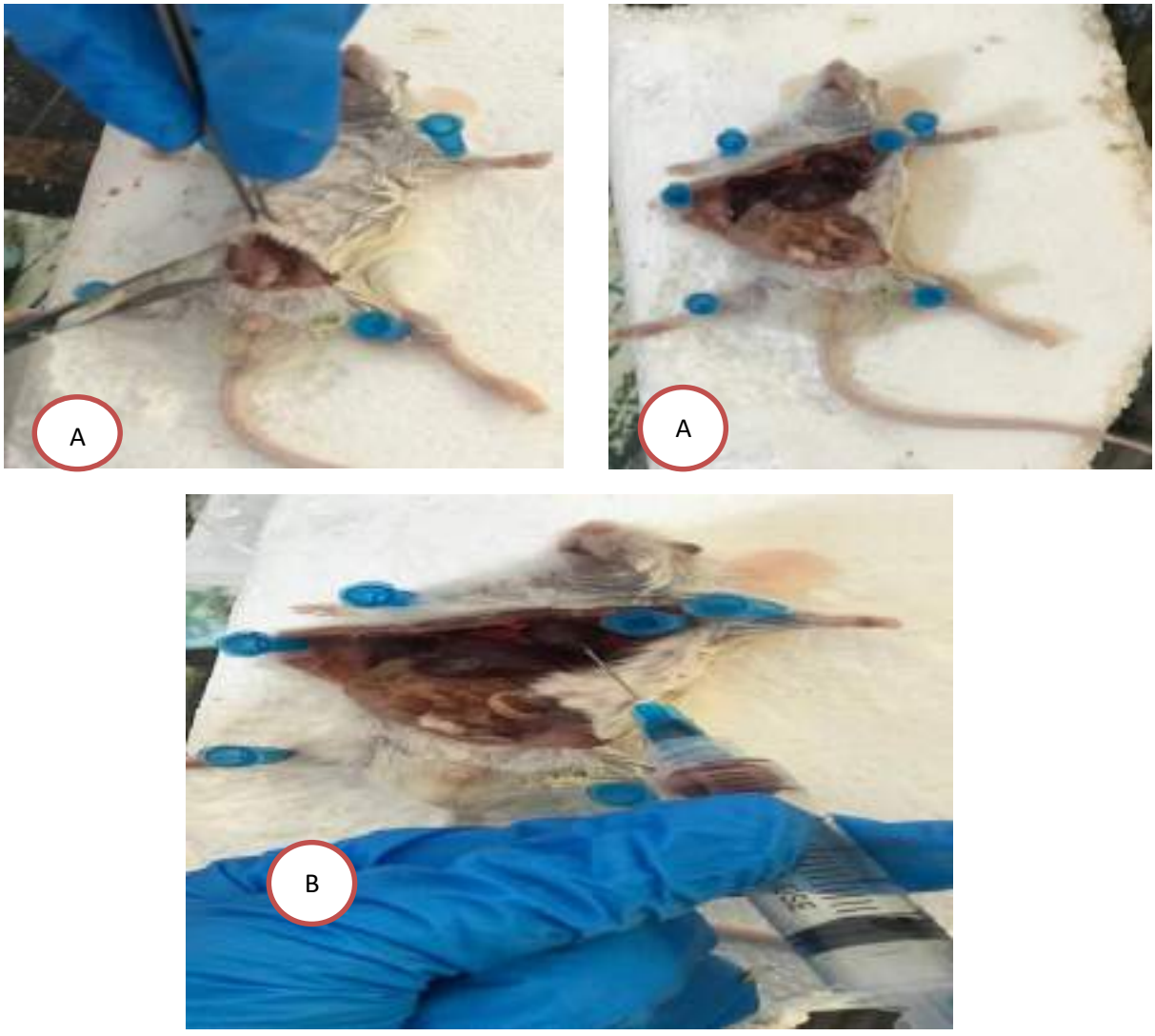

Figure (3-2): The Animal dissection (A) and Blood Withdrawal (B)

3.10 Assessment of experimental parameters

3.10.1 Evaluation of Blood glucose

Glucose in the sample participates in the coupled reaction described below, resulting in the formation of a colored complex that can be quantified using spectrophotometry (Burtis *et al*.,2012). The reagent was provided in a ready-to-use form. The reactions as shown below:

$$\text{Glucose} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Glucose} + H_2O_2$$

$$2H_2O + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

3.10.2 Evaluation of Lipid Profile (TG, TC, HDL, LDL and VLDL)

3.10.2.1 Triglycerides (TG)

Serum TG were measured using an automated spectrophotometric method, with a specific TG kit. Based on a coupled reaction described below, yielding a colored complex that can be measured by spectrophotometry (Fossati and Prencipe, 1982). The reagent was provided in a ready-to-use form.

Lipase

Triglycerides $+H_2O$ ⟶ Glycerol + Fatty acids

Glycerol kinase

Glycerol + ATP ⇌ Glycerol -3-P+ADP

G-3-P-oxidase

Glycerol -3-P $+O_2$ ⇌ Dihydroxyacetone –P$+H_2O_2$

Peroxidase

$2H_2O_2$ Aminoantiphynine + 4-Chlorophenol ⟶

Quinonemine $+4H_2O_2$

3.10.2.2 Total Cholesterol (TC)

TC was measured using an automated spectrophotometric assay with a specific TC kit. Free and esterified cholesterol in the sample originates, through the coupled reaction below, a colored complex that can be measured by spectrophotometry (Burtis *et al*., 2012). The reagent was provided in a ready-to-use form.

Chol. Esterase

Cholesterol ester $+H_2O$ ⟶ Cholesterol + Fatty acid

Chol. Oxidase

Cholesterol ester + ½ O_2 $+H_2O$ ⟶ Cholestenone + H_2O_2

Peroxidase

$2H_2O_2$ + 4 – Aminoantipyrine + Phenol ⟶ Quinoneimine + 4 H_2O

3.10.2.3 High-Density Lipoprotein (HDL)

HDL was measured using an automated spectrophotometric assay with a specific HDL kit. The cholesterol from low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and chylomicrons was broken down by the cholesterol oxidase in an enzymatic accelerated, non-color-forming reaction. The detergent present in the reagent B solubilizes cholesterol from high-density lipoprotein (HDL) in the sample. And then the HDL cholesterol spectrophotometrically measured using the coupled reaction below (Warnick *et al*., 2001). The reagent was provided in a ready-to-use form.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Chol. Esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol ester} + 1/2\ O_2 + H_2O \xrightarrow{\text{Chol. Oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + 4 - \text{Aminoantipyrine} + \text{DSBmT} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4\ H_2O$$

3.10.2.4 Low-Density Lipoprotein (LDL)

LDL was measured using an automated spectrophotometric assay with a specific LDL kit. A specific detergent solubilizes the cholesterol from high-density lipoproteins (HDL), very low-density lipoproteins (VLDL), and chylomicrons. The cholesterol esters were broken down by cholesterol esterase and cholesterol oxidase in a colorless reaction. The second detergent, present in the reagent B, solubilizes cholesterol from low-density lipoproteins (LDL) in the sample. And then the LDL cholesterol spectrophotometrically

measured employing the coupled reactions described below (Nauck *et al*., 2002). The reagent was provided in a ready-to-use form.

Chol. Esterase

Cholesterol esters + H_2O ⟶ Cholesterol + Fatty acid

Chol. Oxidase

Cholesterol ester + ½ O_2 +H_2O ⟶ Cholestenone + H_2O_2

Peroxidase

$2H_2O_2$ + 4 – Aminoantipyrine + DSBmT ⟶ Quinoneimine +

3 H_2O

3.10.2.5 Very Low-Density Lipoprotein (VLDL)

VLDL is evaluated by using spectrophotometrically automated methods, by using the following equation:

VLDL= TG/5

Estimation of the concentration of very low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. (Friedewald *et al*., 1972). The VLDL cholesterol was then spectrophotometrically measured utilizing the coupled reactions. The reagent was provided in a ready-to-use form.

3.10.3 Evaluation of Liver activity (ALT, AST, and ALP):

3.10.3.1 Alanine aminotransferase (ALT)

The ALT activity was measured using an automated Bio System spectrophotometer, with a commercial spesific ALT kit. (ALT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration was determined from the rate of decrease of NADH, measured at 340 nm, based on the lactate dehydrogenase (LDH) coupled reaction, (Gella *et al*., 1985). The reagent was provided in a ready-to-use form.

$$\text{Alanine + 2 – Oxoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate + Glutamate}$$

$$\text{Pyruvate + NADH + H}^{+} \xrightarrow{\text{LDH}} \text{Lactate + NAD}^{+}$$

3.10.3.2 Aspartate transaminase (AST)

The AST activity was measured using an automated Bio System spectrophotometer, with a commercial specific AST kit. Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxaloacetate and glutamate, the catalytic concentration was determined from the rate of decrease of NADH, measured at 340 nm, based on malate dehydrogenase (MDH) coupled reaction, (Gella *et al*., 1985). The reagent was provided in a ready-to-use form.

AST

Aspartate + 2 – Oxoglutarate ⟶ Oxaloacetate + Glutamate

MDH

Oxaloacetate + NADH + H^+ ⟶ Malate + NAD^+

3.10.3.3 Alkaline phosphatase (ALP)

The ALP activity was measured using an automated Bio System spectrophotometer, with a commercial specific ALP kit. ALP catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol, the catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm (Rosalki, 1993). The reagent was provided in a ready-to-use form.

ALP

4 Nitrophenylphosphate + AMP ⟶ AMP – phosphate + 4 Nitrophenol

3.10.4 Evaluation of hormones (Insulin, Leptin and Ghrelin):

The kit employs a sandwich ELISA format to quantitative detect (INS, LEP, GHRL, and GSH) levels in various mouse biological samples, including serum , plasma, and tissue homogenates , in an in vitro setting.

The technique:

1- Standard solutions dilution: (This kit was a standard of original concentration, which could be diluted in small tubes by the user independently) following the instructions:

3.10.4.1 Insulin:

24 MIU/L	Standard NO.5	120µL Original Standard + 120µL Standard diluents
12 MIU/L	Standard NO.4	120µL Standard NO.5 + 120µL Standard diluents
6 MIU/L	Standard NO.3	120µL Standard NO.4 + 120µL Standard diluents
3 MIU/L	Standard NO.2	120µL Standard NO.3 + 120µL Standard diluents
1.5 MIU/L	Standard NO.1	120µL Standard NO.2 + 120µL Standard diluents

Tube	**Standard**	**S5**	**S4**	**S3**	**S2**	**S1**
MIU/L	48	24	12	6	3	1.5

3.10.4.2 Leptin:

1200 ng/l	Standard NO.5	120µL Original Standard + 120µL Standard diluents
600 ng/l	Standard NO.4	120µL Standard NO.5 + 120µL Standard diluents
300 ng/l	Standard NO.3	120µL Standard NO.4 + 120µL Standard diluents
150 ng/l	Standard NO.2	120µL Standard NO.3 + 120µL Standard diluents
75 ng/l	Standard NO.1	120µL Standard NO.2 + 120µL Standard diluents

Tube	**Standard**	**S5**	**S4**	**S3**	**S2**	**S1**
ng/l	2400	1200	600	300	150	75

3.10.4.3 Ghrelin:

400 ng/l	Standard NO.5	120µL Original Standard + 120µL Standard diluents
200 ng/l	Standard NO.4	120µL Standard NO.5 + 120µL Standard diluents
100 ng/l	Standard NO.3	120µL Standard NO.4 + 120µL Standard diluents
50 ng/l	Standard NO.2	120µL Standard NO.3 + 120µL Standard diluents
25 ng/l	Standard NO.1	120µL Standard NO.2 + 120µL Standard diluents

Tube	**Standard**	**S5**	**S4**	**S3**	**S2**	**S1**
ng/l	800	400	200	100	50	25

3.10.5 Evaluation of antioxidant (glutathione):

1000 ng/l	Standard NO.5	120µL Original Standard + 120µL Standard diluents
500 ng/l	Standard NO.4	120µL Standard NO.5 + 120µL Standard diluents
250 ng/l	Standard NO.3	120µL Standard NO.4 + 120µL Standard diluents
125 ng/l	Standard NO.2	120µL Standard NO.3 + 120µL Standard diluents
62.5 ng/l	Standard NO.1	120µL Standard NO.2 + 120µL Standard diluents

Tube	**Standard**	**S5**	**S4**	**S3**	**S2**	**S1**
ng/l	2000	1000	500	250	125	62.5

2- The number of stripes needed is determined by that of samples to be tested added by that of standards. It is suggested that each standard solution and each blank well should be arranged with three or more wells as much as possible.

3- Sample injection:

A: Blank well: Add

- chromogen solutions A and B
- Stop solution.

B: Standard solution well: Add

- 50µL standard
- Streptavidin-HRP 50µL.

C: Sample well to be tested: Add

- 40µL sample
- 10µL (INS, LEP, GHRL or GSH) antibodies.
- 50µL streptavidin-HRP
- Cover with seal plate membrane
- Mix gently
- Incubate at 37℃ for 60 minutes

4- Preparation of washing solution: Dilute the washing concentration (30x) with distilled water for later use.

5- Washing:

- Remove the seal plate membrane
- Drain the liquid
- Shake of the remaining liquid
- Fill each well with washing solution
- Drain the liquid after 30 seconds standing
- Then repeat this procedure five times and blot the plate

6- Color development:

- Add 50µL chromogen solution A
- Add 50µL chromogen solution B to each well

- Shake gently to mix them up
- Incubate for 10 minutes at 37℃ away from light for color development

7- Stop: Add 50µL stop solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment).

8- Assay:

- Take a blank well as zero
- Measure the absorbance (OD) of each well one at 450nm wavelength, which should be carried out within 10 minutes after having added the stop solution.

9-According to standards: concentration and the corresponding OD values, calculate the linear regression equation of the standard curve. Then, according to the OD value of the samples, calculate the concentration of the corresponding sample. Special software could be employed to calculate as well.

3.11 Body Weight

The body weights of the mice were monitored weekly using a high-precision electronic balance. Initial weights were recorded when the animals reached 20–23 g, whereas weights in the range of 35–40 g were considered indicator of obesity development. After establishing this stage, body weights continued to be recorded on a weekly basis. The animals were then sacrificed at two predetermined time points: one week and three weeks after confirmation of obesity, in order to document the final body weights and perform subsequent experimental analyses.

3.12 Statistical Analysis

Statistical analysis was performed using tow-way Analysis of Variance (ANOVA) followed by the Least Significant Difference (LSD) post-hoc test to compare means between the different treatment groups at each time point (1 and 3 weeks). Data are expressed as mean ± standard deviation of the mean. A p-value less than 0.05 ($P < 0.05$) was considered statistically significant. Analysis was conducted using Statistical Product and Service Solutions (SPSS) software (Steel *et al*., 1997).

Chapter Four

Results

4. Results

4.1 According to the groups

4.1.1 Body weight in male mice

The initial weight results showed a significant ($P<0.05$) increase in the B, C, D, and E groups compared to the A group. While non-significant ($P>0.05$) differences among B, C, D, and E groups as shown in Table 4.1.

The results of the weight gain showed a significant ($P<0.05$) increase in the B, C, D, and E groups compared to the A group, and in the D group compared to the B, C, and E groups. While non-significant ($P>0.05$) differences among the B, C, and E groups as shown in Table 4.1.

The results of the Final weight showed a significant ($P<0.05$) increase in B, C, D, and E groups compared to the A group, and in the B group compared to the D and E groups. While non-significant ($P>0.05$) between the B and C groups, also differences among the C, D, and E groups, as shown in Table 4.1.

Table (4.1): The body weights in male mice in different treatment groups (Mean ± SD).

Groups	Body weight (gram)		
	Initial weight	Weight gain	Final weight
A (Control)	a 19.318 ± 1.422	a 21.077 ± 1.355	a 22.585 ± 1.769
B (Obese mice)	b 21.087 ± 1.314	b 35.894 ± 1.243	b 37.317 ± 1.596
C (Orlistat)	b 21.351 ± 1.324	b 36.137 ± 1.180	bc 36.064 ± 1.980
D (Iron oxide)	b 21.664 ± 1.582	c 37.827 ± 3.249	c 35.591 ± 2.589
E (Orlistat + Iron oxide)	b 21.813 ± 1.741	b 37.172 ± 1.292	c 35.111 ± 1.721

Different letters refer to a significant difference among groups at the level of ($P<0.05$).

Similar letters refer to non-significant differences among groups.

4.1.2 Body Mass Index (BMI) and Lee Index in male mice

The results of the BMI values showed a significant ($P<0.05$) increase in B, C, D, and E groups compared to the A group. While non-significant ($P>0.05$) differences among B, C, D, and E groups as shown in Table 4.2.

The results of the Lee Index showed non-significant ($P>0.05$) differences between all groups, as shown in Table 4.2.

Table (4.2): The body Mass Index and Lee Index values in male mice in different treatment groups (Mean ± SD).

Groups	Indicators	
	BMI (g/cm^2)	Lee index (g/cm)
A (Control)	a 0.301 ± 0.036	a 326.357 ± 19.161
B (Obese mice)	b 0.369 ± 0.023	a 330.357 ± 10.262
C (Orlistat)	b 0.373 ± 0.031	a 331.428 ± 13.832
D (Iron oxide)	b 0.367 ± 0.014	a 326.357 ± 5.956
E (Orlistat + Iron oxide)	b 0.366 ± 0.023	a 327.214 ± 10.154

Different letters refer to a significant difference among groups at the level of (P<0.05). Similar letters refer to non-significant differences among groups.

4.1.3 Biochemical parameters in mal mice

4.1.3.1 The glucose values

The results of the glucose value showed a significant (P<0.05) increase in the B, C, D, and E groups compared to the A group, and the B group compared to D and E groups. While non-significant (P>0.05) differences between the B and C groups, also among the C, D, and E groups, as shown in Table 4.3.

Table (4.3): The glucose values in male mice in different treatment groups (Mean ± SD).

Groups	Glucose (mg /dl)
A (Control)	a 167.428 ± 33.806
B (Obese mice)	b 288.428 ± 47.243
C (Orlistat)	bc 264.500 ± 49.985
D (Iron oxide)	c 236.071± 32.638
E (Orlistat + Iron oxide)	c 238.285 ± 26.733

Different letters refer to a significant difference among groups at the level of ($P<0.05$).

Similar letters refer to non-significant differences among groups.

4.1.3.2 The lipid profile values

The results of the TG values appeared there was a significant ($P<0.05$) increase in B, C, D, and E groups compared to the A group, also B group compared to the D group, while non-significant ($P>0.05$) differences among B, C and E groups, also among C, D and E groups as shown in Table 4.4.

The results of the TC values showed a significant ($P<0.05$) increase in the B, C, D, and E groups compared to the A group. While non-significant ($P>0.05$) differences among B, C, D, and E groups as shown in Table 4.4.

The results of the HDL values appeared there was a significant ($P<0.05$) decrease in the B, D and E groups compared to the A group, also the D group compared to the C group. While non-significant ($P>0.05$) differences between A and C groups, B, C and E groups also among B, D and E groups as shown in Table 4.4.

The results of the LDL values showed a significant ($P<0.05$) increase in the B, C, D, and E groups compared to the A group, also the D group compared to the B group. While non-significant ($P>0.05$) differences were observed among B, C, and E groups, and also among C, D, and E groups, as shown in Table 4.4.

The results of the VLDL values appeared non-significant ($P>0.05$) differences among all groups, as shown in Table 4.4.

Table (4.4): The lipid profile values in male mice in different treatment groups (Mean ± SD).

Groups	TG (mg /dl)	TC (mg /dl)	HDL (mg /dl)	LDL (mg /dl)	VLDL (mg /dl)
A (Control)	a 78.928 ± 15.657	a 91.142 ± 15.144	a 49.571 ± 14.113	a 47.857 ± 16.686	a 27.285 ± 10.476
B (Obese mice)	b 151.571 ± 27.340	b 128.285 ± 20.261	bc 35.928 ± 6.005	b 73.785 ± 11.510	a 29.857 ± 5.627
C (Orlistat)	bc 136.214 ± 32.581	b 142.000 ± 29.573	ab 43.357 ± 12.055	bc 87.857 ± 19.728	a 26.928 ± 7.065
D (Iron oxide)	c 125.857 ± 31.768	b 135.000 ± 31.194	c 34.714 ± 7.162	c 89.928 ± 21.688	a 24.928 ± 6.603
E (Orlistat + Iron oxide)	bc 133.714 ± 30.251	b 144.571 ± 35.485	bc 39.142 ± 8.592	bc 86.214 ± 23.383	a 28.642 ± 9.443

Different letters refer to a significant difference among groups at the level of ($P<0.05$).

Similar letters refer to non-significant differences among groups.

4.1.3.3 The liver enzyme activity

The results of the ALT value showed a significant ($P<0.05$) increase in the D group compared to the A, B, C, and E groups, both groups A and C increased significantly ($P<0.05$) compared to the B and E groups. While non-significant ($P>0.05$) differences were observed between the A and C groups, also between the B and E groups, as shown in Table 4.5.

The results of the AST value showed a significant ($P<0.05$) increase in the C, D, and E groups compared to the A and B groups. While non-significant ($P>0.05$) differences were observed between the A and B groups, and also among the C, D, and E groups, as shown in Table 4.5.

The results of the ALP value showed a significant ($P<0.05$) decrease in B, C, D, and E groups compared to the A group, and the B group compared to C, D, and E groups. While non-significant ($P>0.05$) differences among C, D, and E groups as shown in Table 4.5.

Table (4.5): The liver enzyme values in male mice in different treatment groups (Mean ± SD).

Groups	ALT (U /L)	AST (U /L)	ALP (U /L)
A (Control)	a 49.428 ± 19.429	a 143.071 ± 28.712	a 148.785 ± 25.780
B (Obese mice)	b 30.142 ± 7.684	a 164.428 ± 49.563	b 67.428 ± 15.330
C (Orlistat)	a 45.928 ± 14.376	b 205.785 ± 61.087	c 91.571 ± 14.058
D (Iron oxide)	c 74.428 ± 17.028	b 224.071 ± 36.067	c 87.928 ± 30.700
E (Orlistat + Iron oxide)	b 37.214 ± 10.856	b 201.571 ± 58.682	c 91.428 ± 17.032

Different letters refer to a significant difference among groups at the level of ($P<0.05$).

Similar letters refer to non-significant differences among groups.

4.1.4 The hormonal parameters in male mice:

The results of the insulin value appeared there are non-significant ($P>0.05$) difference among all groups as shown in Table 4.6.

The results of the leptin value showed a significant ($P<0.05$) increase in the C and E groups compared to the A and D groups. While non-significant ($P>0.05$) differences between A and D groups, between A and B groups, and also among B, C, and E groups as shown in Table 4.6.

The results of the ghrelin value showed a significant (P<0.05) increase in the B group compared to the A and E groups. While non-significant (P>0.05) differences were observed among A, C, D, and E groups, also among B, C, and D groups as shown in Table 4.6.

Table (4.6): The hormone values in male mice in different treatment groups (Mean ± SD).

Groups	Insulin (µu/ml)	Leptin (ng/ml)	Ghrelin (ng/ml)
A (Control)	a 1.412 ± 0.483	ac 42.509 ± 10.717	a 36.634 ± 13.958
B (Obese mice)	a 1.573 ± 0.531	ab 54.459 ± 16.370	b 49.771 ± 14.064
C (Orlistat)	a 1.263 ± 0.721	b 56.460 ± 16.463	ab 40.847 ± 10.230
D (Iron oxide)	a 1.386 ± 0.576	c 33.764 ± 11.670	ab 42.322 ± 13.192
E (Orlistat + Iron oxide)	a 1.476 ± 0.488	b 56.569 ± 21.655	a 37.800 ± 17.868

Different letters refer to a significant difference among groups at the level of (P<0.05).

Similar letters refer to non-significant differences among groups.

4.1.5 The antioxidant (Glutathione) in male mice

The results of the glutathione value showed a significant (P<0.05) increase in the B group compared to A, C, and E groups, and the D group compared to C and E groups. Non-significant (P>0.05) differences were observed among A, C, and E groups and between B and D groups, as shown in Table 4.7.

Table (4.7): The antioxidant (Glutathione) values in male mice in different treatment groups (Mean ± SD).

Groups	Glutathione (ng/ml)
A (Control)	ac 46.164 ± 17.428
B (Obese mice)	b 60.892 ± 14.616
C (Orlistat)	c 43.852 ± 19.734
D (Iron oxide)	ab 57.942 ± 14.743
E (Orlistat + Iron oxide)	c 44.022 ± 17.531

Different letters refer to a significant difference among groups at the level of (P<0.05).

Similar letters refer to non-significant differences among groups.

4.2 According to the periods

4.2.1 Body weight in male mice

The results of the initial weight after the first week of treatment appeared there was a significant (P<0.05) increased in D group compared to A and B groups, while non-significant (P>0.05) differences compared to C, D and E groups, also among A, B, C and E groups Table 4.8. After the third week of treatment appeared there was a significant (P<0.05) increased in B, C, D, E groups compared to the A group, also E group compared to D group, while non-significant (P>0.05) differences between B, C and E groups, also among B, C and D groups Table 4.8.

The results of the weight gain after the first week of treatment appeared there was a significant (P<0.05) increase in groups B, C, D, E compared to the A group, also between D group compared to B, C and E groups, while non-significant (P>0.05) differences among B, C and E groups Table 4.8. After the third week of treatment appeared of treatment appeared there was a significant (P<0.05) increased in B, C, D and E groups compared to A group, and E group compared of B, C and D groups, also C group compared to B and D groups, while non-significant (P>0.05) differences between B and D groups Table 4.8.

The results of the final weight after the first week of treatment appeared there was a significant (P<0.05) increase in B, C, D, and E groups compared to the A group, while non-significant (P>0.05) differences among B, C, D, and E groups Table 4.8. After the third week of treatment appeared there was a significant (P<0.05) increased in B, C, D and E groups compared to the A

group, also B and C groups compared of D and E groups, while non-significant (P>0.05) differences between D and E groups and also between B and C groups as shown in Table 4.8.

Table (4.8) The body weights during two periods in male mice (Mean ± SD).

Groups	Week	Body weight (gram)		
		Initial weight	Weight gain	Final weight
A (Control)	1 week	a 20.124 ± 1.373	a 21.445 ± 1.549	a 22.235 ± 1.690
	3 weeks	a 18.512 ± 0.993	a 20.710 ± 1.125	a 22.934 ± 1.908
B (Obese mice)	1 week	a 21.241 ± 1.177	b 36.655 ± 1.398	b 38.242 ± 1.613
	3 weeks	bc 20.932 ± 1.517	b 35.132 ± 0.202	b 36.391 ± 0.960
C (Orlistat)	1 week	ab 21.428 ± 1.418	b 36.001 ± 1.448	b 36.110 ± 2.732
	3 weeks	bc 21.274 ± 1.331	c 36.274 ± 0.936	b 36.018 ± 1.012
D (Iron oxide)	1 week	b 22.854 ± 1.376	c 40.480 ± 2.525	b 37.310 ± 2.479
	3 weeks	c 20.474 ± 0.476	b 35.175 ± 0.306	c 33.872 ± 1.217
E (Orlistat +Iron oxide)	1 week	ab 21.650 ± 1.648	b 37.202 ± 1.350	b 36.521 ± 1.109
	3 weeks	b 21.977 ± 1.946	d 37.142 ± 1.338	c 33.701 ± 0.741

Different letters refer to a significant difference among groups at the level of (P<0.05). Similar letters refer to non-significant differences among groups.

4.2.2 Body Mass Index (BMI) and Lee Index in male mice

The results of the BMI after the first and third weeks of treatment appeared there was a significant (P<0.05) increased in B, C, D and E groups compared

to the A group while non-significant (P>0.05) differences among B, C, D and E groups Table 4.9.

The results of the lee index after the first week of treatment appeared non-significant (P>0.05) differences among all groups, Table 4.9. After the third week of treatment appeared there was a significant (P<0.05) increased in C group compared to A group, while non-significant (P>0.05) differences between B, C, D and E groups, also among A, B, D and E groups Table 4.9.

Table (4.9) : The body Mass Index and Lee Index values during two periods in male mice (Mean ± SD).

Groups	Week	Indicators	
		BMI (g/cm^2)	Lee index (g/cm)
A (Control)	1 week	a 0.320 ± 0.030	a 335.000 ± 15.663
	3 weeks	a 0.282 ± 0.032	a 317.714 ± 19.388
B (Obese mice)	1week	b 0.383 ± 0.024	a 335.142 ± 11.582
	3 weeks	b 0.356 ± 0.013	ab 325.571 ± 6.373
C (Orlistat)	1 week	b 0.371 ± 0.037	a 331.142 ± 16.159
	3 weeks	b 0.374 ± 0.028	b 331.714 ± 12.378
D (Iron oxide)	1 week	b 0.377 ± 0.008	a 326.857 ± 6.067
	3 weeks	b 0.357 ± 0.013	ab 325.857 ± 6.283
E (Orlistat + Iron oxide)	1 week	b 0.382 ± 0.020	a 334.285 ± 8.159
	3 weeks	b 0.351 ± 0.013	ab 320.142 ± 6.335

Different letters refer to a significant difference among groups at the level of (P<0.05). Similar letters refer to non-significant differences among groups.

4.2.3 Biochemical parameters in male mice:

4.2.3.1 The glucose values

The results of the glucose value after the first week of treatment appeared there was a significant (P<0.05) increased in B, C and D groups compared to the A group, while non-significant (P>0.05) differences between A and E groups, also among B, C, D and E groups Table 4.10. After the third week of treatment appeared there was a significant (P<0.05) increased in B, C, D and E groups compared to the A group, also in B and C groups compared to D and E groups, while non-significant (P>0.05) differences between B and C groups, also between D and E groups Table 4.10.

Table (4.10): The glucose values during two periods in male mice (Mean ± SD)

Groups	Week	Glucose (mg /dl)
A (Control)	1 week	a 189.000 ± 28.225
	3 weeks	a 145.857 ± 24.368
B (Obese mice)	1 week	b 275.714 ± 64.121
	3 weeks	b 301.142 ± 18.631
C (Orlistat)	1 week	b 239.000 ± 50.358
	3 weeks	b 290.000 ± 36.882
D (Iron oxide)	1 week	b 238.142 ± 26.748
	3 weeks	c 234.000 ± 39.782
E (Orlistat + Iron oxide)	1 week	ab 235.571 ± 31.658
	3 weeks	c 241.000 ± 23.000

Different letters refer to a significant difference among groups at the level of (P<0.05). Similar letters refer to non-significant differences among groups.

4.2.3.2 The lipid Profile values

The results of the TG value appeared after the first week of treatment appeared there was a significant (P<0.05) increased in B, C, D and E groups compared to the A group, also B group compared to C group, while non-significant (P>0.05) differences among B, D and E groups, also among C, D and E groups Table 4.11. After the third week of treatment appeared there was a significant (P<0.05) increased in B, C, D and E groups compared to the A group, and significant (P<0.05) decreased in D and E groups compared to B and C groups and non- significant (P>0.05) difference between B and C groups, also between D and E groups Table 4.11.

The results of the TC value appeared after the first week of treatment. There was a significant (P<0.05) increase in B, C, D, and E groups compared to the A group, while non-significant (P>0.05) differences among B, C, D, and E groups Table 4.11. After the third week of treatment appeared there was a significant (P<0.05) increase in C, D and E groups compared to A group, while non-significant (P>0.05) differences between A and B groups, also among B, C, D and E groups Table 4.11.

The results of the HDL value appeared after the first week of treatment appeared there was a significant (P<0.05) decreased in B, D and E groups compared to the A group, while non-significant (P>0.05) differences between A and C groups, also among B, C, D and E groups Table 4.11. After the third week of treatment appeared there was a significant (P<0.05) decreased in B

and D groups compared to A group, while non-significant (P>0.05) differences were observed among A, C and E groups, also among B, C, D and E groups as shown in Table 4.11.

The results of the LDL value appeared after the first week of treatment appeared there was a significant (P<0.05) increased in B, C and D groups compared to the A group, while non-significant (P>0.05) differences between A and E groups, also among B, C, D and E groups Table 4.11. After the third week of treatment appeared there was a significant (P<0.05) increased in B, C, D and E groups compared to the A group, and significant (P<0.05) decreased in B group compared to D and E groups, while non-significant (P>0.05) differences between B and C groups, also among C, D and E groups as shown in Table 4.11.

The results of the VLDL value appeared after the first week of treatment appeared there was a significant (P<0.05) increased in B, D and E groups compared to the A group, also E group compared to C group, while non-significant (P>0.05) differences between A and C groups, and B, D and E groups, also among B, C and D groups Table 4.11. After the third week of treatment appeared there was a significant (P<0.05) decreased in D and E groups compared to A group, while non-significant (P>0.05) differences among A, B and C groups, also among B, C, D and E groups as shown in Table 4.11.

Table (4.11): The lipid Profile values during two periods in male mice (Mean ± SD).

Groups	Week	TG (mg /dl)	TC (mg /dl)	HDL (mg /dl)	LDL (mg /dl)	VLDL (mg /dl)
A (Control)	**1 week**	a 87.285 ± 11.600	a 84.000 ± 14.696	a 54.571 ± 17.057	a 59.285 ± 16.770	a 19.142 ± 3.716
	3 week	a 70.571 ± 15.284	a 98.285 ± 12.724	a 44.571 ± 9.071	a 36.428 ± 4.157	a 35.428 ± 8.323
B (Obese mice)	**1week**	b 158.428 ± 33.876	b 129.571 ± 28.359	b 35.571 ± 7.764	b 76.428 ± 14.211	bc 30.714 ± 6.237
	3 week	b 144.714 ± 19.032	ab 127.000 ± 9.018	b 36.285 ± 4.191	b 71.142 ± 8.295	ab 29.000 ± 5.291
C (Orlistat)	**1 week**	c 123.428 ± 23.936	b 139.857 ± 14.450	ab 46.142 ± 16.077	b 85.000 ± 9.729	ab 24.428 ± 5.349
	3 week	b 149.000 ± 36.683	b 144.142 ± 40.932	ab 40.571 ± 6.187	bc 90.714 ± 27.010	ab 29.428 ± 8.059
D (Iron oxide)	**1 week**	bc 139.285 ± 35.523	b 131.000 ± 30.670	b 34.714 ± 8.260	b 80.000 ± 15.459	bc 27.714 ± 7.319
	3 weeks	c 112.428 ± 22.448	b 139.000 ± 33.620	b 34.714 ± 6.550	c 99.857 ± 23.455	b 22.142 ± 4.775
E (Orlistat + Iron oxide)	**1week**	bc 150.857 ± 25.281	b 135.714 ± 28.587	b 38.857 ± 8.591	ab 74.571 ± 20.040	c 34.142 ± 7.925
	3 weeks	c 116.571 ± 25.650	b 153.428 ± 41.568	ab 39.428 ± 9.271	c 97.857 ± 21.605	b 23.142 ± 7.733

Different letters refer to a significant difference among groups at the level of ($P<0.05$). Similar letters refer to non-significant differences among groups.

4.2.3.3 Liver enzyme values.

The results of the ALT value appeared after the first week of treatment appeared there was a significant ($P<0.05$) increased in C and D groups compared to the A, B and E groups, while non-significant ($P>0.05$) differences between C and D groups, also among A, B and E groups Table 4.12. After the third week of treatment appeared there was a significant ($P<0.05$) increased in D group compared to A, B, C and E groups also A group compared to B, C and E groups, while non-significant ($P>0.05$) differences among B, C and E groups Table 4.12.

The results of the AST value appeared after the first week of treatment appeared there was a significant ($P<0.05$) increased in C, D and E groups compared to the A and B groups, while non-significant ($P>0.05$) differences among C, D and E groups, also between A and B groups Table 4.12. After the third week of treatment appeared there was a significant ($P<0.05$) increased in D group compared to A, B, C and E groups, while non-significant ($P>0.05$) differences among A, B, C and E groups Table 4.12.

The results of the ALP value appeared after the first week of treatment appeared there was a significant ($P<0.05$) decreased in B, C, D and E groups compared to the A group, also B and D groups compared to C and E groups, while non-significant ($P>0.05$) differences between B and D groups, also between C and E groups Table 4.12. After the third of treatment appeared week there was a significant ($P<0.05$) decreased in B, C, D and E groups compared to the A group, also B group compared to C, D and E groups, while non-significant ($P>0.05$) differences among C, D and E groups Table 4.12.

Table (4.12) Liver enzyme values during two periods in male mice (Mean ± SD).

Groups	Week	ALT (U /L)	AST (U /L)	ALP (U /L)
A (Control)	**1 week**	a 42.428 ± 14.432	a 152.571 ± 30.215	a 142.714 ± 24.770
	3 weeks	a 56.428 ± 22.255	a 133.571 ± 25.741	a 154.857 ± 27.211
B (Obese mice)	**1 week**	a 32.428 ± 7.412	a 162.142 ± 46.344	bc 72.428 ± 14.998
	3 weeks	b 27.857 ± 7.798	a 166.714 ± 56.236	c 62.428 ± 15.031
C (Orlistat)	**1 week**	b 57.285 ± 10.672	b 253.285 ± 30.148	cd 86.142 ± 16.180
	3 weeks	b 34.571 ± 5.740	a 158.285 ± 43.725	b 97.000 ± 9.882
D (Iron oxide)	**1 week**	b 64.571 ± 15.219	b 222.714 ± 33.335	b 62.857 ± 6.986
	3 weeks	c 84.285 ± 13.034	b 225.428 ± 41.266	b 113.000 ± 22.949
E (Orlistat + Iron oxide)	**1 week**	a 39.857 ± 11.922	b 230.428 ± 53.717	d 90.571 ± 12.067
	3 weeks	b 34.571 ± 9.846	a 172.714 ± 51.308	b 92.285 ± 21.937

Different letters refer to a significant difference among groups at the level of ($P<0.05$).

Similar letters refer to non-significant differences among groups.

4.2.4 The hormonal parameters in male mice.

The results of the insulin value appeared after the first week of treatment appeared there is non-significant (P>0.05) difference among all groups A, B, C, D, and E Table 4.13. After the third week of treatment appeared there was a significant (P<0.05) increased in E group compared to the A group, while non-significant (P>0.05) differences among A, B, C and D groups, also among B, C, D and E groups Table 4.13.

The results of the leptin value appeared after the first week of treatment appeared there was a significant (P<0.05) increased in B and C groups compared to A, D and E groups, while non-significant (P>0.05) differences between B and C groups, also among A, D and E groups Table 4.13. After the third week of treatment appeared there was a significant (P<0.05) increased in E group compared to the A, B, C and D group, while non-significant (P>0.05) differences among A, B and C groups, also among A, B and D groups Table 4.13.

The results of the ghrelin value appeared after the first week of treatment appeared there was a significant (P<0.05) increased in B group compared to A and E groups and significant (P>0.05) decreased in E group compared to C and D groups, while non-significant (P>0.05) differences among B, C and D groups, and between A and E groups, also among A, C and D groups Table 4.13. After the third week of treatment appeared there is non-significant (P>0.05) difference among A, B, C, D and E groups Table 4.13.

Table (4.13): The hormones during the two periods in male mice (Mean ± SD).

Groups	Week	Insulin (µu/ml)	Leptin (ng/ml)	Ghrelin (ng/ml)
A (Control)	1 week	a 1.645 ± 0.436	a 45.215 ± 5.348	ac 35.998 ± 16.707
	3 weeks	a 1.178 ± 0.435	ab 39.802 ± 14.254	a 37.270 ± 11.919
B (Obese mice)	1 week	a 1.405 ± 0.496	b 60.815 ± 16.224	b 57.511 ± 9.110
	3 weeks	ab 1.741 ± 0.548	ab 48.102 ± 14.938	a 42.031 ± 14.345
C (Orlistat)	1 week	a 1.150 ± 0.621	b 58.895 ± 15.345	ab 45.610 ± 8.036
	3 weeks	ab 1.377 ± 0.842	a 54.025 ± 18.383	a 36.085 ± 10.452
D (Iron oxide)	1 week	a 1.522 ± 0.724	a 35.352 ± 8.566	ab 46.680 ± 14.539
	3 weeks	ab 1.250 ± 0.389	b 32.175 ± 14.690	a 37.964 ± 11.018
E (Orlistat + Iron oxide)	1 week	a 1.118 ± 0.340	a 40.388 ±10.820	c 28.167 ± 9.688
	3 weeks	b 1.834 ± 0.318	c 72.750 ± 16.974	a 47.434 ± 19.528

Different letters refer to a significant difference among groups at the level of ($P<0.05$).

Similar letters refer to non-significant differences among groups.

4.2.5 The antioxidant (glutathione) in male mice.

The results of the glutathione value appeared after the first week of treatment appeared there was a significant (P<0.05) decreased in E group compared to A, B, C and D groups, while non-significant (P>0.05) differences among A, B, C and D groups Table 4.14. After the third week of treatment appeared there was a significant (P<0.05) decreased in C group compared to B, D and E groups, also A group compared to B and E groups while non-significant (P>0.05) differences between A and C groups, and between A and D groups, also among B, D and E groups as shown in Table 4.14.

Table (4.14): The antioxidant during two periods in male mice (Mean ± SD).

Groups	Week	Glutathione (ng/ml)
A (Control)	**1 week**	a 51.100 ± 21.057
	3 weeks	ac 41.228 ± 12.564
B (Obese mice)	**1 week**	a 64.842 ± 15.933
	3 weeks	b 56.942 ± 13.137
C (Orlistat)	**1 week**	a 52.428 ± 18.484
	3 weeks	c 35.277 ± 18.181
D (Iron oxide)	**1 week**	a 62.100 ± 18.121
	3 weeks	ab 53.785 ± 10.111
E (Orlistat + Iron oxide)	**1 week**	b 30.915 ± 12.599
	3 weeks	b 57.128 ± 10.312

Different letters refer to a significant difference among groups at the level of ($P<0.05$). Similar letters refer to non-significant differences among groups.

4.3 Toxicity Assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay revealed that nanoparticle toxicity increased with concentration, but surface coating reduced this effect. The coated nanoparticles consistently showed higher cell viability across all concentrations, indicating better biocompatibility. The effect was most significant at 10 mg/mL, where coating improved viability by 15% compared to bare particles as shown in Table 4.15.

Table (4.15): Effect of bare and coated Iron Oxide Nanoparticles on COS-7 Cell viability assessed by MTT assay.

Concentration (mg/mL)	Bare NPs Cell Viability (%)	Coated NPs Cell Viability (%)
0.1	95% ± 2%	98% ± 1.5%
1	75% ± 3%	85% ± 2.5%
10	50% ± 4%	65% ± 3.5%

Chapter Five

Discussion

5. Discussion

5.1 According to the groups:

5.1.1 Body weight values in male mice

A significant (P<0.05) increase in body weight was observed following obesity induction (Table 4.1), consistent with studies conducted on male rats (Othman *et al*., 2021; Kobi *et al*., 2023), the increased body weight is attributed to the fact that the high-fat diet (HFD) is high in calories. Excess calories are stored in adipose tissue as a source of energy reserves. Energy from fat has a greater effect on weight gain compared with non-fat energy sources (Warwick *et al*., 2002).

Orlistat administration lead to a non-significant (P > 0.05) decrease in body weight compared with the obese group (Table 4.1), consistent with studies conducted on male rats (Othman *et al*., 2021; Zakaria *et al*., 2021), this decrease in body weight is attributed to the action of orlistat in the intestinal lumen, where it responsible for inhibiting Pancreatic lipase the absorption of fat from the intestine, thus reducing fat mass (Heck *et al*., 2012).

Treatment with IONPs led to a significant (P<0.05) decrease in body weight compared with the obese group and a non-significant (P>0.05) decrease compared to the orlistat treated group (Table 4.1). This is consistent with a study conducted on male rats (Alsenousy *et al*., 2022). Contradicts a study conducted on male rats which showed that orlistat had a better effect on body weight than IONPs (Refaat *et al*., 2024), Both studies showed that a combination of orlistat and IONPs produced better results than the effect of either alone. However, this contradicted our results. The effect of IONPs on body weight is attributed to the

particles' ability to inhibit angiogenesis in white adipose tissue (WAT) and convert WAT into brown adipose tissue (BAT) (Li *et al*., 2019).

5.1.2 The Body Mass Index (BMI) and Lee Index values in male mice

The obese group showed a significant ($P<0.05$) increase in BMI values compared with the control group, which may indicate the success of obesity induction (Table 4.2). This is consistent with studies conducted on rats (Thelappilly *et al*., 2024; Selima *et al*., 2025), and the increased BMI values are attributed to the increase in body weight resulting from energy storage in adipose tissue (Warwick *et al*., 2002).

The results of the above studies showed a decrease in BMI with orlistat treatment, but these results contradicted our results, as we did not observe an effect of orlistat on BMI (Table 4.2). This may be due to the short duration of treatment, the concentration of the drug, or the method of administration.

A non-significant ($P>0.05$) decrease in BMI values was observed with treatment with IONPs compared to the obese group. This is attributed to the ability of nanoparticles to induce browning of WAT (Li *et al*., 2019). The greatest improvement in BMI values was observed with treatment with a combination of IONPs and orlistat, possibly due to the increased efficacy of orlistat when combined with IONPs. Our results are consistent with the results of a study conducted by Refaat and colleagues (2024) on male rats, differing only in that the effect of IONPs was superior to orlistat. This may be due to the lack of effect of orlistat on BMI due to the short treatment period, the concentration, and the method of administration used.

The Lee index is used to measure obesity in rats only, as shown in studies (Bastias-Perez *et al*., 2020; Quiros Cognuck *et al*., 2020; Zakaria *et al*., 2021). We used it to measure obesity in rats, and it yielded good results (Table 4.).

5.1.3 Biochemical Parameters in male mice:

5.1.3.1 The lipid profile values

Significant ($P<0.05$) increases in TG, TC, and LDL levels, and a non-significant ($P>0.05$) increase in VLDL levels, and significant ($P<0.05$) decrease in HDL values, were observed in obese male mice compared with the control group (Table 4.4), this is consistent with studies conducted on rats (El-Shehawi *et al*., 2021; Wasim *et al*., 2024). Obesity is associated with increased FFA flux from adipose tissue to the liver, leading to enhanced hepatic VLDL production and impaired clearance of triglyceride-rich lipoproteins. This results in elevated TG, TC, LDL, and VLDL levels, while HDL decreases due to CETP-mediated triglyceride exchange and accelerated catabolism by hepatic lipase (Klop *et al*.,2013).

Studies conducted on rats by (Othman *et al* 2021; Javed *et al* 2023) showed improvement in dyslipidemia after treatment with orlistat, including decreased TG, TC, and LDL levels and increased HDL levels, whereas the differences observed in our study were only in TC and LDL (Table 4.4), which were not affected by orlistat. This may be due to the short duration of treatment or the concentration of the treatment used in our study. The anti-hyperlipidemic property of orlistat is due to its inhibition of pancreatic lipase, which in turn reduces the breakdown of dietary triglycerides by preventing their hydrolysis in the intestine, thereby reducing the transport of dietary fatty acids from the small

intestine to the lymph (Mahmoud and Elnour, 2013). Orlistat also stimulates the maturation of HDL particles and increases HDL-associated proteins (Nakou *et al*., 2008).

Studies conducted on male rats showed improved dyslipidemia with treatment using IONPs, but treatment with orlistat was even better (Al-Senousy *et al*., 2022; Refaat *et al*., 2024). This improvement is attributed to the role of nanoparticles in reducing the lipid content in adipocytes by promoting WAT browning, thus improving dyslipidemia (Li *et al*., 2019). The results of our study differed from those mentioned, as a slight improvement in blood lipids was observed, in TG and VLDL values (Table 4.4), and to a greater extent than with orlistat treatment. TC, HDL and LDL values were not affected by IONPs, which may be due to the short duration of treatment with IONPs, the difference in concentration, and the routes of administration.

A study conducted on male rats showed that a combination of orlistat and IONPs improved dyslipidemia better than either orlistat alone or IONPs alone (Refaat *et al*., 2024). The results of our study differed, as TC and LDL values were unaffected and the combined treatment with IONPs and Orlistat did not result in the most favorable effects (Table 4.4).

5.1.3.2 The liver enzyme activuty

A significant (P<0.05) decrease in ALT and ALP values, a non-significant (P>0.05) increase in AST values were observed in obese male mice compared with the control group (Table 4.5) The elevation of AST may be attributed to muscle stress or systemic inflammation rather than hepatic inflammation (Ndrepepa, 2021). ALT and ALP values decreased despite the existence of reasons for their elevation of this enzymes. The decrease in ALT and ALP levels contrasts with the results of previous studies conducted on male mice, which showed elevated levels of ALT, AST, and ALP in obese mice (Abbas *et al*., 2022; Baz *et al*., 2022).

A significant (P<0.05) increase in ALT, AST, and ALP levels was observed in male mice treated with orlistat compared with the obese group (Table 4.5). Our findings are consistent with those of (Aparicio *et al*., 2024), who reported similar increases in liver enzymes in male mice, attributing this elevation to the hepatotoxic effects of orlistat. This finding contradicts study in mice that reported a decrease in liver enzymes with orlistat treatment (Thelappilly *et al*., 2024).

Our results showed an increase in liver enzyme levels after treatment with IONPs and a combination of IONPs and orlistat compared to the obese group, which contradicts the findings of a study by Al-Senosy and colleagues (2022) which demonstrated a reduction in elevated liver enzymes ALT and AST in obesity following treatment with iron oxide nanoparticles (IOPNs) and a combination of orlistat with IOPNs.

5.1.4 The hormonal parameters in male mice:

5.1.4.1 The glucose and insulin values

A significant (P<0.05) increase in glucose and a non-significant (P>0.05) increase in insulin levels were observed in obese male mice compared with the control group (Table 4.3 and 4.6). This is consistent with studies conducted on male rats (El-Shehawi *et al*., 2021; Abbas *et al*., 2022), where the increased plasma glucose and insulin levels were attributed to the development of insulin resistance. Insulin resistance is caused by obesity, as the enlargement of adipose tissue, or what is known as obesity-induced hyperplasia, leads to increased FFA and decreased FFA clearance. This, in turn, inhibits the anti-lipolytic effect of insulin, increasing the release of FFA into the circulation. Increased plasma FFA reduces whole-body glucose uptake, thus leading to insulin resistance. Insulin resistance is also linked to oxidative stress caused by obesity (Boden, 2011).

Orlistat administration led to a non-significant (P>0.05) decrease in glucose and insulin levels compared with the obese group (Table 4.3 and 4.6). This is consistent with studies conducted on male rats and mice (Zakaria *et al*., 2021; Xu *et al*., 2023). This result is attributed to the effect of orlistat on insulin resistance. It improves insulin resistance by reducing fat mass (Ke *et al*., 2020).

Treatment with IONPs resulted in a significant (P<0.05) decrease in glucose and a non-significant (P>0.05) decrease in insulin levels compared with the obese group (Table 4.3 and 4.6). Our results are consistent with studies conducted on male rats (Alsenousy *et al*., 2022; Refaat *et al*., 2024). Enhancing the formation of brown adipocytes by IONPs reduces energy storage, thus reducing fat mass and improving glucose and insulin balance in obese rats (Li

et al., 2019). Our results showed that the reduction in glucose levels in the IONPs-treated group was better than in the orlistat-treated group. These results are consistent with the results of the study by Alsenousy and colleagues (2022). Meanwhile, the reduction in insulin levels in the orlistat-treated group was better than in the IONPs-treated group, and this is consistent with the study conducted by Refaat and colleagues (2024) and the study by Alsenousy and colleagues (2022). The two studies above showed that male rats showed a greater improvement in glucose and insulin levels with a combination of orlistat and IONPs than with orlistat alone or IONPs alone, however, our findings were not consistent with these results.

5.1.4.2 The leptin values

A non-significant ($P > 0.05$) increase in leptin was observed in the obese group compared with the control group (Table 4.6). This result is consistent with several studies conducted on male mice (Song *et al*., 2020; Chularojmontri *et al*., 2022), the increased plasma leptin concentrations were attributed to the fact that high insulin levels weaken the hypothalamus's physiological response to leptin to regulate appetite (Lustig *et al*., 2004). With increasing obesity, serum leptin levels also increase, potentially leading to the development of resistance at the blood-brain barrier (Banks *et al*., 1999), this means that less leptin reaches the brain, leading to a decreased feeling of fullness (Izquierdo *et al*., 2019).

Orlistat administration led to a decrease in leptin levels compared with the obese mice group in studies conducted on rats (Caroline *et al*., 2021; Yilmaz *et al*., 2024). The results of our study differed, as orlistat did not affect leptin levels. (Table 4.6), this may be attributed to the short treatment period with orlistat, as orlistat can reduce leptin levels if fat loss is substantial and maintained for a sufficient period (Ozcelik *et al*., 2004).

Treatment with IONPs led to a significant ($P<0.05$) decrease in leptin levels compared with the obese group (Table 4.6). This is consistent with the results of studies conducted on male rats (Alsenousy *et al*., 2022; Refaat *et al*., 2024). Both studies showed that a combination of orlistat and IONPs produced better results than the effect of either alone. However, this contradicted our results, which showed that a combination of orlistat and IONPs had no effect, (Table 4.6). The reduction in leptin secretion induced by IONPs is attributed to the these nanoparticles enhance energy metabolism and promote the browning of white adipose tissue, leading to lower leptin levels in the blood with improved insulin resistance and metabolic profiles (Gao *et al*., 2015; Wu *et al*., 2017).

5.1.4.3 The ghrelin values

Our results showed a significant ($P<0.05$) increase in ghrelin levels in the obese group, while a non-significant ($P>0.05$) decrease was observed following treatment with orlistat (Table 4.6). This finding contrasts with studies in mice that reported decreased ghrelin levels in obese mice and increased levels following orlistat treatment (Mashmoul *et al*., 2017; Caroline *et al*., 2021). The initial reduction in ghrelin in obese state is attributed to energy surplus, where ghrelin secretion decreases as a natural mechanism to reduce appetite. After

weight loss, whether through dietary intervention alone or combined with orlistat, the body compensates by increasing ghrelin secretion to restore energy balance (Pamuk *et al*., 2018).

A systematic review of the literature did not identify any published studies investigating the effects of IONPs, alone or in combination with orlistat, on circulating ghrelin levels in obese animal models.

5.1.5 The antioxidant (glutathione) values in male mice

In contrast to our findings, where glutathione levels were significantly ($P<0.05$) elevated in obese mice and significantly ($P<0.05$) decreased following orlistat treatment (Table 4.7), previous studies have demonstrated reduced glutathione levels in obese models and a subsequent increase after orlistat administration (Morakinyo *et al*., 2020; Othman *et al*., 2021). The decline in glutathione reported in obese state is thought to result from rapid consumption and depletion of its stores to counteract excessive free radical production (Noeman *et al*., 2011).

A comprehensive search yielded no studies evaluating the effects of IONPs alone or in conjunction with orlistat on glutathione levels in obese animal models.

5.2 According to the periods

5.2.1 Body weight in male mice

A significant (P<0.05) increase in body weight was observed during the first and third weeks of obesity induction compared with the control group, and during the first and third weeks of orlistat treatment, a non-significant (P>0.05) decrease in body weight was observed compared with the obese group (Table 4.8). This is consistent with a study conducted by Othman and colleagues (2021) on male rats fed a HFD for 6 weeks, and another study conducted by Selima and colleagues (2025) on male rats given orlistat for 9 weeks. The increase in body weight during the obesity induction period is attributed to excess fat stored in adipose tissue (Warwick *et al*., 2002). The decrease in body weight with orlistat treatment is due to the treatment's action of preventing fat absorption in the intestine (Heck *et al*., 2012).

Treatment with IONPs demonstrated a non-significant (P>0.05) decrease in body weight during the first week compared with the obesity group, followed by a significant (P<0.05) decrease in the third week compared with both the obesity group and the orlistat-treated group (Table 4.8). These findings in the third week are consistent with the study by Alsenousy and colleagues (2022), which reported that the effect of IONPs was superior to that of orlistat. The reduction in body weight observed with IONPs is primarily associated with their capacity to suppress angiogenesis in WAT and promote the browning of WAT into metabolically active BAT (Li *et al*., 2019).

Treatment with the combination of IONPs and orlistat resulted in a non-significant (P>0.05) decrease in the first week compared with the obesity group and the IONPs-treated group, followed by a significant (P<0.05) decrease in the third week compared with the obesity group and non-significant (P>0.05) decrease compared with the IONPs group (Table 4.8). The third-week findings were consistent with those of Alsenousy and colleagues (2022) and Refaat and colleagues (2024), who reported that the combination of IONPs and orlistat exhibited a superior effect compared to either IONPs or orlistat alone.

5.2.2 The Body Mass Index (BMI) and Lee Index values in male mice

In the first and third weeks of obesity induction, a significant (P<0.05) increase in BMI values was observed compared with the control group, indicating successful obesity induction. Orlistat treatment for one week led to an improvement in BMI values (Table 4.9). This is consistent with a study conducted by Suleiman and colleagues (2020) on male rats fed a HFD for 12 weeks, and a study conducted by Selima and colleagues (2025) on male rats fed a HFD for 9 weeks. The above studies were consistent with our results, as the non-significant (P>0.05) decrease in BMI values was observed with Orlistat treatment compared with the obese group, but contradicted the lack of effect in BMI values after orlistat treatment for three weeks, this may be due to the mice being affected by the experimental conditions. The increase in BMI values is attributed to increased body weight (Warwick *et al*., 2002), and the improvement in BMI values with Orlistat treatment is due to the treatment's ability to control fat absorption from the diet (Heck *et al*., 2012).

A non-significant (P>0.05) decrease in BMI values was observed with treatment with IONPs for one week compared with obese group (Table 4.16). This is due to the ability of nanoparticles to stimulate WAT browning (Li *et al*., 2019). The greatest improvement in BMI values was observed with treatment with a combination of IONPs and orlistat (Table 4.9). Perhaps due to the increased efficacy of orlistat when combined with IONPs. Our results are consistent with those of a study conducted by Refaat and colleagues (2024) on male rats, differing only in that three weeks of treatment with IONPs did not affect BMI values.

The Lee index is used to measure obesity in rats only, as studies have shown (Bastias Perez *et al*., 2020; Quiros Cognuck *et al*., 2020; Zakaria et al., 2021). We used it to measure obesity in mice, and it gave good results (Table 4.9).

5.2.3 Biochemical parameters in male mice

5.2.3.1 The lipid profile values

In the first week of obesity induction, a significant (P<0.05) increase in TG, TC, LDL, and VLDL levels, along with a significant (P<0.05) decrease in HDL, was observed. In the third week, a significant (P<0.05) increase TG and LDL levels, a non-significant (P>0.05) increase in TC levels, and a significant (P<0.05) decrease in HDL levels were observed (Table 4.11), this result are consistent with a study conducted by El-Shehawi and colleagues (2021) on rats fed a HFD for 14 weeks to induce obesity. This finding is also supported by a study conducted by Wasim and colleagues (2024) on male rats fed a HFD for 28 days to induce obesity. Our results differed from the above studies only in showing decreased VLDL levels in the obese group compared with the control group in the third week. Generally, such lipid abnormalities in obesity are

attributed to increased FFA flux to the liver, which enhances hepatic VLDL production and impairs TRL clearance, leading to elevated TG, TC, LDL, VLDL, and reduced HDL (Klop *et al*.,2013).

In the first week of treatment with orlistat, a significant ($P<0.05$) decrease in TG, a non-significant ($P>0.05$) decrease in VLDL, and a non-significant ($P> 0.05$) increase in HDL values were observed compared with the obese group. The treatment did not affect TC and LDL values. After three weeks of orlistat treatment, no significant effect was observed on lipid levels, except for a non-significant ($P>0.05$) increase in HDL compared with the obese group (Table 4.11). The observed improvements in TG, HDL and VLDL levels may be attributed to orlistats mechanism of action, which involves reducing fat absorption in the digestive system (Mahmoud and Elnour, 2013). These findings contradict studies by (Javed *et al*., 2023; Wasim *et al* .,2024), which reported improvements in all lipid profile components following orlistat treatment. These discrepancies may be attributed to the short duration of treatment with orlistat.

IONPs treatment caused a non-significant ($P>0.05$) decrease in TG and VLDL during the first week, followed by a significant ($P<0.05$) decrease in TG and a non-significant ($P>0.05$) decrease in VLDL during the third week compared with the obese group (Table 4.11). No notable changes were observed in other lipid profile components. The potential mechanism involves the ability of nanoparticles to promote WAT browning and reduce lipid accumulation in adipocytes, thereby improving dyslipidemia (Li *et al*., 2019). Previous studies (Alsenousy *et al*., 2022; Refaat *et al*., 2024) reported that both IONPs alone and their combination with orlistat significantly improved lipid profiles, which contradicts the findings of the current study. These discrepancies may be

explained by differences in treatment duration, IONP and orlistat dosages, or the routes of administration.

5.2.3.2 The liver enzyme activity

In the first week of obesity induction, a non-significant (P>0.05) decrease in ALT values, a significant (P<0.05) decrease in ALP, and a non-significant (P>0.05) increase in AST were observed. In the third week, a significant (P<0.05) decrease in ALT and ALP values and a non-significant (P>0.05) increase in AST were observed compared with the control group (Table 4.12). The increase in AST is consistent with a study conducted by Abbas and colleagues (2022) on male rats fed a HFD for 8 weeks to induce obesity, and another study conducted by Baz and colleagues (2022) on male rats fed a HFD for 12 weeks to induce obesity. However, the decrease in ALT and ALP values contradicted the studies mentioned above. The elevation of AST may be attributed to muscle stress or systemic inflammation rather than hepatic inflammation (Ndrepepa,2021).

In the first week of treatment with orlistat, a significant (P<0.05) increase in ALT and AST values and a non-significant (P>0.05) increase in ALP were observed. In the third week, a non-significant (P>0.05) increase in ALT, a significant (P<0.05) increase in ALP, and a non-significant decrease in AST were observed compared with the obese group (Table 4.12). These results contradicted a study conducted by Thelappilly and colleagues (2024) on male rats given orlistat for 15 weeks, and another study conducted by Zakaria and colleagues (2021) on male rats given orlistat for 6 weeks. The increase in liver enzymes with orlistat treatment may be attributed to the toxic effect of the drug (Aparicio *et al*., 2024).

Our results differ from those of the study by Al-Senosy and colleagues (2022), which showed an improvement in liver enzyme levels following the use of IONPs, and this improvement was more pronounced when combined with orlistat..

5.2.4 The Hormonal Parameters in mal mice

5.2.4.1 The glucose and insulin values

In the first and third weeks of obesity induction, a significant ($P<0.05$) increase in glucose values was observed, and in the third, a non-significant ($P>0.05$) increase in insulin values was observed in obese male mice compared with the control group (Tables 4.10 and 4.13). This result was consistent with study conducted by El-Shehawi and colleagues (2021) on rats fed a HFD for 14 weeks, and another study conducted by Abbas and colleagues (2022) on male rats fed a HFD for 8 weeks. Elevated glucose and insulin levels are attributed to the development of insulin resistance caused by obesity (Boden *et al*., 2011). Our study differed from the aforementioned studies only in reporting a non-significant ($P>0.05$) decrease in insulin levels during the first week of obesity induction.

A non-significant ($P>0.05$) decrease in insulin and glucose levels was observed after one and three weeks of orlistat treatment (Table 4.10 and 4.13), This finding is consistent with a study conducted by Zakaria and colleagues (2021) on male rats given orlistat for 12 weeks and another study conducted by Xu and colleagues (2023) on mice given orlistat for four weeks. These results are attributed to orlistat's effect on improving insulin resistance (Ke *et al*., 2020).

Treatment with IONPs for one week resulted in a non-significant ($P>0.05$) decrease in glucose levels compared with the obese group and the orlistat treated group. In the third week of treatment with IONPs, a significant ($P<0.05$) decrease in glucose values and a non-significant ($P>0.05$) decrease in insulin values were observed compared with the obese group and the group treated with orlistat (Table 4.10 and 4.13). Our findings were consistent with those of (Refaat *et al*., 2024) in terms of the reduction in blood glucose and insulin levels observed with IONPs treatment compared to the obese group. However, their results contradicted ours when comparing IONPs with orlistat treatment, as their study reported a greater effect of orlistat than IONPs after 4 weeks of administration in male rats. The positive effect of IONPs is due to their ability to enhance the formation of brown adipocytes (Li *et al*., 2019).

A non-significant ($P>0.05$) decrease in glucose levels was observed after the first week of treatment with the combination of IONPs and orlistat compared with the obesity group, orlistat alone, and IONPs alone. However, a significant ($P<0.05$) decrease was recorded after three weeks of combination treatment compared with the obesity group, and orlistat alone. These results are consistent with the findings of Refaat and colleagues (2024) and Al-Senousy and colleagues (2022) on male rats, which showed that the combination was more effective than either treatment alone. However, our findings differed from the aforementioned studies in that insulin levels were not affected by treatment with the combination of IONPs and Orlistat.

5.2.4.2 The leptin values

In the first week of obesity induction, a significant (P<0.05) increase in leptin values was observed, and in the third week, non-significant (P>0.05) increase was observed compared with the control group (Table 4.13). This finding is consistent with a study conducted by Song and colleagues (2020) on male rats fed a HFD for 12 weeks and another study conducted by Chularojmontri and colleagues (2022) on male rats fed a HFD for 16 weeks. This result is attributed to elevated plasma insulin levels (Lustig *et al*., 2004) and the development of leptin resistance (Banks *et al*., 1999).

A study conducted by Caroline and colleagues (2021) on rats given orlistat for 5 weeks and another study conducted by Yilmaz and colleagues (2024) on male rats given orlistat for 12 weeks, showed an improvement in leptin levels. This finding is consistent with our treatment results during the one-week treatment period; However, it contrasted with the three-week treatment period, where orlistat showed no effect (Table 4.13). This discrepancy may be due to the experimental conditions, or the method of treatment administration.

During the first week of treatment with IONPs, a significant (P<0.05) decrease in leptin levels was observed compared with the obese group and the orlistat-treated group. During the third week of treatment with IONPs, non-significant (P>0.05) decrease in leptin levels was observed compared with the obese group and a significant (P<0.05) decrease was observed compared with the orlistat-treated group (Table 4.13). This finding is consistent with the results of a study conducted by Al-Senousy and colleagues (2022) on male rats given IONPs for eight weeks. IONPs lower leptin secretion by promoting white fat browning, which improves insulin sensitivity and metabolic profile (Gao *et al*., 2015; Wu *et al*., 2017)

Our results showed a significant (P<0.05) decrease in leptin levels following one week of treatment with the combination of IONPs and orlistat compared with the obesity group and the group treated with orlistat alone. However, leptin levels were not affected after three weeks of treatment with the same combination. This finding contrasts with the results of Refaat and colleagues (2024) and Al-Senousy and colleagues (2022), who reported that treatment with the combination of IONPs and orlistat was more effective than either IONPs or orlistat alone.

5.2.4.3 The ghrelin values

The results demonstrated a significant (P<0.05) increase in ghrelin levels during the first week of obesity induction, whereas a non-significant (P>0.05) increase was observed in the third week compared with the control group. Moreover, treatment with orlistat resulted in a non-significant (P>0.05) decrease in ghrelin levels after one and three weeks of therapy (Table 4.13). This finding contrasts with studies conducted in mice that reported decreased ghrelin levels during obesity and increased levels following orlistat treatment (Mashmoul *et al*., 2017; Caroline *et al*., 2021). In obesity, ghrelin decreases due to energy surplus, while weight loss through diet or orlistat triggers a compensatory rise to restore energy balance. (Pamuk *et al*.,2018)

A systematic review of the literature did not identify any published studies investigating the effects of IONPs, either alone or in combination with orlistat, on circulating ghrelin levels in obese animal models.

5.2.5 The Antioxidant (glutathione) values in male mice

The results revealed a non-significant (P>0.05) increase in glutathione levels during the first week of obesity induction, followed by a significant (P<0.05) increase in the third week compared with the control group. In contrast, a non-significant (P>0.05) decrease in glutathione levels was observed after one week of orlistat treatment, followed by a significant (P<0.05) decrease in the third week compared with the obesity group (Table 4.14). Our results contradicted studies conducted on rats which have shown a decrease in glutathione levels in obese rats and an increase following orlistat treatment (Morakinyo *et al*., 2020; Othman *et al*., 2021). The decreased glutathione levels in obese rats are attributed to the rapid consumption and depletion of glutathione stores in combating free radicals resulting from the development of obesity (Noeman *et al*., 2011).

A systematic review of the literature did not identify any published studies investigating the effects of IONPs, either alone or in combination with orlistat, on circulating glutathione levels in obese animal models.

5.2.6 Effect of PEG Functionalization on Magnetite Nanoparticle Toxicity

Using an MTT viability assay, we examined how uncoated and polyethylene-glycol-(PEG)-functionalised magnetite (Fe_3O_4) nanoparticles affect COS-7 cells. As nanoparticle concentration rose, cell survival declined in a dose-responsive fashion an observation that echoes earlier reports attributing high-dose nanomaterial toxicity to oxidative stress and membrane injury. Applying a PEG shell markedly lessened this toxicity across the full concentration range. At 10 mg mL^{-1}, PEGylated particles preserved roughly 15 % more viable cells than their uncoated counterparts. The improvement is likely due to PEG's hydrophilic, biocompatible character, which dampens direct particle-cell contact, curbs protein-corona formation, and limits aggregation, thereby lowering the chance of oxidative or mechanical damage. Similar protective effects of polymer coatings are well documented in the nanotoxicology literature. Enhanced compatibility points to PEG-coated iron-oxide nanoparticles as promising candidates for biomedical uses such as targeted drug delivery or imaging. Nevertheless, *in vitro* data alone cannot capture pharmacokinetics or long-term safety; comprehensive *in vivo* investigations on distribution, clearance, and chronic effects remain essential. Overall, these results reinforce the value of surface engineering, particularly PEGylation, for reducing the cytotoxic footprint of iron-oxide nanoparticles and improving their integration with biological systems.

Chapter Six

Conclusions and Recommendations

6.1. Conclusions

The results of the current study included the following conclusions:

1-The process of inducing obesity in mice may have led to disorders in the measured parameters through differnt period in expermint

2-The therapeutic intervention used in the study resulted in a clear improvement in glucose and insulin disturbances, and its positive effect was most evident during the three-week of treatment period.

3-The use of iron oxide nanoparticles (IONPs) alone or in combination with orlistat reduced triglyceride (TG) levels throughout all periods, and the effectiveness of orlistat was most evident during the first week of treatment. while orlistat alone or in combination with IONPs had the best effect in improving HDL cholesterol levels throughout all periods.

4-The treatments strategies used in the study might have had negative effects on hepatic function, reflected in increased liver enzyme levels through experment periods

5- Iron Oxid nanoparticles (IONPs) were the most effective in decreasing leptin concentrations through experiment periods

6-Except for the three-week combination therapy of IONPs and Orlistat, the therapeutic interventions applied in the study seemed to lower in both the ghrelin and glutathione levels.

6.2. Recommendation

The current study recommended the following:

1. Conducting the study on other animals, such as rats.

2. Prolong the treatment period after induced obesity with orlistat or iron oxide nanoparticles (IONPs).

3. Stoping high-fat feeding after inducing obesity in animals and replace it with normal feeding during the treatment period.

4. Studying other parameters in obese animals, such as adiponectin, insulin resistance, Liptin resistance, lipase and Lipoprotien Lipase (LPL).

5. Conducting histological studies on organs (liver, heart, and kidneys) to demonstrate the effects of obesity and treatment on these organs.

6. Using other anti-obesity medications or other nanomaterials for treatment or using slimming herbs and comparing them with anti-obesity medications.

References

References

- Abbas, M. A; Boby, N; Lee, E; Hong, J and Park, S. (2022). Anti-Obesity Effects of Ecklonia cava Extract in High-Fat Diet-Induced Obese Rats. *Antioxidants*, 11(2), 310.
- Abdul-Ghani, M. and DeFronzo, R. A. (2023). Fasting hyperinsulinemia and obesity: Cause or effect. *The Journal of Clinical Endocrinology & Metabolism*, 108(10): 1151-1152.
- Aberg, F; Kantojärvi, K; Männistö, V; But, A; Salomaa, V; Niiranen, T; Farkkila, M; Luukkonen, P; Manbisto, S; Lundqyist, A; Perola, M. and Jula, A. (2022). Association between arterial hypertension and liver outcomes using polygenic risk scores: a population-based study. *Scientific Reports*, 12(1): 15581.
- Agjei, R. O; Balogun, O. S; Olaleye, S. A; Adoma, P. O; Afari-Baidoo, M. and Adusei-Mensah, F. (2025). The impact of ultra-processed foods on obesity risk: A comprehensive bibliometric analysis. Clinical Nutrition Open Science, 61, 26-43.
- Ahmed, B; Sultana, R. and Greene, M. W. (2021). Adipose tissue and insulin resistance in obese. *Biomedicine & Pharmacotherapy*, 137: 111315.
- Aleksandrova, K; Egea Rodrigues, C; Floegel, A. and Ahrens, W. (2020). Omics biomarkers in obesity: novel etiological insights and targets for precision prevention. *Current obesity reports*, 9: 219-230.
- Almeanazel, O; Alanazi, F; Alsarra, I; Alshora, D; Shakeel, F; Almnaizel, A; Alahmed, M. and Fouad, E. (2020). Nanotechnology as a tool to overcome the bariatric surgery malabsorption. *Saudi Pharmaceutical Journal*, 28(5): 565-573.

- Al-senousy, A. H. A; El-tahan, R. A; Ghazal, N. A; Pinol, R; Millan, A; Ali, L. M. A. and Kamel, M. A. (2022). The Anti-Obesity Potential of Superparamagnetic Iron Oxide Nanoparticles against High-Fat Diet-Induced Obesity in Rats: Possible Involvement of Mitochondrial Biogenesis in the Adipose Tissues. *Pharmaceutics*,14: 2134.
- Aoyama, K. (2021). Glutathione in the Brain. *International journal of molecular science*. 22(9): 5010.
- Aparicio, J. L; Salinas, P. M; Arias-Chavez, D. J; Campos-Perez, E; Calixto-Tlacomulco, S; Cruz-Rangel, A; Reyes-Grajeda, J. P. and Bravo, G. (2024). Transcriptomic Analysis of the Protective Effect of Piperine on Orlistat Hepatotoxicity in Obese Male Wistar Rats. *Jornal OF Biochemical and Molecular Toxicology*, 38(11): 70040.
- Arias, L. S; Pessan, J. P; Vieira, A. P. M; Lima, T. M. T. D; Delbem, A. C. B. and Monteiro, D. R. (2018). Iron oxide nanoparticles for biomedical applications: a perspective on synthesis, drugs, antimicrobial activity, and toxicity. *Antibiotics*, 7(2): 46.
- Banack, H. R; Bea, J. W; Chen, Z; Blew, R. M; Nicholas, S; Stefanick, M; Wild, R. A; Manson, J. E. and Odegaard, A. O. (2023). Longitudinal patterns of abdominal visceral and subcutaneous adipose tissue, total body composition, and anthropometric measures in postmenopausal women: results from the Women's Health Initiative. *International Journal of Obesity*, 47(4): 288-296.
- Banks, W. A; DiPalma, C. R. and Farrell, C. L. (1999). Impaired transport of leptin across the blood-brain barrier in obesity. *Peptides*, 20(11): 1341-1345.

- Bao, Y; Sherwood, J. A. and Sun, Z. (2018). Magnetic iron oxide nanoparticles as T 1 contrast agents for magnetic resonance imaging. *Journal of Materials Chemistry C*, 6(6): 1280-1290.
- Barchetta, I; Cimini, F. A; Ciccarelli, G; Baroni, M. G; and Cavallo, M. G. (2019). Sick fat: the good and the bad of old and new circulating markers of adipose tissue inflammation. *Journal of endocrinological investigation*, 42(11): 1257-1272.
- Bastias-Perez, M; Serra, D. and Herrero, L. (2020). Dietary options for rodents in the study of obesity. *Nutrients*, 12(11), 3234.
- Basu, R; Singh, R. J; Basu, A; Chittilapilly, E. G; Johnson, M. C; Toffolo, G; Cobelli,C. and Rizza, R. A. (2005). Obesity and type 2 diabetes do not alter splanchnic cortisol production in humans. *The Journal of Clinical Endocrinology & Metabolism*, 90(7): 3919-3926.
- Bays, H. E; Toth, P. P; Kris-Etherton, P. M; Abate, N; Aronne, L. J; Brown, W. V; Gonzalez-Campoy, J. M; Jones, S. R; Kumar, R; La Forge, R. and Samuel, V. T. (2013). Obesity, adiposity, and dyslipidemia: a consensus statement from the National Lipid Association. *Journal of clinical lipidology*, 7(4): 304-383.
- Baz, L; Algarni, S; Al-thepyani, M; Aldairi, A; and Gashlan, H. (2022). Lycopene Improves Metabolic Disorders and Liver Injury Induced by a Hight-Fat Diet in Obese Rats. *Molecules*, 27(22), 7736.
- Bianchi, V. E; and Locatelli, V. (2018). Testosterone a key factor in gender related metabolic syndrome. *Obesity Reviews*, 19(4): 557-575.
- Bjorbaek, C. and Kahn, B. B. (2004). Leptin signaling in the central nervous system and the periphery. *Recent progress in hormone research*, 59: 305-332.

- Bjornson, E; Adiels, M; Taskinen, M. R. and Borén, J. (2017). Kinetics of plasma triglycerides in abdominal obesity. *Current opinion in lipidology*, 28(1): 11-18.
- Bluher, M. (2020). Metabolically healthy obesity. *Endocrine reviews*, 41(3), 004.
- Bobo, D; Robinson, K. J; Islam, J; Thurecht, K. J. and Corrie, S. R. (2016). Nanoparticle-based medicines: a review of FDA-approved materials and clinical trials to date. *Pharmaceutical research*, 33: 2373-2387.
- Boden, G. (2011). Obesity, insulin resistance and free fatty acids. Current Opinion in Endocrinology, Diabetes and Obesity, 18(2), 139-143.
- Burtis, C. A; Ashwood, E. R. and Bruns, D. E. (2012). Tietz textbook of clinical chemistry and molecular diagnostics (5th ed.). St. Louis, MO: Elsevier Saunders.
- Canfora, E. E; Meex, R. C; Venema, K. and Blaak, E. E. (2019). Gut microbial metabolites in obesity, NAFLD and T2DM. *Nature Reviews Endocrinology*, 15(5): 261-273.
- Caroline, O. B; Ebuehi, O. A. T; Cecilia, O. A. and Kayode, O. A. (2021). Effect of Allium sativum extract in combination -with orlistat on insulin resistance and disrupted metabolic hormones in high fat diet induced obese rats. *Scientific African*, 14: 00994.
- Censin, J. C; Peters, S. A; Bovijn, J; Ferreira, T; Pulit, S. L; Mägi, R; Mahajan, A; Holmes, M. V. and Lindgren, C. M. (2019). Causal relationships between obesity and the leading causes of death in women and men. *PLoS genetics*, 15(10): 1008405.
- Chacon, D; Bernardino, T; Geraghty, F; Rodriguez, A. C; Fiani, B; Chadhaury, A; Pierre-Louis, M. and Rodriguez, A. I. C. (2022). Bariatric

surgery with Roux-en-Y gastric bypass or sleeve gastrectomy for treatment of obesity and comorbidities: current evidence and practice. *Cureus*, 14(6): 25762.

- Chandrasekaran, P. and Weiskirchen, R. (2024). The role of obesity in type 2 diabetes mellitus—An overview. *International journal of molecular sciences*, 25(3), 1882.
- Chen, Y. Y., Tsai, M. G., Chi, M. C., Wang, T. F. and Lin, L. L. (2013). Covalent immobilization of Bacillus licheniformis γ-glutamyl transpeptidase on aldehyde-functionalized magnetic nanoparticles. *International Journal of Molecular Sciences*, 14(3), 4613-4628.
- Chularojmontri, L; Nanna, U; Tingpej, P; Hansakul, P; Jansom, C; Wattanapitayakul, S and Naowaboot, J. (2022). Raphanus sativus L. var. caudatus Extract Alleviates Impairment of Lipid and Glucose Homeostasis in Liver of High-Fat Diet-Induced Obesity and Insulin Resistance in Mice. *Preventive Nutrition and Food Science (PNF)*, 27(4):399–406.
- Cinti, S. (2007). The adipose organ. *Adipose tissue and adipokines in health and disease*, 3-19.
- Cinti, S. (2018). Pink adipocytes. *Trends in Endocrinology & Metabolism*, 29(9): 651-666.
- Colak, E. and Pap, D. (2021). The role of oxidative stress in the development of obesity and obesity-related metabolic disorders. Journal of Medical Biochemistry, 40(1), 1.
- Commandeur, J. N; Stijntjes, G. J. and Vermeulen, N. P. (1995). Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Role in bioactivation and detoxication mechanisms of xenobiotics. *Pharmacological reviews*, 47(2): 271-330.

- Couto, D; Freitas, M; Costa, V. M; Chisté, R. C; Almeida, A; Lopez-Quintela, M. A; Rivas, J; Freitas, P; Silva, P; Carvalho, F. and Fernandes, E. (2016). Biodistribution of polyacrylic acid-coated iron oxide nanoparticles is associated with proinflammatory activation and liver toxicity. *Journal of Applied Toxicology*, 36(10): 1321-1331.
- Dallman, M. F; Pecoraro, N; Akana, S. F; La Fleur, S. E; Gomez, F; Houshyar, H; Bell, M. E; Bhatnagar, S; Laugero, K. D. and Manalo, S. (2003). Chronic stress and obesity: a new view of "comfort food". *Proceedings of the National Academy of Sciences*, 100(20): 11696-11701.
- Dallman, M. F. (2010). Stress-induced obesity and the emotional nervous system. *Trends in Endocrinology & Metabolism*, 21(3): 159-165.
- Dandona, P; Ghanim, H; Chaudhuri, A; Dhindsa, S. and Kim, S. S. (2010). Macronutrient intake induces oxidative and inflammatory stress: potential relevance to atherosclerosis and insulin resistance. *Experimental & molecular medicine*, 42(4): 245-253.
- Dhilip Kumar, S. S. and Abrahamse, H. (2022). Recent advances in the development of biocompatible nanocarriers and their cancer cell targeting efficiency in photodynamic therapy. Frontiers in chemistry, 10, 969809.
- Di Lorenzo, M; Cacciapuoti, N; Lonardo, M. S; Nasti, G; Gautiero, C; Belfiore, A; Guida, B. and Chiurazzi, M. (2023). Pathophysiology and nutritional approaches in polycystic ovary syndrome (PCOS): a comprehensive review. *Current nutrition reports*, 12(3), 527-544.
- Doaei, S; Mosavi Jarrahi, S. A; Sanjari Moghadam, A; Akbari, M. E; Javadi Kooshesh, S; Badeli, M; Azizi Tabesh, G. H; Abbas Torki, S; Gholamalizadeh, M; Zhu, Z. H; Montazeri, F. and Mirzaei Dahka, S.

(2019). The effect of rs9930506 FTO gene polymorphism on obesity risk: a meta-analysis. *Biomolecular concepts*, 10(1): 237-242.

- Dragano, N. R., Ferno, J; Diéguez, C; Lopez, M. and Milbank, E. (2020). Reprint of: recent updates on obesity treatments: available drugs and future directions. *Neuroscience*, 447: 191-215.
- Drew, B. S; Dixon, A. F. and Dixon, J. B. (2007). Obesity management: update on orlistat. *Vascular health and risk management*, 3(6), 817-821.
- Du, B; Yu, M. and Zheng, J. (2018). Transport and interactions of nanoparticles in the kidneys. *Nature Reviews Materials*, 3(10): 358-374.
- Dubern, B. and Clement, K. (2012). Leptin and leptin receptor-related monogenic obesity. *Biochimie*, 94(10): 2111-2115.
- Duckworth, W. C; Bennett, R. G. and Hamel, F. G. (1998). Insulin degradation: progress and potential. *Endocrine reviews*, 19(5): 608-624.
- Dulinska-Litewka, J; Lazarczyk, A; Halubiec, P; Szafranski, O; Karnas, K. and Karewicz, A. (2019). Superparamagnetic iron oxide nanoparticles current and prospective medical applications. *Materials*, 12(4), 617.
- Duntas, L. H. and Biondi, B. (2013). The interconnections between obesity, thyroid function, and autoimmunity: the multifold role of leptin. *Thyroid*, 23(6): 646-653.
- Eivazzadeh-Keihan, R., Bahreinizad, H., Amiri, Z., Aliabadi, H. A. M., Salimi-Bani, M., Nakisa, A., Davoodi, F; Tahmasebi, B; Ahmadpour, F; Radinekiyan, F; Maleki, A; Hamblin, M. R; Mahdavi, M. and Madanchi, H. (2021). Functionalized magnetic nanoparticles for the separation and purification of proteins and peptides. *TrAC Trends in Analytical Chemistry*, 141: 116291.

- El-Shehawi, A. M; Alkafafy, M; El-Shazly, S; Sayed, S; Farouk, S; Alotaibi, S; Madkour, D. A; Khalifa, H. K; and Ahmed, M. M. (2021). Moringa oleifera leaves ethanolic extract ameliorates high fat diet-induced obesity in rats. *Jornal of king saud unevercity- scince*, 33(6): 101552.
- Estelrich, J; Escribano, E; Queralt, J. and Busquets, M. A. (2015). Iron oxide nanoparticles for magnetically-guided and magnetically-responsive drug delivery. *International journal of molecular sciences*, 16(4): 8070-8101.
- Fahed, M; Abou Jaoudeh, M. G; Merhi, S; Mosleh, J. M. B; Ghadieh, R; Al Hayek, S. and El Hayek Fares, J. E. (2020). Evaluation of risk factors for insulin resistance: a cross sectional study among employees at a private university in Lebanon. *BMC endocrine disorders*, 20: 1-14.
- Farahmand, M; Tehrani, F. R; Rahmati, M. and Azizi, F. (2021). Anthropometric indices and age at natural menopause: a 15-year follow-up population-based study. *International Journal of Endocrinology and Metabolism*, 19(4): 109285.
- Fasipe, O. J. (2018). Recent advances and current trend in the pharmacotherapy of obesity. *Archives of Medicine and Health Sciences*, 6(1): 99-116.
- Feng, Q; Liu, Y; Huang, J; Chen, K., Huang, J. and Xiao, K. (2018). Uptake, distribution, clearance, and toxicity of iron oxide nanoparticles with different sizes and coatings. *Scientific reports*, 8(1): 2082.
- Fernandez, C. J; Chacko, E. C. and Pappachan, J. M. (2019). Male obesity-related secondary hypogonadism–pathophysiology, clinical implications and management. *European endocrinology*, 15(2), 83.

- Fitch, A., Fox, C., Bauerly, K., Gross, A., Heim, C., and Judge-Dietz, J. (2013). Institute for Clinical Systems Improvement. Prevention and Management of Obesity for Children and Adolescents.
- Fjalldal, S; Follin, C; Gabery, S; Sundgren, P. C; Bjorkman-Burtscher, I. M; Latt, J; Nordstrom, C. H; Rylander, L; Ekman, B; Cheong, R; Palsson, A; Petersen, A. and Erfurth, E. M. (2019). Detailed assessment of hypothalamic damage in craniopharyngioma patients with obesity. *International Journal of Obesity*, 43(3): 533-544.
- Flehmig, G; Scholz, M; Kloting, N; Fasshauer, M; Tonjes, A; Stumvoll, M; Yong, B. S. and Blüher, M. (2014). Identification of adipokine clusters related to parameters of fat mass, insulin sensitivity and inflammation. *PloS one*, 9(6): 99785.
- Fossati, P. and Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical chemistry, 28(10): 2077-2080.
- Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry, 18(6): 499-502.
- Gao, Y; Li, Z; Gabrielsen, J. S; Simcox, J. A; Lee, S. H; Jones, D; Cooksey, B; Stoddard, G; Cefalu, W. T. and McClain, D. A. (2015). Adipocyte iron regulates leptin and food intake. The Journal of Clinical Investigation, 125(9), 3681-3691.
- Gar, C; Rottenkolber, M; Haenelt, M; Potzel, A. L; Kern-Matschilles, S; Then, C; Seissler,J; Bidlingmaier,M. and Lechner, A. (2020). Altered metabolic and hormonal responses to moderate exercise in overweight/obesity. *Metabolism*, 107: 154219.

- Garcia-Eguren, G; Giró, O; del Mar Romero, M; Grasa, M. and Hanzu, F. A. (2019). Chronic hypercortisolism causes more persistent visceral adiposity than HFD-induced obesity. Journal of Endocrinology, 242(2), 65-77.
- Gastaldelli, A; Abdul Ghani, M. and DeFronzo, R. A. (2021). Adaptation of insulin clearance to metabolic demand is a key determinant of glucose tolerance. *Diabetes*, 70(2): 377-385.
- Ge, X; Yang, H; Bednarek, M. A; Galon-Tilleman, H; Chen, P; Chen, M; Lichtman, J. S; Wang, Y; Dalmas, O;Yin, Y; Tian, H; Jermutus, L; Grimsby, J. H; Rondinone, C. N; Konkar, A; Kaplan, D. D. and Kaplan, D. D. (2018). LEAP2 is an endogenous antagonist of the ghrelin receptor. *Cell metabolism*, 27(2): 461-469.
- Gella, FJ, Olivella T, Cruz Pastor M, Arenas J, Moreno R, Durban R, Gomez JA.(1985) . A simple procedure for the routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. Clin Chim Acta, 153(3): 241-247 .
- Genchi, V. A; D'Oria, R; Palma, G; Caccioppoli, C; Cignarelli, A; Natalicchio, A; Laviola, L; Giorgino, F. and Perrini, S. (2021). Impaired leptin signalling in obesity: is leptin a new thermolipokine?. *International journal of molecular sciences*, 22(12), 6445.
- Gentile, C. L. and Weir, T. L. (2018). The gut microbiota at the intersection of diet and human health. *Science*, 362(6416): 776-780.
- Giordano, A; Frontini, A. and Cinti, S. (2016). Convertible visceral fat as a therapeutic target to curb obesity. *Nature Reviews Drug Discovery*, 15(6): 405-424.

- Glastras, S. J; Chen, H; Pollock, C. A. and Saad, S. (2018). Maternal obesity increases the risk of metabolic disease and impacts renal health in offspring. *Bioscience reports*, 38(2): 20180050.
- Gobbo, O. L. (2024). Tackling obesity from a nanomedicine perspective. *Review of Clinical Pharmacology and Pharmacokinetics*,38, 15-17.
- Goens, D; Virzi, N. E; Jung, S. E; Rutledge, T. R. and Zarrinpar, A. (2023). Obesity, chronic stress, and stress reduction. *Gastroenterology Clinics of North America*, 52(2), 347-362.
- Gomes, A. C; Hoffmann, C. and Mota, J. F. (2018). The human gut microbiota: Metabolism and perspective in obesity. *Gut microbes*, 9(4): 308-325.
- Gruzdeva, O; Borodkina, D; Uchasova, E; Dyleva, Y. and Barbarash, O. (2019). Leptin resistance: underlying mechanisms and diagnosis. Diabetes, metabolic syndrome and obesity: *targets and therapy*, 12: 191-198.
- Guerreiro, V. A; Carvalho, D. and Freitas, P. (2022). Obesity, adipose tissue, and inflammation answered in questions. *Journal of obesity*, 2022(1), 2252516.
- Gupta, D; Ogden, S. B; Shankar, K; Varshney, S. and Zigman, J. M. (2021). A LEAP 2 conclusions? Targeting the ghrelin system to treat obesity and diabetes. *Molecular Metabolism*, 46, 101128.
- Hardy, O. T; Perugini, R. A; Nicoloro, S. M; Gallagher-Dorval, K; Puri, V; Straubhaar, J. and Czech, M. P. (2011). Body mass index-independent inflammation in omental adipose tissue associated with insulin resistance in morbid obesity. *Surgery for Obesity and Related Diseases*, 7(1): 60-67.
- Heck, A. M; Yanovski, J. A. and Calis, K. A. (2000). Orlistat, a new lipase inhibitor for the management of obesity. Pharmacotherapy: *The Journal of Human Pharmacology and Drug Therapy*, 20(3): 270-279.

- Herhaus, B; Ullmann, E; Chrousos, G. and Petrowski, K. (2020). High/low cortisol reactivity and food intake in people with obesity and healthy weight. *Translational psychiatry*, 10(1): 40.
- Hewagalamulage, S. D; Lee, T. K; Clarke, I. J. and Henry, B. A. (2016). Stress, cortisol, and obesity: a role for cortisol responsiveness in identifying individuals prone to obesity. *Domestic animal endocrinology*, 56: 112-120.
- Hinney, A; Körner, A; and Fischer-Posovszky, P. (2022). The promise of new anti-obesity therapies arising from knowledge of genetic obesity traits. *Nature Reviews Endocrinology*, 18(10): 623-637.
- Hjelholt, A. J; Søndergaard, E; Pedersen, S. B; Møller, N; Jessen, N. and Jørgensen, J. O. L. (2020). Growth hormone upregulates ANGPTL4 mRNA and suppresses lipoprotein lipase via fatty acids: Randomized experiments in human individuals. *Metabolism*, 105: 154188.
- Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature*, 444(7121), 860-867.
- Inaishi, J. and Saisho, Y. (2020). Beta-cell mass in obesity and type 2 diabetes, and its relation to pancreas fat: a mini-review. *Nutrients*, 12(12), 3846.
- Iriadam M. Musa D. Gumushan H and Baba, F. (2006). Effects of two Turkish medicinal plants Artemisia herba alba and Teucrium polium on blood glucose levels and other biochemical parameters in rabbits. *Cellular and Molecular Biology*, 5: 19-24
- Izquierdo, A. G; Crujeiras, A. B; Casanueva, F. F and Carreira, M. C. (2019). Leptin, Obesity, and Leptin Resistance: Where Are We 25 Years Later. *Nutrients*, 11(11):2704.

- Jagot, S. A. and Webb, G. P. (1980). Fat distribution in genetically-obese (OB-OB) mice. *Irsc medecal science-biochemestry*, 8(9): 633-634.
- Jalili, V; Poorahmadi, Z; Hasanpour Ardekanizadeh, N; Gholamalizadeh, M; Ajami, M; Houshiarrad, A; Hajipour, A; Shafie, F; Alizadeh, A; Mokhtari, Z; Shafaei, H. and Doaei, S. (2022). The association between obesity with serum levels of liver enzymes, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transferase in adult women. *Endocrinology, diabetes & metabolism*, 5(6), 367.
- Javed, M; Ahmed, W; Khan, A. and Rabbani, I. (2023). Comparison of Efficacy of Fermented Garlic and Orlistat (Lipase Inhibitor) in Obesity Management Using an Experimental Rodent Model. *Foods*, 12(21), 3905.
- Jawad, A.H. (1996). Ethological studies in assessing the anti-aggressive effect of some Iraqi medicinal plant in laboratory mice. Ph. D. thesis. College of education, Basrah University
- Jensen, M. D., Ryan, D. H., Apovian, C. M., and Yanovski, S. Z. (2014). 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation*, 129(25_suppl_2), S102-S138.
- Jin, X; Qiu, T; Li, L; Yu, R; Chen, X; Li, C; Proud, C.G. and Jiang, T. (2023). Pathophysiology of obesity and its associated diseases. *Acta Pharmaceutica Sinica* B, 13(6), 2403-2424.
- Jonklaas, J; Bianco, A. C; Bauer, A. J; Burman, K. D; Cappola, A. R; Celi, F. S; Cooper,D.S; Kim,B.W; Peeters,R.P; Rosenthal,M.S. and Sawka, A. M. (2014). Guidelines for the treatment of hypothyroidism: prepared by the

american thyroid association task force on thyroid hormone replacement. *thyroid*, 24(12): 1670-1751.

- Kappel, S; Hawkins, P.and Mendl, M. Y. (2017). To Group or Not to Group? Good Practice for Housing Male Laboratory Mice. *Animals*, 7(12): 88.
- Kapoor, D; Goodwin, E; Channer, K. S. and Jones, T. H. (2006). Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. *European journal of endocrinology*, 154(6): 899-906.
- Katsnelson, B. A; Degtyareva, T. D; Minigalieva, I. I; Privalova, L. I; Kuzmin, S. V; Yeremenko, O. S; Kireyeva, E. P; Sutunkova, M. P; Valamina, I. I; Khodos, M. Y; Kozitsina, A. N; Shur, V. Y; Vazhenin, V. A; Potapov, A. P. and Morozova, M. V. (2011). Subchronic systemic toxicity and bioaccumulation of Fe3O4 nano-and microparticles following repeated intraperitoneal administration to rats. *International Journal of Toxicology*, 30(1): 59-68.
- Kawai, T; Autieri, M. V. and Scalia, R. (2021). Adipose tissue inflammation and metabolic dysfunction in obesity. *American Journal of Physiology-Cell Physiology*, 320(3), 375-391.
- Ke, J; An, Y; Cao, B; Lang, J; Wu, N. and Zhao, D. (2020). Orlistat-Induced Gut Microbiota Modification in Obese Mice. *Evidence-Based Complementary and Alternative Medicine*, 2020(1): 9818349.
- Kennett, G. A. and Clifton, P. G. (2010). New approaches to the pharmacological treatment of obesity: can they break through the efficacy barrier?. *Pharmacology Biochemistry and Behavior*, 97(1): 63-83.
- Kenneth, R. and Feingold, M.D.(2023).Obesity and Dyslipidemia.*Endotext*

- Kelly, D. M. and Jones, T. H. (2015). Testosterone and obesity. *Obesity Reviews*, 16(7): 581-606.
- Kinshella, M. L. W; Moore, S. E. and Elango, R. (2021). The missing focus on women's health in the First 1,000 days approach to nutrition. *Public Health Nutrition*, 24(6): 1526-1530.
- Klop, B; Elte, J. W. F. and Castro Cabezas, M. (2013). Dyslipidemia in obesity: mechanisms and potential targets. *Nutrients*, 5(4), 1218-1240.
- Kobi, J. B. B. S; Matias, A. M; Gasparini, P. V. F; Torezani-Sales, S; Madureira, A. R; da Silva, D. S; Correa, C. R; Garcia, J. L; Haese, D; Nogueira, B. V; de Assis, A. L. E. M; Lima-Leopoldo, A. P; Correa, C. R; Garcia, J. L; Haese, D; Nogueira, B. V; and Leopoldo, A. S. (2023). High-fat, high-sucrose, and combined high-fat/high-sucrose diets effects in oxidative stress and inflammation in male rats under presence or absence of obesity. *Physiological Reports*, 11(7): 15635.
- Kohane, D. S. (2007). Microparticles and nanoparticles for drug delivery. *Biotechnology and bioengineering*, 96(2): 203-209.
- Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, 402(6762): 656-660.
- Koutouratsas, T; Kalli, T; Karamanolis, G. and Gazouli, M. (2019). Contribution of ghrelin to functional gastrointestinal disorders' pathogenesis. *World journal of gastroenterology*, 25(5): 539.
- Kumar, S. and Kelly, A. S. (2017, February). Review of childhood obesity: from epidemiology, etiology, and comorbidities to clinical assessment and treatment. *In Mayo Clinic Proceedings*, 92 (2): 251-265.

- Kurylowicz, A. and Puzianowska-Kuznicka, M. (2020). Induction of adipose tissue browning as a strategy to combat obesity. *International journal of molecular sciences*, 21(17): 6241.
- Lam, B. C. C; Lim, A. Y. L; Chan, S. L; Yum, M. P.S; Koh, N. S. Y. and Finkelstein, E. A. (2023). The impact of obesity: a narrative review. *Singapore Med Jornal*, 64(3):163–171.
- Lauby-Secretan, B; Scoccianti, C; Loomis, D; Grosse, Y; Bianchini, F. and Straif, K. (2016). Body fatness and cancer—viewpoint of the IARC Working Group. *New England journal of medicine*, 375(8): 794-798.
- Li, J.J; Cha, R.T; Luo, H.Z; Hao, W.S; Zhang, Y; Jiang, X.Y. (2019). Nanomaterials for the theranostics of obesity. *Biomaterials*, 223: 119474–119488.
- Li, Y; Ma, J; Yao, K; Su, W; Tan, B; Wu, X; Huang, X; Li ,T; Yin,Y; Tosini ,G. and Yin, J. (2020). Circadian rhythms and obesity: Timekeeping governs lipid metabolism. *Journal of pineal research*, 69(3): 12682.
- Li, J; Duan, H; Liu, Y; Wang, L. and Zhou, X. (2022). Biomaterial-based therapeutic strategies for obesity and its comorbidities. Pharmaceutics, 14(7), 1445.
- Lin, X. and Li, H. (2021). Obesity: epidemiology, pathophysiology, and therapeutics. *Frontiers in endocrinology*, 12: 706978.
- Ling, C. and Ronn, T. (2019). Epigenetics in human obesity and type 2 diabetes. *Cell metabolism*, 29(5): 1028-1044.
- Litzenburger, T; Huber, E. K; Dinger, K; Wilke, R; Vohlen, C; Selle, J; Kadah, M; Persigehl, T; Heneweer, C; Dötsch, J. and Alejandre Alcazar, M. A. (2020). Maternal high-fat diet induces long-term obesity with sex-dependent metabolic programming of adipocyte differentiation.

hypertrophy and dysfunction in the offspring. Clinical Science, 134(7): 921-939.

- Liu, T. T; Liu, X. T; Chen, Q. X. and Shi, Y. (2020). Lipase inhibitors for obesity: A review. *Biomedicine and Pharmacotherapy*, 128, 110314.
- Liu, B. N; Liu, X. T; Liang, Z. H. and Wang, J. H. (2021). Gut microbiota in obesity. *World journal of gastroenterology*, 27(25): 3837.
- Liu, J; Li, J; Xia, C; He, W; Li, X; Shen, S; Zhou, X; Tong, N. and Peng, L. (2024). The effect of hyperlipidemia and body fat distribution on subclinical left ventricular function in obesity: a cardiovascular magnetic resonance study. *Cardiovascular Diabetology*, 23(1), 120.
- Loftus, J; Camacho-Hubner, C; Hey-Hadavi, J. and Goodrich, K. (2019). Targeted literature review of the humanistic and economic burden of adult growth hormone deficiency. *Current Medical Research and Opinion*, 35(6): 963-973.
- Lopomo, A. N. G. E. L. A; Burgio, E. and Migliore, L. U. C. I. A. (2016). Epigenetics of obesity. *Progress in molecular biology and translational science*, 140: 151-184.
- Lu, X. M; Zhao, H. and Wang, E. H. (2013). A high-fat diet induces obesity and impairs bone acquisition in young male mice. *Molecular medicine reports*, 7(4), 1203-1208.
- Lustig, R. H; Sen, S; Soberman, J. E. and Velasquez-Mieyer, P. A. (2004). Obesity, leptin resistance, and the effects of insulin reduction. *International Journal of obesity*, 28(10): 1344-1348.
- Lv, Y; Liang, T; Wang, G. and Li, Z. (2018). Ghrelin, a gastrointestinal hormone, regulates energy balance and lipid metabolism. *Bioscience reports*, 38(5): 20181061.

- Maffei, M. and Giordano, A. (2022). Leptin, the brain and energy homeostasis: From an apparently simple to a highly complex neuronal system. *Reviews in Endocrine and Metabolic Disorders*, 23(1): 87-101.
- Mahmoud, R. H. and Elnour, W. A. (2013). Comparative evaluation of the efficacy of ginger and orlistat on obesity management, pancreatic lipase and liver peroxisomal catalase enzyme in male albino rats. *European Review for Medical & Pharmacological Sciences*, 17(1): 75-83.
- Makris, M. C; Alexandrou, A; Papatsoutsos, E. G; Malietzis, G; Tsilimigras, D. I; Guerron, A. D. and Moris, D. (2017). Ghrelin and obesity: identifying gaps and dispelling myths. A reappraisal. *in vivo*, 31(6): 1047-1050.
- Mancuso, P. (2016). The role of adipokines in chronic inflammation. *ImmunoTargets and therapy*, 5: 47-56.
- Mani, B. K; Puzziferri, N; He, Z; Rodriguez, J. A; Osborne-Lawrence, S; Metzger, N. P; Chhina, N; Gaylinn, B; Thorner, M. O; Thomas, E. L; Bell, J. D; Williams, K. W; Goldstone, A. P. and Zigman, J. M. (2019). LEAP2 changes with body mass and food intake in humans and mice. *The Journal of clinical investigation*, 129(9): 3909-3923.
- Margetic, S; Gazzola, C; Pegg, G. G. and Hill, R. A. (2002). Leptin: a review of its peripheral actions and interactions. *International journal of obesity*, 26(11): 1407-1433.
- Marlatt, K. L; Pitynski-Miller, D. R; Gavin, K. M; Moreau, K. L.; Melanson, E. L. Santoro, N. and Kohrt, W. M. (2022). Body composition and cardiometabolic health across the menopause transition. *Obesity*, 30(1): 14-27.

- Martinez, J. A; Milagro, F. I; Claycombe, K. J. and Schalinske, K. L. (2014). Epigenetics in adipose tissue, obesity, weight loss, and diabetes. *Advances in nutrition*, 5(1): 71-81.
- Martínez-Sánchez, N. (2020). There and back again: leptin actions in white adipose tissue. *International journal of molecular sciences*, 21(17), 6039.
- Martyn, J. J; Kaneki, M. and Yasuhara, S. (2008). Obesity-induced insulin resistance and hyperglycemia: etiological factors and molecular mechanisms. *Anesthesiology*, 109(1): 137.
- Mashmoul, M; Azlan, A; Mohtarrudin, N; Nisak, P; Yusof, M and Khaza'ai, H. (2017). Saffron Extract and Crocin Reduced Biomarkers Associated with Obesity in Rats Fed a High-Fat Diet. *Mal J Nutr*, 23(1): 117 – 127.
- Mehran, L; Amouzegar, A; Bakhtiyari, M; Mansournia, M. A; Rahimabad, P. K; Tohidi, M. and Azizi, F. (2017). Variations in serum free thyroxine concentration within the reference range predicts the incidence of metabolic syndrome in non-obese adults: a cohort study. *Thyroid*, 27(7): 886-893.
- Michalaki, M. A; Vagenakis, A. G; Leonardou, A. S; Argentou, M. N; Habeos, I. G; Makri, M. G; Psyrogiannis, Psyrogiannis,A. I; Kalfarentzos, F.E. and Kyriazopoulou, V. E. (2006). Thyroid function in humans with morbid obesity. *Thyroid*, 16(1), 73-78.
- Mihalache, L; Arhire, L. I; Giuşcă, S. E; Gherasim, A; Niţă, O; Constantinescu, D; Constantinescu, R. N; Padureanu, S. and Danciu, M. (2019). Ghrelin-producing cells distribution in the stomach and the relation with Helicobacter pylori in obese patients. *Rom Journal Morphol Embryol*, 60(1): 219-225.

- Molina-Vega, M; Muñoz-Garach, A; Damas-Fuentes, M; Fernández-García, J. C. and Tinahones, F. J. (2018). Secondary male hypogonadism: A prevalent but overlooked comorbidity of obesity. *Asian journal of andrology*, 20(6), 531-538.
- Morakinyo A.E; Fasakin W.O; Fajobi A.O; Akinpelu B.A; and Oyedapo O.O. (2020). Effects of aframomum melegueta leafextract on the antioxidant status of obese rats. *African Journal of Biology and Medical Research*, 3(3): 22-31.
- Mu, X; Li, J; Yan, S; Zhang, H; Zhang, W; Zhang, F. and Jiang, J. (2018). siRNA delivery with stem cell membrane-coated magnetic nanoparticles for imaging-guided photothermal therapy and gene therapy. *ACS biomaterials science & engineering*, 4(11): 3895-3905.
- Nakou, E. S; Filippatos, T. D; Kiortsis, D. N; Derdemezis, C. S; Tselepis, A. D; Mikhailidis, D. P. and Elisaf, M. S. (2008). The effects of ezetimibe and orlistat, alone or in combination, on high-density lipoprotein (HDL) subclasses and HDL-associated enzyme activities in overweight and obese patients with hyperlipidaemia. *Expert Opinion on Pharmacotherapy*, 9(18): 3151-3158.
- Nauck, M., Warnick, G. R. and Rifai, N. (2002). Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. Clinical chemistry, 48(2): 236-254.
- Ndrepepa, G. (2021). Aspartate aminotransferase and cardiovascular disease—a narrative review. *Journal of Laboratory and Precision Medicine*, *6*.

- Nimptsch, K; Konigorski, S. and Pischon, T. (2019). Diagnosis of obesity and use of obesity biomarkers in science and clinical medicine. *Metabolism*, 92: 61-70.
- Noeman, S. A; Hamooda, H. E. and Baalash, A. A. (2011). Biochemical Study of Oxidative Stress Markers in the Liver, Kidney and Heart of High Fat Diet Induced Obesity in Rats. *Diabetology & Metabolic Syndrome*, 3(17).
- Nowak-Jary, J; Defort, A. and Kozioł, J. J. (2020). Modified physicochemical properties of acidic model drugs immobilized on Fe 3 O 4 magnetic iron oxide nanoparticles. *Pharmaceutical Chemistry Journal*, 53: 1025-1035.
- Nowak-Jary, J; Machnicka, B. and Kozioł, J. J. (2021). Cytotoxicity of chlorambucil immobilized on magnetic iron oxide nanoparticles Fe3O4. *Micro & Nano Letters*, 16(10): 515-523.
- Olateju, I. V; Ogwu, D; Owolabi, M. O; Azode, U; Osula, F; Okeke, R; and Akabalu, I. (2021). Role of behavioral interventions in the management of obesity. *Cureus*, 13(9): 18080.
- Othman, Z. A; Zakaria, Z; Suleiman, J. B; Ghazali, W. S. W. and Mohamed, M. (2021). Anti-Atherogenic Effects of Orlistat on Obesity-Induced Vascular Oxidative Stress Rat Model. *Antioxidants*, 10(2): 251.
- Ozcelik, O; Dogan, H. and Kelestimur, H. (2004). Effects of a weight-reduction program with orlistat on serum leptin levels in obese women: a 12-week, randomized, placebo-controlled study. Current therapeutic research, 65(2), 127-137.
- Palzer, J; Eckstein, L; Slabu, I; Reisen, O; Neumann, U. P. and Roeth, A. A. (2021). Iron oxide nanoparticle-based hyperthermia as a treatment

option in various gastrointestinal malignancies. *Nanomaterials*, 11(11): 3013.

- Pamuk, B; Yilmaz, H; Kebapçilar, L; Kirbiyik, H; Alacacioğlu, A; Bozkaya, G; Pamuk, G. and Demirpence, M. (2018). The effect of orlistat and weight loss diet on plasma ghrelin and obestatin. Journal of Research in Medical Sciences, 23(1), 95.
- Parente, E. B; Mutter, S; Harjutsalo, V; Ahola, A. J; Forsblom, C; and Groop, P. H. (2020). Waist-height ratio and waist are the best estimators of visceral fat in type 1 diabetes. *Scientific reports*, 10(1): 18575.
- Peng, X. and Chen, Y. (2023). The emerging role of circadian rhythms in the development and function of thermogenic fat. *Frontiers in Endocrinology*, 14, 1175845.
- Poulain-Godefroy, O; Lecoeur, C; Pattou, F; Fruhbeck, G. and Froguel, P. (2008). Inflammation is associated with a decrease of lipogenic factors in omental fat in women. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 295(1): R1-R7.
- Powles, L., Wilson, K. L., Xiang, S. D., Coppel, R. L., Ma, C., Selomulya, C. and Plebanski, M. (2020). Pullulan-coated iron oxide nanoparticles for blood-stage malaria vaccine delivery. *Vaccines*, 8(4), 651.
- Qiu, Y; Liu, Q; Luo, Y; Chen, J; Zheng, Q; Xie, Y. and Cao, Y. (2024). Causal association between obesity and hypothyroidism: a two-sample bidirectional Mendelian randomization study. *Frontiers in endocrinology*, 14, 1287463.
- Quiros Cognuck, S; Reis, W. L; Silva, M; Debarba, L. K; Mecawi, A. S; de Paula, F. J; Franci, C. R; Elias, L. L. K. and Antunes-Rodrigues, J. (2020). Sex differences in body composition, metabolism-related hormones, and

energy homeostasis during aging in Wistar rats. *Physiological Reports*, 8(20), e14597.

- Rahman, M. S; Hossain, K. S; Das, S; Kundu, S; Adegoke, E. O; Rahman, M. A; Abdul Hannan, M; Uddin, M.J. and Pang, M. G. (2021). Role of insulin in health and disease: an update. *International journal of molecular sciences*, 22(12): 6403.
- Ramamoorthy, S. and Cidlowski, J. A. (2016). Corticosteroids-mechanisms of action in health and disease. *Rheumatic diseases clinics of North America*, 42(1): 15.
- Rangel-Huerta, O. D; Pastor-Villaescusa, B. and Gil, A. (2019). Are we close to defining a metabolomic signature of human obesity? A systematic review of metabolomics studies. *Metabolomics*, 15: 1-31.
- Refaat, H; Dowidar, M. F; Ahmed, A. I; Khamis, T; Abdelhaleem, S. E; and Abdo, S. A. (2024). The corrective role of superparamagnetic iron oxide nanoparticles for the genes controlling hypothalamus-pituitary-testis-axis in male obesity-associated secondary hypogonadism. *Open veterinary jornal*,14(1):428–437.
- Regan, J. A. and Shah, S. H. (2020). Obesity genomics and metabolomics: a nexus of cardiometabolic risk. *Current cardiology reports*, 22: 1-19.
- Rodriguez, A. C. I; Epel, E. S; White, M. L; Standen, E. C; Seckl, J. R., and Tomiyama, A. J. (2015). Hypothalamic-pituitary-adrenal axis dysregulation and cortisol activity in obesity: a systematic review. *Psychoneuroendocrinology*, 62: 301-318.
- Rosalki, S. B., Foo A.Y.and Burlina, A. (1993). Multicenter evaluation of iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. Clin chem, (39): 648- 652.

- Rosenbaum, M; Knight, R. and Leibel, R. L. (2015). The gut microbiota in human energy homeostasis and obesity. *Trends in Endocrinology & Metabolism*, 26(9): 493-501.
- Rotondi, M; Leporati, P; La Manna, A; Pirali, B; Mondello, T; Fonte, R; Magri, F. and Chiovato, L. (2009). Raised serum TSH levels in patients with morbid obesity: is it enough to diagnose subclinical hypothyroidism?. *European journal of endocrinology*, 160(3): 403-408.
- Rucker, D; Padwal, R; Li, S. K; Curioni, C. and Lau, D. C. (2007). Long term pharmacotherapy for obesity and overweight: updated meta-analysis. *Bmj*, 335(7631), 1194-1199.
- Ruggiero, M. R; Crich, S. G; Sieni, E; Sgarbossa, P; Forzan, M; Cavallari, E; Stefania, R; Dughiero, F. and Aime, S. (2016). Magnetic hyperthermia efficiency and 1H-NMR relaxation properties of iron oxide/paclitaxel-loaded PLGA nanoparticles. *Nanotechnology*, 27(28): 285104.
- Russell, E; Dunne, V; Russell, B; Mohamud, H; Ghita, M; McMahon, S. J; Butterworth, K. T; Schettino, G; McGarry, C. K. and Prise, K. M. (2021). Impact of superparamagnetic iron oxide nanoparticles on in vitro and in vivo radiosensitisation of cancer cells. *Radiation Oncology*, 16(1), 104.
- Saltiel, A. R. and Olefsky, J. M. (2017). Inflammatory mechanisms linking obesity and metabolic disease. *The Journal of clinical investigation*, 127(1): 1-4.
- Savulescu-Fiedler, I; Mihalcea, R; Dragosloveanu, S; Scheau, C; Baz, R. O; Caruntu, A; Ssheau, A; Caruntu, C. and Benea, S. N. (2024). The interplay between obesity and inflammation. *Life*, 14(7), 856.
- Sbraccia P; Finer N. Obesity: Pathogenesis, Diagnosis, and Treatment. Springer International Publishing; Berlin/Heidelberg, Germany: 2019.

- Seki, Y., Morimoto, S., Bokuda, K., Watanabe, D., Yamashita, K., Takano, N., Amano, K; Kawamata, T; Ichihara,A. and Ichihara, A. (2023). Effect of GH deficiency caused by nonfunctioning pituitary masses on Serum C-reactive protein levels. *Journal of the Endocrine Society*, 7(12), bvad137.
- Selima, R. M; Saleem, I. A; Shawki, M. M; Darwish, A. A; Yehia, M. A. and Mohamed, E. I. (2025). Effect of gold nanoparticles treatment on rats-induced obesity by evaluating body-composition directly and indirectly via bioelectric impedance analysis. *Scientific Reports*,
- Seth, A; Orkin, S; Yodoshi, T; Liu, C; Fei, L., Hardy, J; Trout, A. T; Clachar, A. A; Bramlage, K; Xanthaakos, S. and Mouzaki, M. (2020). Severe obesity is associated with liver disease severity in pediatric non-alcoholic fatty liver disease. *Pediatric Obesity*, 15(2): 12581.
- Sharifi, S; Daghighi, S; Motazacker, M. M; Badlou, B; Sanjabi, B; Akbarkhanzadeh, A; Rowshani, A.T; Laurent, S; Peppelenbosch, M. P. and Rezaee, F. (2013). Superparamagnetic iron oxide nanoparticles alter expression of obesity and T2D-associated risk genes in human adipocytes. *Scientific Reports*, 3(1): 2173.
- Shirai, K; Fujita, T; Tanaka, M; Fujii, Y; Shimomasuda, M; Sakai, S. and Samukawa, Y. (2019). Efficacy and safety of lipase inhibitor orlistat in Japanese with excessive visceral fat accumulation: 24-week, double-blind, randomized, placebo-controlled study. *Advances in therapy*, 36: 86-100.
- Sibuyi, N. R. S; Moabelo, K. L; Meyer, M; Onani, M. O; Dube, A. and Madiehe, A. M. (2019). Nanotechnology advances towards development of targeted-treatment for obesity. *Journal of nanobiotechnology*, 17: 1-21.
- Skotland, T; Iversen, T. G. and Sandvig, K. (2010). New metal-based nanoparticles for intravenous use: requirements for clinical success with

focus on medical imaging. Nanomedicine: Nanotechnology, *Biology and Medicine*, 6(6): 730-737.

- Song, J; Kim, J; Park, H. J. and Kim, H. (2020). Anti-Obesity Effects of a Prunus persica and Nelumbo nucifera Mixture in Mice Fed a High-Fat Diet. *Nutrients*, 12(11), 3392.
- Sovetkina, A; Nadir, R; Fung, J. N. M; Nadjarpour, A. and Beddoe, B. (2020). The physiological role of ghrelin in the regulation of energy and glucose homeostasis. *Cureus*, 12(5): 7941.
- Srivastava, G. and Apovian, C. M. (2018). Current pharmacotherapy for obesity. *Nature Reviews Endocrinology*, 14(1): 12-24.
- Stanislawski, M. A; Dabelea, D; Lange, L. A; Wagner, B. D. and Lozupone, C. A. (2019). Gut microbiota phenotypes of obesity. *NPJ biofilms and microbiomes*, 5(1): 18.
- Steel , R.G.D., Torrie, J.H. and Dicky, D.A. (1997) . Principles and Procedures of Statistics , A Biometrical Approach. 3rd Edition, McGraw Hill, Inc. Book Co., New York, 352-358.
- Subramaniyan, V. and Hanim, Y. U. (2025). Role of pancreatic lipase inhibition in obesity treatment: Mechanisms and challenges towards current insights and future directions. *International Journal of Obesity*, 1-15.
- Suleiman, J. B; Nna, V. U; Othman, Z. A; Zakaria, Z; Bakar, A. B. A. and Mohamed, M. (2020). Orlistat attenuates obesity-induced decline in steroidogenesis and spermatogenesis by up-regulating steroidogenic genes. *Andrology*, 8(5), 1471-1485.
- Tahir, N. T; ALfatlawi, W. R. and Jedda, W. A. A. (2023). Ghrelin and obestatin levels as a novel marker in iraqi obese children. *Baghdad Science Journal*, 20(5): 1654-1654.

- Tan, H. C; Hsu, J. W; Tai, E. S; Chacko, S; Kovalik, J. P. and Jahoor, F. (2024). The impact of obesity-associated glycine deficiency on the elimination of endogenous and exogenous metabolites via the glycine conjugation pathway. *Frontiers in Endocrinology*, 15: 1343738.
- Tanenbaum, L. and Tuffanelli, D. L. (1980). Antimalarial agents: chloroquine, hydroxychloroquine, and quinacrine. *Archives of dermatology*, 116(5): 587-591.
- Taylor, P. N; Richmond, R; Davies, N; Sayers, A; Stevenson, K; Woltersdorf, W; Taylor, A; Groom, A; Northstone, K; Ring, S; Okosieme, O; Rees, A; Nitsch, D; Williams, G. R Smith, G. D; Gregory, J. W; Timpson, N. J; Tobias, J. H. and Dayan, C. M. (2016). Paradoxical relationship between body mass index and thyroid hormone levels: a study using Mendelian randomization. *The Journal of Clinical Endocrinology & Metabolism*, 101(2): 730-738..
- Teskey, G; Abrahem, R; Cao, R ; Gyurjian, K; Islamoglu, H; Lucero, M; Martinez, A; Paredes, E; Salaiz, O; Robinson, B. and Venketaraman, V. (2018). Glutathione as a Marker for Human Disease. *Advances in Clinical Chemistry*. 87: 141-159.
- Thaker, V. V. (2017). Genetic and epigenetic causes of obesity. Adolescent medicine: *state of the art reviews*, 28(2): 379.
- Thelappilly, B. B; Murali, R; Suriyaprakash, T; Venkatachalam, V; Anbiah, S. V; Srinivasan, N. and Suhail, P. (2024). Interrogating the Antiobesity Efficacy of Flacourtia inermis Roxb. Fruits in High Fat Diet-induced Obese Rats. *Sage Jornals*, 16(1).

- Thovhogi, N; Sibuyi, N; Meyer, M; Onani, M. and Madiehe, A. (2015). Targeted delivery using peptide-functionalised gold nanoparticles to white adipose tissues of obese rats. *Journal of Nanoparticle Research*, 17: 1-8.
- Torres, L; Miranda, M. C. G; Martins, V. D; Caixeta, F; de Almeida Oliveira, M; Trindade, L. M; de Assis, H. C; Nascimento, V; Rosa, N. P; Gomes, E; Almeida, S. O; Marquet, F; Genser, L; Marcelin, G; Clément, K; Russo, M; Caetano Faria, A. M. and Maioli, T. U. (2023). Obesity-induced hyperglycemia impairs oral tolerance induction and aggravates food allergy. *Mucosal Immunology*, 16(4): 513-526.
- Tsou, Y. H; Wang, B; Ho, W; Hu, B; Tang, P; Sweet, S; Zhang, X. and Xu, X. (2019). Nanotechnology-mediated drug delivery for the treatment of obesity and its related comorbidities. *Advanced Healthcare Materials*, 8(12): 1801184.
- Uskoković, V; Tang, S. and Wu, V. M. (2019). Targeted magnetic separation of biomolecules and cells using earthicle-based ferrofluids. *Nanoscale*, 11(23): 11236-11253.
- Uzun, H; Konukoglu, D; Gelisgen, R; Zengin, K. and Taskin, M. (2007). Plasma protein carbonyl and thiol stress before and after laparoscopic gastric banding in morbidly obese patients. *Obesity Surgery*, 17: 1367-1373.
- van den Berg, E. H; van Tienhoven-Wind, L. J; Amini, M; Schreuder, T. C; Faber, K. N; Blokzijl, H. and Dullaart, R. P. (2017). Higher free triiodothyronine is associated with non-alcoholic fatty liver disease in euthyroid subjects: the Lifelines Cohort Study. *Metabolism*, 67: 62-71.
- van den Berg, R., Kooijman, S., Noordam, R., Ramkisoensing, A., Abreu-Vieira, G., Tambyrajah, L. L., Dijk, W; Ruppert, P; Mol, I. M; Kramar, B;

Caputo, R; Puig, L. S; de Ruiter, E. M; Kroon, J; Hoekstra, M; van der Sluis, R. J; Meijer, O.C; van Dijk, K. W; van Kerkhof, L. W.M; Christodoulides, C; Karpe, F; Gerhart-Hines, Z; Kersten, S; Meijer, G. H; Coomans, C. P; Heemst, D.V; Biermasz, N. R. and Rensen, P. C. (2018). A diurnal rhythm in brown adipose tissue causes rapid clearance and combustion of plasma lipids at wakening. *Cell reports*, 22(13): 3521-3533.

- Van der Valk, E. S; Savas, M. and van Rossum, E. F. (2018). Stress and obesity: are there more susceptible individuals?. *Current obesity reports*, 7(2): 193-203.
- van Vliet, S; Koh, H. C. E; Patterson, B. W; Yoshino, M; LaForest, R; Gropler, R. J; Klein, S. and Mittendorfer, B. (2020). Obesity is associated with increased basal and postprandial β-cell insulin secretion even in the absence of insulin resistance. *Diabetes*, 69(10): 2112-2119.
- Vangijzegem, T; Lecomte, V; Ternad, I; Van Leuven, L; Muller, R. N; Stanicki, D; and Laurent, S. (2023). Superparamagnetic iron oxide nanoparticles (SPION): From fundamentals to state-of-the-art innovative applications for cancer therapy. *Pharmaceutics*, 15(1): 236.
- Vekic, J; Zeljkovic, A; Stefanovic, A; Jelic-Ivanovic, Z. and Spasojevic-Kalimanovska, V. (2019). Obesity and dyslipidemia. *Metabolism*, 92: 71-81.
- Vitarius, J. A; Sehayek, E. and Breslow, J. L. (2006). Identification of quantitative trait loci affecting body composition in a mouse intercross. *Proceedings of the National Academy of Sciences*, 103(52): 19860-19865.
- Vettor, R; Di Vincenzo, A; Maffei, P; and Rossato, M. (2020). Regulation of energy intake and mechanisms of metabolic adaptation or maladaptation

after caloric restriction. *Reviews in Endocrine and Metabolic Disorders*, 21(3): 399-409.

- Wahajuddin, N. and Arora, S. (2012). Superparamagnetic iron oxide nanoparticles: magnetic nanoplatforms as drug carriers. *International journal of nanomedicine*, 7: 3445-3471.
- Wang, J; Wu, D; Guo, H. and Li, M. (2019). Hyperandrogenemia and insulin resistance: The chief culprit of polycystic ovary syndrome. *Life sciences*, 236: 116940.
- Warnick, G. R; Nauck, M. and Rifai, N. (2001). Evolution of methods for measurement of HDL-cholesterol: from ultracentrifugation to homogeneous assays. Clinical chemistry, 47(9): 1579-1596.
- Warwick, Z. S; Synowski, S. J. and Bell, K. R. (2002). Dietary fat content affects energy intake and weight gain independent of diet caloric density in rats. *Physiology and behavior*, 77(1), 85-90.
- Wasim, R; Mahmood, T; Shamim, A; Ahsan, F. and Singh, A. (2024). Metaphorical investigation of aqueous distillate of Cichorium intybus, Foeniculum vulgare and Solanum nigrum along with atorvastatin and orlistat in experimental rodent models of dyslipidaemia and obesity. Intelligent Pharmacy, 2(1), 1-11.
- Weetman, A. P. (2021). An update on the pathogenesis of Hashimoto's thyroiditis. *Journal of endocrinological investigation*, 44(5): 883-890.
- Wharton, S; Lau, D. C; Vallis, M; Sharma, A. M; Biertho, L; Campbell-Scherer, D; Adamo, K; Alberga, A; Bell, R; Boulé, N; Boyling, E; Brown, J; Calam, B; Clarke, C; Crowshoe, L; Divalentino, D; Forhan, M; Freedhoff, Y; Gagner, M; Glazer, S; Grand, C; Green, M; Hahn, M; Hawa, R; Henderson, R; Hong, D; Hung, P; Janssen, I; Jacklin, K; Johnson-

Stoklossa, C; Kemp, A; Kirk, S; Kuk, J; Langlois, M; Lear, S; McInnes, A; Macklin, D; Naji, L; Manjoo, P; Morin, M; Nerenberg, K; Patton, I; Pedersen, S; Pereira, L; Piccinini-Vallis, H; Poddar, M; Poirier, P; Prud'homme, D; Salas, X. R; Rueda-Clausen,C; Russell-Mayhew, S; Shiau, J; Sherifali, D; Sievenpiper, J; Sockalingam, S; Taylor, V; Toth, E; Twells, L; Tytus, R; Walji, S; Walker, L. and Wicklum, S. (2020). Obesity in adults: a clinical practice guideline. *Cmaj*, 192(31): 875-891.

- Wild, S; Roglic, G; Green, A; Sicree, R. and King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030 Diabetes Care 27: 1047–1053.
- World Health Organization. (2015). Country factsheet insufficient physical activity: Bahrain. In Country factsheet insufficient physical activity: Bahrain.
- Wu, Q; Jin, R; Feng, T; Liu, L; Yang, L; Tao, Y; Ai, H. and Li, H. (2017). Iron oxide nanoparticles and induced autophagy in human monocytes. International journal of nanomedicine, 3993-4005.
- Wu, Y; Duan, H; Tian, X; Xu, C; Wang, W; Jiang, W; Pang, Z; Zhang, D. and Tan, Q. (2018). Genetics of obesity traits: a bivariate genome-wide association analysis. *Frontiers in genetics*, 9: 179.
- Wyatt, H. R. (2013). Update on treatment strategies for obesity. *The Journal of Clinical Endocrinology & Metabolism*, 98(4): 1299-1306.
- Xiao, C; Dash, S; Morgantini, C; Hegele, R. A. and Lewis, G. F. (2016). Pharmacological targeting of the atherogenic dyslipidemia complex: the next frontier in CVD prevention beyond lowering LDL cholesterol. *Diabetes*, 65(7): 1767-1778.

- Xu, G; Zhao, Z; Wysham, W. Z; Roque, D. R; Fang, Z; Sun, W; Yin, Y; Deng, B; Shen, X; Zhou, C. and Bae-Jump, V. (2023). Orlistat exerts anti-obesity and anti-tumorigenic effects in a transgenic mouse model of endometrial cancer. Frontiers in Oncology, 13, 1219923.
- Yazdi, F. T., Clee, S. M., and Meyre, D. (2015). Obesity genetics in mouse and human: back and forth, and back again. *PeerJ-life & environment*, 3: 856.
- Yilmaz, A; Toraman, M. N; Karakas, S. M; Ozden, Z; Pinarbas, E. and Mercantepe, T. (2024). Effect of White Tea on Leptin and Asprosin Levels in Rats Feeding a High-Fat Diet. Life, 14(12), 1548.
- Ylli, D., Sidhu, S., Parikh, T., & Burman, K. D. (2022). Endocrine Changes in Obesity. In K. R. Feingold (Eds.) et. al., Endotext. MDText.com, Inc.
- Young, E. R., & Jialal, I. (2023). Biochemistry, Ghrelin. In StatPearls. StatPearls Publishing.
- Yuen, K. C; Biller, B. M; Radovick, S; Carmichael, J. D; Jasim, S; Pantalone, K. M. and Hoffman, A. R. (2019). American Association of Clinical Endocrinologists and American College of Endocrinology guidelines for management of growth hormone deficiency in adults and patients transitioning from pediatric to adult care. *Endocrine Practice*, 25(11): 1191-1232.
- Zakaria, Z; Othman, Z. A; Bagi Suleiman, J; Jalil, N. A. C; Ghazali, W. S. W. and Mohamed, M. (2021). Protective and therapeutic effects of orlistat on metabolic syndrome and oxidative stress in high-fat diet-induced metabolic dysfunction-associated fatty liver disease (MAFLD) in rats: Role on Nrf2 activation. Veterinary sciences, 8(11), 274.
- Zhu, J; Zhou, Y; Jin, B. and Shu, J. (2023). Role of estrogen in the regulation of central and peripheral energy homeostasis: from a

menopausal perspective. Therapeutic Advances in Endocrinology and Metabolism, 14, 20420188231199359.

- Zhuo, C; Xu, Y; Liu, S., Li, J; Zheng, Q; Gao, X; Li, S; Jing, R; Song, X; Yue, W; Zhou, C. and Upthegrove, R. (2018). Topiramate and metformin are effective add-on treatments in controlling antipsychotic-induced weight gain: a systematic review and network meta-analysis. *Frontiers in pharmacology*, 9: 1393.

الخلاصة

أُجريت هذه الدراسة في بيت الحيوان التابع لقسم علم الأحياء، كلية العلوم، من ٢٠٢٣/١١/١٥ إلى٢٠٢٥/٥/٢٥ ، جامعة ميسان، للتحقق من تأثيرات الإعطاء الفموي لأورليستات، وجسيمات أكسيد الحديد النانوية (IONPs)، وتركيبهما معًا على مجموعة من المؤشرات الأيضية والكبدية والهرمونية ومضادات الأكسدة في الفئران البيضاء الذكور المصابة بالسمنة. تم استخدام ما مجموعه ٧٠ فأرًا، حيث شكل١٤ فأرًا مجموعة التحكم، وتم توزيع الـ ٥٦ فأرًا السمين المتبقيين عشوائيًا على أربع مجموعات تجريبية: العلاج بأورليستات، العلاج بـ IONPs، العلاج المشترك بأورليستات وIONPs، والمجموعة السمنة بدون علاج.

نتائج الدراسة وفق المجموعات:

أدى تحفيز السمنة في الفئران الذكور إلى زيادة معنوية (أ<٠،٠٥) في مستوى السكر في الدم، ملف الدهون (TG, TC, LDL)، الجريلين، الجلوتاثيون، مؤشر كتلة الجسم، والوزن النهائي، مع انخفاض معنوي في HDL، ALT، وALP

أدى العلاج بأورليستات إلى انخفاض معنوي (أ<٠،٠٥) في الجلوتاثيون، لكنه حسّن فقط بعض المؤشرات الأيضية بشكل طفيف دون فقدان وزن معنوي.

أدى العلاج بـ IONPs إلى انخفاض معنوي (أ<٠،٠٥) في السكر في الدم، TG، اللبتين، والوزن النهائي، رغم أنه تسبب في زيادة معنوية (أ<٠،٠٥) في مستويات إنزيمات الكبد.

أدى العلاج المشترك لأورليستات وIONPs إلى انخفاض معنوي (أ<٠،٠٥) في السكر في الدم، الجريلين، الجلوتاثيون، والوزن، مما يشير إلى تأثيرات تآزرية محتملة رغم الزيادات المعنوية (أ<٠،٠٥) في AST وALP.

نتائج الدراسة وفق الفترات:

الأسبوع الأول:

أدّى استحداث السمنة إلى ارتفاعات معنوية (أ<٠،٠٥) في مستوى سكر الدم، ومؤشرات الدهون (TG، TC، LDL، VLDL)، واللبتين، والغرلين، ومؤشر كتلة الجسم (BMI)، والوزن النهائي، ترافق ذلك مع انخفاضات معنوية (أ<٠،٠٥) في كل من HDL وALP مقارنةً بمجموعة السيطرة.

أظهر العلاج بالأورليستات تحسناً أساسياً من خلال انخفاض معنوي (أ<٠،٠٥) في مستوى TG، لكنه ارتبط أيضاً بارتفاعات معنوية (أ<٠،٠٥) في إنزيمات الكبد (ALT، AST)بينما سبّب العلاج بجسيمات الحديد النانوية (IONPs) انخفاضاً معنوياً (أ<٠،٠٥) في مستوى اللبتين، لكنه ترافق أيضاً بارتفاعات معنوية (أ<٠،٠٥) في إنزيمات الكبد (ALT، AST) .أما العلاج المدمج (أورليستات + جسيمات الحديد النانوية) فقد أدّى إلى انخفاضات معنوية (أ<٠،٠٥) في كل من اللبتين، والغرلين، والغلوتاثيون، لكنه ارتبط أيضاً بارتفاعات معنوية (أ<٠،٠٥) في إنزيمات الكبد (AST وALP)

الأسبوع الثالث:

أدى تحفيز السمنة إلى زيادة معنوية (أ<٠،٠٥) في السكر في الدم، TG، LDL، الجلوتاثيون، مؤشر كتلة الجسم، والوزن النهائي، مع انخفاض معنوي (P<0.05) في HDL، ALT، وALP مقارنة بمجموعة التحكم.

أدى العلاج بأورليستات إلى انخفاض معنوي (أ<٠،٠٥) في الجلوتاثيون، لكنه تسبب في زيادة معنوية (أ<٠،٠٥) في .ALP

أدى العلاج بـ IONPs إلى انخفاض معنوي (أ<٠،٠٥) في السكر في الدم، TG، والوزن النهائي، لكنه تسبب في زيادة معنوية (أ<٠،٠٥) في ALT، AST، ALP، وLDL

أظهر العلاج المشترك (أورليستات + IONPs) انخفاضات معنوية (أ<٠،٠٥) في السكر في الدم، TG، والوزن النهائي، لكنه تسبب أيضًا في زيادات معنوية (أ<٠،٠٥) في LDL، ALP، ومستوى اللبتين

أدى استحداث السمنة إلى اضطراب واضح في معايير الدراسة. أظهر العلاج بأورليستات تحسنًا في مستويات TG خلال الأسبوع الأول، في حين أدى العلاج بجسيمات النانو وحده أو بالاشتراك مع أورليستات إلى تحسين مستويات TG في الأسبوع الثالث، ومستويات اللبتين في الأسبوع الأول، ووزن الجسم في الأسبوع الثالث. تشير هذه النتائج إلى الدور الإيجابي المحتمل لجسيمات النانو في مكافحة السمنة وتعزيز المؤشرات الأيضية والهرموني

وزارة التعليم العالي والبحث العلمي

جامعة ميسان/ كلية العلوم

قسم علوم الحياة

تاثير الاورليستات واوكسيد الحديد النانوي المضادين للسمنه في بعض المعايير الهرمونيه والكيموحيويه في ذكور الفئران

رسالة مقدمة الى

مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

صفا عبد الكريم رمضان

بكالوريوس علوم /علوم الحياة (2018)

بأشراف **أ.د زينب عبد الجبار رضا العلي**

و

أ.م.د. اسوان كاظم جبر العبودي

أغسطس ٢٠٢٥ م **ربيع الاول 1447هـ**