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Exploring the therapeutic potential of sesamol and daidzein in polycystic ovarian syndrome: An *in-silico* approach

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Abstract

Polycystic Ovary Syndrome (PCOS) is a prevalent endocrine disorder in women, characterized by hormonal imbalances, including elevated androgen levels. In the search for potential therapeutic agents, phytocompounds such as sesamol and daidzein have gained attention due to their antioxidant and oestrogenic properties. This study investigated the binding affinity and pharmacokinetic properties of sesamol and daidzein as potential treatments for PCOS through molecular docking and *in silico* analysis. Molecular docking studies were conducted using AutoDock Vina, targeting oestrogen receptor beta (ERβ), androgen receptor (AR) and aromatase (CYP19A1) as key macromolecules involved in PCOS pathophysiology. The docking results revealed significant binding affinities, with daidzein showing a binding energy of -8.9 kcal/mol to ERβ, -5.2 kcal/mol to AR and -9.2 kcal/mol to CYP19A1. Sesamol exhibited binding energies of -5.9 kcal/mol to both ERβ and AR and -5.8 kcal/mol to CYP19A1. These values, although lower than the respective endogenous ligands (Oestradiol: -11.0 kcal/mol, Testosterone: -12.0 kcal/mol and -11.3 kcal/mol), indicate a favorable interaction with the target receptors. Additionally, pharmacokinetic properties, including absorption, distribution, metabolism, excretion and toxicity (ADMET), were analyzed using SwissADME and AdmetSAR tools. The analysis demonstrated favorable drug-likeness and ADMET profiles for both sesamol and daidzein, reinforcing their suitability as therapeutic candidates. The findings from this study suggested that sesamol and daidzein possess promising pharmacological profiles and could be considered for further *in vivo* and clinical studies as potential therapeutic agents for managing PCOS.

Keywords

daidzein; molecular docking; pharmacokinetic profiling; sesamol

Introduction

Polycystic Ovarian Syndrome (PCOS) is recognized as one of the most prevalent endocrine disorders affecting women of reproductive age, with a global prevalence estimated at 11-13% of this population (1). Countries with the highest prevalence of Polycystic Ovary Syndrome (PCOS) include Kuwait, Qatar and Saudi Arabia within the Middle East and North Africa (MENA) region, where age-standardized point prevalence rates are notably high. For instance, Kuwait has a prevalence rate of 2,838.1 per 100,000 women, followed closely by Qatar at 2,748.1 and Saudi Arabia at 2,692.0 per 100,000 women (2). The syndrome is intricately linked to various metabolic disturbances, such as insulin resistance, obesity and an elevated risk of developing type 2 diabetes and cardiovascular diseases. The multifactorial etiology of PCOS, involving genetic predispositions, environmental influences and lifestyle factors, further complicates its management (3), necessitating a comprehensive understanding of its underlying mechanisms.

Conventional therapeutic approaches for PCOS predominantly focus on symptomatic relief through the use of hormonal contraceptives, anti-androgens and insulin-sensitizing agents (4). While these treatments can mitigate the immediate symptoms, they do not address the root causes of the syndrome and are often associated with undesirable side effects. Among the alternative therapies gaining attention are phytoestrogens and phytochemicals, plant-derived compounds known for their ability to mimic or modulate oestrogenic activity in the body. These compounds are increasingly being investigated for their potential therapeutic roles in managing PCOS. Daidzein, a prominent soy isoflavone, has shown promise in modulating oestrogen receptor activity, improving insulin sensitivity and reducing oxidative stress, all of which are critical in the pathophysiology of PCOS (5). Similarly, sesamol, a lignan and potent antioxidant found in sesame seeds is being explored for its potential to alleviate oxidative stress and inflammation, thereby improving ovarian function and overall metabolic health.

Recent advancements *in silico* molecular docking studies have provided valuable insights into the interaction profiles and binding affinities of these phytoestrogens with key macromolecules involved in endocrine regulation. The structural similarity of daidzein to oestrogen enables it to bind effectively to oestrogen receptors, exerting oestrogenic effects (6), while sesamol has demonstrated significant antioxidant and anti-inflammatory properties (7). Pharmacokinetic profiling and molecular docking are integral components of the drug discovery process, essential for evaluating the efficacy and safety of potential therapeutic candidates. Pharmacokinetic profiling encompasses the analysis of absorption, distribution, metabolism and excretion (ADME) properties of a compound, providing critical insights into its bioavailability, half-life and potential for adverse effects. These parameters are crucial for predicting the *in vivo* behavior of a drug and ensuring its therapeutic efficacy and safety. Molecular docking, a computational technique, is employed to predict the binding affinity and interactions of small molecules with specific target proteins or enzymes. By simulating these molecular interactions, it is possible to identify compounds with strong and selective binding to the target, which is a pivotal step in the rational design of new therapeutic agents (8). Together, pharmacokinetic profiling and molecular docking provide a comprehensive framework for the evaluation and optimization of drug candidates, thereby enhancing the likelihood of success in subsequent stages of drug development. Hence this study was undertaken to find out the binding affinity of sesamol and daidzein against AR, ERβ and CYP19A1 and thus evaluate the treatment potential of the phytochemicals in PCOS.

Materials and Methods

Preparation of ligand structure

Sesamol (PubChem CID: 68289) and daidzein (PubChem CID:5281708) were selected as the ligands for the study. The chemical structures of these ligands were obtained from the PubChem database. The molecular structures were retrieved in SDF format and converted to PDBQT format using OpenBabel (version 3.1.1). Ligand structures were then subjected to energy minimization using the Universal Force Field (UFF) in OpenBabel to achieve a stable conformation for molecular docking studies.

Target Protein Preparation

The target proteins selected for this study included the oestrogen receptor beta (ERβ, PDB ID: 1HJ1, 1L2J), androgen receptor (AR, Homo sapiens PDB ID: 1E3G) and aromatase (CYP19A1, Homo sapiens PDB ID: 3EQM) from *Rattus norvegicus* and *Homo sapiens.* Due to the unavailability of rat-specific crystal structures for AR and aromatase in the Protein Data Bank (PDB), a homology modeling approach was employed.

Sequence Retrieval and homology modelling

The amino acid sequences for rat AR (UniProt ID: P15207) and rat aromatase (UniProt ID: P22443) were retrieved from the UniProt database (https://www.uniprot.org). The retrieved sequences were used to generate homology models of rat AR and aromatase using the SWISS-MODEL web server (https://swissmodel.expasy.org). Templates with the highest sequence identity and coverage were selected for modeling. Structural assessment of the models was done by calculating the percentage of residues in the most favored regions, allowed regions and disallowed regions using the Ramachandran plot statistics and MolProbity score provided by SWISS-MODEL.

Validation of Homology models

The homology modeling validation was conducted using PyMOL by calculating the RMSD between the model and reference structures (PDB ID: 3RLJ, 1E3G for AR and PDB ID: 4GL7, 3EQM for aromatase). The structures were first loaded into PyMOL and the homology model was aligned to the reference structure using the 'align' command. Following the alignment, the root mean square deviation (RMSD) value was computed.

Structure Preparation

The generated homology models and the retrieved crystal structures of other macromolecules were prepared for docking. Preparation steps included removing water molecules, adding polar hydrogens and assigning Kollman charges using AutoDockTools (version 1.5.7). Non-essential ligands and ions were also removed to ensure a clean docking environment.

Molecular Docking Protocol

Molecular docking was performed to predict the binding affinity and interaction patterns of sesamol and daidzein with the selected macromolecules (ERβ, AR and aromatase) using AutoDock Vina (version 1.1.2) (9). The prepared protein structures were converted to the PDBQT format using AutoDockTools. The grid box was centered on the active site of each macromolecule, with dimensions large enough to accommodate the entire ligand within the binding pocket. The grid box parameters were optimized based on the known active site residues. The docking protocol was executed with the default settings of AutoDock Vina, allowing the ligand to explore multiple binding poses. Each ligand was docked into the active sites of ERβ, AR and aromatase of rat and human separately. The binding affinities were recorded as the predicted binding energy (kcal/mol) for each ligand-macromolecule complex and were compared to the natural ligands and clomiphene citrate.

ADMET Analysis

The pharmacokinetic properties of sesamol and daidzein were predicted using *in silico* ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) tools. The ADME properties, including water solubility, gastrointestinal absorption, blood-brain barrier (BBB) permeability and drug-likeness, were predicted using the SwissADME web tool (http://www.swissadme.ch). The SMILES notation of sesamol and daidzein was used as input to generate the pharmacokinetic profile. Toxicological properties, including human hepatotoxicity and carcinogenicity, were assessed using the admetSAR tool (http://lmmd.ecust.edu.cn/admetsar2) and were compared to the known selective oestrogen receptor modulator, Clomiphene citrate (PubChem CID: 3033832). The combined data from molecular docking and ADMET analysis provided insights into the potential efficacy and safety of sesamol and daidzein as modulators of ERβ, AR and aromatase.

Results

The structure of proteins obtained from SWISS-MODEL aligned with reference structures is represented in Fig. 1 and the Ramachandran plot and 3D structure are given in Fig. 2. The structural alignment of rat aromatase with its human template showed a root mean square deviation (RMSD) of 0.316 Å and for the pig template, it was 0.496 Å. Rat AR had RMSD values of 0.637 Å and 1.282 Å for mouse and human AR respectively. The values suggest that the model aligns quite well with the reference structure in those regions, indicating a high level of accuracy, as RMSD values below 1 Å are typically indicative of very good structural similarity. Although the value of 1.282 Å is slightly higher, it still falls within a reasonable range.

The quality of the homology models was further validated by Ramachandran plot analysis. For the rat aromatase model, 95.41% of the residues were found in the most favored regions, while 4.59% were in the allowed regions, with no residues in the disallowed regions. Similarly, for the rat AR

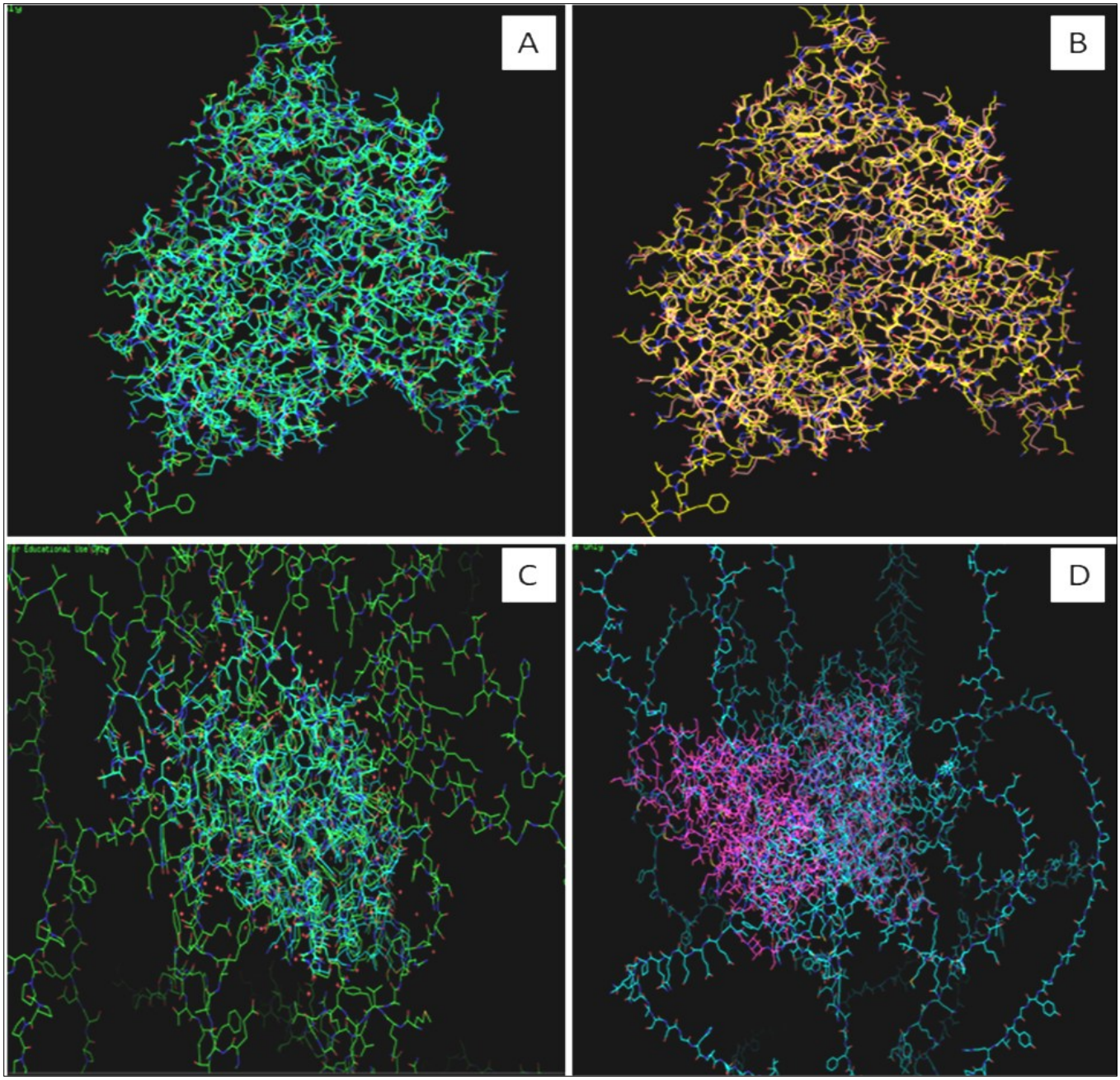

Fig. 1. Structure of homology models aligned with reference structures. **A**. Rat aromatase with *Sus scrofa* (pig) aromatase (PDB ID: 4GL7); **B**. Rat aromatase with *Homo sapiens* aromatase (PDB ID: 3EQM); **C**. Rat AR with *Mus musculus* AR (PDB ID: 3RLJ); **D**. Rat AR with *Homo sapiens* AR (PDB ID: 1E3G).

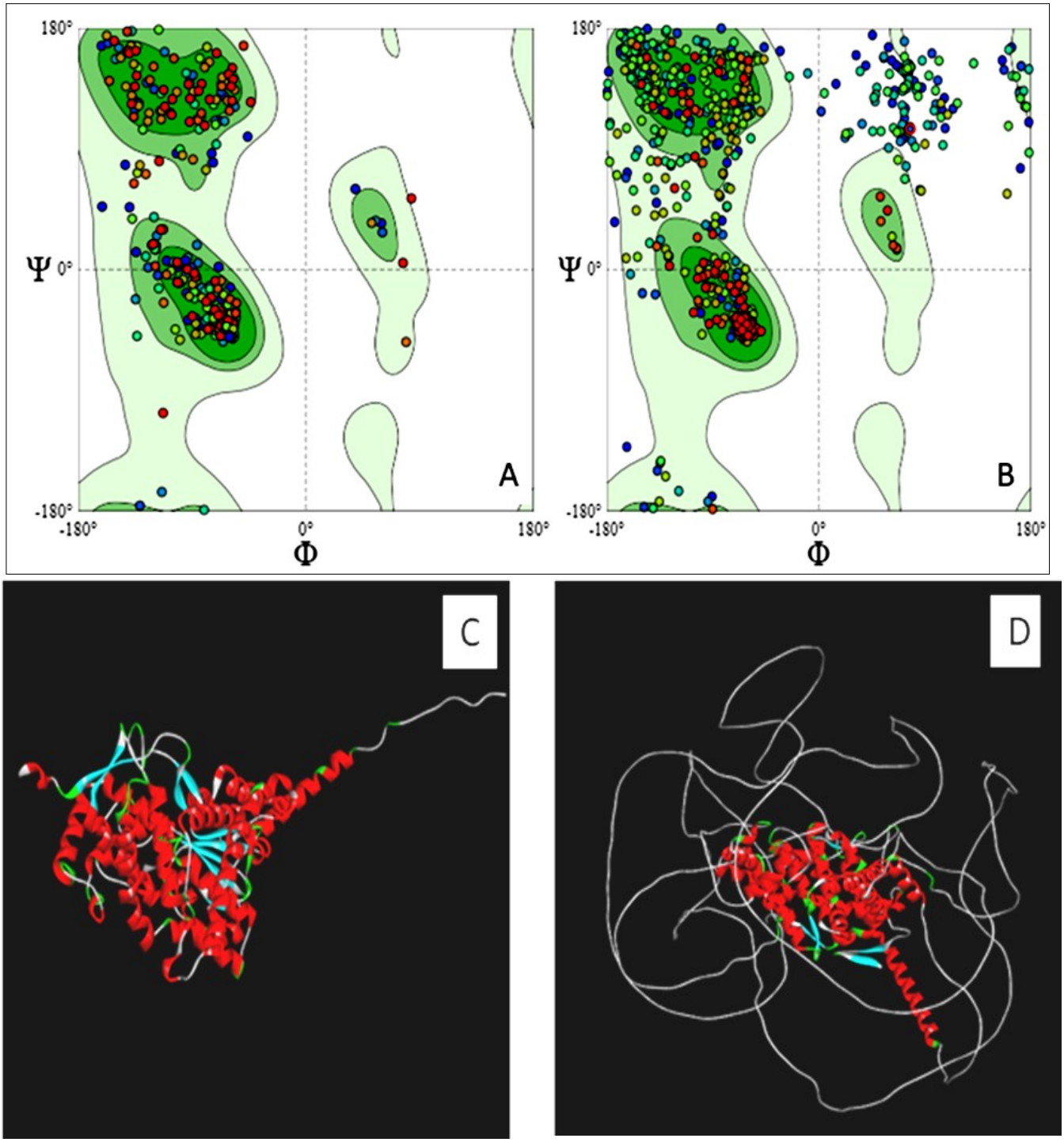

Fig. 2. Structure of proteins after homology modeling. **A**. Ramachandran plot for aromatase; **B**. Ramachandran plot for AR; **C**. Structure of aromatase; **D**. Structure of AR.

model, 71.11% of the residues were in the most favored regions, 27.89% in the allowed regions and 1% in the disallowed regions. These statistics confirm that the majority of the amino acids are in energetically favorable conformations, supporting the reliability of the models for subsequent docking studies. The results also revealed that majority of the amino acids are in a phi –psi distribution consistent with a right-handed -helix and reliable to be a good quality model. The colored dots correspond to individual residue angles, significantly clustering the allowed regions. For both Aromatase (A) and AR (B) most of the amino acids are seen in that region, hence that the protein adopts stable and energetically favorable conformations and the MolProbity score for aromatase and AR was found to be 1.36 and 2.14 respectively.

The *in-silico* analysis revealed distinct differences in the binding affinities of sesamol and daidzein to key macromolecular targets associated with PCOS, including oestrogen receptor beta (ERβ), androgen receptor (AR) and cytochrome P_{450} 19A1 (CYP19A1), in both *Rattus norvegicus* and *Homo sapiens*. The binding energies obtained are presented in Table 1. The native ligands, oestradiol for ERβ and testosterone for AR and CYP19A1, were used as reference compounds to gauge the relative affinities of sesamol and daidzein. In rats, oestradiol exhibited a strong binding energy of -11.0 kcal/mol with ERβ, whereas sesamol showed a significantly lower binding energy of -5.9 kcal/mol and daidzein presented a stronger interaction with a binding energy of -8.9 kcal/mol which is higher than clomiphene (-7.8). For the AR, testosterone showed

Table 1. Binding energies of sesamol and daidzein against ERβ, AR and Aromatase enzyme

Species	Macro - molecule	ligand	Binding energy (Kcal/mol)
Rattus norvegicus	ERβ	Oestradiol*	-11.0
		Sesamol	-5.9
		Daidzein	-8.9
		Clomiphene	-7.8
	AR	Testosterone*	-12.0
		Sesamol	-5.9
		Daidzein	-10.3
		Clomiphene	-8.1
	CYP19A1	Testosterone*	-11.3
		Sesamol	-5.8
		Daidzein	-9.2
		Clomiphene	-7.7
Homo sapiens	ERβ	Oestradiol*	-10.8
		Sesamol	-5.8
		Daidzein	-9.2
		Clomiphene	-9.1
	AR	Testosterone*	-11.3
		Sesamol	-5.5
		Daidzein	-9.1
		Clomiphene	-8.2
	CYP19A1 (Human)	Testosterone*	-11.5
		Sesamol	-5.8
		Daidzein	-9.0
		Clomiphene	-8.0

*Natural ligands.

a high binding affinity of -12.0 kcal/mol. Sesamol displayed a binding energy of -5.9 kcal/mol, while daidzein exhibited a notably stronger binding energy of -10.3 kcal/mol. Similarly, for CYP19A1, testosterone demonstrated a binding energy of -11.3 kcal/mol. Sesamol showed a binding energy of -5.8 kcal/mol and daidzein had a significantly higher binding affinity at -9.2 kcal/mol which can be attributed to the structural similarity of daidzein to that of oestrogen. The docked images are given in Fig. 3.

In humans, the binding energy of oestradiol with ERβ was recorded at -10.8 kcal/mol. Daidzein and clomiphene demonstrated a significantly stronger affinity, with a binding energy of -9.2 kcal/mol and -9.1 kcal/mol respectively which is comparable to that of oestradiol while sesamol exhibited a lower binding energy of -5.8 kcal/mol. For the androgen receptor (AR), testosterone displayed a binding energy of -11.3 kcal/mol. In comparison, sesamol showed a binding energy of -5.5 kcal/mol, whereas daidzein again exhibited a higher binding affinity at -9.1 kcal/mol. Regarding the CYP19A1 enzyme, testosterone had a binding energy of -11.5 kcal/mol, with sesamol binding at -5.8 kcal/mol and daidzein displaying a notably strong binding energy of -9.0 kcal/mol. The docked images are given in Fig. 4.

Comparatively, daidzein consistently demonstrated stronger binding affinities across all targets in both species, with binding energies closer to those of the native ligands. This suggests that daidzein may have a more favorable interaction profile and could be a more potent therapeutic agent in the management of PCOS. Sesamol, while exhibiting moderate binding affinities, may still have therapeutic value, potentially in combination with other agents, due to its interaction with the same molecular targets. The differences in binding energies between sesamol and daidzein underscore the potential of daidzein as a more effective candidate for PCOS treatment, while the role of sesamol may be supportive in a combined therapeutic strategy. Compared to sesamol, daidzein has closer binding energy to that of oestradiol with ERβ. Against AR both the ligands are showing less affinity compared to ERβ, even though they are known to bind with AR. Against CYP19A1 the binding affinity of sesamol was determined to be -5.8 kcal/mol, while daidzein exhibited a significantly higher binding affinity of -9.2 kcal/mol. The standard drug, clomiphene has binding energy values in between that of sesamol and daidzein for all the macromolecules. These findings indicate that daidzein binds more strongly to the active site of aromatase compared to sesamol. The enhanced binding affinity of daidzein may be attributed to its structural similarity to the endogenous substrates of aromatase, which facilitates more favorable interactions with the enzyme. The docked images of clomiphene with both the rat and human proteins are given in Fig. 5.

Drug likeness as per Lipinki's Rule of Five is given in Table 2. Both sesamol and daidzein adhere to Lipinski's Rule of Five (MW ≤ 500 Da, H-bond donors ≤ 5, H-bond acceptors ≤ 10 and log $P_{O/W}$ ≤ 5), indicating favorable drug-like properties, while clomifene has 1 violation (MW ≥ 500 Da). The pharmacokinetic profiles of daidzein and sesamol, compared to clomiphene citrate, which serves as the standard, revealed both similarities and distinctions in their absorption and distribution properties as indicated in Fig. 6. Clomiphene citrate, a well-established therapeutic agent, exhibits a blood-brain barrier permeability of 0.7974, an intestinal absorption rate of 0.7351 and a Caco-2 permeability of 0.592. Additionally, it shows a strong affinity as a P-glycoprotein substrate (0.9351) and interacts with the renal organic cation transporter (0.7452). In comparison, both daidzein and sesamol demonstrate efficient intestinal absorption, with sesamol potentially surpassing clomiphene in absorption efficiency. While all three compounds effectively reach peripheral tissues, sesamol (0.93) may exhibit superior blood-brain barrier penetration compared to clomiphene. These comparisons highlight the pharmacokinetic advantages and potential limitations of daidzein and sesamol relative to the standard drug, clomiphene citrate, particularly in terms of absorption and tissue distribution. Metabolically, both are primarily processed in the liver through conjugation reactions, but daidzein shows a stronger potential to inhibit key cytochrome P450 (CYP) enzymes. Specifically, daidzein has higher probabilities of inhibiting CYP4501A2 (0.91 vs. 0.85), CYP4502C9 (0.97 vs. 0.89), CYP4502D6 (0.91 vs. 0.69) and CYP4502C19 (0.89 vs. 0.83) compared to sesamol and both are having similar values to that of clomiphene citrate. The findings suggest that daidzein may have a higher likelihood of drug-drug interactions due to its more significant impact on these CYP enzymes. In comparison to clomiphene citrate, daidzein and sesamol exhibit distinct toxicity profiles across various parameters. clomiphene citrate, with an

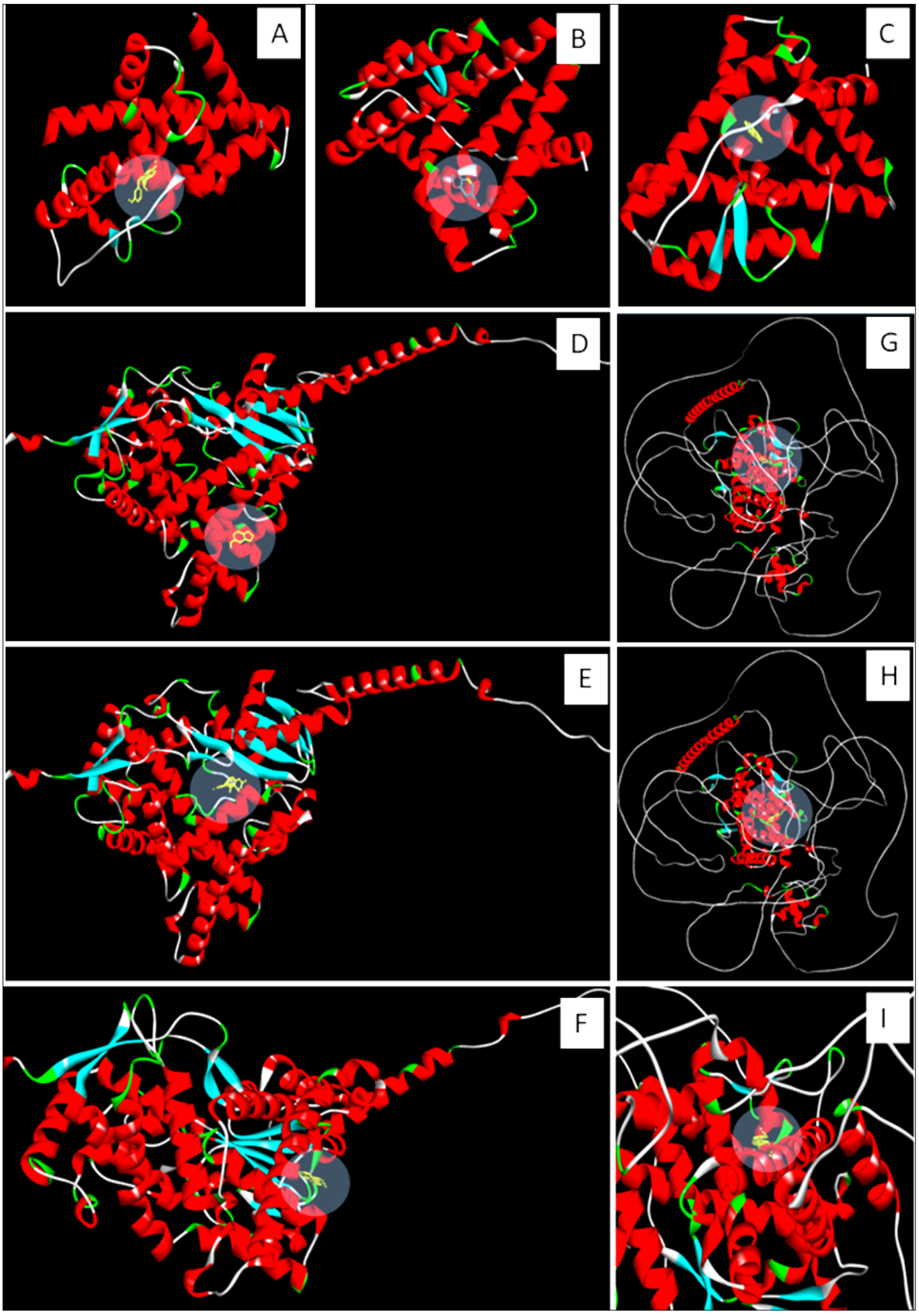

Fig. 3. Docked images (Rat). **A**. Daidzein against ERβ; **B**. Sesamol against ERβ; **C**. Oestradiol against ERβ; **D**. Sesamol against aromatase; **E**. Daidzein against aromatase; **F**. Testosterone against aromatase; **G**. Sesamol against AR ; **H**. Daidzein against AR; **I**. Testosterone against AR.

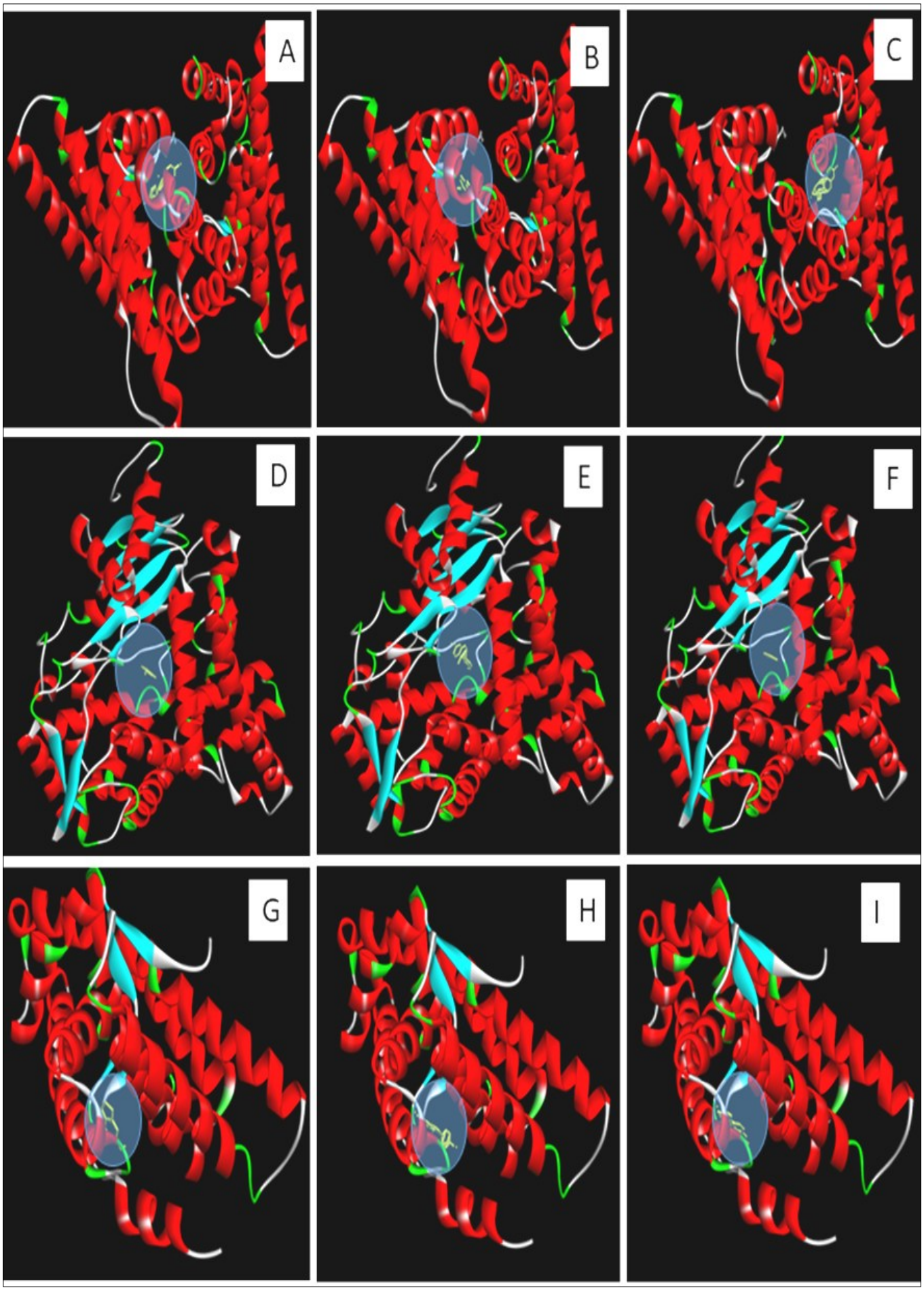

Fig. 4. Docked images (Human). **A**. Daidzein against ERβ; **B**. Sesamol against ERβ; **C**. Oestradiol against ERβ; **D**. Sesamol against aromatase; **E**. Daidzein against aromatase; **F**. Testosterone against aromatase; **G**. Sesamol against AR ; **H**. Daidzein against AR; **I.** Testosterone against AR.

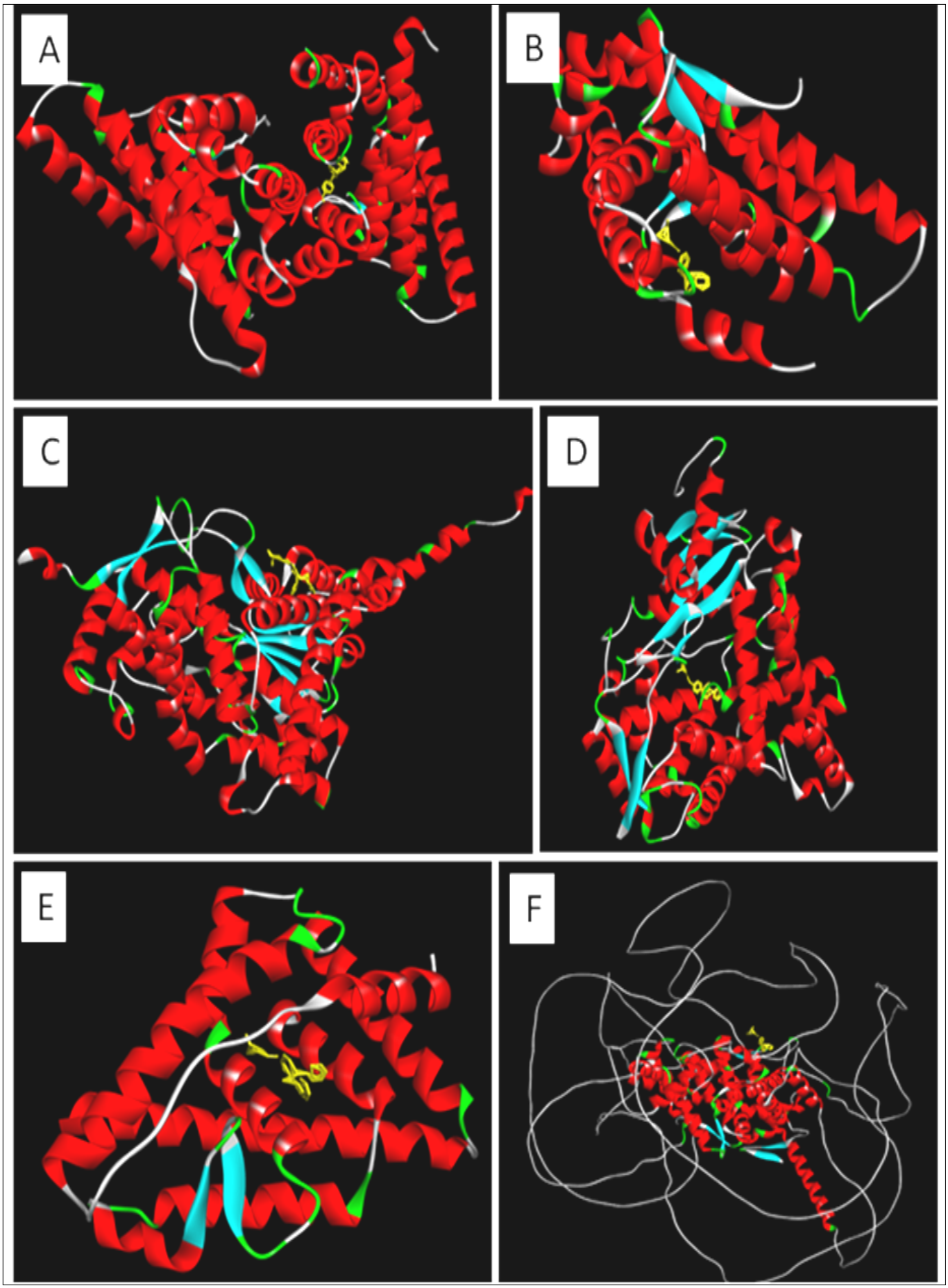

Fig. 5. Docked images of clomiphene with macromolecules. **A**. With human ER; **B**. With human AR; **C**. With rat aromatase; **D**. With human aromatase; **E**. With rat ERβ; **F**. With rat AR.

Table 2. Drug-likeness as per Lipinki's Rule of Five

Compound	Lipinski's Rule of Five			
	Molecular weight (g/mol)	H-bond donors	H-bond acceptors	Octanol-Water partition co-efficient
Sesamol	138.12	1	3	1.19
Daidzein	254.24	2	4	2.24
Clomiphene citrate	598.08	4	9	4.45

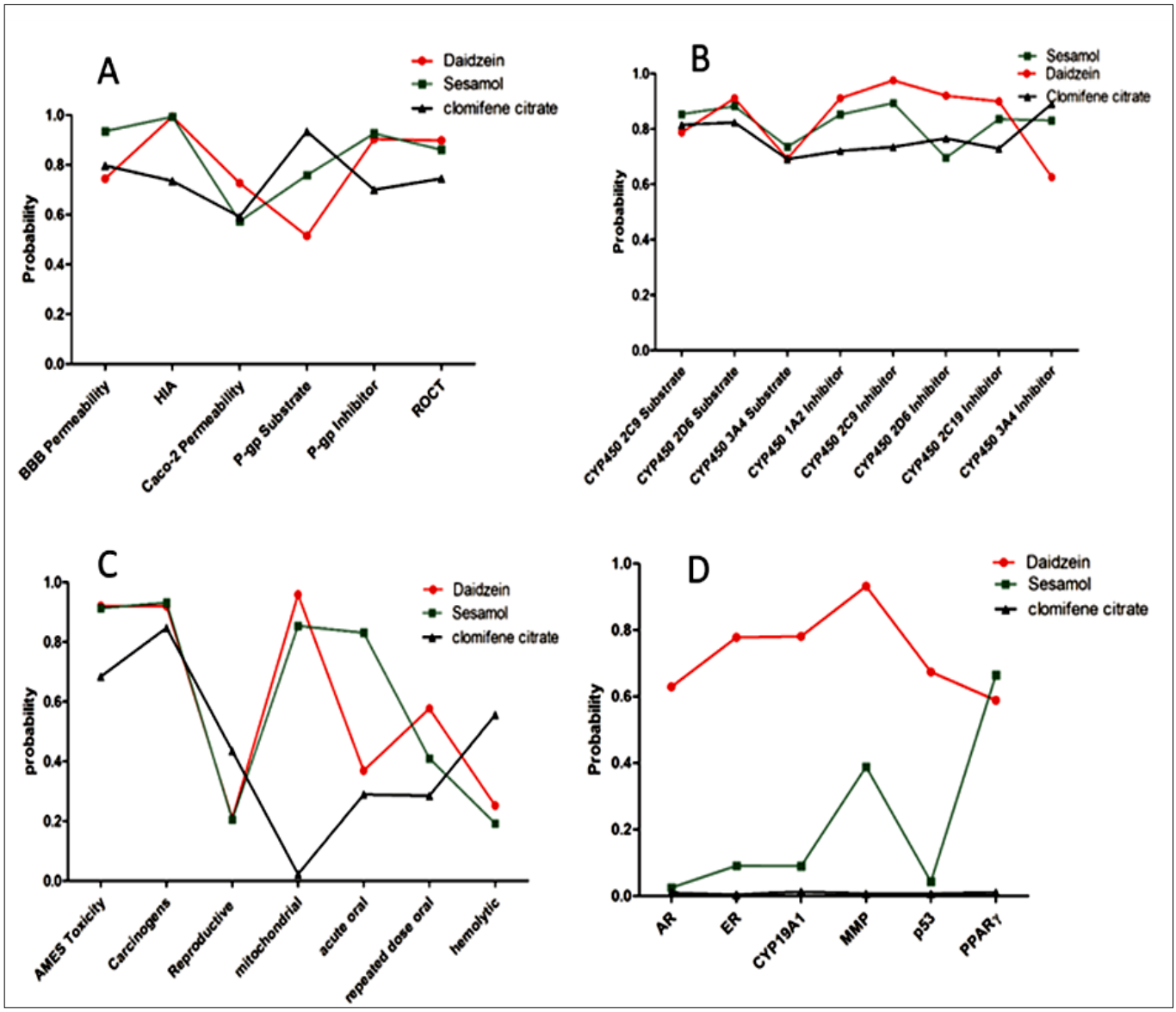

Fig. 6. ADMET properties of sesamol, daidzein and clomiphene citrate represented as probability (0-1). **A**. Factors affecting absorption; **B**. Effect on metabolizing enzymes; **C**. Toxicities produced; **D**. Endocrine disruption.

Ames toxicity probability of 0.6841, presents a moderate risk of mutagenicity, which is notably lower than the probabilities observed for daidzein (0.92) and sesamol (0.91), indicating a relatively reduced likelihood of DNA mutations and potential carcinogenicity. The carcinogenicity probability for clomiphene citrate stands at 0.8473, reflecting a substantial risk that aligns with the high mutagenic potential inferred from the Ames test results for daidzein and sesamol.

Regarding reproductive toxicity, clomiphene citrate exhibits a probability of 0.435, significantly higher than the low probabilities observed for daidzein (0.21) and sesamol (0.20), suggesting a greater potential for adverse reproductive effects. In contrast, clomiphene citrate demonstrates an exceptionally low probability of mitochondrial toxicity (0.022), which starkly contrasts with the elevated risks associated with daidzein (0.959) and sesamol (0.854), both of which indicate considerable potential for mitochondrial dysfunction that could impair cellular energy metabolism. clomiphene citrate also presents a probability of 0.289 for acute oral toxicity, indicating a moderate potential for harm upon single-dose ingestion. This is significantly lower than the higher risk associated with sesamol (0.831) but closer to the lower risk observed for daidzein (0.369). Finally, in terms of repeated dose oral toxicity, clomiphene citrate shows a probability of 0.285, reflecting a modest risk associated with chronic exposure. This is somewhat lower than the moderate probability of daidzein (0.577) but comparable to the lower probability of sesamol (0.41). These findings suggest that while clomiphene citrate poses certain safety concerns, particularly about reproductive toxicity, daidzein and sesamol present heightened risks for mitochondrial toxicity, with Sesamol

also associated with a higher likelihood of acute oral toxicity. As evidenced by their interaction with key endocrine components (Fig. 4D), daidzein demonstrates a moderate probability of disrupting androgen receptor (AR) signaling (0.629) compared to sesamol's lower probability (0.25), indicating a higher likelihood of interfering with androgen-related processes. In terms of oestrogen receptor (ER) binding, daidzein shows a significantly higher potential for disruption (0.778) relative to sesamol (0.091), suggesting a greater risk of oestrogenic effects, such as altered reproductive health or increased cancer susceptibility. Similarly, daidzein exhibits a high probability of inhibiting aromatase (0.781), which could lead to hormonal imbalances by affecting the conversion of androgens to oestrogens, whereas sesamol presents a much lower risk (0.09) in this regard. Additionally, daidzein has a very high likelihood of disrupting matrix metalloproteinases (MMPs) (0.932), suggesting significant potential to affect tissue remodeling and inflammation, whereas sesamol shows a lower, though still notable, probability (0.389). In all these endocrine targets clomiphene citrate presents a minimal risk for endocrine disruption, with very low probabilities for disrupting AR signaling (0.009), ER binding (0.004), aromatase inhibition (0.013), matrix metalloproteinases activity (0.007),and PPARy activation (0.001). Overall, daidzein presents a substantially higher risk of endocrine disruption across multiple pathways compared to clomiphene and sesamol, which exhibit minimal to moderate potential for such effects.

Discussion

The interplay between oestrogen receptors (ER), androgen receptors (AR), and the enzyme aromatase is crucial in the pathophysiology of PCOS. Androgens exert their effects predominantly through AR, while oestrogens act via the oestrogen receptors, ERα and ERβ, which are the two distinct isoforms. The roles of oestrogen receptor beta (ERβ), androgen receptor (AR) and aromatase (CYP19A1) in Polycystic Ovary Syndrome (PCOS) are critical for understanding the pathophysiology of this common endocrine disorder. Each of these components contributes to the hormonal imbalance and reproductive issues characteristic of PCOS, making them important targets for molecular docking studies aimed at developing therapeutic interventions. Oestrogen receptors, particularly ERα and ERβ, are nuclear hormone receptors that mediate the biological effects of oestrogens by binding to oestrogen response elements (EREs) on DNA. In the context of PCOS, the dysregulation of oestrogen signaling plays a significant role. Both ERα and ERβ are expressed in ovarian tissues, where they regulate folliculogenesis, ovulation and steroidogenesis (10). In PCOS, altered levels of oestrogens, especially an increased ratio of estrone to oestradiol, may influence ER signalling (11). This dysregulation can lead to impaired feedback mechanisms on the hypothalamus and pituitary, exacerbating the hyperandrogenic state and menstrual irregularities seen in PCOS. In the case of PCOS, ERβ is particularly significant because it mediates the effects of oestrogen on ovarian function and endometrial health (12). This disruption can contribute to ovulatory dysfunction, which is a hallmark of PCOS and highlights the potential of ERβ as a therapeutic target, as modulating its activity could restore normal ovarian function (Yang et al., 2018; Xu et al., 2021). The androgen receptor (AR) is crucial in mediating the effects of androgens, which are often elevated in PCOS. The overactivation of AR in ovarian tissues can promote follicular arrest and inhibit normal ovulation (15). This overexpression can lead to increased androgenic activity, further perpetuating the cycle of hormonal imbalance. Targeting AR through molecular docking studies could provide insights into developing selective antagonists that may alleviate hyperandrogenic symptoms and restore normal ovarian function. *CYP19A1* encodes the aromatase enzyme, which is responsible for converting androgens into oestrogens (16). In PCOS, the expression of *CYP19A1* is often reduced in granulosa cells, leading to decreased oestrogen production and an accumulation of androgens, such as testosterone (17). As aromatase plays a pivotal role in oestrogen biosynthesis, targeting CYP19A1 in molecular docking studies could help identify compounds that enhance its activity, potentially restoring normal oestrogen levels and improving reproductive outcomes in PCOS (18).

The approach of *in silico* screening is particularly useful in the context of Polycystic Ovary Syndrome (PCOS), as it allows researchers to explore the therapeutic potential of phytochemicals, which are naturally occurring compounds found in plants. Several studies have employed in-silico screening to identify phytochemicals that may be effective against PCOS by targeting key enzymes and receptors involved in ovarian steroidogenesis such as human aromatase (CYP19A1), human 17β-hydroxysteroid dehydrogenase type 1 (HSD17B1), human androgen receptor and oestrogen receptor (19,20). Docking studies targeting the androgen receptor (AR) aim to identify molecules that modulate its activity as antagonists or agonists to mitigate hyperandrogenism. For oestrogen receptors (ER), these studies seek compounds that can either mimic or block oestrogenic effects. In the context of PCOS, selective oestrogen receptor modulators (SERMs) like clomiphene are particularly valuable for their ability to selectively modulate oestrogen signaling, potentially restoring hormonal balance and improving ovulatory function (21). Aromatase inhibitors, which reduce oestrogen production and alleviate feedback inhibition on the hypothalamus and pituitary, are also a focus, with docking studies aiming to design more effective inhibitors with high binding affinity to the enzyme's active site. By targeting key enzymes and receptors involved in ovarian steroidogenesis, such as CYP19A1, HSD17B1, AR and ER, these studies offer insights into the potential therapeutic applications of phytochemicals in PCOS management.

In the context of PCOS, phytoestrogens have been proposed to exert several beneficial effects. They may reduce hyperandrogenism by downregulating the expression of key enzymes involved in androgen biosynthesis (22), such as 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase. Additionally, by modulating insulin sensitivity, a common metabolic disturbance in PCOS phytoestrogens may indirectly influence androgen production and ovulatory function. Since persistent low-grade inflammation is becoming more widely acknowledged as a contributing element to the pathogenesis of PCOS, the possible anti-inflammatory qualities of

phytoestrogens also merit attention (23, 24). By attenuating inflammatory signaling pathways, phytoestrogens might further ameliorate the metabolic and reproductive disturbances in PCOS.

Daidzein structurally resembles oestradiol, which is a primary form of oestrogen in the body. Both compounds feature a phenolic structure, which is crucial for their interaction with oestrogen receptors. This similarity allows daidzein to bind to oestrogen receptors (ERα and ERβ) and mimic some of the biological effects of oestradiol, albeit with varying potency (25). Daidzein despite being a phytoestrogen can also act on AR (26) and also has anti-inflammatory, anti-oxidant and anti-diabetic activities (27). Sesamol is a naturally occurring organic compound found in sesame seeds and sesame oil. It is a type of phenolic antioxidant, which is known for its Antioxidant (28), anti-inflammatory, cardioprotective effects (29) and neuroprotective effects (30). In this study, sesamol showed similar binding energies with ERβ and AR. This dual affinity can be attributed to several structural and biochemical characteristics of sesamol, as well as the nature of the receptors themselves. Oestrogen receptors, particularly ERα and ERβ, exhibit a degree of flexibility in their ligand-binding domains, allowing them to accommodate various ligands (31), including non-steroidal compounds like sesamol. Sesamol may act as a xenoestrogen (32), a compound that can bind to oestrogen receptors and elicit oestrogen-like effects. The ability of sesamol to bind to both ER and AR suggests that it may influence pathways involved in hormonal regulation, potentially modulating the effects of endogenous hormones and the structural similarity between sesamol and known AR ligands allows sesamol to interact with the receptor and potentially influence its transcriptional activity (33). The accuracy of homology modeling in predicting the structures of AR and CYP19A1 is highly dependent on the sequence identity between the target and template proteins. Sequence identities below 50% can result in significant inaccuracies, particularly in regions with substantial divergence, affecting binding site predictions and overall structural conformation. Even with higher sequence identity, structural differences, especially in loop regions or ligand-binding domains, may lead to errors in docking studies, potentially misidentifying key interactions between sesamol, daidzein and their targets. The quality of the homology models is also contingent on the resolution of the template structures; low-resolution templates or those with experimental errors can propagate inaccuracies in secondary structures and protein folding (34).

Furthermore, without proper validation and refinement, such as energy minimization and molecular dynamics simulations, the models may not accurately represent the native protein conformation, leading to unreliable *in silico* predictions. These limitations underscore the need for structural alignment comparisons with known human or mouse structures and highlight the importance of acknowledging homology modeling's inherent constraints in drug discovery. Additionally, translating *in silico* findings to *in vivo* studies presents challenges, as structural differences between species, such as rats and humans, could impact the therapeutic efficacy of compounds like sesamol and daidzein. Experimental validation, including site-directed mutagenesis or crystallography, is essential to confirm the *in-silico* predictions and ensure the reliability of these models in therapeutic applications.

The *in silico* ADMET analysis identified a high probability of mutagenicity for both sesamol and daidzein, which raises concerns regarding their long-term safety, particularly in terms of potential carcinogenic effects. This mutagenic potential warrants further investigation through a comprehensive battery of genotoxicity tests, including in vitro micronucleus assays and *in vivo* comet assays, to confirm these findings and assess the risk in a biological context (35). These additional tests will help to confirm or refute the mutagenic potential identified *in silico* and determine the safety of sesamol and daidzein for long-term therapeutic applications. Mitigating this risk may involve structural modifications to these compounds to reduce their mutagenicity while retaining therapeutic efficacy.

The observed mitochondrial toxicity of daidzein is particularly concerning given the crucial role of mitochondria in cellular energy metabolism. Mitochondrial dysfunction could exacerbate the metabolic disturbances already present in PCOS, potentially leading to more severe complications (36).

To mitigate this risk, future studies should explore the dose-dependent effects of these compounds on mitochondrial function in cell-based models, focusing on parameters such as ATP production and mitochondrial membrane potential (37). Additionally, the development of derivatives with reduced mitochondrial toxicity could enhance the safety profile of these compounds.

Daidzein's higher probability of endocrine disruption, particularly its interaction with oestrogen and androgen receptors, poses a significant concern. Such disruption could potentially worsen the hormonal imbalances characteristic of PCOS (12). It is imperative to conduct *in vivo* studies to assess the endocrine effects of prolonged exposure to these compounds, focusing on reproductive health and overall hormonal balance. Furthermore, designing analogs with lower affinities for these endocrine targets may help in reducing the risk of endocrine-related adverse effects.

Given the moderate probability of chronic toxicity for daidzein, particularly with prolonged use, it is essential to evaluate the potential cumulative effects in long-term studies. Chronic exposure could lead to unforeseen toxicities that are not immediately evident in short-term studies. Establishing a clear dose-response relationship through animal studies will be critical in defining safe therapeutic windows (27). Modifications to dosing regimens or the development of combination therapies might also mitigate chronic toxicity risks.

Conclusion

In conclusion, daidzein and sesamol demonstrate promising therapeutic potential in addressing hormonal imbalances associated with PCOS. Daidzein, an isoflavone, modulates oestrogen signaling and reduces hyperandrogenism through selective oestrogen receptor affinity and anti-androgenic properties, while sesamol, a phenolic compound, mitigates hormonal disturbances via its antioxidant activity and

interaction with hormone receptors. Despite these benefits, their safety profiles necessitate further investigation. While clomiphene citrate primarily raises concerns of reproductive toxicity, daidzein and sesamol present heightened risks of mitochondrial toxicity, with sesamol also associated with acute oral toxicity. Furthermore, in silico analyses highlight potential risks, including mutagenicity, mitochondrial toxicity and endocrine disruption, emphasizing the importance of thorough preclinical safety assessments.

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Authors' contributions

AS carried out the research work and prepared the article. BJK conceptualized, designed the experiment and done the article correction. SS assisted in the docking studies.

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