CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

To Explore the *Insilico* Potential of *Olea Europaea* Fruit for Drug Development in PCOS

by

Ayesha Hussain

A thesis submitted in partial fulfillment for the degree of Master of Science

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Dedicated to ALLAH Almighty, Hazrat Muhammad (PBUH), my parents, my other family members, my respected teachers for their encouragement, guidance, motivation during my research work and supporting me spiritually throughout my life.

CERTIFICATE OF APPROVAL

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Abstract

In-silico structural drug designing against polycystic ovarian syndrome (PCOS) was done in order to get the best bioactive compound for further clinical trials. Polycystic ovary syndrome is associated with many metabolic disorders and is being characterized by many stalled follicles in the ovaries, anovulation and hormonal imbalance. The main causal agent of PCOS is androgen excess and a cluster of other morbidities. These morbidities may include insulin resistance, type II diabetes, obesity, cancer, metabolic disorder, cardiovascular disease and depression etc. There are many standard drugs available that are used today to treat this disease. The need of the hour is to develop drugs that have the herbal origin with least side effects. Olive fruit plants are rich source of bioactive compounds like Sterol, Phytosterol, Phenol, Polyphenol, Dialcohol, Glucosinolates, Carotenoids, Tocopherol, Alkaloids and Squalene. In order to identify natural, plant-based drug these ligands are being docked against best target protein UCP2 which is the main cause of pathogenesis of PCOS. UCP2 Protein was selected as the target proteins against ligands Sterol, Phytosterol, Phenol, Polyphenol, Dialcohol, Glucosinolates, Carotenoids, Tocopherol, Alkaloids and Squalene. Out of all, Sterol was selected as a lead bioactive compound for the current study. Molecular Docking was used to estimate the strength of a bond between a ligand and a target protein through a special scoring function and to determine the correct structure of the ligand within the target binding site. The 2D structure of the target proteins and the ligands was taken as the input for docking. The best ligands were selected on the basis of best docking score, log p value, hydrogen bond acceptor, hydrogen bond donor and molecular weight. The selection of most efficient already available PCOS drug Berberine was done on the basis of physiochemical and ADMET properties in order to compare with the bioactive ligand sterol. The comparison between Sterol and drug Berberine help us to identify the better treatment for PCOS. Comparison was being performed through patch dock and Sterol was found to be the most reliable bioactive compound that can be used for drug development against PCOS.

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Abbreviations

ADMET: Absorption Distribution Metabolism Excretion Toxicity

AMH: Anti Mullerian Hormone

AR: Androgen Receptor

BBR: Berberine

BMI: Body Mass Index

CVD: Cardio Vascular Disease

CRP: C Reactive Protein

E2: Estrogen

ER: Estrogen Receptor

FSH: Follicle-Stimulating Hormone

GnRH: Gonadotrophin Releasing Hormone

HRT: Hormone Replacement Therapy

IBS: Irritable Bowel Syndrome

IVS: Invivo Fertilization

IL: Interleukin

LH: Luteinizing Hormone

MCP: Monocyte Chemotactic Protein

NAFLD: Non Alcoholic Fatty Liver Disease

OLF : Olive Leaf Extract

PCOS: Polycystic Ovary Syndrome

PCOD: Polycystic Ovarian Disorder

T2D: Type 2 Diabetes

TNF: Tumor Necrosis Factor

TNF: Tumor Necrosis Factor

UCP: Uncoupling Protein

VD: Vitamin D

WHO: World Health Organization

Chapter 1

Introduction

One of the most prevalent hormone diseases in women of reproductive age is Polycystic Ovarian Syndrome (PCOS), which accounts for between 5 and 10 percent of all problems in this age group[[1], [2]. Other names for the condition include multicystic ovaries, schlerocystic ovaries, and Stein Leventhal syndrome. Polycystic ovarian syndrome is sometimes referred to as PCOS or PCOD (Polycystic Ovarian Disorder). Infertility, obesity, anovulation, and insulin resistance (IR) are also linked to a longer incidence of cysts. It has been established that the disorders of the metabolic syndrome and PCOS are connected. Rotterdam criterion is the most widely used diagnosis standard for PCOS [3]. The typical form of PCOS was initially described by Ashtyn and Leventhal in 1935 [4]. Ovulatory dysfunction, the clinical manifestations of polycystic ovaries and hyperandrogenism features of polycystic ovarian syndrome. Ovarian dysfunction continues to be the primary symptom, making this disease the primary contributor to ovulatory-related infertility. A woman's hormone levels may be impacted by PCOS, a polygenic and multifactorial syndromic condition. It is the most prevalent endocrine condition in women of reproductive age and is frequently accompanied by comorbidities, such as obesity, hyperinsulinemia, and infertility [5]. In accordance with other studies, 10% of PCOS patients also had type 2 diabetes (T2D), while 77% of patients with PCOS had IR [6]. While some women may only experience a few or moderate PCOS-related symptoms, others may experience many or all of these symptoms.

Thus, each woman may experience PCOS symptoms differently. Many PCOS patients exhibit unhappiness and a lower quality of life, and some of them also have breathing problems when they sleep (sleep apnea). Hormonal dysregulation, menstrual abnormalities, hair loss (alopecia), abundant terminal hair (hirsutism), and metabolic abnormalities, and an ovarian cystic appearance are all symptoms of PCOS [7]. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are produced in response to GnRH, are the two main pituitary hormones that are crucial for the reproductive system of women. In women with PCOS, gonadotrophin-releasing hormone (GnRH) causes an excess of LH and a deficiency of FSH. According to Kalro et al., women with PCOS accumulate antral follicles (2 to 8 mm in size) that can be identified early and experience premature development arrest. These women have altered LH and FSH levels compared to normal women. Women with PCOS frequently have increased levels of pituitary-derived hormones, which promote the overproduction of androgens in the ovaries. One of the most significant indicators produced by granulose cells, which play a crucial role in folliculogenesis, is anti-Mullerian hormone (AMH). It plays a significant function in the development of the ovaries in PCOS-affected women and is directly associated to a higher proportion of immature oocytes and lower fertilization rates. Women with PCOS may have abnormal early gonadotropin-independent follicle development, and genes involved in follicle development may also play a role in the etiopathogenesis of this condition.

Compared to follicle-stimulating hormones, luteinizing hormone (LH) production levels are higher in this condition (FSH). The LH surge activates the ovarian follicular cells, increasing androgen production and release. Additionally, it raises the levels of testosterone and androstenedione, two estrogen precursors. During the ovulation period, increased androgens cause the creation of a lot of follicles [8].

Chronic diseases, particularly cardiovascular disease, are more likely to affect those with polycystic ovarian syndrome (CVD). Additionally, PCOS raises the chance of dyslipidemia. Despite the fact that not all women with PCOS have IR, dyslipidemia in this condition is linked to widespread insulin resistance [9]. The abnormal

metabolism of biological macromolecules, including amino acids, carbohydrates, lipids, nucleic acids, fatty acids, glycophospholipids, sphingolipids, and hormones, is a hallmark of PCOS. Women with PCOS frequently exhibit depression and a lower quality of life. PCOS is influenced by both hereditary and environmental factors. PCOS is influenced by a number of factors, including genetics, lifestyle choices, and hormonal changes. Perhaps as a result of their sedentary, unhealthy indoor lifestyle, obese women are more likely to develop PCOS and other hormone disorders [10]. The majority of investigations on CVD risk factors have been done on women with traditional PCOS. Women with PCOS have IR that is both independent of and additive to their weight, and PCOS and obesity work together to reduce insulin sensitivity. Effects of IR and hyperandrogenism in combination with hereditary and environmental variables (diet, exercise) [11]. A key component of PCOS is Hyperandrogenism [12].

It is crucial that women exercise to meet their bodies' physiological needs. Exercise and physical activity cause various hormone levels to rise or fall in comparison to resting levels. The production of Estrogen and Steroid hormones is decreased by exercise. Increased physical activity combined with calorie restriction in lifestyle intervention studies has been shown to improve ovulatory function, circulating androgen levels, inflammatory pattern, and insulin sensitivity in PCOS-afflicted women [13].

The precise cause of PCOS is unknown. IR in women with PCOS is frequently more severe than in women with weight-matched PCOS. Regardless of weight or age, women with PCOS have a higher risk of T2DM and decreased glucose tolerance. To reduce PCOS's clinical symptoms and the risk of T2DM and cardiovascular disease (CVD), women with PCOS concentrate on weight loss through consistent exercise and diet [14]. Infertility (71.87%), Oligo Menorrhagia (79.68%), Obesity (3.70%), Oligo Menorrhagia (79.68%) & Hyperandrogenism (62.49%) are among the consequences of PCOS, although they can vary based on the region of evaluation. China had a PCOS prevalence of 2.2%, Mexico had a PCOS prevalence of 6%, and all of these women were of reproductive age. The prevalence of PCOS among Omani women who are trying to conceive is unaffected. Women of

childbearing age have PCOS, yet some nations have no evidence of it. The World Health Organization (WHO) describes PCOS as a chronic illness and a pressing public health concern. A woman may experience physical and physiological effects. Other problems brought on by obesity include a rise in estrogen levels, a fall in a high dietary fat intake, sex hormone, and a rise in opioid activity. PCOS is most frequently diagnosed in adolescent girls between the ages of 12 and 25. These individuals had Amenorrhea, Hirsutism, Acne, and Obesity [15].

It's crucial to remember that PCOS can occur in thin women as well as fat ones. Conclusion: Non-obese PCOS patients have a similar metabolic risk to obese patients because they have a similar amount of Visceral Adipose Tissue. Obese and slim women with PCOS are metabolically worse and have higher visceral adiposity compared to the control group. It is obvious that environmental factors, like eating habits, are crucial in both preventing and treating PCOS in women. Given that obesity exacerbates PCOS's clinical manifestation, international recommendations point to weight restriction as one of the primary therapeutic approaches [16].

The concentration of androgens like testosterone, androstenedione, and the precursor to androgen Dehydroepiandrosterone sulphate (DHEA -S) can be used to identify the main characteristics of PCOS, while the symptoms of Hyperandrogenism may be caused by inherited flaws in ovarian steroidogenesis, excessive steroidogenesis by the ovaries as a result of Hyperinsulinemia, and excessive LH stimulation [17]. A ketogenic diet's impact on PCOS outcomes has also been studied. The typical components of a ketogenic diet include high fat, moderate protein, and very low (40–50 g/day) CHO intake. Evidence suggests that this particular pattern can enhance anthropometric and biochemical measurements (such as LH, FSH, and SHBG) and decrease aberrant estrogen synthesis resulting from androgen aromatization in adipose tissue, which improves the LH/FSH ratio. Considering that in people with PCOS, a persistent inflammatory condition that affects the uterus and the ovaries makes anovulation and infertility worse. Animal models of PCOS have shown that the molecular and morphological abnormalities in ovarian tissue that are typical of the condition can be linked to epigenetic modifications [18]. Genes that are involved in the development of the condition are the key genetic

indicators associated with PCOS. 770 proteins in all were found, and 186 of them had differential abundance between controls and PCOS-positive women. Amphiregulin, heparin sulphate proteoglycan 2, tumor necrosis factor-alpha-induced protein 6, plasminogen, and lymphatic vessel endothelial hyaluronan receptor 1 were discovered to be unregulated in PCOS. These proteins are involved in a variety of follicular development processes. The follicular fluid of women with PCOS was revealed to have variable expression of proteins necessary for follicular growth [19]. PHYTESTROGENS are plant chemicals with biological activity similar to that of estrogen.

The estrogenic characteristics of some plants may explain their use in folklore and traditional medicine. Flavones, coumestans, and lignans are the three primary types of Phytoestrogens, and they can be found in both plants and their seeds. In the urine of nonhuman primates in 1979 and in the urine of people in 1982, Phytoestrogens were found. According to epidemiological research, eating a diet high in Phytoestrogens, like those seen in traditional Asian countries, may reduce your risk of developing so-called "Western" ailments including breast and prostate cancer and cardiovascular disease. With the outbreak of infertility in sheep grazing on pastures rich in underground clover in Western Australia in the 1940s, later known as "Clover Disease," Phytoestrogens attained biological and economic significance [20]. Phytoestrogens are non-steroidal substances that are found naturally in plants and have estrogenic-like actions in a variety of target tissues [21]. Herbal estrogens known as Phytoestrogens come from many Herbal preparations. When compared to Steroidal Estrogens, Phytoestrogens are thought to have less of an impact on the endocrine system, nevertheless, they are still capable of having both physiologically estrogenic and anti-estrogenic effects. Contrary to estrogen receptors (ER), Phytoestrogens have a higher affinity for binding to ER. According to research. However, compared to endogenous estrogens like 17-beta estradiol, phytestrogens are often less powerful. When used with clomiphene citrate, phytestrogens improve endometrial thickness, reduce the chance of miscarriage by reversing the anti-estrogenic effects of clomiphene on the endometrium and increase conception rates which play important role in the estrogen receptor.

For the purpose of ovulation induction in patients with polycystic ovarian disease (PCO), phytestrogens can be used instead of clomiphene [22]. Due to the presence of Lignans and Phenolic compounds, the olive (olea europea), a member of the oleaseae family, is regarded as a phytestrogen plant chemical. Oleuropein, which is present in olives, has antioxidant, anti-hyperlipidemia, and anti-ischemic properties. Oleuropein also contains stilbenes, phenolic acid, and flavonoids. Its laxative effects make it effective in treating digestive issues as well. The olive, which is one of the naturally occurring plants rich in Phytestrogens and is a member of the Lignans, includes Phenol chemicals. The plant can significantly lessen women's Menopausal Syndrome [23].

Numerous hazardous pests and dangerous diseases can have a negative impact on olive trees, endangering production. Green olives have a greater flavour and more chlorophyll. The value of chlorophyll's presence in virgin olive oil has increased as a result of growing evidence that it provides health benefits, including antioxidant, antimutagenic, and chemo protective characteristics. The amount of chlorophyll in virgin olive oil is determined by the amount of chlorophyll in each fruit (Olea europaea L.) [24].

Higher quality olive oil is produced when olive plants are grown at higher altitudes. The amounts of antioxidants, including phenolic and other active biomolecules, can also be impacted by altitude and other environmental conditions, such as cold damage (particularly freezing) during fruit ripening [25]. Olive tree (Olea europaea) leaves are commonly utilised in the Mediterranean region's traditional medicine. Numerous references to the therapeutic properties of the olive plant are found.

The leaf's bioactive qualities have established a basis for usage as an anti- inflammatory, anti-atherogenic, anti-hypertensive, antioxidant & treatment for hypoglycemia and low cholesterol. Similar polyphenols to those in EVOO or the fruit itself can be found in olive tree leaves, however in much higher concentrations. As a result, olive leaf extract (OLE) may have even greater nutritional potential than extra virgin olive oil (EVOO) for enhancing health outcomes in the PCOS [26].

1.1 Problem Statement

Metabolic syndrome, which encompasses type 2 diabetes and cardiovascular disease, is more likely to develop in PCOS individuals. Since many antibiotics, including metformin, clomiphene citrate, and Berberine can have negative side effects, including antibiotic gene resistance, olive fruit extract should be taken into consideration as a PCOS treatment.

1.2 Aims & Objectives

The Aim of this study is the in-silico evaluation of olive fruit as a therapeutic agent against PCOS.

1. To determine various bioactive compounds of *Olea europaea* as potential inhibitors of UCP2 protein.
2. To Scrutinize the interaction between targeted UCP2 protein and bioactive compound of *Olea europaea*.
3. To recognize the best interacting molecule that exhibits inhibitory effects against the disease.

Chapter 2

Review of Literature

Reproductive age of women is affected 6% to 10% by the prevalent and complicated condition known as polycystic ovarian syndrome (PCOS). Due to its effects on Reproduction, Metabolism, and Mental Health, PCOS affects many aspects of health and wellbeing across the lifespan. The most frequent cause of Anovulatory Infertility is PCOS [27]. The name PCOS was given to the condition after ovarian morphological alterations were noticed in women with monthly irregularities and symptoms of hyperandrogenism. Follicular cysts, numerous immature follicles, numerous atretic follicles, and other morphological abnormalities that point to the failure of folliculogenesis are among these changes [28]. Reproductive, Metabolic, and Psychological characteristics make PCOS a complex condition [29].

The Rotterdam criteria state that polycystic ovaries, clinical or biochemical Hyperandrogenism, and persistent ovulatory dysfunction are all necessary for the diagnosis of polycystic ovary syndrome (PCOS). Insulin resistance (IR) has not been included in the criteria even though abnormalities in glucose metabolism and insulin sensitivity are frequently observed in patients with PCOS.

The major treatment is changing one's diet and way of life. In some people, these changes can completely restore ovarian function and prevent PCOS sequelae. However, it's frequently necessary to get a prescription for drugs or dietary supplements. PCOS affects two to four times as many women as the general

population do who have metabolic syndrome; this is most pronounced in women between the ages of 30 and 40, have higher prevalence rate 50% [30].

2.1 Main Problem in PCOS

Chronic infections, such those that characterise PCOS, are linked to elevated levels of oxidative stress, systemic inflammation, inflammatory cytokines, adhesion molecules, blood lymphocytes, and monocytes, as well as myeloperoxidase, c-reactive protein (CRP), and lipid peroxidation markers [31]. One of the main side effects of PCOS is infertility in female patients due to anovulation [32]. Anovulation brought on by a hormone imbalance is the primary issue with PCOS. When compared to healthy women, Patients with PCOS have greater luteinizing hormone (LH) levels but decreased follicle-stimulating hormone levels (FSH). Some of the striking symptoms of ovaries with PCOS are the buildup of follicles, the lack of the corpus luteum, and an increase in ovarian volume [33]. PCOS has a wide range of effects, including psychiatric, gynecological, and metabolic conditions like insulin resistance, type II diabetes, cardiovascular risks, and obesity. Additionally, PCOS is one of the reasons of infertility in women because of the ovulatory abnormalities it brings on. The symptoms of hyperandrogenism, reestablishing the menstrual cycle to improve chances of fertility, and addressing metabolic irregularities are all addressed by the recommended treatments for PCOS, which are symptomatic in nature [34].

2.2 Metabolic Disorder

Women with PCOS frequently have metabolic problems that can have a long-term negative impact on their health, such as elevated levels of prolactin, testosterone, and luteinizing hormone (LH) in their serum [35].

At least two of the following conditions must be present in order to diagnose the syndrome:

1. Amenorrhea or Oligo menorrhea linked to lowered ovulation. The most frequent factor causing anovulatory infertility is PCOS.

2. In the absence of other underlying illness conditions, Hyperandrogenaemia or other clinical signs of androgen excess

3. Increased ovarian volume or abnormal ovarian ultrasonography with more than 12 follicles per ovary, each measuring 2 to 9 mm in diameter. A rise in LH and a higher LH/FSH ratio [36].

2.3 Symptoms of PCOS

Anovulation-related infertility, Obesity, Irregular Menstrual cycles, and Symptoms brought on by an excess of Androgen, such as Hirsutism, are the chief signs of the syndrome. Although the pathogenesis of PCOS is thought to be multifactorial, Insulin resistance plays a significant role [37]. Additionally, the illness may be accompanied by other chronic metabolic conditions, such as elevated insulin resistance, necessitating the use of the right therapies to avoid consequences.

2.3.1 Obesity

One of the most prevalent issues among PCOS patients is obesity. Additionally, there is a strong link between PCOS prevalence and obesity. In women with a body mass index (BMI) of less than or equal to 25 kg/m2 and more than 30 kg/m2, the prevalence of PCOS is 4.3% and 14%, respectively. Patients with PCOS have a four times higher rate of obesity than healthy controls [38].

2.3.2 Hyperandrogenism

A diagnosis of PCOS is made in 70–80% of women who have hyperandrogenism, which is characterised by increased LH secretion compared to FSH. In the etiology of PCOS, hyperandrogenism is associated with inflammation. Inflammatory

markers like C-reactive protein (CRP), Tumor Necrosis Factor (TNF), interleukin-6 (IL-6), interleukin-18 (IL-18), monocyte chemotactic protein-1 (MCP-1), and acute phase serum amyloid A (APSAA) also seem to be elevated in PCOS-affected women, according to recent studies [39].

2.4 Pathogenetic Role of Hyperandrogenism in PCOS

Despite having many follicles, women with PCOS simply do not ovulate [40]. Hyperandrogenism in PCOS may result from impaired intrinsic steroidogenesis in ovarian theca cells or from high LH levels brought on by abnormal hypothalamic-pituitary axis control, which is also regulated by insulin. Hyperandrogenism is one of the acknowledged potential reasons of insulin resistance in PCOS if it results in hyperandrogenism and anovulation. It has been demonstrated that having too much androgen during pregnancy or the first few months after birth makes visceral obesity and insulin resistance worse. Metabolic issues and insulin resistance are more prevalent in hyper androgenic PCOS phenotypes. Insulin resistance is improved by the use of medicines with anti-androgenic action.

2.5 Pathogenetic Role of Insulin Resistance in PCOS

Insulin regulates glucose homeostasis by decreasing hepatic glucose synthesis and by promoting glucose absorption by insulin-sensitive tissues like adipose tissue, skeletal muscle, the liver, and the heart. Additionally, insulin has the ability to inhibit lipolysis, which lowers levels of free fatty acids and may be how insulin affects hepatic glucose generation. If pancreatic function is normal, insulin resistance is described as a decline in insulin's capacity to carry out the metabolic processes that are inherent in glucose uptake, generation, and lipolysis.

As a result, large levels of compensatory insulin are produced both at rest and after a glucose load. Regardless of body mass index, there is still no agreement on the precise mechanism that causes insulin resistance in PCOS (BMI). An ancient study claimed that the mechanism causing insulin resistance in PCOS diminished the insulin receptor's own auto phosphorylation after insulin binding [41].

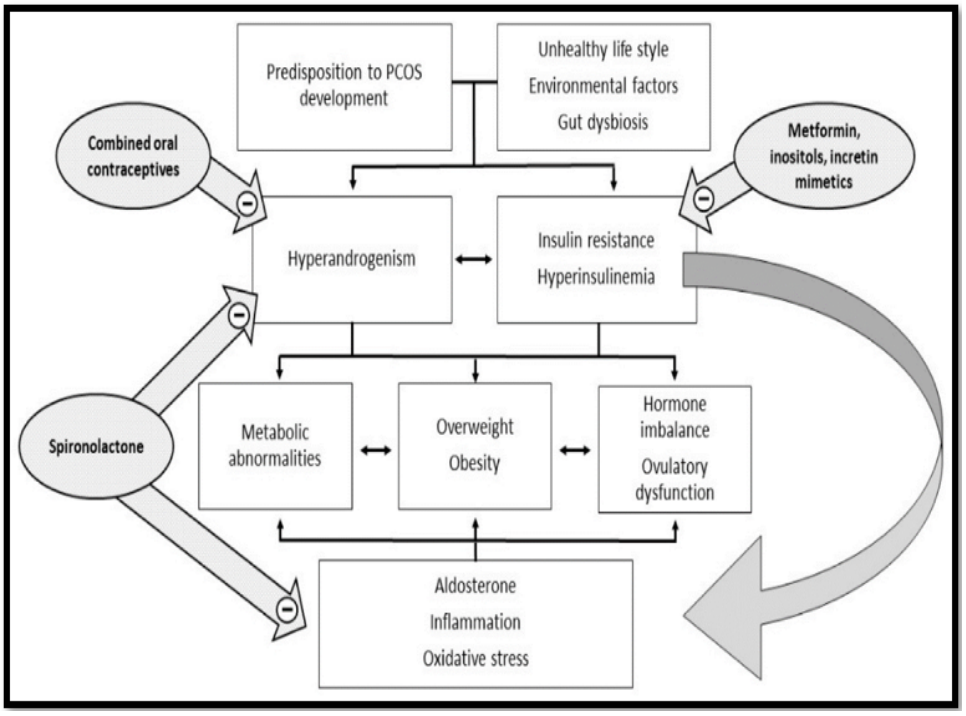


FIGURE 2.1: Pathophysiology and potential therapeutic targets of PCOS [37].

2.6 Obesity, Insulin Resistance, and Inflammation

PCOS primarily manifests as an excess of androgen, polycystic ovarian morphology, and ovulatory dysfunction (which manifests as oligo-ovulation, menstruation disorder, and subfertility) (PCOM). Because insulin functions as a co-gonadotropin on the ovary, enhances androgen production from the adrenal glands, and modifies the release of luteinizing hormone, it is thought that hyperinsulinemia is the primary cause of enhanced androgen secretion (LH). These factors contribute to an increased frequency of PCOS in women with elevated insulin levels [42]. In

more than half of PCOS patients, obesity is present. Insulin resistance is largely caused by obesity [43]. PCOS is regarded as an inflammatory condition. Obesity is characterised by a state of chronic systemic inflammation. For instance, systemic levels of free fatty acids are increased in obese people. These compounds act as Toll-like receptors' main ligands, which are important controllers of the innate immune response. Thus, there is a clear connection between the systems that control inflammation and fat. Obesity, Insulin resistance, and Inflammation are essential characteristics of PCOS [44]. The crucial role insulin resistance plays in PCOS is well-known. Although little is known about the molecular mechanisms behind insulin resistance in PCOS, several studies have shown that the skeletal muscles' defective insulin signaling is the root cause of the IR phenotype in the women of PCOS. The study, which included slim PCOS women, found that there may be no disruption of insulin signaling at the proximal end. About 50 to 80 percent of PCOS-afflicted women have insulin resistance [45].

2.7 Factors Involved in PCOS

PCOS may be related to: even if its pathophysiology is not fully understood.

- Genetic factors
- Lifestyle
- Inadequate intake of vital micronutrients in individuals with insulin resistance
- Oxidative stress.

Since an unbalanced element status is a crucial underlying cause of insulin resistance in PCOS, lifestyle modifications such as food and activity changes are typically the first-line therapy for the condition. A rising number of disorders with aberrant metabolism are being treated with various combinations of dietary supplements, such as magnesium and vitamin E, due to their potential to enhance

metabolic profiles [46]. Although the precise cause of PCOS is unknown, genetic history and lifestyle variables are known to contribute. Since PCOS is seen as a lifestyle disease, genetic predispositions may in reality be triggered by a person's lifestyle choices. Lack of physical activity and busy schedules contribute to stress. Sleep patterns are messed up [47]. Polygene interactions with environmental factors control the multifactorial characteristic of PCOS. Nutritional, physical behavioral problems, chemical are a few examples of environmental factors that could be connected to PCOS.

Exposure to organic solvent like ether, di-isobutyl phthalate, toluene pesticide, carbon disulfide, ethylene glyalcohol, pesticide, carbon disulfide, di-isononyl phthalate (DINP), areca nut chewing, and cigarette smoke are examples of chemical variables. Mood swings and stress, difficulties losing weight growth, sleep apnea, weight, and consumption of high-calorie foods, indoor decoration & use of plastic dishes are some other external causes.

Numerous diversely functioning genes have been linked to PCOS, including those that encode the androgen receptor, sex hormone-binding protein (SHBG), and the genes that control adrenal and ovarian steroidogenesis. More than 100 PCOS susceptibility genes have been discovered, including genes associated to metabolism, such as insulin growth factors and obesity genes, as well as genes involved in the synthesis or mediation of steroid hormones, such as androgen-related genes. PCOS is a complex and multi-cause disease [48].

2.8 Role of Food in PCOS

In PCOS patients, food is vital. Avoiding foods with too much greasy, white flour spices, strong fried food, sugar, spices, white flour, coffee, refined grains, and fat or tea is advised. Get eight to ten glasses of water a day minimum. Young girls simply devour these ready-made goods that are so easily accessible in today's modern lifestyle, which should be completely avoided.Chronic inflammation is accompanied with genes. Consume nutritious food; this has already been mentioned [49].

2.9 Role of Exercise in PCOS

Lack of physical activity is one of the main contributors of PCOS's sedentary lifestyle. According to study, PCOS affects 50% of overweight women. This is a serious worry, which is why exercising regularly is crucial to enhancing reproductive health.

2.9.1 Best Exercise for PCOS

1. HIIT (High Intensity Interval Training)
2. Strength training.
3. Yoga [5].

2.10 Types of PCOS

There are four main categories for PCOS. Period irregularities are a result of the hormonal condition PCOS.

1. Insulin Resistant of PCOS: In this instance, preventing insulin levels from rising reduced the body's capacity to control sugar levels. High levels of sugar directly reduced insulin levels. It's a major factor in PCOS's insulin resistance.
2. Inflammatory of PCOS: Inflammatory effects, such as inflammation in the digestive system, leading to conditions including irritable bowel syndrome (IBS), joint discomfort, and skin issues, are present in certain forms of PCOS. The hsCRP test is effective for determining the body's level of inflammation.
3. Post pills of PCOS: If your periods were regular before to using hormonal birth control and you now meet the PCOS diagnostic criteria, you may have the post-pill version of the condition. It's possible that you had PCOS before

using the pill if you experienced symptoms before taking the pill but did not acquire a formal diagnosis.

4. Hidden cause of PCOS: Thyroid dysfunction is one of the hidden medical causes of PCOS because hypothyroidism interferes with ovulation and results in vitamin deficiencies. Elevated prolactin because it can raise DHEA [50], vitamin D, zinc, or iodine since your ovaries require these nutrients.

2.11 Physiological Basis

The physiological basis of PCOS has four primary causes, which are as follows:

1. Disorders of gonadotropin hormonal synthesis;
2. The emergence of insulin resistance;
3. The impact of the current excessive body fat; and finally, The metabolic pathways involved in PCOS including those for steroidogenesis, insulin secretion and activity, and other metabolic and hormonal pathways [51].

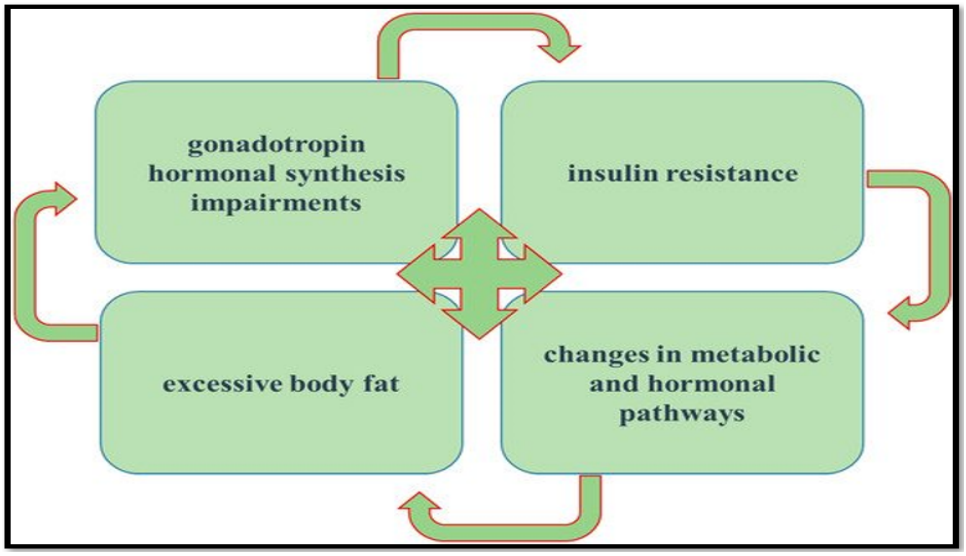


FIGURE 2.2: The primary pathophysiological causes of polycystic ovarian syndrome (PCOS) are abnormalities in gonadotropin hormone production, the development of insulin resistance, the impact of current excess body fat, and PCOS-related oblique metabolic pathway.

2.12 Evolutionary Theory of the Pathogenesis of PCOS

According to a comprehensive evolutionary explanation of PCOS etiology, genetic variations from prehistoric times that were compatible with the environment at the time gave kids in ancestral populations an adaptive survival advantage. Maladaptive physiological responses happen when these identical genetic polymorphisms are exposed to effects from the environment and modern lifestyle. Improved energy storage, decreased fertility in ancestor's population, insulin resistance & hyperandrogenism become pathological and cause the PCOS symptoms seen in modern women [52].

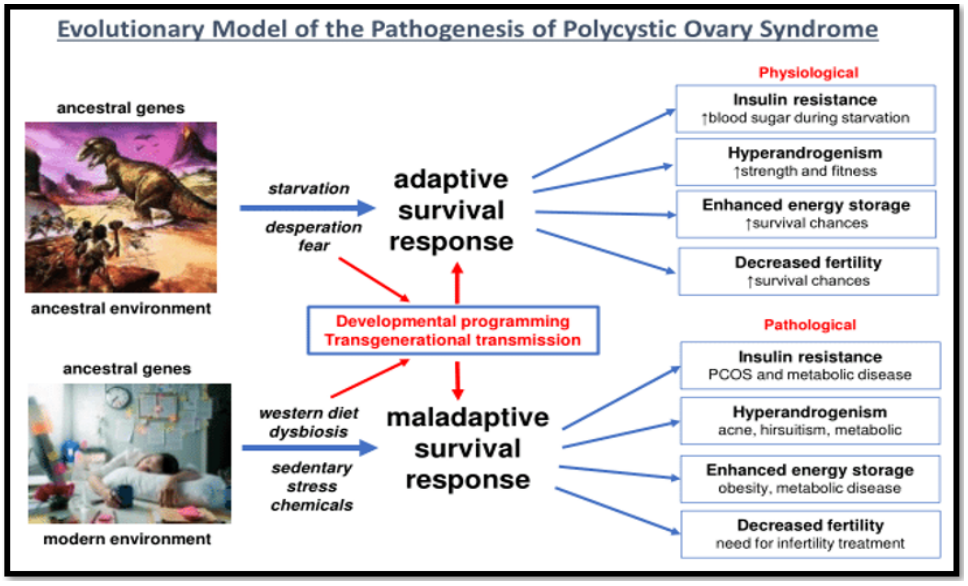


FIGURE 2.3: Evolutionary model of the pathogenesis of polycystic ovary syndrome.

2.13 Bi-Directional Relationship Between Obesity and PCOS

Obesity and PCOS have a reciprocal relationship in which they both increase one another in an endless cycle. Fewer metabolic risk factor and less insulin resistance

factors are present in lean PCOS patients, which raises the possibility that their etiology may differ from that of obese PCOS patients [53]. According to reports, 30–75% of PCOS women are obese. We evaluated a number of clinical, metabolic, and hormonal markers across the two groups. The main cause of excess androgen such as acne, excessive hair growth was included in the clinical factors. The modified Gallwey and Ferriman score was used to identify if hair growth was too much [54]. IGT occurs in 31% of obese PCOS patients, and 7.5% of them meet the requirements for type 2 diabetes mellitus. IGT and type two diabetes mellitus are three times more common in non-obese PCOS patients than in the general population, with prevalence rates of 10.3% and 1.5%, respectively. IGT and type 2 diabetes mellitus are substantially more likely to develop in PCOS women at any weight and at a young age [55].

2.14 Role of Vitamin D in PCOS

The human body requires vitamin D to function properly. There is also proof of a strong correlation between it and female infertility issues. This review examines the connection between vitamin D deficiency and conditions that affect women's fertility, including polycystic ovarian syndrome (PCOS), uterine leiomyomas, and endometriosis. Vitamin D affects how the female reproductive system functions and has been linked to PCOS, uterine leiomyomas, endometriosis, and the success of in vitro fertilization (IVF) [56]. The ovary, placenta, and endometrium all contain vitamin D receptors. Follicular growth, normal menstruation, and fertility are all affected by changes in calcium absorption brought on by vitamin D insufficiency. Numerous studies demonstrate that 65-86% of individuals with PCOS have serum vitamin D concentrations below 20 ng/mL, and that treatment with vitamin D supplementation may have beneficial effects on insulin resistance and menstrual irregularities. On the other hand, the precise process by which vitamin D creates these qualities is not well understood, and too much vitamin D causes intoxication [57]. The development and implantation of follicles depend on vitamin D's immunological effects as well as its involvement in controlling calcium hemostasis

and bone mineralization. It is now widely acknowledged that there may be a link between low vitamin D levels and an increased risk of PCOS. In PCOS-afflicted women, VD replacement increases the ability of the follicle to grow and to synthesis estrogen and progesterone. In PCOS, VD replacement lowers serum androgen levels while enhancing follicle morphology [58]. Circadian rhythm disruption has been linked to an increased risk of PCOS and irregular estrous cycles, according to some research [59].

2.15 Estrogen

A steroid hormone, estrogen is principally released by the ovaries. It affects the physiological operations of reproductive organs such the mammary gland, uterus, oviduct, and vagina as well as their proliferation and differentiation. Additionally, estrogen has a number of significant roles in the metabolism of non-reproductive organs and tissues include the skeletal, immunological, cardiovascular, and central neurological systems. The physiological operations of these non-reproductive systems can be impacted by the low estrogen state that women experience after menopause or bilateral oophorectomy, specifically causing osteoporosis, lipid problems, obesity, atherosclerosis, and dementia [60].

2.16 Phytoestrogens (Medicinal Plant)

Medical plants, also known as medicinal herbs, are those plants with curative qualities and beneficial medicinal effects on the human or animal body. Drug development employs a variety of plant parts, including leaves, seeds, roots, flowers, and sometimes the entire plant. Most compounds contain bioactive substances that are employed as therapeutic agents because they have direct or indirect therapeutic effects. Therefore, these plants are used as complementary medicine all over the world. But today, most people use therapeutic plants. Prenylated flavonoids, isoflavones, coumestans, and lignans are examples of the wide class

of nonsteroidal, diphenolic, estrogenic plant chemicals known as phytestrogens. They are nonsteroidal chemicals generated from plants that share structural or functional similarities with mammalian estrogen (E2), particularly 17β-estradiol. Poly-phenolic substances known as phytestrogens contain more than 100 molecules [61].

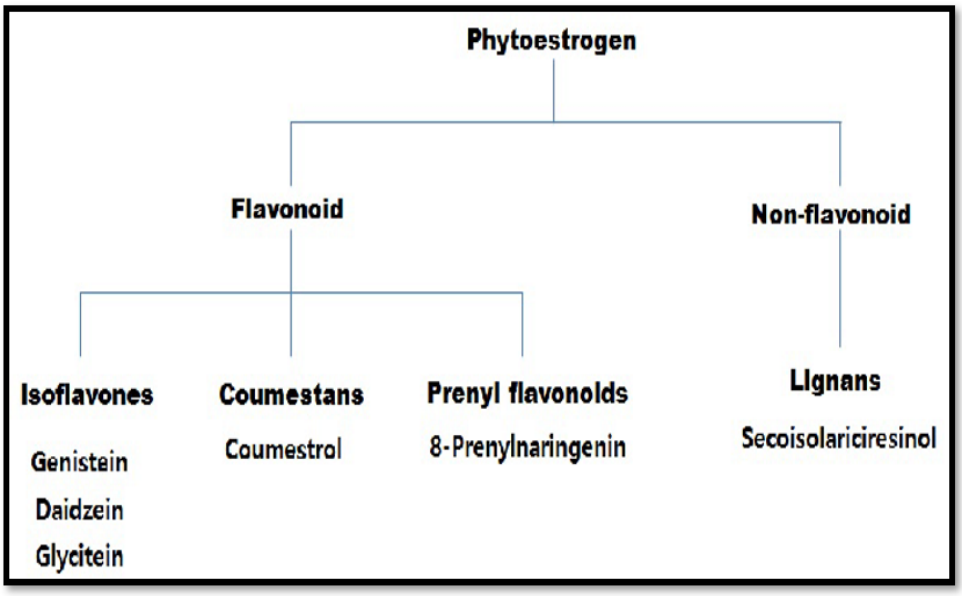


FIGURE 2.4: Classification and metabolism of phytoestrogens.

The phytoestrogens, which can be derived from a variety of plant tissues, has already been shown to have anti-oxidative and cancer-preventive characteristics. A naturally occurring organic substance known as Coumestrol (CMT) belongs to the Coumestans class of phytochemicals. Ladino clover and several other sprouting plants, like alfalfa, contain this phytoestrogen. CMT has been developed as a potent anti-inflammatory drug in addition to having anti-oxidative and anti-catabolic properties. Controlling auto-immune reactions is another pharmacological advantage of CMT [62]. Foods containing phytoestrogens and to compile quantitative information on these substances into a database of nutrients. This will enable research to clarify the potential impact dietary phytoestrogens may have in cancer, menopause, and other aspects of health, as well as the ability to examine American diets for phytoestrogen intake [63].

Due in part to their capacity to serve as estrogen agonists and antagonists, phytoestrogens exhibit a wide range of biological action. Phytoestrogens imitate natural

estrogens and have estrogenic effects since they are estrogen agonists. They may change or block estrogen receptors (ER), which would inhibit estrogenic action and result in antiestrogen effects [64]. Plant-based foods including fruits and vegetables are the primary dietary sources of phytoestrogens. These compounds operate as antioxidants, light filters, and—most importantly—defensive agents against predators in plants where they are primarily found as glycosides (Mazur & Adlercreutz, 1998a). Isoflavones, which are primarily found in legumes like lignans, chickpeas, lignans & soybeans which are found in linseed, cereals and other vegetables, fruits and coumestans, which are present in young sprouting legumes like alfalfa or clover sprouts, are the important classes of phytestrogens. The phytestrogen content of foods depends on a wide range of environmental and genetic factors, including variety, proceedings and harvest [65].

Natural compounds known as phytoestrogens can behave similarly to estrogen because of their having a similar chemical composition as 17–estradiol. Numerous advantages of foods containing phytoestrogens have been revealed by international study during the last four decades. On fact, studies on participants who consumed moderate amounts of soy foods found that phytoestrogens had a generally beneficial effect in preventing heart disease and menopausal symptoms.

2.16.1 Phytoestrogen Classification

The three types of phytoestrogens are lignans, coumestans, and isoflavones (plant lignans: enterolignans: enterodiolm, enterolactone). Because they contain phenolic groups in their structure, they are also categorised under the more general structural class of polyphenols. Isoflavones and lignans, which are mostly found in legumes, especially soy, and in smaller amounts in fruits, vegetables, and grains, are the two types of phytoestrogens that are most commonly found in the diet [66]. According to several reports, phytoestrogens can help prevent certain disorders such different cancers [67]. Some degenerative processes connected to nonalcoholic fatty liver disease can be mitigated by phytoestrogens (NAFLD). Urinary phytoestrogen concentrations were assessed in urine samples, and they

were then classified into tertiles in accordance with the concentration distributions [68]. Several chronic diseases and death have previously been linked to dietary intake of phytoestrogens. In addition to increasing the production of neurotrophins, phytoestrogens are also associated with increased anti-inflammatory and antioxidant activity, hippocampal synaptic modulation, and increased nitric oxide bioavailability, all of which are crucial for maintaining cognitive function [69]. As a phytoestrogen, resveratrol (1, RSV) exhibits varied degrees of estrogen receptor expression in various cell types. RSV exhibits anticancer properties [70]. Herbal estrogens known as phytoestrogens come from many herbal preparations. When compared to steroidal estrogens, phytoestrogens are thought to have less of an impact on the endocrine system, but they still have the ability to have both anti-estrogenic effects and physiological estrogenic. Phytoestrogens have a higher affinity for attaching to estrogen receptors (ER) than do estrogen receptors (ER), according to research. However, compared to endogenous estrogens like 17-beta estradiol, phytoestrogens are often less powerful. It is possible to improve endometrial thickness, risk of miscarriage rate lower and increase conception rates by combining phytoestrogens with clomiphene citrate to reverse the anti-estrogenic effects of the drug on the endometrium [71]. Herbal substances known as phytoestrogens estrogenic effects caused by mimic estrogen when estrogen receptors attached. Shaoxing duck laying effectiveness increased during the post-peak laying period, a natural phytoestrogen, dietary daidzein. Egg growth and Eggshell thickness increased by Daidzein supplementation, according to research by Dusza et al while decreasing the rate of broken eggs. Antioxidants help maintain an animal's health, immune system supported by Antioxidants & animal's capacity also increased by Antioxidants for output [72].

2.17 Proteins Involved in PCOS

A desirable large-scale analysis for searching proteins for complicated disorders is proteomics. Proteins have a major role in PCOS. The current investigation discovered that certain proteins, which are important for cell proliferation and

death, were expressed regularly in PCOS follicular fluid. The biological roles played by the 20 proteins that have been identified were compiled. We discovered that these proteins are crucial for insulin resistance, cell proliferation, lipoprotein metabolism, glucose metabolism, and other processes. The molecular mechanism of PCOS may be discovered by doing a thorough analysis of the structure, function, and role of these proteins in the occurrence and development of PCOS [73]. SET Protein, UCP protein (Mitochondrial uncoupling proteins. The bulk of PCOS-associated loci still need to have their mediating genes and/or functional effects determined. Understanding the biology of a disease risk locus can be aided by Colocalization analysis of the illness and intermediate cellular phenotypes, such as the expression of gene & in diverse tissues the levels of protein. The hypothalamic-pituitary-gonadal signaling pathway has been linked to C8 or f49 of these possible genes & effector proteins opening a channel for functional follow-up to show a causative role in the pathophysiology of PCOS [74].

2.17.1 SET Protein

The SET protein, also referred to by the names TAF-1, I2PP2A, and INHAT, belongs to the family of multitasking proteins. A strong possibility for involvement in PCOS' hyperandrogenism would be the SET Protein. We started by looking into the SET protein's cellular localization in human ovaries and how it differs from normal human expression in polycystic ovaries in adult females. It would give us a foundational understanding of the pathophysiology of hyperandrogenism in PCOS as well as basic knowledge regarding the regulation of androgen production in the ovary [75].

2.18 Genomics Biomarkers

Finding biomarkers and understanding the molecular pathophysiology of PCOS have benefited from research on genetic and epigenetic patterns and their impact

on genetic profiles. Researchers have investigated a few suitable candidate genes to identify the genetic basis of PCOS and its associated symptoms in order to disclose its basic molecular mechanisms. Researchers can comprehend the molecular causes of sickness using this method. There is strong evidence that the disorder PCOS has a hereditary component. Genes that are involved in the development of the condition are the key genetic indicators associated with PCOS.

TABLE 2.1: Table of Genes & their Biological function that are involved in PCOS.

Gene	**Biological Function**
1.GATA4	In PCOS patients androgen biosynthesis effect
2. FTO	Type 2 diabetes mellitus and obesity-related genes.
3. KHDRBS3	Leads to PCOS and the syndrome's clinical manifestations and is connected to telomerase function.
4. MTHFR	Ovarian function.
5. CYP17/P450c17	Hyperandrogenism causes androgen secretion.

The literature has discussed several gene-to-gene, protein-to-gene, gene-to- environment, and gene-to-environment interactions as well as the significance of genetic predisposition in the development of PCOS [76].

2.19 Proteomics Biomarkers

The identification of biomarkers is significantly impacted by proteomics in relation to genetics and phenotype. The structure, modification, and interactions of proteins and peptides are studied through proteomics, a high-throughput method of studying these molecules. With the aid of proteomics research, numerous unique

proteins that may be implicated in the pathophysiology of PCOS have been identified. Understanding dynamic variations in protein expression at a broad scale and systematic level is crucial for understanding complex diseases. Huge potential for unravelling the illness process and revealing novel insights into PCOS is capped by variations in protein function with broad alterations in protein expression and posttranslational variation. The following techniques, such as protein array, 2D PAGE, mass spectroscopy, and other protein separation techniques, as well as proteomic methodologies, can be utilised to profile the protein expression from cells and tissues [77].

TABLE 2.2: Proteins and their Biological function that are involved in PCOS.

Proteins	**Biological Function**
LH	Increase amounts of LH help explain why PCOS has high levels of androgens.
Resistin	Encourages inflammation and can make PCOS worse
FSH	Follicle growth, steroidogenesis and oocyte maturation are all increasing
Homocysteine	IR
UCP2	The production of androgen by granulose cells

2.20 Molecular Docking

In structural molecular biology and computer-assisted drug creation, molecular docking is a crucial tool. Predicting the typical binding mode(s) of a ligand with a protein whose three-dimensional structure is known is the aim of ligand-protein docking. A scoring system is used by efficient docking approaches to quickly explore high-dimensional spaces and evaluate candidate dockings. Docking can

be used to perform virtual screening on large libraries of compounds, score the results, and offer structural hypotheses for how the ligands block the target in order to optimise leads. [78].

2.21 Target Protein: UCP2

With 309 amino acids and a molecular weight of 33 kDa, UCP2 has an amino acid sequence that is 59% identical to UCP1. In humans, the UCP2 gene is found on chromosome 11q13, which is associated with obesity and energy homeostasis. Control on the transport and metabolism of free fatty acids. Inhibition of insulin secretion [79]. The transport of C4 metabolites into the mitochondria for the generation of NADPH, which is necessary for the development of cancer cells, is facilitated by UCP2. In orthotopic xenograft models, UCP2 over-expression also promotes the growth of tumors [80].

Uncoupling proteins (UCPs), a family of mitochondrial carrier proteins found in the inner membrane of mitochondria, may play a role in the development of follicles in PCOS. UCPs may also be linked to diseases other than metabolic illness. For instance, greater levels of UCP2 expression may promote the formation of follicles in polycystic ovarian syndrome. Patients with PCOS were impacted by the uncoupling protein, which is in charge of the granulose cells' androgen synthesis. The part UCP2 plays in the typical hyperandrogenism associated with PCOS. UCP2 may play a role in regulating follicle growth as well as the maturity and quality of oocytes [81].

2.22 Olive Plant

Olives (*Olea europaea, Oleaseae*) are a long-lived tree that contain Phytoestrogens and are valuable to Mediterranean residents economically and culturally. Numerous flavonoid and polyphenolic chemicals with anti-inflammatory, anticancer, antidiabetic, gastro protective, and wound-healing effects are present in this plant's

leaves [82]. The Oleaceae family member olive (Oleaeuropea), which contains lignans and phenolic compounds, is regarded as a Phytoestrogen plant chemical. Oleuropein and various forms of flavonoids including rutin, apigenin, and luteolin are only a few of the many components found in olive leaves. Olive leaves are a plentiful by-product of olive tree cultivation and olive mills, and they have historically been linked to a variety of medical claims, albeit few of these have been supported by experimental research. Stilbenes, phenolic acid, and flavonoids are found in olive. Oleuropein possesses anti-inflammatory, antioxidant, and anti-hyperlipidemia properties. Due to its laxative effects, it is also helpful in treating digestive issues [83].

All olive byproducts include polyphenols and other antioxidant chemicals, with olive leaves being the main source. *Olea europaea L.*, an evergreen tree with a total land area of 10.8 million ha and a member of the Oleaceae family, has been cultivated in 41 different nations, particularly those in the Mediterranean basin. There are 21.6 million tons of olives and 3.2 million tons of olive oil produced worldwide, respectively. Spain is the world's top producer of olive oil, with approximately 23% of the world's olive tree acreage. Over 1.6 million acres of olive trees are present in Tunisia, which has recently generated 196,000 tons of olive oil [84]. Due to its strong endurance and high adaptability to poor soils, drought, salinity, and excess boron and chlorine, the olive plant can grow in a wide range of settings of soil pH (5.5-8.5) and humidity, from arid to semi-arid locations. The improvement of soil fertility and the satisfaction of its water needs are essential components to achieving high output. It is well known that the olive tree has a significant propensity for alternating bearing, with bigger yields appearing every other year. The gram-negative bacteria Pseudomonas savastanoi pv is the cause of olive knot disease. Another prevalent and significant disease, savastanoi, affects the aerial sections of the olive plant and results in severe damage and significant production losses. Anthracnose disease, which is brought on by the pathogenic fungus Colletotrichum acutatum and Colletotrichum gloeosporioides, may also have similar effects. It affects a number of olive tree components, including the flowers, leaves, shoots, and fruits, resulting in significant yield reductions [85] [86].

2.23 Bioactive Compounds in *Olea europaea* as Inhibitors

Remember that there are two classes of biological plant system molecules for a better understanding of the genesis of bioactive compounds (BC). Primary metabolites, such as carbohydrates, amino acids, proteins, and lipids, are chemical compounds with a function in development and growth and are included in the first class. While the second class consists of secondary metabolites, which support the plant's ability to thrive, overcome regional challenges, and interact with its environment. Vegetables, fruits, oils, cereals, and nuts all contain minor levels of BC, which are secondary molecules, metabolites, or chemical compounds. These compounds have anti-inflammatory, antibacterial, anticarcinogenic, and antioxidant properties [87]. Additionally, many vital nutrients in plants contain a variety of physiologically active non-nutrient groups. These compounds are known as "bioactive compounds," and they have been defined as substances that cause a particular biological reaction in humans and animals. The following discussion is about olive fruit bioactive chemicals that have inhibitory effects.

2.23.1 Squalene

A Japanese industrial engineer named Tsujimoto initially extracted the chemical known as "squalene" (SQ) from Squalus species. Shark oil from deep water. The richest and main source of squalene in nature, with an abundance of 80%, is thought to be shark liver oil. SQ was discovered in leaves and olive oil. It has a variety of purposes in plants, such as acting as a nutrient, reducing the risk of cancer, and serving as a precursor for the synthesis of various bioactive molecules. SQ is a medicinal reagent with enormous promise. It is found throughout nature, in both plants and animals as well as bacteria. The occurrence, structure, biosynthesis, physical and chemical characteristics, and biological importance of SQ with regard to food (dietary supplements), human health care, cosmetic and pharmaceutical industries other fields were assembled in this review [88].

2.23.2 Tocopherol & Carotenoids

A toxicant that are naturally lipid-soluble include tocopherols (α, β and γ). They are a subset of the vitamin E family and are found in the majority of plant and vegetable oils. According to a number of studies, they help keep olive oil from oxidising. Due to their antioxidant activity, pigments, tocopherols, and the hydrocarbon component of olive oil have attracted a lot of interest. Because only plants and their derivatives can produce carotenoids, the only route for them to enter the human body is through a healthy diet. Natural antioxidants included in olive oil, carotenoids are sufficient and advantageous to human health [89].

2.23.3 Phytosterol & Sterol

At normal temperature, Phytosterol is a solid crystalline form. They are insoluble in water but soluble in nonpolar solvents including hexane, iso-octane, and 2-propanol as well as in vegetable oils and fats. The three distinct crystal forms of -sitosterol, the most prevalent Phytosterol, are anhydrate, hemihydrate, and monohydrate. The stability of the phospholipid bilayer, which is typically characterised by a large proportion of unsaturated fatty acyl chains, is provided by sterols, which are essential parts of the cell membrane. There are more than 100 different forms of sterols found in plant species, although campesterol, stigma sterol, and -sitosterol are the most prevalent ones. 5α-cholestan-3β-ol is the fundamental sterol that other sterol structures are derived from. Sterol reduces the risk of cardiovascular illnesses by inhibiting the absorption of cholesterol from the gut [90].

2.23.4 Alkaloids

Alkaloids, which are typically extracted from plants, are primarily biosynthesized from amino acids and result in a range of chemical configurations. About 20% of plant species contain some form of alkaloid, and research and development

continue to be heavily focused on alkaloid production, extraction, and processing. For example, genetic manipulation of alkaloid biosynthesis pathways can increase alkaloid output levels. Both human treatment and an organism's natural defence depend heavily on alkaloids [91].

2.23.5 Polyphenol

A class of chemical compounds known as poly phenol is primarily obtained from plants. These polyphenolic chemicals exhibit potent anti-inflammatory, antioxidant, anticancer, and antidiabetic capabilities in addition to having a variety of other important traits. Based on the quantity of phenol rings, polyphenols are categorised in terms of their chemical makeup [92]. The phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and flavonoids (flavones, flavonoids flavones, isoflavones, and anthocyanin) are two families of polyphenols that are widely distributed in plants and are primarily present as glycosides.

2.23.6 Phenol

A common organic contaminant is phenol. Phenols are important antioxidants and anticancer agents. Phenol, an aromatic semi-volatile hydrocarbon by nature, can be found in wastewater at concentrations ranging from as little as 1 mg/L to as much as 7000 mg/L in most sectors. According to the United States Environmental Protection Agency's list of 126 compounds, phenol makes up 11 of them, making it a major contaminant. It aids in the synthesis of substances during the oxidation and disinfection processes [93].

Chapter 3

Research Methodology

3.1 Methodology Flow Chart

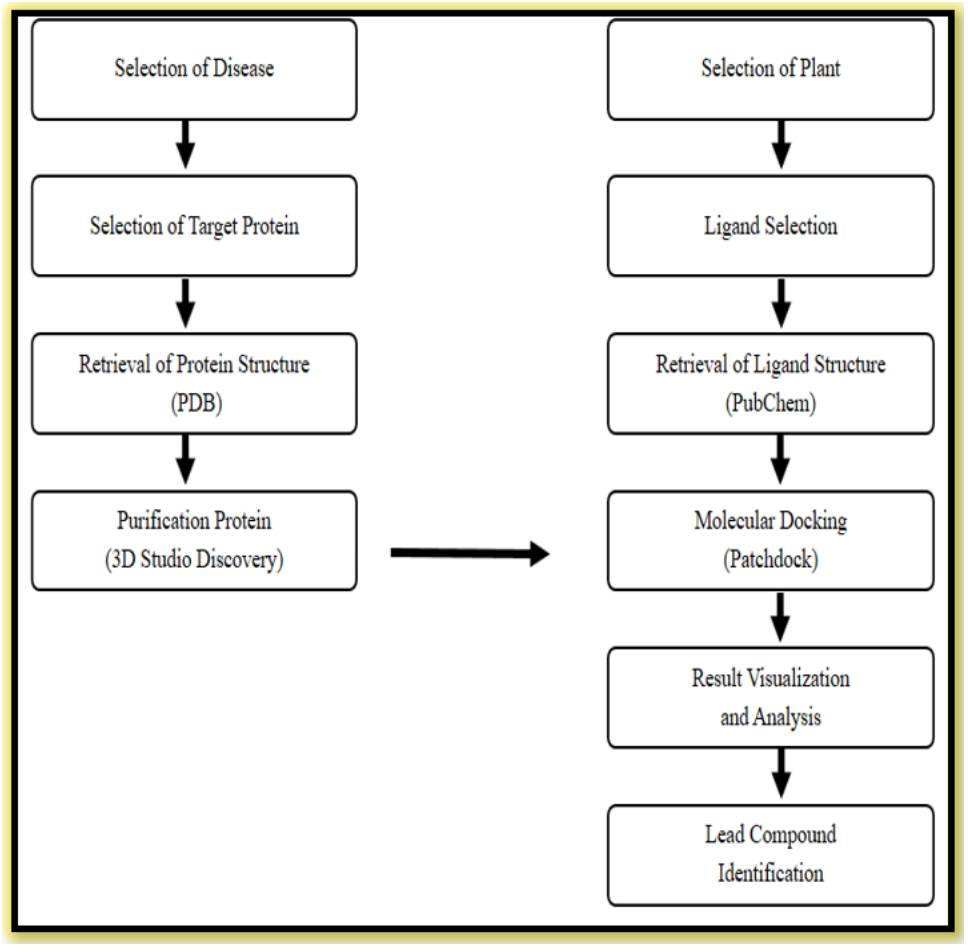


FIGURE 3.1: Flow chart of reserach methodology

3.2 Selection of Disease

Menstrual disorders and infertility are the main symptoms of polycystic ovary syndrome (PCOS), an endocrine disease typically affecting women of reproductive age [94]. Stein and Leventhal proposed the concept of polycystic ovary syndrome (PCOS) in 1935. Stein-Leventhal syndrome is characterized by bilateral polycystic ovary enlargement, obesity, symptoms, hypertrichosis, amenorrhea, and infertility. PCOS is a type of endocrine disorder that occurs frequently among females of childbearing age. Polycystic ovary (PCO) is characterized by high androgen level. In addition to being a disease of the reproductive system PCOS is also a metabolic disorder. Human health may also be adversely affected by cardiovascular diseases, diabetes, hyperinsulinemia, hyperlipidemia, and endometrial cancer [95]. In young women of reproductive age (20-30%) it is prevalent. Around 20% of women with normal ovaries have polycystic ovary disease. There are multiple factors involved in PCOS which is a polygenic disorder [96].

3.3 Primary Sequence Retrieval

Primary Sequence of target protein (UCP2) was taken in FASTA format from protein sequence database UniProt ID (`http://www.uniprot.org`) under accession number AOAO24R5N5.

3.4 Analysis of Physiochemical Properties of Protein

Physiochemical Properties play an important role in determining the function of protein. ProtParam was used to predict the property of UCP2. The number of positive and negative charged residues, Theoretical Pi, Molecular weight, Ext coefficient (Cys included), Ext coefficient (Cys not included), Instability index,

Aliphatic Index and Grand average of Hydrophobicity were computed through ProtParam.

3.5 Selection of Target Protein

In PCOS UCP2 may mediate follicle development. UCP2 is part of the mitochondrial carrier protein family. UCPs may be associated with diseases separate from metabolic diseases. The expression of UCP2 is elevated in patients with polycystic ovary syndrome [97]. Uncoupling protein which is responsible for androgen synthesis in granulose cells from PCOS patients are also elevated [98]. In PCOS UCP2 plays a role in Hyperandrogenism [99].

3.6 Retrieval of Protein Structure (PDB)

Molecular structure information about macromolecular structures can be found in the Protein Data Bank (PDB) [100]. Using the Protein Data Bank (PDB) web server you can access structural information about entries. PDBs contain 3D structural models of proteins that have been obtained through experimentation. A number of schematic diagrams are provided to visualize different aspects of the protein molecules and their interactions with other molecules [101]. The current research uses a structure taken from the Protein Data Bank under job id AF-AOAO24R5N5-F1 that is mainly involved in PCOS.

3.7 Purification of Protein Structure (3D Studio Discovery)

The 3D structure of protein UCP2 and its interaction with several ligands can be predicted using Discovery Studio [102]. The purification of protein was performed using a program called 3D Discovery Studio. By using 3D Studio Discovery, we

were able to purify the water molecule and other small molecules such as Ligands present in the UCP2 protein. Docking results should be purified to avoid unusual results.

3.8 Ligand Structure (PubChem)

PubChem provides information about chemical substances and their biological activities. PubChem has grown rapidly into a major resource for chemical information serving communities in many scientific fields including cheminformatics, chemical biology, medicinal chemistry, and drug discovery. A large amount of chemical information is available on PubChem [103]. Several hundred thousand of compounds was tested against different biological targets in public databases such as PubChem [104]. PubChem chemical library and bioassay data was used for data mining, virtual screening, SAR, and in silico design in a growing number of research publications [105].

3.9 Analysis of Ligands & Toxicity Measurement

Using the PubChem database, we selected ligands that are chemical substances. In some compounds, the Lipinski rule of five can be observed and they may be exploited as pharmaceutical active ingredients. Lipinski's RO5 physicochemical property guidelines would describe an ideal drug molecule. In order to be considered a drug-like compound, it must have

1. Molecular weight should be under 500
2. Maximum H- Bond acceptor should be 10.
3. No more than 5 H-Bond donors.
4. Log P Value less than 5 [7].

3.10 Molecular Docking (Patch Dock)

Molecular docking is used in drug designing. In the process of elaborating biological and molecular mechanisms, molecular docking techniques have become the most critical and widely used method [106]. Based on the complementarity and preorganization, molecular docking can be used to predict and obtain the binding affinity and interaction mode between the ligand and receptor [107].

In docking two essential components are the target protein and the ligands. UCP2 is used as the target protein while the ligands used are (tocopherols, polyphenols, squalene, sterols, phenols, Phytosterol, Glucosinolates, alcohol, alkaloids). In Patch Dock binding sites are automatically identified for docking and docking is performed online. By predicting the binding sites of target protein it simplifies docking procedures and improves accuracy.

3.10.1 Process of Molecular Docking

The study of intermolecular interactions and prediction of complex structures using ligand-receptor molecular docking is based on the principles of shape complementarity and property complementarity. To discover new drugs, molecular docking is required [108].

The first step is to create PDB files for the ligand and target protein. Patch dock received a PDB file of target protein (UCP2) and ligand file. A complex of protein and ligand is formed for further processing.

3.11 Active Site Identification

The Ligand interacts most strongly with the protein at the active site of the Target Protein. Amino acids play an important role in the formation of complexes between ligands and proteins. Using the online software CASTp, binding pockets for proteins were identified [107].

3.12 Ligand ADMET Properties

The lead needs to be more like the drug in order to be more successful in drug discovery. Additionally, the compounds were evaluated for drug-likeness, toxicity, and drug score. Molecule toxicity can be predicted using the pkCSM tool [108].

3.13 Results Visualized & Analysis (3D Studio Discovery)

Discovery Studio is commercial software and the installation cost of it is pretty high comparing to the free of charged Patch Dock. But Discovery Studio provides detailed tutorials for users to get familiar with its functions and the technical support team from the Accelrys Company is very helpful with troubleshooting of Discovery Studio. Discovery Studio is used to predict the 3D structure of protein UCP2 and its interaction with several ligands. It plays an important role in lipid metabolic pathway [109].

3.14 Lead Compound Identification

After a detailed analysis of protein and ligand interactions, docking scores and toxicity studies, the most active inhibitor was identified. (Phenol, Polyphenol, Sterol, Dialcohol, Phytosterol, Glucosinolates, Carotenoids, Squalene, Alkaloids, Tocopherol) are the Ligands which were used for current research. The selected compound was our lead compound.

3.15 Drug Identification

In the identification of Berberine drugs, we refer to drugs that were used to treat PCOS diseases. In order to identify drugs, we used Drug Bank databases, which

provide a detailed analysis of the disease, its pathway, and the drugs that are used in treating it [110].

3.16 Drug Selection

To select the most effective drug, the identified drugs must be filtered. This is achieved by examining all identified drugs closely and identifying the most effective drug based on several parameters, such as its physiochemical properties, its ADMET properties, its mechanism of action, and its minimal side effects. PKCSM, Drug Bank, and PubChem were consulted for physical chemical properties, ADMET properties, and mechanisms of action related to drug side effects [111].

Chapter 4

Results and Discussions

This chapter will explain the result that were obtained by following our methodological steps. The structure of protein and ligands was taken as input. After checking physiochemical properties of proteins were docked against the selected ligands. ADMET properties and lipin ski rule helped in prediction of drug-like features of compounds. Further the validation of selected compound was checked by comparing its properties with available antibiotic drug. All these steps are described under headings sequentially.

4.1 Structure Modeling

Structure modeling includes primary sequence retrieval, physiochemical properties prediction, 2D structures prediction and identification of proteins.

4.1.1 Primary Sequence Retrieval

Primary sequence of target proteins (UCP2) was taken in FASTA format from UniProt database (http://www.uniprot.org) under accession number AOAO24R5.
>trA0A024R5N5A0A024R5N5-HUMAN OS=Homo sapiens OX=9606 GN=UCP2 PE=3 SV=1

MVGFKATDVPPTATVKFLGAGTAACIADLITFPLDTAKVRLQIQGESQGPV
RATASAQYRGVMGTILTMVRTEGPRSLYNGLVAGLQRQMSFASVRIGLYDS
VKQFYTKGSEHASIGSRLLAGSTTGALAVAVAQPTDVVKVRFQAQARAGG
GRRYQSTVNAYKTIAREEGFRGLWKGTSPNVARNAIVNCAELVTYDLIKDA
LLKANLMTDDLPCHFTSAFGAGFCTTVIASPVDVVKTRYMNSALGQYSSAG
HCALTMLQKEGPRAFYKGFMPSFLRLGSWNVVMFVTYEQLKRALCTSRE
APF

4.1.2 Physiochemical Characterization of UCP2

ProtParam is an online tool which allows the calculation of different physical and chemical parameters for a given protein stored in Swiss-Prot or for protein sequence entered by user. The various parameters computed by Prot-Param are molecular weight, theoretical PI, amino acid composition (positive and negative charge), extinction coefficient, instability index, aliphatic index and grand average of Hydropathicity (GRAVY). The calculated PI greater than 7 represents the basic nature of protein while less than 7 represents the acidic nature of protein. Light absorption is represented by extinction coefficient.

Table 4.1: Physiochemical properties of UCP2

Model Name	**Predicted Values**
Ext.Co1	9970
Ext.Co2	9970
Instability index	33.33
Aliphatic index	89.00
GRAVY	0.082
Molecular Weight	9495.79
Theoretical PI	10.12
PR	12
NR	06

Instability index which is less than 40 indicates the stability of protein while greater than 40 indicates the instability of protein. Low GRAVY shows better interaction with water molecules. The Physiochemical properties of UCP2 were shown in Table 4.1. NR indicate total number of negatively charged residues.PR indicate total number of positively charged residues. Ext.Co1 indicates all Cys residues are reduced, Ext.Co2 indicate all Cys residues are reduced. GRAVY indicates (Grand average of Hydropathicity).

4.2 Retrieval of Structure of Protein

PDB is a web server that offers structural details on the Protein Data Bank entries (PDB) Protein secondary structure, protein-ligand, and protein-DNA interactions are all included in the analyses, which are predominantly image-based. PDB is a database of proteins for which an experimentally discovered 3D structural model has been created. It offers many schematic representations for each to show various features of the protein molecule(s) in the structure, as well as the molecules they interact with (such as bound ligands or RNA/DNA) [112]. The target protein UCP2 structure was retrived through PDB under ID AF AOAO24R5N5-F1 for current research.

FIGURE 4.1: Protein UCP2 structure

Uncoupling Protein Structure was retrieved by using PDB under ID AF AOAO24R5N5-F1 for current study. The PDB is managed by a company known as the Worldwide Protein Data Bank, or www.PDB.com

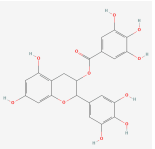
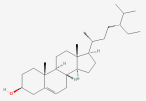
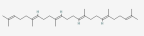
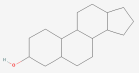
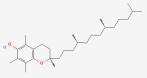
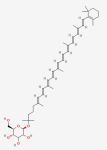
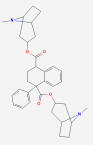
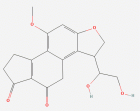
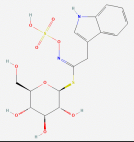
4.3 Purification of Protein

For effective drug design and protein modelling research, Discovery Studio® offers a single integrated, user-friendly graphical interface. The 3D structure of the protein UCP2 and its interactions with several ligands are predicted using Discovery Studio. Both of these applications are included in Discovery Studio. A full molecular modelling tool called Discovery Studio Standalone was created for independent modelers. For computational chemists and computational biologists, the software suite Discovery Studio provides molecular design solutions for the life sciences. It makes it simpler to research systems, find leads, discover candidates, and examine the characteristics of both large and tiny molecule [113]. Discovery Studio was used to purify target proteins such as to remove water molecule and small molecules such as Ligands. The purification of protein was done to avoid the unusual results of docking.

4.4 Structure of Ligands

Popular chemical information site PubChem caters to both the general public and the scientific community. In several fields of biomedical research, including cheminformatics, chemical biology, medicinal chemistry, and drug development, PubChem serves as a vital source of chemical knowledge. Furthermore, PubChem is frequently used as a "big data" source in data science and machine learning studies for metabolite discovery, drug repurposing, chemical toxicity prediction, pharmacological side effect prediction, and virtual screening. Substance, Compound, and Bio Assay are the three databases that PubChem uses to arrange its data [114]. The structure of Ligands were retrieved through PubChem.

TABLE 4.2: Structure of Ligands along with molecular weight & molecular formula

Ligand Name	**Molecular Weight**	**Molecular Formula**	Structure
Polyphenol	458.4g/mol	$C_{22}H_{18}O_{11}$	
Phytosterol	414.7g/mol	$C_{29}H_{50}O$	
Phenol	94.11g/mol	C_6H_5OH	
Squalene	410.7g/mol	$C_{30}H_{50}O$	
Sterol	248.4g/mol	$C_{17}H_{28}O$	
Tocopherol	430.7g/mol	$C_{29}H_{50}O_2$	
Carotenoids	717.0g/mol	$C_{46}H_{68}O_6$	
Alkaloids	542.7g/mol	$C_{34}H_{42}O_4$	
Dialcohol	330.3g/mol	$C_{18}H_{18}O_6$	
Glucosinolates	448.5g/mol	$C_{16}H_{20}N_2O_9S_2$	

The selective Ligands such as Sterol, Phenol, Polyphenol, Glucosinolates & Di-alcohol used for current research.The structure of ligands was retrieved through PubChem. The selective ligands such as Phenol, Polyphenol, Sterol, Phytosterol, Glucosinolates, Dialcohol for current research which are given in the above table 4.2 for the current result.

4.5 Molecular Docking

The Purpose of molecular docking is to find the best conformational interaction Between Target Protein and compound. The two essential requirement for docking are the target protein and the candidate ligand. UCP2 is used as the target protein and selected ligands are Sterol, Phenol, Dialcohol. Patch dock is an online docking server which automatically identifies binding sites and is used to perform docking .It can simplify docking procedure and improve accuracy by predicting target protein binding sites [115].

Results of Patch Dock with ligands name, binding score, cavity size, Grid, Binding Energy Map and Max. and mini. Energy value as shown in below table 4.3.

TABLE 4.3: Shows results of Patch Dock with Ligands

Models Name	**Binding Score**	**Cavity Size**	**Grid Map**	**Minimum Energy**	**Maximum Energy**
Phenol	-4.2	5574	33	0.00	1.6E+00
Sterol	-8.4	5574	33	0.00	1.6E+00
Dialcohol	-7.4	5574	33	0.00	1.6E+00
Glucosinolates	-8.1	5574	31.33	0.00	1.6E+00
Phytosterol	-8.2	361	26	0.00	1.6E+00

4.6 Active site Identification

The Ligand interacts most strongly with the protein at the active site of the Target Protein. Amino acids play an important role in the formation of complexes between ligands and proteins. Using the online software CASTp, binding pockets for proteins were identified.

TABLE 4.4: This table shows the Area and Volume of UCP2 obtained by CASTp.

Pocket Id	**Area (SA)**	**Volume (SA)**
1	4172.032	5002.033
2	128.953	43.618
3	44.007	17.644
4	18.484	9.847
5	14.817	6.131
6	11.091	1.535
7	8.287	0.700
8	5.064	0.381
9	3.470	0.235
10	2.163	0.231
11	1.433	0.076
12	0.329	0.034
13	1.152	0.027
14	0.637	0.016
15	0.280	0.003
16	0.115	0.002
17	0.001	0.000
18	0.001	0.000

Red color showing the available binding pocket for protein. Binding pocket is the region where ligand can bind.

The number of pockets with size and volume is already shown in above table 4.4.

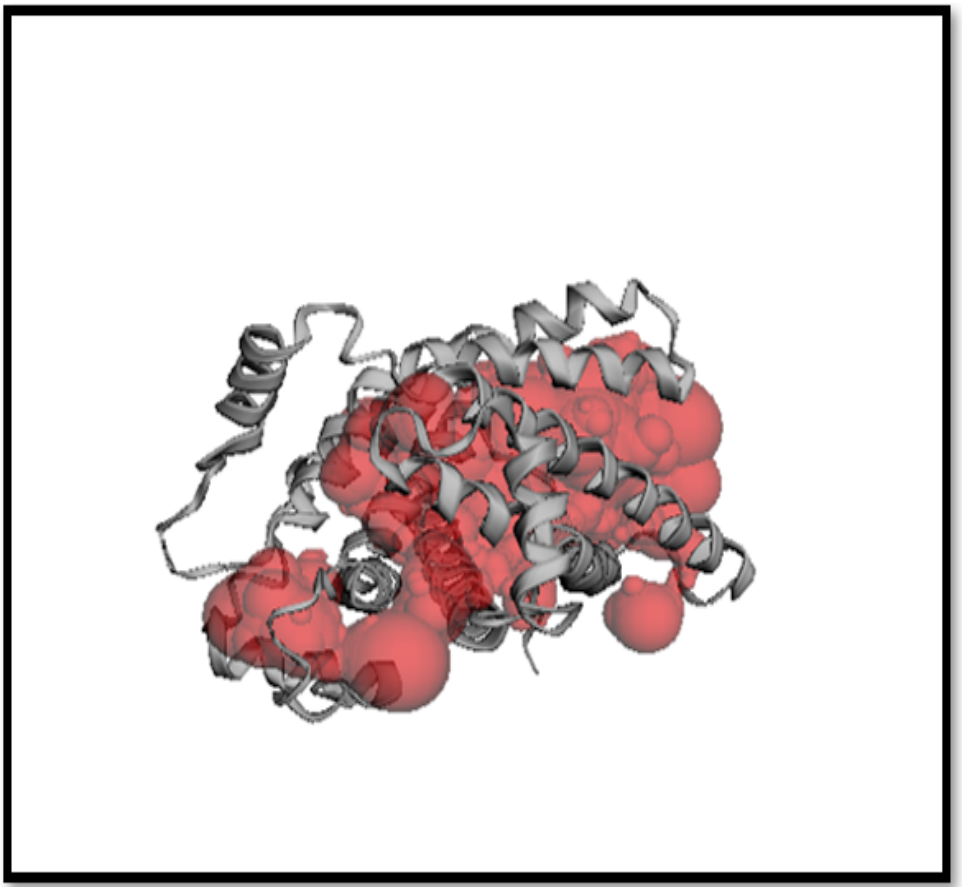


FIGURE 4.2: Structure of UCP2 shows available pockets for ligands.

4.7 Interaction of Ligands and Target Proteins

Small molecules and macromolecules are both transcriptionally combined using a program called Discovery Studio. It was made by Dassault Systems BIOVIA (Accelrys). Discovery Studio, a single integrated graphical user interface, handles both advanced drug design and research on protein modelling. There are several plot viewers and data visualization viewers available in this application.

This study improved people's understanding of how to use the Discovery Studio tool to access, distribute, and analyses data on proteins and small molecules. Numerous disciplines, such as quantum physics, molecular dynamics, and mechanism, can benefit from the DS program. Software also has the ability to do hybrid QM/MM calculations. Applications involving macromolecules and small molecules both employ it. Calculations and structural editing can be utilized to ascertain the molecular properties.

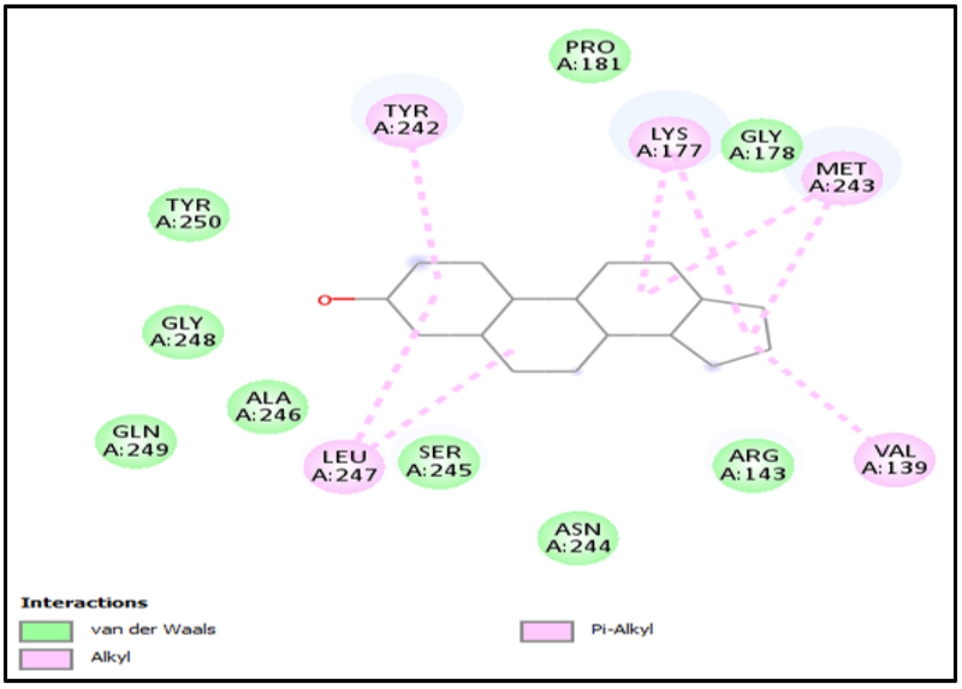


FIGURE 4.3: Sterol interaction

The figure 4.3 interaction of Sterol by 3D Studio Discovery.

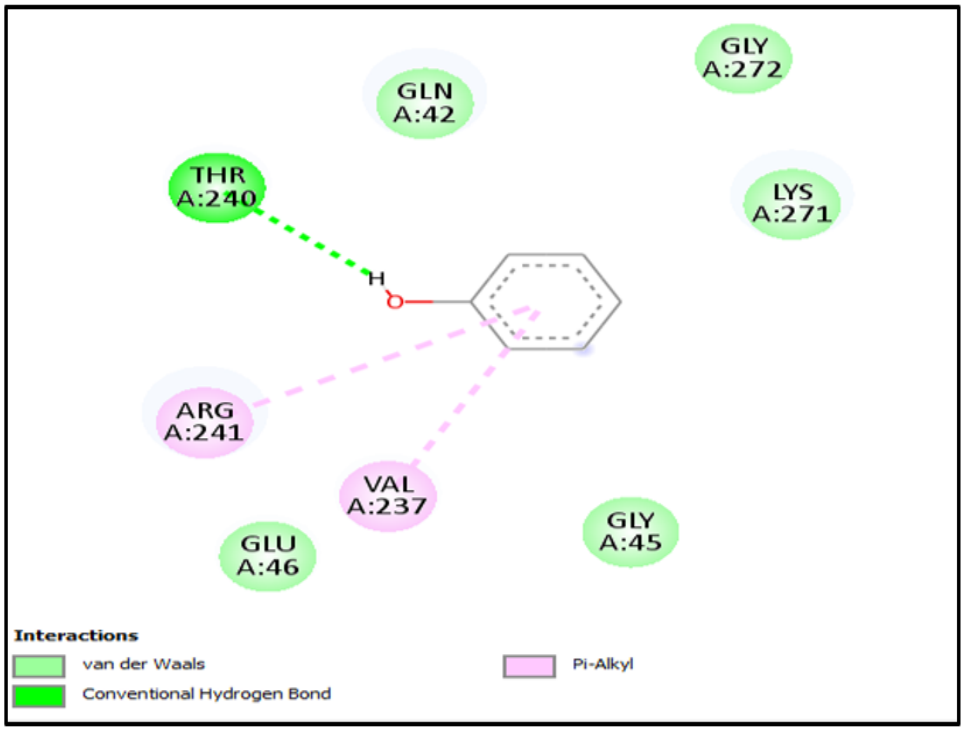


FIGURE 4.4: Phenol interaction

The figure 4.4 interaction of Phenol by 3D Studio Discovery.

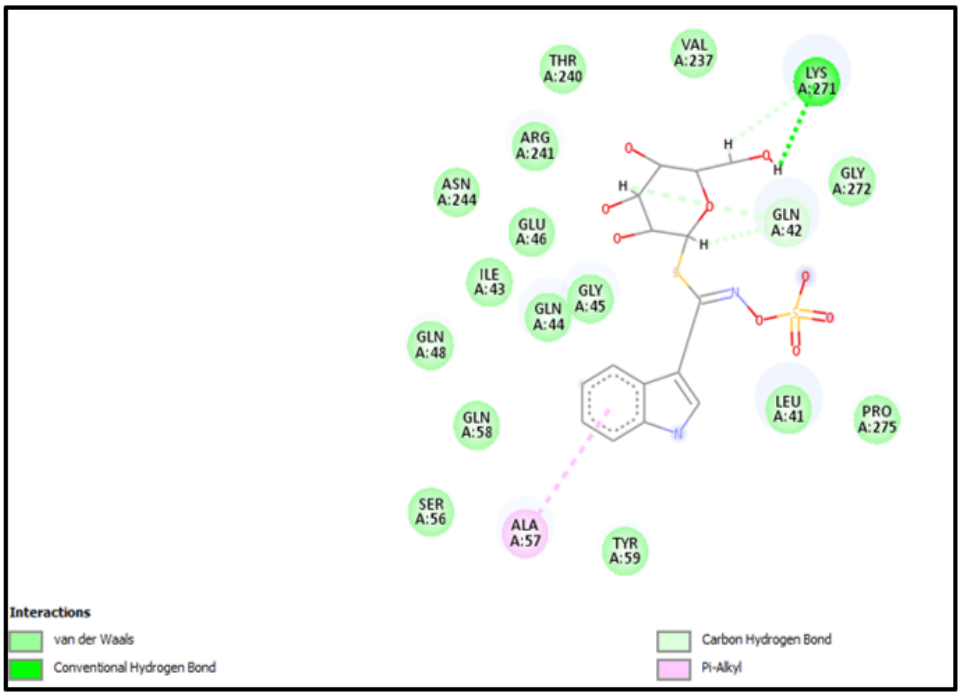


FIGURE 4.5: Glucosinolates interaction

The figure 4.5 interaction of Glucosinolates by 3D Studio Discovery.

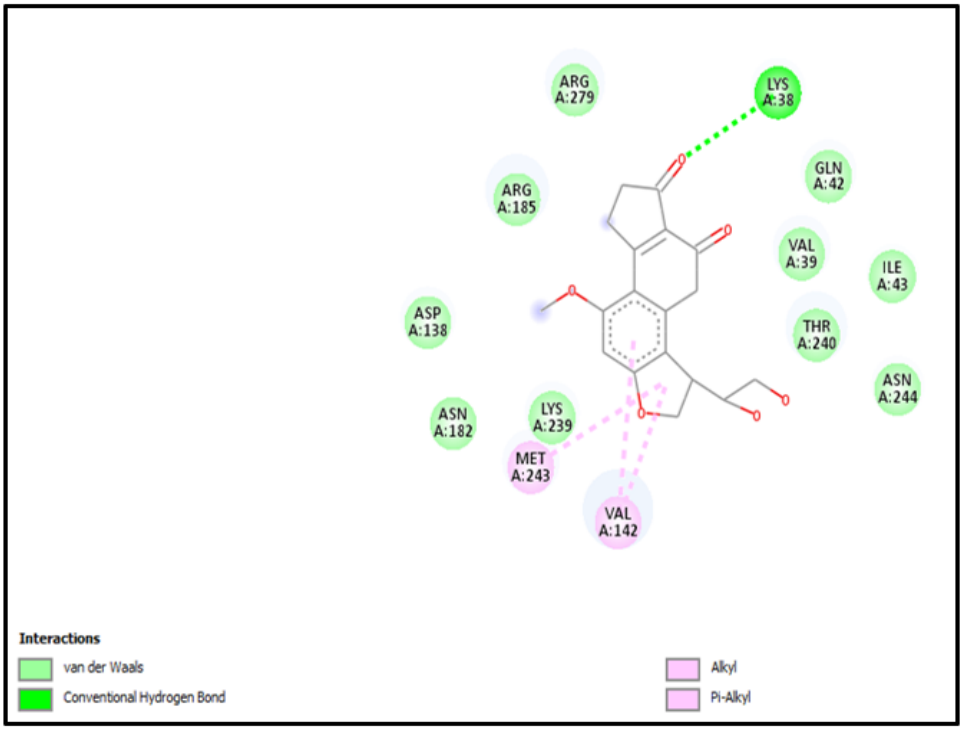


FIGURE 4.6: Dialcohol interaction

The figure 4.6 interaction of Dialcohol by 3D Studio Discovery.

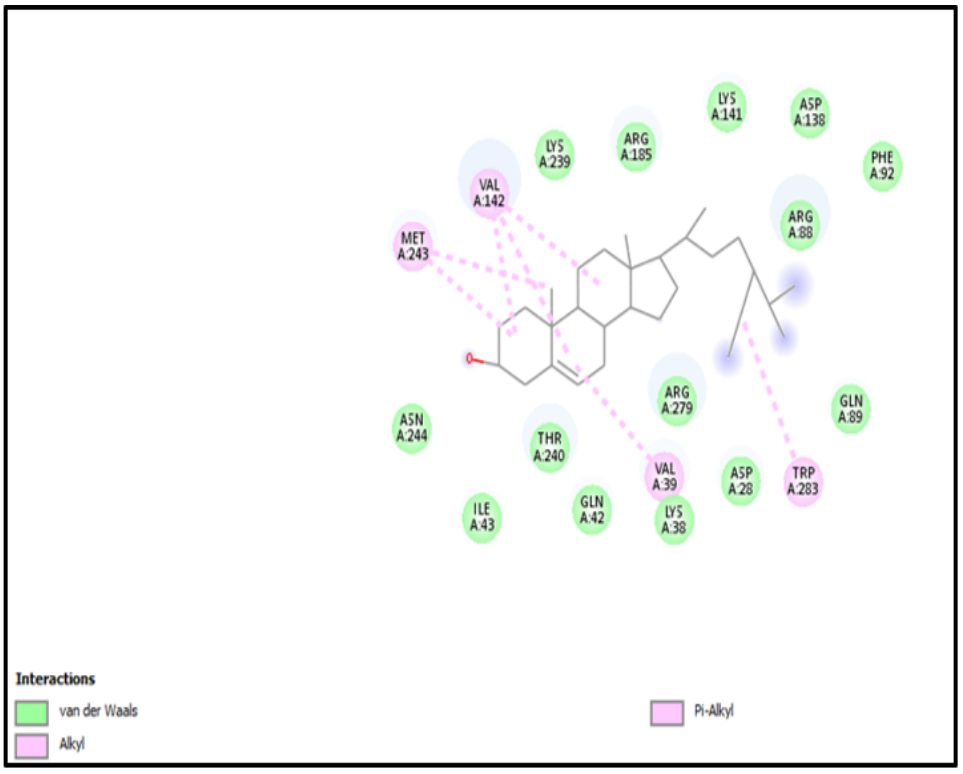


FIGURE 4.7: Phytosterol interaction

The figure 4.7 interaction of Phytosterol by 3D Studio Discovery.

4.7.1 Results of Protein and Ligand Interaction

Software called Discovery Studio combines transcription of small compounds and macromolecules. Dassault Systems BIOVIA created it (Accelrys). Discovery Studio, a single integrated graphical user interface, handles both advanced drug design and research on protein modelling. There are numerous plot viewers and data visualization viewers available in this application.

For computational chemists and computational biologists, the software suite Discovery Studio provides molecular design solutions for the life sciences. It makes it simpler to analyse systems, find leads, discover candidates, and examine the properties of both large and tiny molecules. The 3D structure of the protein UCP2 and its interactions with several ligands are predicted using Discovery Studio. Both of these applications are included in Discovery Studio. A full molecular modelling tool called Discovery Studio Standalone was created for independent modelers.

TABLE 4.5: Values of interaction of Protein Ligand complex.

Name of Compound	**Vander Waals aa**	**Pi-Alkayl**	**Pi-Sulfur**	**Alkyl**	**Conventional H bond**	**Pi-Cation**
Sterol	ARG (A:143)	TYR (A:242)		LEU (A:247)		
	ASN (A:244)	LYS (A:177)		VAL (A:139)		
	SER (A:245)	MET (A:243)				
	ALA (A:246)					
	GLN (A:249)					
	GLY (A:248)					
	TYR (A:250)					
Phenol	PRO (A:181)					LYS (A:177)
	GLY (A:178)					
	PRO (A:181)					
	GLY (A:178)					
	MET (A;243)					
Phytosterol	SER	MET		VAL		

TABLE 4.5: Values of interaction of Protein Ligand complex.

Name of Compound	**Vander Waals aa**	**Pi-Alkayl**	**Pi-Sulfur**	**Alkyl**	**Conventional H bond**	**Pi-Cation**
Glucosinoes	(A:245) LEU (A:247) ALA (A:246) GLY (A:248) TYR (A:250) LYS (A:239) ARG (A:185) LYS (A:141) ASP (A:138) PHE (A:92) ARG (A:88) ASN (A:244) ILE (A:243) THR (A:240)	(A:243) VAL (A:142) ALA (A:57)		(A:39) TRP (A:283)		

TABLE 4.5: Values of interaction of Protein Ligand complex.

Name of Compound	**Vander Waals aa**	**Pi-Alkayl**	**Pi-Sulfur**	**Alkyl**	**Conventional H bond**	**Pi-Cation**
Dialcohol	GLN (A:42) ARG (A:279) LYS (A:38) ASP (A:28) GLN (A:89) VAL (A:237) THR (A:240) ARG (A:241) ILE (A:43) VAL (A:39) GLN (A:145) ASP (A:35)		MET (A:23)	VAL (A:142)	GLN (A:42) LYS (A:271)	

TABLE 4.5: Values of interaction of Protein Ligand complex.

Name of Compound	**Vander Waals**	**Pi-Alkayl**	**Pi-Sulfur**	**Alkyl**	**Conventional H bond**	**Pi-Cation**
	ARG (A:279					
	GLU (A:46)					
	GLN (A:44)					
	GLY (A:45)					
	ILE (A:43)					
	GLN (A:48)					
	ARG (A:88)					
	GLN (A:58)					
	GLN (A:58)					
	SER (A:56)					
	LYS (A:141)					
	ASP (A:138)					
	ASN (A:182)					

The purified structure of protein & ligands was given as a input files to Patch dock in pdb format. The Patch dock automatically predict the interaction of target protein and ligands. The result of Patch dock gives 10 complex interaction between ligand and the Target Protein. These interaction was visualized by 3D Discovery Studio. Some of ligands such as sterol, Phenol, Polyphenol, Glucosinolates & Dialcohol give us good results as shown in the above Table. The interaction shows Van der Waals, Conventional Hydrogen Bond, Unfavorable Acceptor-Acceptor, Pi-Sulfur, Alkyl, Pi-Alkyl and Carbon Hydrogen Bond were shown in the above table 4.5.

4.8 ADMET Properties of Ligands

ADME properties of ligands extracted from pkCSM online tool. Toxicity provides insights into the nature of ligands, which must be considered before designing a drug. To use a compound as a chemotherapeutic agent, it must first be tested for toxicity. pkCSM is used to find the toxicity and ADMET properties of the drug. This server takes SMILES as input. The dragged server values are as follow.

4.8.1 Absorption

In pharmacology (specifically pharmacokinetics), the transfer of a drug from the bloodstream into the tissues is called absorption. So the chemical composition of a drug, as well as the environment into which a drug is placed.

TABLE 4.6: Absorption Properties of Ligands [108]

Ligand Name	**Water solubility**	**$CaCO_2$ Permeability**
Phytosterol	-6.773	1.201
Sterol	-5 .086	1.495
Dialcohol	-2.907	0.973
Phenol	-0.723	1.613
Glucosinolates	-2.683	-0.848

TABLE 4.7: Absorption Properties of Ligands [108]

Ligand Name	**Intestinal Absorption**	**Skin Permeability**
Phytosterol	94.464	-2.783
Sterol	94.633	-2.666
Dialcohol	92.84	-3.03
Phenol	93.055	-1.924
Glucosinolates	5.082	-2.735

TABLE 4.8: Absorption Properties of Ligands [108]

Ligand Name	**P-glycoprotein Substrates**	**P-glycoprotein I Inhibitor**	**P-glycoprotein Inhibitor**
Phytosterol	No	Yes	Yes
Sterol	No	No	No
Dialcohol	Yes	No	No
Phenol	No	No	No
Glucosinolates	Yes	No	No

4.8.2 Distribution

Distribution in pharmacology is a branch of pharmacokinetics which deals with the movement of drug within the body from one location to another location.

When a drug enters the systemic circulation by absorption or direct administration, it must be distributed into interstitial and intracellular fluids. The distribution was a properties of ADMET properties.

TABLE 4.9: Distribution Properties of Ligands [109]

Ligand Name	**VDss**	**Fraction Unbound**
Phytosterol	0.193	0
Sterol	0.523	0.088

TABLE 4.9: Distribution Properties of Ligands [109]

Ligand Name	VDss	Fraction Unbound
Dialcohol	-0.27	0.185
Phenol	0.131	0.533
Glucosinolates	-0.281	0.644

TABLE 4.10: Distribution Properties of Ligands [109]

Ligand Name	BBB Permeability	CNS Permeability
Phytosterol	-0.705	-1.705
Sterol	0.699	-1.657
Dialcohol	-0.524	-3.409
Phenol	-0.222	-1.824
Glucosinolates	-1.54	-4.048

4.8.3 Metabolism

Metabolism is the process of converting one compound into another with the help of enzymes. Mostly metabolism occurs in the plasma of blood, liver, intestine and lungs. Generally, the metabolic process will convert the drug into a more water-soluble compound by increasing its polarity.

TABLE 4.11: Metabolism Properties of Ligands [109]

Ligand Name	CYP2D6 Substrate	CYP3A4 Inhibitor	CYP2C19 Inhibitor
Phytosterol	No	Yes	Yes
Sterol	No	No	Yes
Dialcohol	No	No	No
Phenol	No	No	No
Glucosinolates	No	No	No

TABLE 4.12: Metabolism Properties of Ligands [109]

Ligand Name	**CYP3A4 Substrate**	**CYP3A2 Inhibitor**	**CYP2D6 Inhibitor**
Phytosterol	No	Yes	No
Sterol	Yes	No	Yes
Dialcohol	Yes	No	No
Phenol	No	No	No
Glucosinolates	No	No	No

4.8.4 Excretion

The organs involved in drug excretion are the kidneys, which play important role in excretion (renal excretion) and the liver (biliary excretion). Other organs may also be involved in excretion, such as the lungs for volatile or gaseous agents. Drugs can also be excreted in sweat, saliva and tears.

TABLE 4.13: Excretion Properties of Ligands [109]

Ligand Name	Total Clearance	Renal OCT_2 Substrate
Phytosterol	0.628	No
Sterol	0.955	No
Dialcohol	0.422	No
Phenol	0.208	No
Glucosinolates	0.375	No

4.8.5 Toxicity

The level of harm that a chemical compound or specific chemical combination can do to an organism is known as its toxicity. Toxicity can refer to an impact on an

organism as a whole, such as an animal, bacterium, or plant, as well as an impact on an organism's cells or organs, such the liver. Toxicity properties of ligands were shown in table 4.14 and table 4.15 and in table 4.16.

TABLE 4.14: Toxicity Properties of Ligands [109]

Model name	**AMES toxicity**	**Max. tolerated dose (human)**	**hERG 1 inhibitor**	**hERG 11 inhibitor**
Phytosterol	Yes	0.559	No	No
Sterol	No	-0.602	No	No
Dialcohol	Yes	-0.126	No	No
Phenol	No	0.54	No	No
Glucosinolates	Yes	0.438	No	No

TABLE 4.15: Toxicity Properties of Ligands [109]

Ligand Name	**Oral Rat Acute Toxicity**	**Oral Rat Chronic Toxicity**	**Hepatotoxicity**
Phytosterol	2.552	0.855	No
Sterol	1.772	1.292	No
Dialcohol	2.474	2.216	No
Phenol	2.153	2.011	No
Glucosinolates	2.537	3.72	Yes

TABLE 4.16: Toxicity Properties of Ligands [109]

Ligand Name	**Skin Sensitization**	**T. Pyriformis Toxicity**	**Minnow Toxicity**
Phytosterol	No	0.43	-1.802
Sterol	Yes	1.104	0.197
Dialcohol	No	0.365	2.766

TABLE 4.16: Toxicity Properties of Ligands [109]

Ligand Name	**Skin Sensitization**	**T. Pyriformis Toxicity**	**Minnow Toxicity**
Phenol	Yes	-0.198	2.034
Glucosinolates	No	0.285	4.236

4.9 Lipinski rule of 5

The Rule of 5 has the advantage of simplicity and a readily understood physico-chemical basis. Followings rules are:

1. Molecular weight 500
2. log P5
3. Number of H-bond donors 5
4. Number of H-bond acceptors 10.

So, rule was applied to our compound and analyses the different ligands of Olive Fruit extract is checked which are shown in table 4.17 [115].

TABLE 4.17: Toxicity Values of Ligands were identified with the help of Protox Server [110]

Physio-chemical properties	**Phyto-sterol**	**Sterol**	**Phenol**	**Glucosino-lates**	**Dialcohol**
LD_{50} Values	890mg/kg	500mg/kg	270mg/kg	1190 mg/Kg	1190mg/g
Toxicity class	4	4	3	4	4

4.10 Physiochemical Properties of Ligands

PkCSM is an online tool used to find the Absorption, Distribution, Metabolism, Excretion and Toxicity of ligands. In the present research study, the Lipinski rule has been used for filtration. Applicability of Lipinski rule on ligands was shown in below table. All ligands follow Lipinski rule of five but except few ligands such as Phytosterol, Glucosinolates.

4.11 Physiochemical Properties of Ligands

PkCSM is an online tool used to find the Absorption, Distribution, Metabolism, Excretion and Toxicity of ligands. In the present research study, the Lipinski rule has been used for filtration. Applicability of Lipinski rule on ligands was shown in below table. All ligands follow Lipinski rule of five but except few ligands such as Phytosterol, Glucosinolates. Table 4.18 shows the molecular weight, log P, Hydrogen bond acceptor and Hydrogen bond donor values of ligands of Olive Plant. A compound is considered a drug when it follows 3 or more rules and if a compound violates two or more rules it is considered poorly absorbed.

TABLE 4.18: Physicochemical properties of Ligands according to Lipinski rule of 5 [111]

Ligands Name	**Molecular weight**	**Num. H-bond acceptors**	**Num. H-bond donors**	**Log P Value**
Phytosterol	414.71g/mol	1	1	8.02
Sterol	248.40g/mol	1	1	3.99
Dialcohol	330.33g/mol	6	2	0.76
Phenol	94.11g/mol	1	1	1.39
Glucosinolates	437.51g/mol	11	5	6

4.12 Identification of Lead Compound

After a detailed analysis of protein and ligand interactions, docking scores and toxicity studies, the most active inhibitor was identified. The selected compound was our lead compound.

Physiochemical & Pharmacokinetics properties determine the final destiny of compound as drug or non-drug compounds. Physiochemical properties or Lipinski rule of five works as primary filter & pharmacokinetics studies as secondary filter in screening of potential compounds Phytosterol & Glucosinolates did not obey Lipinski rule of five so they knock out in primary screening.

On the basis of Binding Score, ADMET properties, Physiochemical properties & Lipinski rule of five, Sterol was selected as Lead compound which could inhibit target protein Ucp2.

4.13 Identification of Drugs

Berberine is most effective drug used for the PCOS. Drug bank Databases are used to identify this medication.

4.14 Selection of Drug

The most effective medication is chosen based on its Physiochemical and ADMET qualities. Physiochemical Properties are identified by using PubChem online database, while ADMET properties are determined using the PkCSM online tool.

4.15 Physiochemical Properties of Drug

PkCSM is an online tool used to find the Absorption, Distribution, Metabolism, Excretion and Toxicity of Drug. In the present research study, the Lipinski rule

has been used for filtration. Applicability of Lipinski rule on drug was shown in below table 4.19.

TABLE 4.19: Physiochemical Properties of Drug [112]

Properties	**Berberine**
Molecular weight	336.4g/mol
Chemical Formula	$C_{20}H_{18}NO_4$
Absorption	Not Available
Water Solubility	0.000354mg/Ml
Log P	3.0963
H- Bond Donor	0
H- Bond Acceptor	4
Bioavailability	1
Polarizability	136.92A3
Side Effect	Constipation Gas Upset stomach. Diarrhea

4.16 Berberine Drug

Berberine (BBR) an isoquinoline alkaloid, is a main component of many commonly used medicinal plants, such as Coptis chinensis Franch, Hydrastis canadensis L and Coptis japonica Makino. They are all generally believed to have the excellent effects of clearing heat, removing dampness, purging fire and detoxifying Makino. BBR can intervene the pathological process of PCOS by multiple pathways and targets [116].

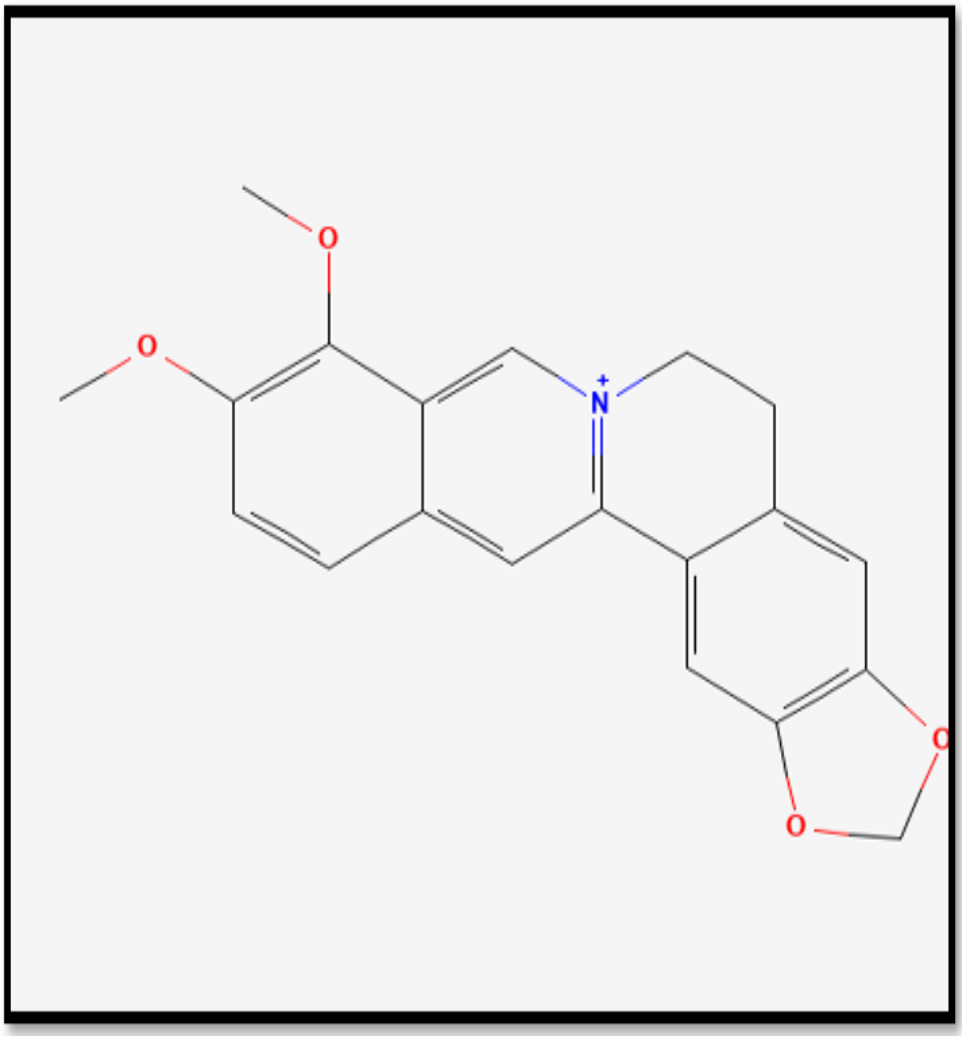


FIGURE 4.8: Berberine as drug.

The study of BBR as a PCOS treatment has gradually grown in recent years. The fundamental mechanism of BBR against PCOS is the reduction of androgen levels, which has been demonstrated in mice. Inhibiting androgen binding to AR and promoting insulin binding to insulin receptor are BBR's two primary PCOS therapeutic strategies [116].

4.17 ADMET Properties of Berberine

ADMET properties of Drug extracted from pkCSM online tool. Toxicity provides insights into the nature of drug, which must be considered before designing a drug. To use a compound as a chemotherapeutic agent, it must first be tested for

toxicity. pkCSM is used to find the toxicity and ADMET properties of the drug. This server takes SMILES as input. The dragged server values are as follow.

4.17.1 Absorption Properties

In pharmacology (specifically pharmacokinetics), the transfer of a drug from the bloodstream into the tissues is called absorption. So the chemical composition of a drug, as well as the environment into which a drug is placed, work together to determine the rate and extent of drug absorption. Shown in table 4.20.

TABLE 4.20: Absorption Properties of Drug [113]

Model Name	**Predicted Values**
Water Solubility	-3.973
CaCO2 Permeability	1.734
Intestinal absorption (human)	97.147
Skin Permeability	-2.576
P-glycoprotein substrate	Yes
P-glycoprotein 1 inhibitor	No
P -glycoprotein 11 inhibitor	Yes

4.17.2 Distribution Properties

Distribution in pharmacology is a branch of pharmacokinetics which deals with the movement of drug within the body from one location to another location. When a drug enters the systemic circulation by absorption or direct administration, it must be distributed into interstitial and intracellular fluids as shown in table 4.21 .

TABLE 4.21: Distribution Properties of Drug [113]

Model Name	**Predicted Values**
VDss(Human)	0.58

TABLE 4.21: Distribution Properties of Drug [113]

Model Name	**Predicted Values**
Fraction Unbound (human)	0.262
BBB Permeability	0.198
CNS Permeability	-1.543

4.17.3 Metabolic Properties

Metabolism is the process of converting one compound into another with the help of enzymes. Mostly metabolism occurs in the plasma of blood, liver, intestine and lungs.

Generally, the metabolic process will convert the drug into a more water-soluble compound by increasing its polarity in table 4.22.

TABLE 4.22: Metabolic Properties of Drug [114]

Model Name	**Predicted Values**
CYP2D6 substrate	No
CYP3A4 substrate	Yes
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
CYP2C9 inhibitor	No

4.17.4 Excretion Properties

The organs involved in drug excretion are the kidneys, which play important role in excretion (renal excretion) and the liver (biliary excretion). Other organs may also be involved in excretion, such as the lungs for volatile or gaseous agents. Drugs can also be excreted in sweat, saliva and tears. As shown in the table 4.23.

TABLE 4.23: Excretion Properties of Drug [114]

Model Name	**Predicted Values**
Total Clearance	1.27
Renal OCT2 Substrate	No

4.17.5 Toxicity Properties

The level of harm that a chemical compound or specific chemical combination can do to an organism is known as its toxicity. Toxicity can refer to an impact on an organism as a whole, such as an animal, bacterium, or plant, as well as an impact on an organism's cells or organs, such the liver. Toxicity properties of Drug were shown in Table 4.24.

TABLE 4.24: Toxicity Properties of Drug [114]

Model Name	**Predicted Values**
AMES toxicity	Yes
Max. tolerated dose (human)	0.144
hERG 1 inhibitor	No
hERG 11 inhibitor	No
Oral Rat Acute Toxicity (LD_{50})	2.571
Oral Rat Chronic Toxicity (LOAEL)	1.89
Hepatotoxicity	Yes
Skin Sensitization	No
T. Pyriformis toxicity	0.354
Minnow toxicity	-0.277

Toxicity Values of Drug were identified with the help of Protox Server in below Table 4.25.

TABLE 4.25: Toxicity Values of Drug were identified with the help of Protox Server [115]

Physiochemical properties	**Predicted values of Berberine**
LD_{50} Values	200mg/kg
Toxicity class	3

4.18 Drug Docking

Results of Patch Dock with Drug name, Binding Energy, Cavity Size, Grid Map & Max. And Mini. Energy Values as shown in below table 4.26.

TABLE 4.26: Shows results of CB Dock with drug [115]

Drug Name	**Binding Energy**	**Cavity Size**	**Grid Map**
Berberine	-7.3	700	22

4.19 Drug and Lead Compound Comparison on the Bases of ADMET Properties

The comparison between physiochemical properties of Berberine and Sterol.

4.19.1 Absorption Properties Comparison

The Absorption properties of drug and lead compound.

TABLE 4.27: Absorption properties of drug and lead compound [115]

Model name	**Berberine**	**Sterol**
Water Solubility	-3.973	-5.086

TABLE 4.27: Absorption properties of drug and lead compound [115]

Model name	**Berberine**	**Sterol**
$CaCO_2$ Permeability	1.734	1.495
Intestinal Absorption	97.147	94.633
Skin Permeability	-2.576	-2.666
P-glycoprotein substrate	Yes	No
P-glycoprotein 1 inhibitor	No	No
P-glycoprotein 11 inhibitor	Yes	No

4.19.2 Distribution Properties of Comparison

The Distribution properties of drug and lead compound.

TABLE 4.28: Distribution properties of drug and lead compound [115]

Model Name	**Berberine**	**Sterol**
VDSS	0.58	0.523
Fraction unbound	0.262	0.088
BBB permeability	0.198	0.699
CNS	-1.543	-1.657

4.19.3 Metabolic Properties of Comparison

The Metabolic properties of drug and lead compound.

TABLE 4.29: Metabolic properties of drug and lead compound [115]

Model name	**Berberine**	**Sterol**
CYP2D6 substrate	No	No
CYP3A4 substrate	Yes	No
CYP1A2 inhibitor	Yes	Yes
CYP2C19 inhibitor	No	No

TABLE 4.29: Metabolic properties of drug and lead compound [115]

Model name	**Berberine**	**Sterol**
CYP2D6 inhibitor	Yes	Yes
CYP3A4 inhibitor	Yes	No
CYP2C9 inhibitor	No	No

4.19.4 Excretory Properties of Comparison

The organs involved in drug excretion are the kidneys, which play important role in excretion (renal excretion) and the liver (biliary excretion).

TABLE 4.30: Excretory properties of drug and lead compound [115]

Model Name	**Berberine**	**Sterol**
Total Clearance	1.27	0.955
Renal OCT_2 Substrate	No	No

4.19.5 Toxicity Properties of Comparison

The Toxicity properties of drug and lead compound.

TABLE 4.31: Toxicity properties of drug and lead compound [115]

Model Name	**Berberine**	**Sterol**
AMES toxicity	Yes	No
Max. tolerated dose (human)	0.144	-0.602
hERG 1 inhibitor	No	No
hERG 11 inhibitor	No	No
Oral Rat Acute Toxicity (LD50)	2.571	1.772
Oral Rat Chronic Toxicity (LOAEL)	1.89	1.292
Hepatotoxicity	Yes	No
Skin Sensitization	No	Yes

TABLE 4.31: Toxicity properties of drug and lead compound [115]

Model Name	**Berberine**	**Sterol**
T. Pyriformis toxicity	0.354	1.104
Minnow toxicity	-0.277	0.197

4.20 Comparison of the Drug and Lead Compound on the base of Lipinski rule of 5

The comparison between physiochemical properties of Berberine and Sterol is important steps that help us to find out the drug activity manner and biochemical reactivity portion. So the comparison between physiochemical properties of Berberine and Sterol were shown in Table 4.32.

TABLE 4.32: Comparison of Physiochemical Properties of Drug and Lead Compound [115]

Model Name	**Berberine**	**Sterol**
Molecular Weight	336.4g/mol	248.40g/mol
H-donor	0	1
H-Acceptor	4	1
Log P value	3.096	3.99

The comparison between physiochemical properties of Berberine and Sterol is important steps that help us to find out the drug activity manner and biochemical reactivity portion. So the comparison between physiochemical properties of Berberine and Sterol were shown in table 4.32. So from physiochemical properties

it is determine that the log P value, hydrogen bond acceptor, hydrogen bond donor and molecular weight of Sterol is less as compere to Berberine So it means that Sterol can act as inhibitor for inhibition of protein such as UCP2 which involved in PCOS.

4.21 Discussion

One of the most prevalent hormone diseases in women of reproductive age is Polycystic Ovarian Syndrome (PCOS), which accounts for between 5 and 10 percent of all problems in this age group[[1], [2]. Other names for the condition include multicystic ovaries, schlerocystic ovaries, and Stein Leventhal syndrome. Polycystic ovarian syndrome is sometimes referred to as PCOS or PCOD (Polycystic Ovarian Disorder). Infertility, obesity, anovulation, and insulin resistance (IR) are also linked to a longer incidence of cysts. It has been established that the disorders of the metabolic syndrome and PCOS are connected. Rotterdam criterion is the most widely used diagnosis standard for PCOS [3]. The typical form of PCOS was initially described by Ashtyn and Leventhal in 1935 [4]. Ovulatory dysfunction, the clinical manifestations of polycystic ovaries and hyperandrogenism features of polycystic ovarian syndrome. Ovarian dysfunction continues to be the primary symptom, making this disease the primary contributor to ovulatory-related infertility. A woman's hormone levels may be impacted by PCOS, a polygenic and multifactorial syndromic condition. It is the most prevalent endocrine condition in women of reproductive age and is frequently accompanied by comorbidities, such as obesity, hyperinsulinemia, and infertility [5]. Medical plants, also known as medicinal herbs, are those plants with curative qualities and beneficial medicinal effects on the human or animal body. Drug development employs a variety of plant parts, including leaves, seeds, roots, flowers, and sometimes the entire plant. Most compounds contain bioactive substances that are employed as therapeutic agents because they have direct or indirect therapeutic effects. Therefore, these plants are used as complementary medicine all over the world. But today, most people use therapeutic plants. Prenylated flavonoids, isoflavones, coumestans, and lignans are

examples of the wide class of nonsteroidal, diphenolic, estrogenic plant chemicals known as phytestrogens. They are nonsteroidal chemicals generated from plants that share structural or functional similarities with mammalian estrogen (E2), particularly 17β-estradiol. Poly-phenolic substances known as phytestrogens contain more than 100 molecules [6].

Olives (*Olea europaea, Oleaseae*) are a long-lived tree that contain Phytoestrogens and are valuable to Mediterranean residents economically and culturally. Numerous flavonoid and polyphenolic chemicals with anti-inflammatory, anticancer, antidiabetic, gastro protective, and wound-healing effects are present in this plant's leaves [7]. The Oleaceae family member olive (Oleaeuropea), which contains lignans and phenolic compounds, is regarded as a Phytoestrogen plant chemical. Oleuropein and various forms of flavonoids including rutin, apigenin, and luteolin are only a few of the many components found in olive leaves. Olive leaves are a plentiful by-product of olive tree cultivation and olive mills, and they have historically been linked to a variety of medical claims, albeit few of these have been supported by experimental research. Stilbenes, phenolic acid, and flavonoids are found in olive. Oleuropein possesses anti-inflammatory, antioxidant, and antihyperlipidemia properties. Due to its laxative effects, it is also helpful in treating digestive issues [8], [9] and [10].

The insilico results evaluation of Ligands of olive fruit as a therapeutic agent for PCOS. For this purpose six Ligands of olive fruit were identified from literature. The structure of ligands was visualized in Pub chem and patch dock was used by docking purpose. After that the protein ligand interaction of these ligands were analyzed by using 3D discovery studio. After the comprehensive analysis of their physiochemical properties including molecular weight, log p value, H-bond Acceptors, H-bond donors are visualized as potent inhibitors. The above mentioned properties of selected ligands Phenol, Polyphenol, Phytosterol, Sterol, Squalene, Alkaloids shows the best activates but not all follow the Lipinski rule of 5 so they knock out. Among the six above mentioned ligands Sterol can b suggested as Potent lead compound because it follow all the properties of as MW, log P value, H-bond donor, H-bond Acceptors.

Chapter 5

Conclusions and Recommendations

One of the most prevalent hormonal diseases in women of reproductive age is Polycystic Ovarian Syndrome (PCOS), which accounts for between 5 and 10 percent of all problems in this age group. Other names for the condition include multicystic ovaries, schlerocystic ovaries, and Stein Leventhal syndrome. Polycystic ovarian syndrome is sometimes referred to as PCOS or PCOD (Polycystic Ovarian Disorder). Infertility, obesity, anovulation, and insulin resistance (IR) are also linked to a longer incidence of cysts.

It has been established that the disorders of the metabolic syndrome and PCOS are connected. Rotterdam criterion is the most widely used diagnosis standard for PCOS. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are produced in response to GnRH, are the two main pituitary hormones that are crucial for the reproductive system of women. In women with PCOS, gonadotrophin-releasing hormone (GnRH) causes an excess of LH and a deficiency of FSH.

PCOS is influenced by both hereditary and environmental factors which includes genetics, sedentary lifestyle and hormonal changes. It is crucial that women must have to exercise to meet their bodies physiological needs. Exercise and physical

activity cause various hormone levels to rise or fall in comparison to resting levels. The production of Estrogen and Steroid hormones is decreased by exercise. Increased physical activity combined with calorie restriction in lifestyle intervention studies has been shown to improve ovulatory function, circulating androgen levels, inflammatory pattern, and insulin sensitivity in PCOS-afflicted women.

The aim of this study was to find a bioactive compounds found in olive fruit that might potentially be used as an effective medicine to treat PCOS by utilizing different computational tools. Ten ligands were chosen for the current study from the above mentioned fruit for further drug designing against UCP2 Protein which is a key player of PCOS. UCP2 protein also underwent virtual screening before molecular docking. The docking trials were carried out using Patch Dock. By using 3D Discovery studio, the interactions between these ligands and proteins were also explored.

The first objective was to identify the target protein which is involved in PCOS progression from PDB. The 2D structure of UCP2 protein was visualized in PubChem for amino acid residues. The roles of uncoupling protein-2 (UCP2) on the androgen synthesis of granulosa cells derived from patients with polycystic ovary syndrome (PCOS). In humans, the UCP2 gene is found on chromosome 11q13, which is associated with obesity and energy homeostasis. UCP2 proteins belongs to a family of mitochondrial carriers found in the inner membrane of mitochondria which play a major role in the development of follicles in PCOS. Normal expression of the UCP2 protein may play a role in regulating follicle growth as well as the maturity and quality of oocytes.

The second objective was Insilico evaluation of bioactive compounds of Olive Fruit as a therapeutic agent against PCOS for drug development. For this purpose, ten ligands were identified from literature. The structure of ligands was visualized in PubChem and Patch dock used for docking purpose. After that the protein ligand interaction of theses ligands were analyzed by using 2D discovery studio. After the comprehensive analysis of their physiochemical properties including molecular weight, log P value, H-bond Acceptors, H-bond donor are visualized as potent

inhibitors. The above-mentioned properties of Sterol, Dialcohol, Phenol, Polyphenol, Glucosinolates showed the best activates but not all follow the Lipinski rule of 5 so they knock out. Sterol found in Olive fruit was considered as best bioactive compound against UCP2 protein.

Berberine is considered as herbal medicine locally available in the market against PCOS. The efficacy of lead compound Sterol obtained after molecular docking analysis was further compared with already available Berberine drug in market. So that the alternative treatment of PCOS with herbal origin having high efficacy as compared to standard must be further evaluated for drug development and clinical trials.

Future Prospective

In-silico analysis must be evaluated on animal model for further clinical trials and the efficacy must also be evaluated as a potential therapeutic.

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