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Investigation of Phytoestrogens Found in Sesame Seeds via Computational and Translational Approaches

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ABSTRACT

Polycystic Ovarian Syndrome (PCOS) is a common endocrine disorder that primarily impacts reproductive-aged women. Phytoestrogens, also known as plant oestrogens, are naturally occurring compounds with a chemical structure similar to that of human oestrogen. In this study, rational and translational approaches were used to check the efficacy of phytoestrogens found in sesame seeds. The objectives included the prediction and comparison of proteins responsible for PCOS, that is, MADH4 and phytoestrogens (ascorbic acid and thiamine) present in sesame seeds. Moreover, their interaction and inhibition of the target protein through molecular docking as a rational layout were recorded. The effects of these phytochemicals were further confirmed by using PCOS induced *Sprague dawley* rats with standard drug Clomiphene citrate. In the translational study, PCOS was induced in an animal model and ovulation and estrous cycles were observed. Afterwards, the effects of the crude extract of sesame seed were further confirmed by measuring the levels of progesterone, testosterone, and estrogen serum levels in control, positive control, and all the sesame seed and standard drug treated groups. Two lead compounds namely ascorbic acid and thiamine obtained from sesame seeds showed the best results as compared to the standard drug Clomiphene citrate which imparts immunotoxicity against MADH4 protein responsible for the onset of PCOS. Furthermore, different stages of ovulation namely proestrus, metestrus, estrus, and diestrus were observed with vaginal smears obtained in the disease induction time period. The results obtained from the hormonal profile of all the groups indicated that the progesterone, estrogen, and testosterone levels were statistically significant with *p*-values less than 0.05. The findings indicated that sesame seeds possess the capability to suppress the expression of MADH4, while also maintaining the regulated hormone levels.

Keywords: animal model, Clomiphene citrate, hormones, ligands, molecular docking, sesame seeds

Highlights

1. Identifying Inhibitory Compounds: The research discovered the therapeutic potential of sesame seeds that can inhibit the MADH4 protein, a significant factor in PCOS.
2. Integrated Approach: The research combined computational and experimental methods to assess sesame seeds as a potential PCOS treatment, offering both theoretical insights and real-world testing using an animal model.

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3. Positive Treatment Impact: Using sesame seed extract leads to statistically significant improvements in hormone levels, offering promise for innovative PCOS treatment options.

1. INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is a hormonal imbalance condition affecting the women of reproductive age. It is characterized by the formation of multiple cysts in the ovaries, leading to disruptions in menstrual cycle and fertility issues. It is associated with various other conditions, such as hirsutism, acne, insulin resistance, type II diabetes, sleep apnea, anxiety, and depression. The global prevalence of PCOS is estimated to be 5-10% and it remains a significant contributor to endometrial cancer. The condition is also linked to several metabolic disorders, making its diagnosis challenging due to its multifactorial and complex nature [1].

PCOS is influenced by both genetic and environmental factors [2]. An unhealthy lifestyle, diet choices, and infectious mediators can impact hormone levels, such as luteinizing-hormone (LH), follicular stimulating hormone (FSH), prolactin, and gonadotropin-releasing hormone (GnRH). Additionally, a specific gene called 'PCO' located on chromosome 19 has been associated with PCOS [3]. In PCOS patients, elevated androgen levels lead to subcellular aberrations in theca cells, causing increased androgen secretion. This intrinsic activation also affects granulosa cells, resulting in higher levels of serum anti-mullerian hormone as compared to normal women [1].

Moreover, mothers against decapentaplegic homolog 4 (MADH4) protein plays a crucial role in mammalian signaling pathways and is involved in PCOS. This protein is a member of the SAMD family. The SAMD4 protein has two distinct functional domains. The tridimensional structure of MH1 and MH2 is characterized by the presence of regions M and H, which correspond to MAD homology. There exists a notable resemblance between the SAMD4 protein found in mammals and its counterpart in drosophila species. In human genome, the gene is located at chromosome 18 [4].

The Rotterdam criteria are currently used to diagnose PCOS. The fulfillment of Rotterdam's two out of three criteria namely polycystic ovaries, anovulation, and biochemical or chemical indicators of hyperandrogenism is required for diagnosis. PCOS is presumably involved in endogenous insulin resistance, which can be worsened by obesity. Adipocytes from obese PCOS patients may not show significant reductions in insulin receptor number and affinity, although they may exhibit blunted insulin inhibition of lipolysis and reduced maximal glucose utilization. Metformin is the preferred drug for PCOS due to its insulin-sensitizing properties, though Clomiphene citrate and glucocorticoids are also used but with associated side effects [5].

Complementary and alternative medicine (CAM) offers various treatment options including meditation, lifestyle modification, yoga, acupuncture, vitamins, and herbal medicine including phytoestrogens, which can reduce hyperandrogenism, insulin resistance, and ovarian weight in PCOS [6].

Sesame (Sesamum *indicium L.*) is a cultivated oil crop rich in phytochemical compounds, such as lignans, polyphenols, and phytosterols found in its seeds and oil. These components, particularly the phytoestrogens and antioxidants in lignans,

have the potential to influence the pituitary-gonadal axis and enhance fertility indirectly. Sesamin, present in sesame, enhances antioxidant activity by protecting cells from oxidative stress and reducing reactive oxygen species (ROS). The consumption of white sesame oil shows promising effects on heart and kidney, reduces hyperglycemia, and alleviates diabetes-related side effects [2].

The current study aims to explore the potential of phytochemicals present in sesame seeds against PCOS. It also extends the relationship between MADH4 protein and sesame seed. Computational and experimental methods were used to evaluate the efficacy of sesame seeds as a PCOS potent inhibiting drug. Clomiphane citrate is a drug being used for PCOS treatment and it contains bioactive chemical compounds such as ascorbic acid and thiamine. Using the translational approach, female rats were induced with PCOS and their ovulation and estrous cycles were investigated. The effect of crude extract of sesame seeds alone or in combination with a standard drug was examined to find out the desired interactions for PCOS management.

2. MATERIALS AND METHODS

2.1. Retrieval of Sequence and Structure of the Target Protein

The primary sequence of MADH4 was obtained from the UniProt database (https://www.uniprot.org/) under the accession number Q13485 in FASTA format. The 3D structure of the chosen templates was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/) under the identifier 6YIC. Further, ProtParam (https://web.expasy.org/protparam/) was utilised to ascertain the physicochemical properties of the protein of interest.

2.2. Retrieval of Ligand Structure

The ligands derived from sesame seeds were chosen as chemical compounds, sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The ligands chosen for this study included ascorbic acid, folic acid, and thiamine. These compounds were selected for further screening based on Lipinski's rule of five and ADMET characteristics. The analyses were performed using SwissADME (http://www.swissadme.ch/) , whereas toxicity assessment was conducted using ProTox-II (https://tox-new.charite.de/) .

2.3. Molecular Docking of Target Protein

Following the screening process, ligands that did not meet the criteria based on ADMET characteristics and Lipinski's rule of five were excluded. The subsequent phase involved molecular docking. The online docking server PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/) was employed for the docking process of ligands with the target protein MADH4. The software BIOVIA Discovery Studio [3] was employed for visualizing PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/) results and assessing the interaction between protein-ligand and protein-standard drug.

Moreover, Clomiphene citrate (a standard drug) was selected to be compared with the lead compound. The standard drug was assessed through SwissADME (http://www.swissadme.ch/) and ProTox-II (https://tox-new.charite.de/) for pharmacokinetics, physicochemical, and toxicity analysis.

2.4. Ethical Approval and Animals

This study was approved by the Department of Pharmacy, Capital University of Science and Technology

(CUST). Fifteen (15) healthy adult female albino rats, weighing 150-200g and aged 8-9 weeks, were acquired from the animal house of the Pharmacy Department, CUST. These rats were housed under controlled conditions with a temperature of 23-25°C and a natural light/dark period.

2.5. Estrous Cycle Confirmation

Vaginal smear method was used to detect normal estrous cycle. For the assortment of vaginal smear tests, 0.2 - 0.3 ml of typical saline was flushed into the vaginal hole by utilizing a dropper. In order to conduct a microscopic examination, a drop of the sample fluid was taken from the vaginal orifice, placed on the glass slide, air-dried, covered with a cover slip, and stained with Giemsa stain.

2.6. Extract Preparation

In this experiment, locally available white sesame seeds (0.25kg) were ground into fine powder. The powder was mixed with 50% ethanol in a conical flask, covered with cotton plugs and an aluminum foil, and left to shake at 25°C for 48 hours. After filtering and centrifuging, the solution was evaporated to obtain the sesame extract powder, which was then dissolved in saline for the desired concentration [4].

2.7. Standard Drug

The standard drug Clomiphene citrate was used against PCOS in this experiment [5].

2.8. PCOS Induction

PCOS was induced in rats using Estradiol Valerate (0.4ml/200g) through hormonal induction until the disease developed, confirmed by changes in the ovulation cycle [7]. Estradiol Valerate was purchased as Gravibinan 2 ml injections from Al Latif Pharmacy (Pvt) Ltd.

2.9. Experimental Design

The animals were divided into 5 groups with each group containing 3 rats. Group 1 was the control group that was given the normal saline (0.2ml/150g) orally. Group 2 comprised the positive control group treated with about (0.3ml/150g) of EV orally for PCOS induction. Group 3 was treated with the drug Clomiphene citrate (15 mg / 150g), given orally for 2 weeks. Group 4 was the sesame seed group, treated with sesame seeds extract (30mg / 150g) orally for 2 weeks. Group 5 was treated with a combination of sesame seed extract and Clomiphene citrate given orally for 2 weeks in 1:1.

2.10. Biochemical Analysis

Treated rats were anesthetized and 5ml blood samples were collected using 5cc syringes. The samples were stored in yellow-topped gel vials. The serum extracted after centrifugation was utilized for hormonal analysis, including testosterone, progesterone, and estrogen levels measurement.

2.11. Statistical Analysis

The data was analyzed using one-way ANOVA to compare the levels of gonadotropin hormones (progesterone, testosterone, and estrogen) in the five groups. The results were expressed in the form of mean and standard error of mean.

3. RESULTS

3.1. Primary Sequence Retrieval

The primary sequence of the target protein MADH4 was obtained in FASTA format from the UniProt database (https://www.uniprot.org/). The sequence was retrieved using the accession ID Q13485. It had a total length of 552 residues (Figure 1).

>sp|Q13485|SMAD4_HUMAN Mothers against decapentaplegic homolog 4 OS=Homo sapiens OX=9606 GN=SMAD4 PE=1 SV=1
MDNMSITNTPTSNDACLSIVHSLMCHRQGGESETFAKRAIESLVKKLKEKKDELDSLITA
ITTNGAHPSKCVTIQRTLDGRLQVAGRKGFPHVIYARLWRWPDLHKNELKHVKYCQYAFD
LKCDSVCVNPYHYERVVSPGIDLSGLTLQSNAPSSMMVKDEYVHDFEGQPSLSTEGHSIQ
TIQHPPSNRASTETYSTPALLAPSESNATSTANFPNIPVASTSQPASILGGSHSEGLLQI
ASGPQPGQQQNGFTGQPATYHHNSTTTWTGSRTAPYTPNLPHHQNGHLQHHPPMPPHPGH
YWPVHNELAFQPPISNHPAPEYWCSIAYFEMDVQVGETFKVPSSCPIVTVDGYVDPSGGD
RFCLGQLSNVHRTEAIERARLHIGKGVQLECKGEGDVWVRCLSDHAVFVQSYYLDREAGR
APGDAVHKIYPSAYIKVFDLRQCHRQMQQQAATAQAAAAAQAAAVAGNIPGPGSVGGIAP
AISLSAAAGIGVDDLRRLCILRMSFVKGWGPDYPRQSIKETPCWIEIHLHRALQLLDEVL
HTMPIADPQPLD

Figure 1. MADH4 Protein Sequence

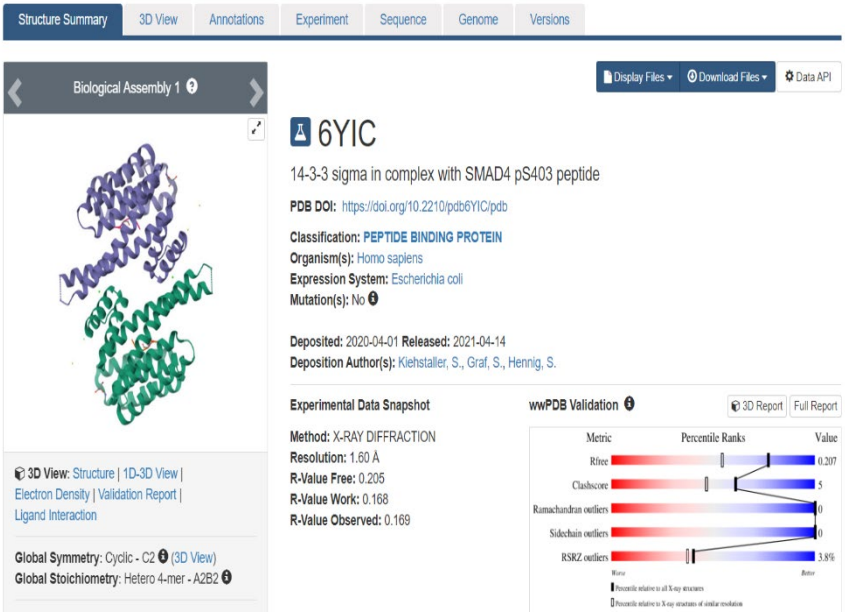


Figure 2. 3D Structure of Target Protein in PDB Database

3.2. Target Protein 3D Structure

The three-dimensional (3D) structure of MADH4 was obtained by retrieving the corresponding data from the Protein Data Bank (PDB) (https://www.rcsb.org/). The selected template was identified as 6YIC (Figure 2). The protein was obtained by using the experimental technique of X-ray diffraction, having a resolution of 1.60 Å.

3.3. Physicochemical Properties of Target Proteins

Physicochemical properties of the MADH4 protein were estimated using the online tool

ProtParam (https://web.expasy.org/protparam/). The online application was utilized for the computation of various physical and chemical properties pertaining to the specific proteins of interest. The calculated parameters covered several aspects, such as theoretical PI, amino acid composition (positive and negative charge), atomic aliphatic index, and grand average of hydropathicity (GRAVY) (Table 1).

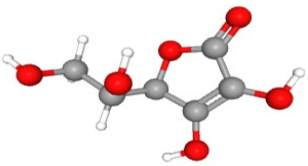
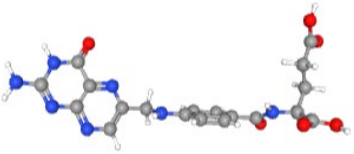
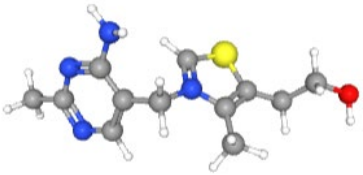
3.4. Retrieval of Ligand

The ligands selected for the current study consisted of ascorbic acid, folic acid, and thiamine. The ligands were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Table 2). molecular weight, instability index, compound, extinction coefficient,

Molecular weight	pI	Negatively charged residues	Positively charged residues	Ext. coefficient 1	Ext. coefficient 2	Instability index	Aliphatic index	Gravy
60439.14	6.5	51	44	71695	70820	51.12	75.49	-0.388

Table 1. Physicochemical Results of MADH4 Protein

Table 2. Ligand 3D Structures Retrieved from PubChem

S #	Ligand Name	Molecular Formula	Molecular Weight	3D Structure
1.	Ascorbic acid	$C_6H_8O_6$	176.12 g/ mol	
2.	Folic acid	$C_{19}H_{19}N_7O_6$	441.4 g/mol	
3.	Thiamine	$C_{12}H_{17}N_4OS^+$	.36 /mol	

3.5. Ligands Screening According to Lipinski's Rule of Five

The Lipinski's rule of five was applied to the selected ligands derived from sesame seeds using SwissADME (http://www.swissadme.ch/). The screening process revealed that folic acid exhibited two violations of the rule. Hence, folic acid was excluded (Table 3).

Table 3. Lipinski's Rule of Five Results

Ligands	Lipinski's Rule Violations
Ascorbic acid	0 violation
Folic acid	2 violations
Thiamine	0 violation

3.6. Ligands Screening for Toxicity

Ascorbic acid and thiamine were analyzed for toxicity through ProTox-II (https://tox-new.charite.de/). Neither of the ligands showed any signs of toxicity (Table 4).

Table 4. Toxicity Analysis of Ligands

Toxicity Targets	Ascorbic acid	Thiamine
Hepatotoxicity	Inactive	Inactive
Carcinogenicity	Inactive	Inactive
Immunotoxicity	Inactive	Inactive
Mutagenicity	Inactive	Inactive
Cytotoxicity	Inactive	Inactive

3.7. Comparison of Leading Compounds and Standard Drug

Physiochemical characteristics including absorption, distribution, metabolism, and elimination, were assessed using the SWISS ADME (http://www.swissadme.ch/) property calculator, an online tool. Based on the calculations performed, it was found that substances with high gastrointestinal (GI) absorption and compliance to Lipinski's rule exhibited more druglikeness, as compared to Clomiphene citrate (Table 5).

Table 5. ADME Properties and Druglikeness of the Selected Chemical Compounds

Chemical Compounds	Molecular Weight	Molecular Refractivity	iLOGP	GI Absorption	Lipinski's Rule
Ascorbic acid	C6H8O6	35.12	0.39	High	0 violation
Thiamine	C12H17N4OS+	73.26	-1.60	High	0 violation
Clomiphene citrate	C32H36ClNO8	161.99	4.53	Low	1 violation

Table 6. Toxicity Comparison

Toxicity Targets	Ascorbic acid	Thiamine	Clomiphene citrate
Hepatotoxicity	Inactive	Inactive	Inactive
Carcinogenicity	Inactive	Inactive	Inactive
Immunotoxicity	Inactive	Inactive	Active
Mutagenicity	Inactive	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive

ProTox-II (https://tox-new.charite.de/) was used to predict outcomes pertaining to toxicity. It was determined that ascorbic acid and thiamine did not exhibit any harmful effects, while the standard drug Clomiphene citrate demonstrated immunotoxicity (Table 6).

3.8. Molecular Docking

The process of molecular docking was performed using PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/) with the aim to analyze the interaction between the protein and the two selected ligands, namely ascorbic acid and thiamine. Prior to docking with the protein, the PDB file of MADH4 was subjected to visualization in BIOVIA Discovery Studio software to eliminate water molecules and undesirable ligands.[8] The ligand structures were converted into PDB format in order to facilitate their input into PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/). Moreover, the file with the highest docking score was acquired for the purpose of visualization and analysis of interactions (Table 5).

Table 7. Docking Scores

Ligands	Docking Score
Ascorbic acid	2384
Thiamine	3550

The visualization and analysis of docking results and ligand interaction with the target protein were recorded using BIOVIA Discovery Studio software (Figure 3 and 4) [3].

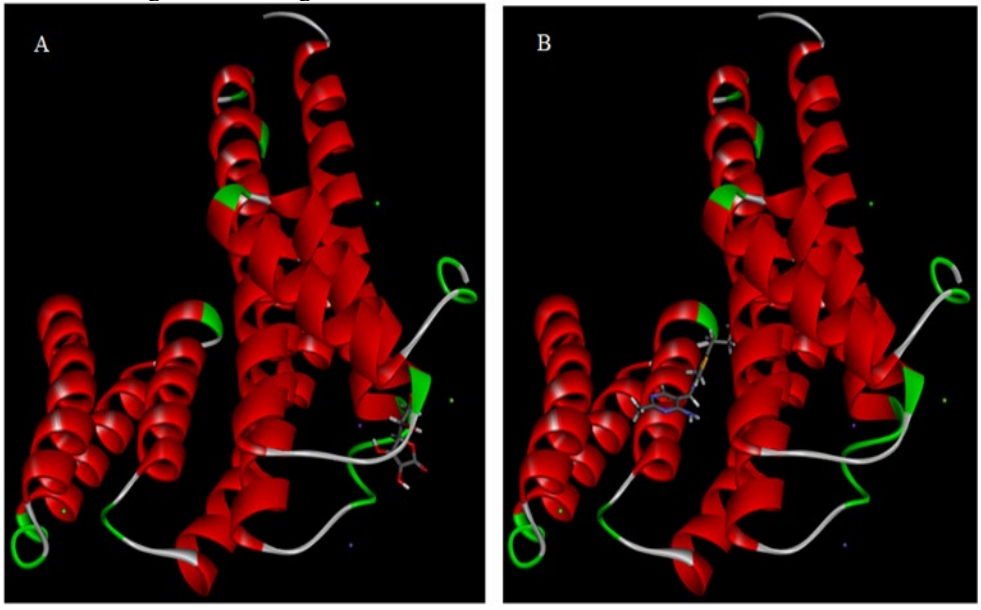


Figure 3. **(A)** Ligand Protein Complex of Ascorbic Acid with MADH4. (B) Ligand-Protein Complex of Thiamine with MADH4

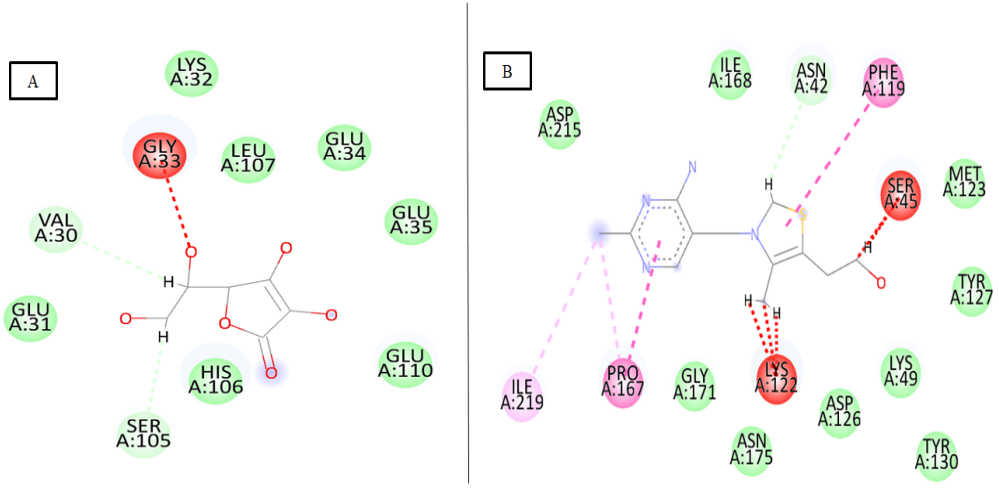


Figure 4. (A) Binding Interaction of Ascorbic Acid with MADH4 Protein (B) Binding Interaction of Thiamine with MADH4 Protein

3.9. Estrous Cycle Detection

The poliestric nature of female rodents, characterised by spontaneous ovulation and successive, regular estrous cycles, can differ among species and age groups. Additionally, light, the seasons, and life circumstances impact these cycles. Conversely, in laboratory settings where environmental control is implemented, estrous cycles transpire in rats irrespective of seasonal variation [8]. Estrous cycles are distinguished by morphological modifications in the vagina, ovaries, and uterus [9]. These modifications transpire throughout distinct phases referred to as proestrus, estrus, metestrus, and diestrus [10]. The identification of these phases is commonly based on the cell types that are observed in vaginal smears.

Therefore, before inducing PCOS, estrous cycle cytology was observed. The cytology of the vaginal smear revealed the ovulation cycle. Stained slides were seen under the microscope at 40X magnification. Different types of cells were tracked in different slides confirming the presence of ovulation, as shown in Figure 5. Figure 5 (A-D) confirms the regularity and normal estrous cycle of ovulating rat and is further guaranteed by the presence of all of kinds of cells and phases.

The proestrus phase was detected by the abundance of round shaped nucleated cells, found individually or in the form of clusters. The estrus phase was detected by the abundance of cornified and anucleated epithelial cells. The metestrus phase was detected by the abundance of leucocytes, while the diestrus phase was detected by the abundance of leucocytes that were polymorphonuclear in structure. However, hormonal profiling was performed in the subsequent week for confirmation, so as the authenticity was not affected.

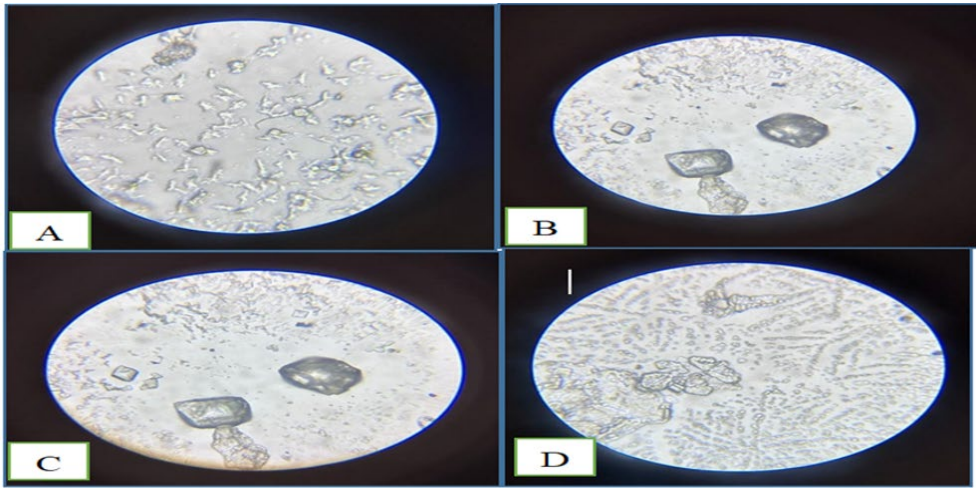


Figure 5. Estrous Cycle Observation: A. Proestrus, B. Metestrus, C. Estrus, and D. Diestrus

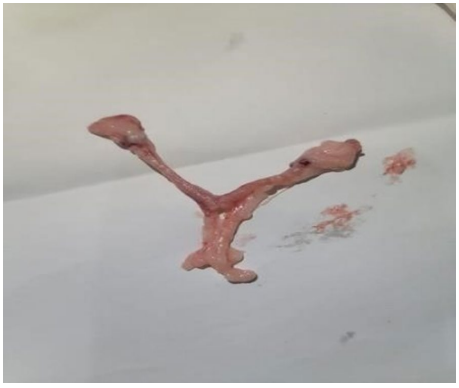


Figure 6. Ovaries with Cysts

3.10. Physical Inspection of Ovarian Cysts

Following the strict ethical guidelines, rats from the positive control group were dissected to confirm the induction of PCOS through hormonal profiling. Using chloroform, rats were anesthetized in a clean environment. The dissection was carried out with a sharp scalpel. On site, the ovaries appeared to have multiple cysts formation (Figure 6) which confirmed PCOS induction.

3.11. Biochemical Analysis

The serum obtained during the process of centrifugation was employed for the purpose of conducting hormonal analysis, which involved the assessment levels of testosterone, progesterone, and estrogen.

3.11.1 Estrogen. The analysis of variance revealed that the groups showed significant variation in serum concentrations of the estrogen hormone. The maximum serum concentration of estrogen hormone (Figure 7) was observed in the positive control group after PCOS induction by Estradiol valerate. The maximum serum concentration of estrogen was 80.73 ± 4.83, as compared to the control group which had a serum concentration of 38.7±1.7. It was followed in the descending order by the extract treated group and standard drug group with serum concentrations of 8.07±7.9 and 7.1±2.2, respectively. As for the combined group, the concentration of estrogen was 0.22±0.03.

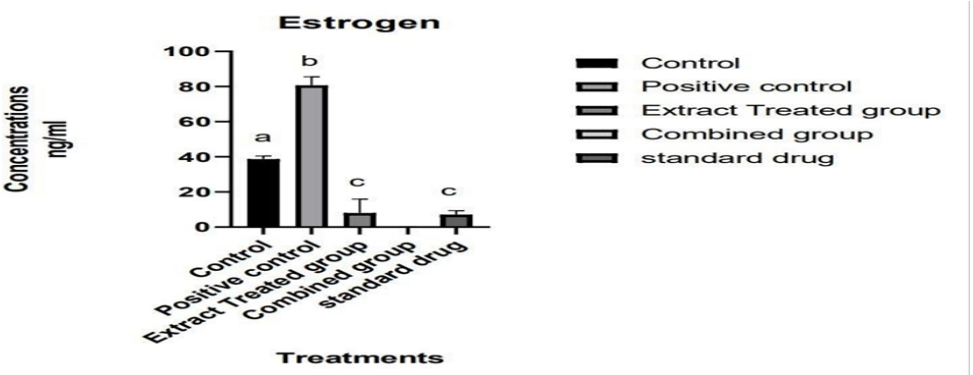


Figure 7. Mean Serum Levels of Estrogen Hormone (Ng/Ml) In Control Group, Positive Control Group (PCOS Induced Group with No Treatment Therapy), Standard Drug Group, Sesame Extract Treated Group, Sesame Seed, And Clomiphene Citrate Combined Therapy Treatment Group

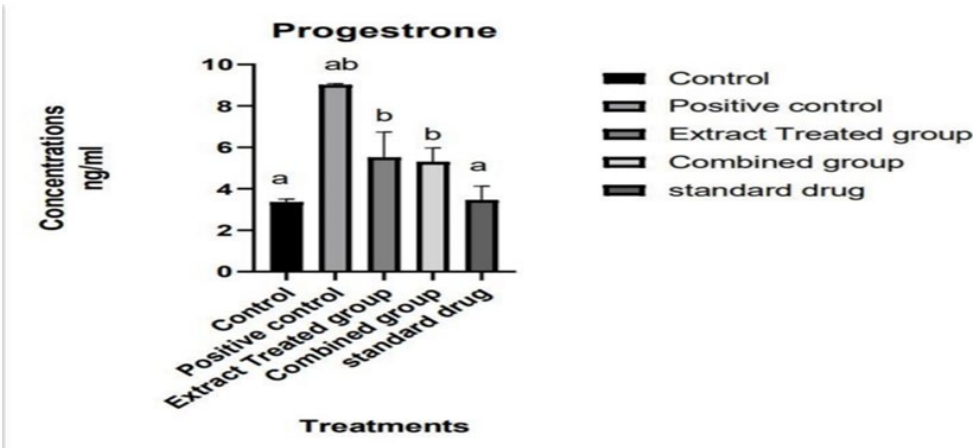


Figure 8. Mean Serum Levels of Progesterone Hormone (Ng/Ml) In Control Group, Positive Control Group (PCOS Induced Group with No Treatment Therapy), Standard Drug Group, Sesame Extract Treated Group, Sesame Seed, And Clomiphene Citrate Combined Therapy Treatment Group

3.11.2. Progesterone. Progesterone is classified as an endogenous steroid and progestogen sex hormone that plays a crucial role in various biological processes such as the menstrual cycle, pregnancy, and embryogenesis in both humans and other species. A high level of progesterone indicates successful ovulation and the release of the egg from the ovary. Conversely, a low progesterone level suggests that the egg was likely not released, resulting in anovulation in individuals with polycystic ovarian syndrome (PCOS) [11].

The analysis of variance revealed that the groups showed significant variation in serum concentrations of progesterone

hormone. The maximum serum concentration of progesterone hormone (Figure 8) was observed in the positive control group after PCOS induction by Estradiol valerate. The maximum serum concentration of progesterone was 9.30±0.05, as compared to control group which had a serum concentration of 3.36±0.13. It was followed in descending order by the extract treated group, combined group, and standard drug group with serum concentrations of 5.52±0.66, 5.32±0.66, and 3.46±0.66, respectively.

3.11.3 Testosterone. Testosterone serves as the principal male sex hormone and functions as an anabolic steroid. Testosterone serves as the principal male sex hormone and functions as an anabolic steroid. Elevated levels of androgens, referred to as hyperandrogenemia, are linked to irregularities in the menstrual cycle and negative metabolic characteristics in premenopausal women. These metabolic features include insulin resistance, central obesity, dyslipidemia, and chronic inflammation, which may contribute to an elevated risk of cardiovascular disease. According to a study, around 80 percent of women diagnosed with hyperandrogenism exhibit the presence of polycystic ovaries [12]. Bartolone et al. [13] showed a significantly elevated concentration of testosterone in individuals with polycystic ovary syndrome (PCOS). This highlights the significance of testosterone in the diagnosis of polycystic ovary syndrome (PCOS).

The analysis of variance revealed that the groups showed significant variation in serum concentrations of testosterone hormone. The maximum serum concentration of testosterone hormone (Figure 9) was observed in the positive control group after PCOS induction by Estradiol valerate. The maximum serum concentration of testosterone was 2.51±0.09, as compared to control group which had a serum concentration of 2.2±0.35. It was followed in descending order by standard drug, combined group, and extract treated group with serum concentrations of 1.81±0.18, 0.56±0.17, and 0.32±0.02, respectively.

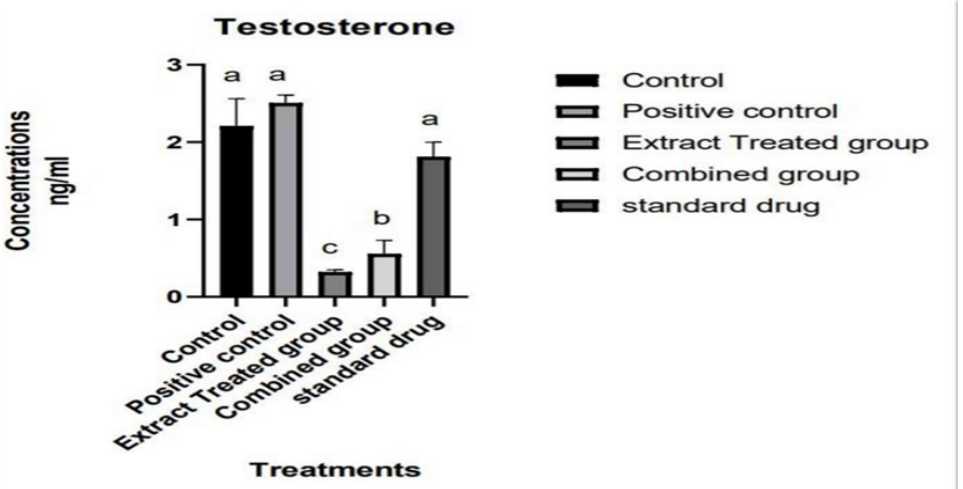


Figure 9. Mean Serum Levels of Progesterone Hormone (Ng/Ml) In Control Group, Positive Control Group (PCOS Induced Group with No Treatment Therapy), Standard

Drug Group, Sesame Extract Treated Group, Sesame Seed, And Clomiphene Citrate Combined Therapy Treatment Group

4. DISCUSSION

The methodology encompasses both predictive and wet lab investigations. Predictive investigations identified a protein that has a crucial role in PCOS. The docking of MADH4 with ligands generated from sesame seeds was conducted subsequently to the assessment of its absorption, distribution, metabolism, excretion (ADME), and toxicity properties. The primary ligands utilized in this study were ascorbic acid and thiamine. Both of the primary ligands, when assessed in terms of drug likeness and toxicity, exhibited superior outcomes as compared to the conventional drug Clomiphene citrate.

Protein-ligand interaction was evaluated, specifically examining the binding of ascorbic acid to 10 amino acids inside the target protein. Among these amino acids, it was observed that GLY33 exhibited an unfavorable bump. In contrast, thiamine exhibited interactions with a total of 15 amino acids. Among these, LYS122 and SER45 displayed negative steric clashes. The remaining amino acids exhibited a high binding affinity with the ligands.

Further, in the wet lab, Estradiol valerate was administered to rats to induce PCOS. Previous studies demonstrated the successful induction of PCOS using Estradiol valerate. The presence of specific cell types at different stages in vaginal smear cytology, as well as the formation of cysts in the ovaries upon dissection, confirmed the onset of PCOS. Additionally, the examination of serum hormone levels further validated the induction of PCOS in the current study [14].

The rats were divided into five different groups. Hormonal levels were assessed before and after treatments. The control group was neither induced with PCOS nor received any other treatments. The positive control group represented the PCOS-induced group without any additional treatment. The results for the positive control group showed elevated levels of progesterone, estrogen, and testosterone, indicating the successful induction of PCOS with increased hormone levels in the bloodstream.

The third group was treated with sesame seed extract as a potential treatment for PCOS. The statistical analysis revealed a decrease in all three hormones. The progesterone level decreased from 9ng/ml to nearly 5ng/ml, which is close to the value observed in the control group. Likewise, both estrogen and testosterone exhibited a significant decline in their concentrations, supporting the efficacy of the PCOS treatment. However, substantial fluctuations in hormone levels might be attributed to other unknown factors. The fourth group received the standard drug, namely Clomiphene citrate.

The results showed a decrease in progesterone and estrogen levels, as well as an increase in testosterone which indicated the morbidity of hypothyroidism. These results are supported by a scientific study illustrating the fact that once the PCOS was induced in young females, it showed an association with other long-term reproductive, metabolic, and oncologic complications [15].

Finally, the fifth group was subjected to a combined treatment of the standard drug and sesame seed extract. The results indicated a decrease in testosterone,

progesterone, and estrogen levels. However, it is worth noting that estrogen levels appeared to be marked as 0, which could potentially be an anomaly or might be attributed to some external factors that remain unknown currently.

Indeed, when conducting a PCOS study using rat models, several factors should be taken into consideration. These factors include the rat strain, gender, diet and duration, age, circadian rhythm, ease of maintenance, and the relevance of the model to the human condition being studied [16].

The variations observed in the results could be attributed to these factors, such as the weight and age of the rats used. Additionally, factors such as the concentration of sesame seed extract administered and the timeframe of its administration could also contribute to the fluctuations in the results. It is possible that sesame seeds contain compounds other than phytoestrogens that may influence hormone levels and, thereby, affect the outcomes of the study. Considering these factors and their potential impact on the study outcomes is important to accurately interpret the results and understanding the observed variations in hormone levels.

4.1. Conclusion

The current situation necessitates a significant demand for effective treatment options for PCOS. The utilization of natural ingredients in research contributes to the advancement of formulation techniques and the potential treatment of various syndromes. The current study employed *in silico* methodology to identify the essential component in sesame seeds that interacts with the designated receptor and yields favorable outcomes. On further examination, it was observed that the ligands exhibited a significantly higher affinity towards the target protein in comparison to Clomiphene citrate. The experiment conducted in the wet lab demonstrated that the extract group exhibited outstanding results, in comparison to both the combined group and the conventional medication group. Moreover, it is crucial to acknowledge the existence of other potential factors that could influence the outcomes in order to accurately interpret the data and comprehend the observed fluctuations in hormone levels.

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