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EFFECTS OF GENISTEIN ON ESTROGEN RECEPTOR AS ANTAGONIST DISRUPTING THE OVULATION IN FEMALE WISTAR RAT

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ABSTRACT

Progesterone and Estrogen are being used to synthesize Combined oral contraceptives (COCs) and serve as most convenient, safe, effective method of contraception. Due to their side effects in most women, herbal medicines have been proposed as alternatives to these contraceptive methods. The present study was aimed to evaluate the antifertility effects of Genistein (isolated from *Glycine max*), via in-silico and in vivo experimentation on female Rattusnorvergicus (Wistar albino rats). Genistein was docked with Estrogen Receptor of rats (ER α and ER β). For, antagonist actions we compared ERA-4-[(2-{4-[(1E)-1-(1H-indazol-5-yl)-2-phenylbut-1-en-1-yl]phenoxy}ethyl)amino]-N,N-dimethylbutanamide complex with ERA-genistein complex and ERB-(R,R)-5,11-cis-diethyl-5,6,11,12-tetrahydro-chrysene-2,8-diol complex (PDB ID: 1l2J) with ERB-genistein complex. To further investigate the in vivo anti-fertility effects of Genistein and its correlation with serumestrogen, adult *Rattus norvergicus* (Wistar albino rats) was administered with 10, 20 and30mg Genistein/kg body weight dissolved in DMSO: PBS (1:4) vehicle for 15 days. Estrogen hormone level in the serum was estimated through ELISA. In-silico observation has shown that Genistein may act as antagonist for Estrogen Receptors. Serum estrogen level was found to be reduced, which can lead to disrupt ovulation. This shows that the Genistein might be working as anti-ovulatory agent by acting as a phytoestrogen.

Keyword: Genistein, estrogen, estrogen receptors, docking study, anti-ovulation, herbal contraceptive

1. INTRODUCTION

Contraceptive pills are considered to be the most common and preferred method of contraception used by women compared to the other methods. These drugs are formulated by synthetic progesterone only or in combination with estrogen **[1, 2]**. Synthetic steroidal contraceptives were initiated early in the twentieth century which was effective but along with these benefits there are many unwanted side effects associated with it such as obesity, nausea, breast and uterus cancer, disorders of cardiovascular system, and dysmenorrheal **[3]**. Research has shown the use of plants/phytochemicals for fertility regulation. Plant kingdom, therefore, holds a great promise for the invention of new and effective antifertility agents **[4, 5]**. Many studies have reported antizygotic, blastocytotoxic, anti-implantation, and abortifacient properties of many commonly used medicinal plants **[6]**. Usually foods containing phytoestrogens are considered safe for prolonged consistent usages on the daily basis but for women who are at the higher risk of developing the estrogen dependent cancers, prolonged daily usage of herbs and supplements containing phytoestrogens is not considered to be safe as they tend to alter the estrogen level in the women body making them more suspectable for inducing estrogen dependent cancer.

Genistein is a Phytoestrogen derived from soy (*Glycine max*), shown to possess estrogenic activities through its ability to bind to the estrogen receptor β **[7]**. ER-alpha (ERA) and ER-beta (ERB), are the two main types of estrogen receptor, which are activated by the sex hormone estrogen. In this study, we compared the docking scores of the ERA-Genistein, and ERB-Genistein complex with their known antagonist fully understand the genistein's mechanism of action against ERA and ERB. For, antagonist actions we compared ERA-4-[(2-{4-[(1E)-1-(1H-indazol-5-yl)-2-phenylbut-1-en-1-yl]phenoxy}ethyl)amino]-N,N-dimethylbutanamide complex with ERA-genistein complex and ERB-(R,R)-5,11-cis-diethyl-5,6,11,12-tetrahydro-chrysene-2,8-diol complex (PDB ID: 1l2J) with ERB-genistein complex **[8]**. Furthermore, the antagonist effect was validated by hormonal analysis. The study aims to determine the effects of Genistein at different doses of 10mg, 20 mg and 30 mg/kg body weight/ day (dissolved in 1:4 DMSO: PBS) upto 15 days and also to correlate the alterations produced with the changes in the circulating levels of Estrogen.

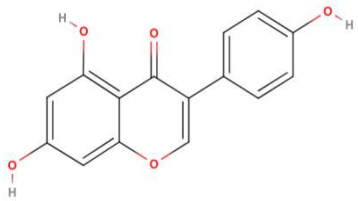

Figure 1.1: ((Chemical Structure of Genistein from PubChem) [9]

2. MATERIAL AND METHODS

2.1 In-Silico Study

2.1.1 Protein Structure Preparation and ligand Preparation

Missing Hydrogens were added to stabilize the PDB files. Potentially transposed heavy atoms in arginine, glutamine, and histidine side chains were corrected and optimization of the protein's hydrogen bond network by means of a systematic, cluster-based approach **[10]**. A restrained minimization was done that allows hydrogen atoms to be freely minimized while allowing for sufficient heavy-atom movement to relax strained bonds, angles, and clashes. The OPLS_2005 force field was used to minimize the Genistein structure. The structures were also desalted and stereoisomers were generated by retaining the specific chirality **[11]**.

2.2. *In-Vivo Study*

2.2.1. Hormonal Analysis

Approximately 7-8 week old thirty female Wistar rats were housed and acclimated at approximately 24 ± 2 °C temperature with 10h : 14h light : dark cycle in the animal house of the Amity Institute of Pharmacology, Amity University Uttar Pradesh, Noida, India, following the guidelines of the Institutional Animal Ethics Committee (IAEC) formed under CPCSEA, Govt. of India. They were provided commercially available feed and water ad libitum. Commercially available 98% pure HPLC grade Genistein powder purchased from Sigma Aldrich was used to prepare a dose containing 10 mg, 20 mg and 30 mgGenistein / kg body weight / day, dissolved in 1: 4 Dimethyl sulfoxide (DMSO): Phosphate buffered saline (PBS).

Thirty females rats, (6 ratsin each groups) were administered orally 10 mg(gp-3), 20 mg(gp-4) and 30 mg(gp-5) Genistein/kg body weight/day, dissolved in 1:4 Dimethyl sulfoxide (DMSO):Phosphate buffered saline (PBS) up to 15 days and remaining six received equal volume of vehicle (1: 4, DMSO : PBS) alone (gp-2) for 15 days and last group of six rats were treated as negative control(gp-1). On 16^{th}day all the rats were sacrificed by cervical dislocation and their blood were collected into sterile collection vials by retro-orbital method. Sera were separated by appropriate technique and estimation of Estrogen (E2) hormone were done using appropriate ELISA assay kits developed by My Bio-Source, Inc., San Diego, USA.

2.3 Statistical analysis

Statistical analysis was carried out by oneway (ANOVA). Results were expressed as mean ±SE and P values $p<0.05$ were considered as significant.

3. RESULTS

3.1. *In-Silico* Study

Shown in **Table 3.1.1.1 and Figure 3.1.1, 3.1.2, 3.1.3.**

3.1.1. Molecular Docking

Glide with XP calculations were carried out to calculate the Glide Score, with ERA & ERB as receptors and Genistein, Estradiol, 4-[(2-{4-[(1E)-1-(1H-indazol-5-yl)-2-phenylbut-1-en-1-yl]phenoxy}ethyl)amino]-N, N-dimethylbutanamide and (R,R)-5,11-cis-diethyl-5,6,11,12-tetrahydro-chrysene-2,8-diol as ligands.The docking grid was constructed around the active site residues of the known antagonists (4-[(2-{4-[(1E)-1-(1H-indazol-5-yl)- 2-phenylbut-1-en-1-yl] phenoxy}ethyl) amino] -N , N-dimethyl-butanamide and (R,R)-5,11-cis-diethyl-5 ,6,11,12-tetrahydro -chrysene- 2,8-diol) and agonists (Estradiol), having dimensions as 15*15*15 Å **[12]**.

3.2. *In-Vivo* Study

3.2.1. Effect of Genistein on Estrogen level

It was observed that the estrogen level was increased in the treatment group -6in comparison with all the other groups. The decreased level of serum estrogen may disrupt the ovulation process by negative feedback, which will further block the secretions of FSH and LH **(Figure 3.2.1)**.

Table 3.1.1: Comparison with known Antagonist

S. No.	Receptor	Ligand	Mechanism	Docking Score
1	Estrogen Receptor α	4-[(2-{4-[(1E)-1-(1H-indazol-5-yl)-2-phenylbut-1-en-1-yl]phenoxy}ethyl)amino]-N,N-dimethylbutanamide	Antagonist	-7.389
2	Estrogen Receptor α	Genistein	Antagonist	-8.692
3	Estrogen Receptor β Chain-A	(R,R)-5,11-cis-diethyl-5,6,11,12-tetrahydro-chrysene-2,8-diol	Antagonist	-8.263
4	Estrogen Receptor β Chain-A	Genistein	Antagonist	-9.11
5	Estrogen Receptor β Chain-B	(R,R)-5,11-cis-diethyl-5,6,11,12-tetrahydro-chrysene-2,8-diol	Antagonist	-7.242
6	Estrogen Receptor β Chain-B	Genistein	Antagonist	-9.073

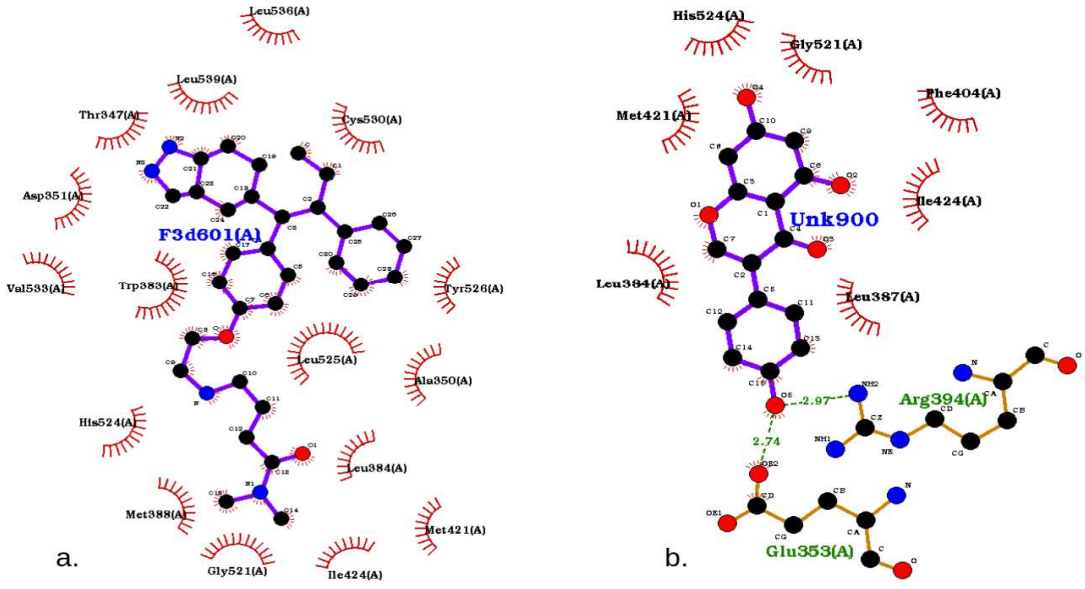

Figure 3.1.1: ((a) ERA-Chain-A in complex with 4-[(2-{4-[(1E)-1-(1H-indazol-5-yl)-2-phenylbut-1 -en-1-yl]phenoxy}ethyl)amino]-N,N-dimethylbutanamide; (b) ERA-Chain-A in complex with Genistein)

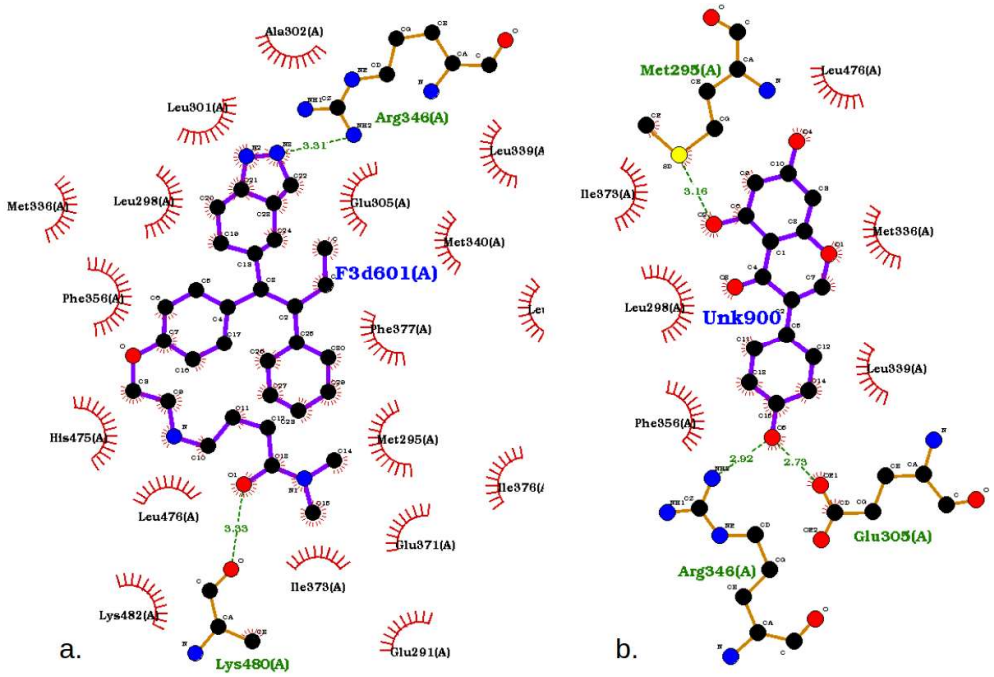

Figure 3.1.2 ((a) ERB-Chain-A in complex with (R,R)-5,11-cis-diethyl-5,6,11,12-tetrahydro- chrysene-2,8-diol; (b) ERB-Chain-A in complex with Genistein)

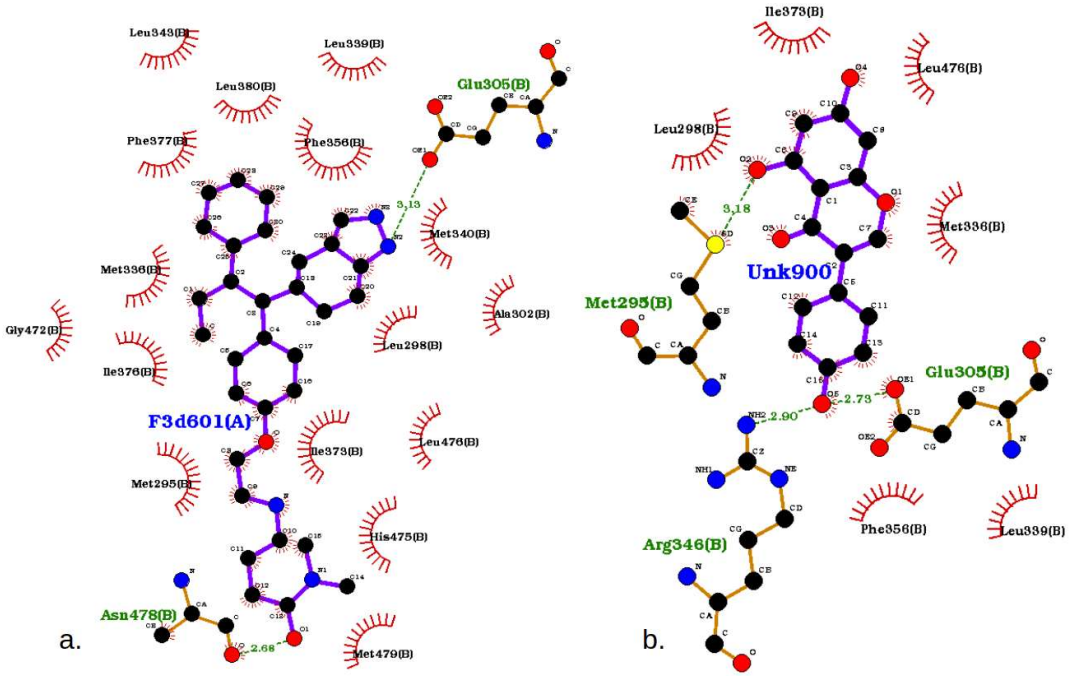

Figure 3.1.3: ((a) ERB-Chain-B in complex with (R,R)-5,11-cis-diethyl-5,6,11,12-tetrahydro- chrysene-2,8-diol; (b) ERB-Chain-B in complex with Genistein)

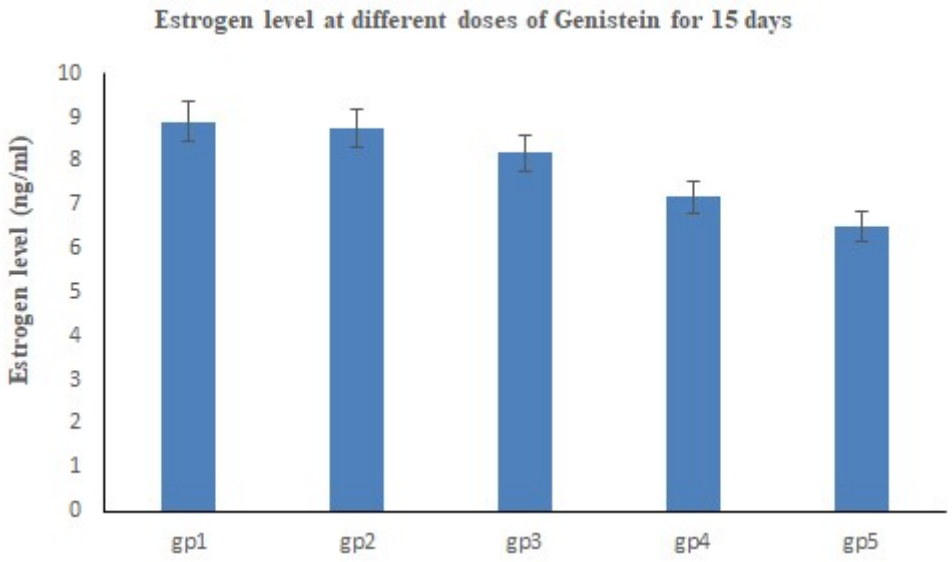

Figure 3.2.1: Comparision of circulating Estrogen levels (ng/ml) in Group 1(negative control), Group 2 (Vehicle), Group 3(Genistein -10 mg/kg) , Group 4 (20mg/kg) and Group 5 (30mg/kg) after 15 days

4. DISCCUSION

Genistein showed better docking affinity (More Negative Docking Score) as compared to the already known antagonist in computational analysis. This confirms the antagonist nature of Genistein against the ERA and ERB. In order to evaluate its antifertility effects, the compound was administered to female wistar rats for 15 days and the blood sample was collected after the last treatment on 16th day for hormone analysis through ELISA. The results depict that the level of estrogen was low in 3rd, 4th and 5thgroups (10 mg/kg, 20 mg/kg and 30 mg/kg, Genistein administered) as compared to all other groups (control, vehicle). Imbalance in the hormones leads to irregularity in the ovarian functions and duration of the estrus cycle **[13-15]**, which indicates an availability of the matured secondary follicles Graafian follicles). Therefore, ovulation may be hindered or inhibited.

The essential role in the regulation of ovarian function is controlled by the hypothalamus- pitutary- gonadal axis. Functioning in a coordinated way with suitable signals provided by ovary through pituitary gland is responsible for the production of gonadotrophins (LH and FSH). However, administration of the Genistein to the female rats showed a remarkable decrease in the level of estradiol hormones. Estradiol is the most potent naturally occurring ovarian and placental estrogen in mammals. It prepares the uterus for implantation of the fertilized ovum and promotes the maturation and maintenance of the female accessory reproductive organs and secondary sexual characters. Plants with estrogenic activity can directly affect pituitary action by

modulating LH and FSH, decreasing secretion of these hormones, and blocking ovulation **[16]**. Thus, decreased serum concentration of estradiol observed in the group may be attributed to a decreased aromatase activity or substrate supplementation during estrogen synthesis **[17]**. Hence, such decreased in estradiol levels may hinder ovulation, preparation of the reproductive tract for zygote implantation, and the subsequent maintenance of pregnancy state **[17]**.

5. CONCLUSION

The present study shows that Genistein has antagonist effects validated through *in-silico* and *in-vivo* study both. The decreased level of estrogen led to interrupt the ovulation process in female rats. Further study needs to be done to evaluate level of other gonadotropins to support this finding.

6. ACKNOWLEDGMENT

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