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ANTI-OVULATORY EFFECTS OF *LEPIDINE* IN FEMALE WISTAR RATS

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ABSTRACT

Combined oral contraceptives (COCs) are prepared using synthetic progesterone, estrogen and serve as most convenient, safe, effective, and reversible 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 at evaluating the antifertility effects of lepidine, isolated from *Lepidium sativum,* via *in-silico* and *in vivo* experimentation on female *Rattus norvergicus* (Wistar albino rats). Human homolog of Progesterone receptor (Pgr), which is 95.4% identical to the rat Pgr, was retrieved from the Protein Data Bank (PDB) for molecular docking evaluation. Schrödinger Suite was used for protein and ligand preparation. Schrödinger Glide with XP calculations were carried out to calculate the Glide Score, with human Pgr as receptor and Lepidine as a ligand. To further investigate the *in vivo* anti-fertility effects of lepidine on estrous cycle and its correlation with serum progesterone, adult *Rattus norvergicus* (Wistar albino rats) was administered with 10, 20 and30mg lepidine/kg body weight dissolved in DMSO: PBS (1:4) vehicle for 15 days. Vaginal smear samples were collected for cytological studies and progesterone hormone level in the serum was estimated through ELISA. *In-silico* observation has shown that lepidine has strong affinity with human progesterone receptors. Serum progesterone level was found to be maintained throughout the estrous cycle. This shows that the lepidine might be working as anti-ovulatory agent by acting as a phyto progesterone.

Key words: Lepidine, herbal contraceptives, anti- ovulation, progesterone receptor, docking study, estrous cycle

1. INTRODUCTION

Combined oral contraceptives (COCs) have become a popular method of birth control due to their contraceptive efficacy and good tolerability profile **[1]**. These medicines are made up of synthetic progesterone and synthetic estrogen that are given as birth control pills. While the efficiency of such drugs is excellent, they are known to have side effects such as nausea, dizziness, headaches, stomachaches, and vomiting. An antifertility compound is considered to be effective in females when it prevents ovulation, fertilization or implantation. In recent years, there has been a considerable interest in plants with possible antifertility effect **[1]**. Many researchers have emphasized the importance of medicinal plants as a source of anti-ovulatory compounds **[3-5]**. The plant-derived compounds can directly influence the pituitary gland by decreasing the secretion of luteinizing hormones (LH) and follicle-stimulating hormones, eventually blocking ovulation or implantation **[6]**. Plant kingdom, therefore, holds a great promise for the discovery of new and effective antifertility agents **[7]**. In present study 4-methylquinoline or Lepidine, commonly known as garden cress, was taken into consideration, which is a heterocyclic aromatic organic compound isolated from *Lepidium sativum* **[8-11]**. This plant has been recognized to possess abortifacient, aphrodisiac, teratogenic antifertility and antiovulatory properties **[12,-16]**. Wistar female rat was used as animal model for the study. The reproductive cycle of female rats is known as estrous cycle and is distinguished as proestrous, estrous, metestrous (or diestrous I) and diestrous (or diestrous II) **[17, 18]**. The ovulation occurs from the beginning of proestrous to the end of diestrous **[19, 20]** and the mean length of the cycle in the female rat is 4 days **[17, 18, 21]**. The short cycle length makes the rat an ideal model for investigation of changes in the reproductive cycle.

2. MATERIAL AND METHODS

2.1 Pairwise Sequence Alignment

Pgr sequences of rat (Q63449) and *Homo sapiens* (P06401) were retrieved from Swiss-ProtKB Database. Local sequence alignment was carried out using the Smith-Watermen algorithm using EMBOSS – Water **[8]**. The alignment showed 98.1% similarity with 95.4% identity with only two inserted gaps **(Figure 3.1)**. Active site residues GLN725A, ASN719A and Met759A of the human Pgr aligns identically to the rat's Pgr **[9]**. Therefore, human Pgr crystallographic structure (3d90) was retrieved from PDB and was used for further molecular docking evaluation.

2.2 Protein Structure and ligand Preparation

Missing hydrogens were added to stabilize the PDB file. Potentially transposed heavy atoms in arginine, glutamine, and histidine side chains were corrected and optimization of the protein's hydrogen bond network was done by means of a systematic, cluster-based approach. 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 **[10]**. The OPLS_2005 force field was used to minimize the lepidine structure and the possible states were generated using Epik at the physiological pH of 7.0 +/- 2.0. The structures were also desalted and stereoisomers were generated by retaining the specific chirality **[11]**.

2.3 Schrödinger Glide with XP for Molecular Docking

Schrödinger Glide with XP calculations were carried out to calculate the Glide Score, with Human Pgr as receptor and Lepidine as a ligand **[11]**. The docking grid was constructed around the active site residues GLN725A, ASN719A, and Met759A, having dimensions as 15*15*15 Å.

2.4 Experimental Animal

The animal study was performed in the Amity Institute of Pharmacology, Amity University, Uttar Pradesh, Noida, India. Animals were procured from animal house of Amity University, Uttar Pradesh, Noida, India. All the experimental protocols were approved by the Institutional Animal Ethics Committee for the Purpose of Control and Supervision of experiment on animal (CPCSEA) guidelines (CPCSEA/IAEC/AIP/2018/05/05).

The animals were housed in the animal facility and were maintained with food and water ad libitum in a temperature-controlled room (23 ±1ºC) with light:dark (12/12 h, lights on: 8:00 AM) cycle. Female albino rats (Wistar strain weighing 150-200 g) were used for the study of anti-ovulatory activity. The rats were acclimatized to laboratory hygienic conditions for 10 days before starting the experiment.

2.6 Effect of Lepidine on the estrous cycle of rats

Six groups, with six animals in each group, were studied for 15 days. Group 1 was control, group 2 was administered vehicle (DMSO: PBS in 1:4), group 3 was given standard drug (OVER ALL®) and group 4, 5 and 6 were administered 10, 20 and 30mg/kg of lepidine respectively. All the compounds were administered orally. The vaginal smears were collected from each animal on daily basis (between 9-10 am) for 15 days to determine the different stages of estrous cycle. Three different types of the cells were observed in the vaginal smear, i.e. nucleated cells, cornified cells and multi nucleated leukocytes. On

the basis of the proportion of these cells, four different stages of estrous cycle of female rats were identified.

2.7 Effect of Lepidine on the serum progesterone level in rats

The blood sample was taken from the rats at the time of sacrifice on the 16th day after the treatment. Blood serum was separated and progesterone was estimated through ELISA (Biomeriux-**VIDAS® Estradiol kit** and **VIDAS® Progesterone kit**).

2.8 Histopathological study of ovarian tissue

This study was done to determine the effects of lepidine on the ovarian tissues at morphological level. After the treatment, rats were sacrificed on 16th day and ovaries were fixed in formalin.Post fixation the tissues were routinely processed, embedded in paraffin, and 5 μm thick sections were cut and stained with haematoxylin and eosin for light microscopic evaluation.

2.9 Statistical analysis

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

3. RESULTS

3.1 Molecular Docking

Our Schrödinger's Glide calculations with XP, with Human Pgr as receptor and Lepidine as ligand computed the Glide score equivalent to -7.716 Kcal/mol. Lepidine binds with the GLN725 of chain A of human Pgr, which is one of the active site residues that are responsible for the activation of the Human Pgr **{Figure 3.1(a) and (b)}**. Our study resulted in the identification of potential hit against the Human Pgr which holds 95.4% identity with the Rat Pgr, therefore, it paves the way for future wet-lab experimentation on rat model.

3.2 Estrous Cycle Analysis

In all the groups animals exhibited normal cyclical oestrus phase throughout the study. Different phases of the estrous cycle are shown in **Figure 3.2**. It is observed that treatment of rats with lepidine prolonged the estrous cycle after 4.5 days on an average. This estrous suppressing effect of lepidine lasted for some period of drug treatment and also showed the reduced duration of estrous and metestrus phases, characterized by a prolongation of the diestrous phase ($P< 0.05$). From the above observations, it is seen that lepidine caused suppression of the estrous phase in female albino rats in a dose-dependent, reversible manner. Since estrous phase in an animal is a manifestation of ovulation, it may be presumed that suppression of estrous phase in albino rats is due to suppression of ovulation, suggesting an anti-ovulatory effect of the drug in the experimental group of animals.

3.3 Effects on Progesterone level

It was observed that the progesterone level was increased in the treatment group -6in comparision with all the other groups. The increased level of serum progesterone may cause delay/reduction in the ovulation process **(Figure 3.3)**.

3.4 Histopathological study of Ovarian Tissue

More primordial follicles formation was observed in the treated group than the control group. That may depict that maturation of the follicles is being hampered **(Figure 3.4)**.

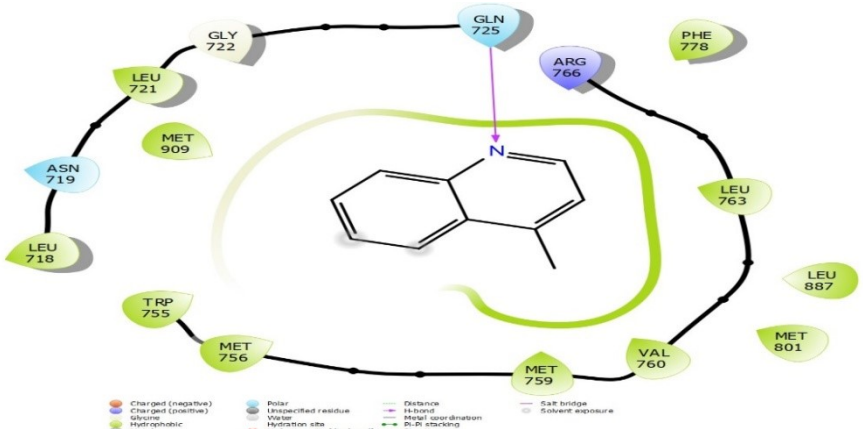


Figure 3.1 (a) 2-D Interaction diagram for Human Pgr as receptor and Lepidine as ligand; Docked Lepidine, forming hydrogen bond with GLN725A i.e. one of the active site residues

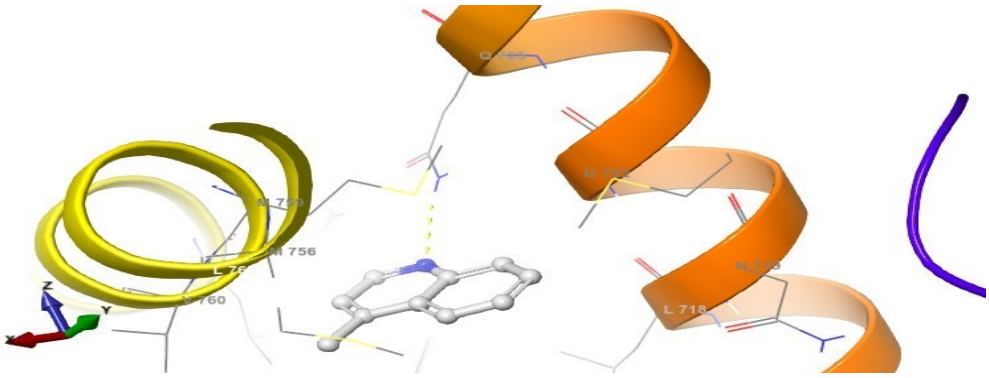
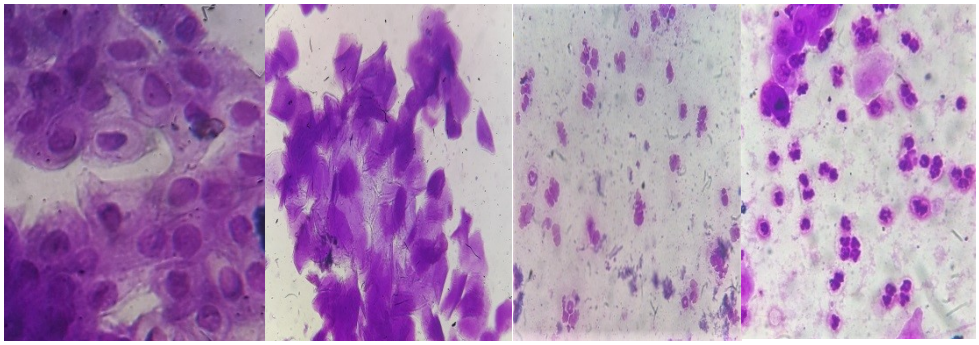


Figure 3.1(b) 3-D Interaction diagram for Human Pgr as receptor and Lepidine as ligand; Docked Lepidine, forming hydrogen bond with GLN725A i.e. one of the active site residues



(a) (b) (c) (d)

Figure 3.2 - Each value represents the mean ± S.E.M. (n=6); * *P* < 0.05 (a) Proestrous- nucleated cells are present, (b) Estrous- all cornified cells are present, (c) Metestrous –nucleated, cornified and leukocyte cells were present and (d) Diestrous- Leukocytes were observed

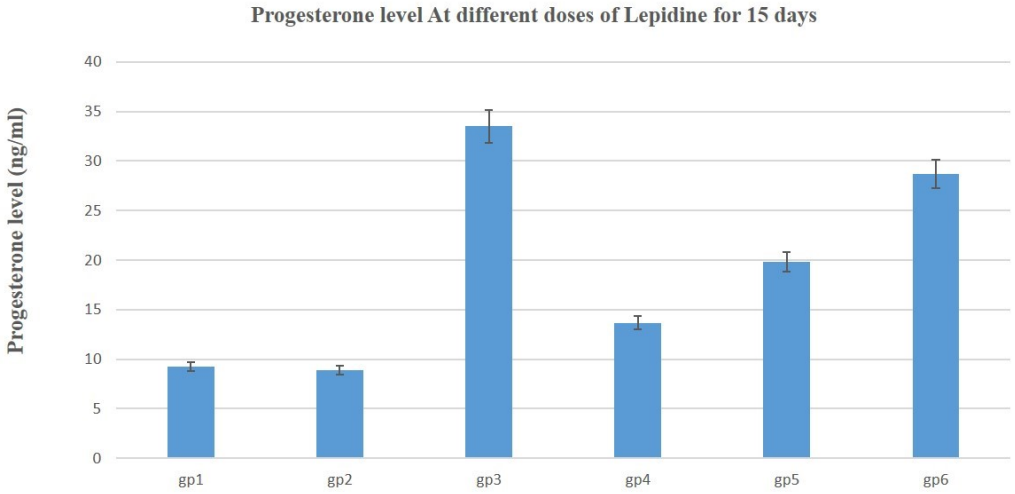
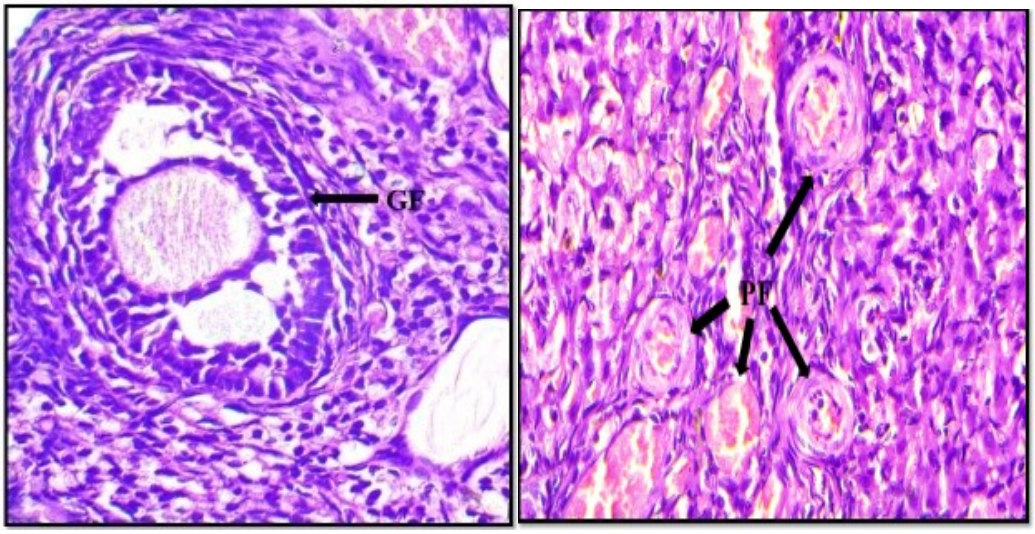


Figure 3.3: Comparision of circulating Progesterone levels (ng/ml) in Group 1(negative control), Group 2(Vehicle), Group 3(Synthetic drug), Group 4(Lepidine-10 mg/kg) , Group 5 (20mg/kg) and (30mg/kg) after 15 days



(a) **(b)**

Figure: 3.4 Longitudinal sectionsof control (a) GF(graffian follicle) and the treated(30 mg/kg) (b) ovary of Wistar Rat (primoidal follicles)

4. DISCUSSION

This study was focused on Lepidine (phytochemical, isolated from *Lepidium sativum* plant). Lepidine has similarity with progesterone molecule ,this was observed with the help of *in-silico* studies. In order to check its antifertility effects, it was adminnistered to wistar rats for 15 days and it was obsereved that diestrous stage of the estrous cycle has been delayed.The blood sample was collected after the last treatment on 16th day for hormone analysis through ELISA. The results depict that the level of progesterone was high in 3rd, 4th and 5th groups (10 mg/kg, 20 mg/kg and 30 mg/kg, lepidine administered) as compared to all other groups (control, vehicle). According to the observations we may state that, Lepidine might be working indirectly as an anti-ovulatory/anti-fertility agent at the dose of 30mg/kg, as it has been found to increase the progesterone level in the

blood serum. There could be a reason that the high level of progesterone in the blood serum may be inhibiting the FSH and LH release in the treated group respectively, which in turn blocking the development of the follicles and further its release. The higher level of progesterone results in endometrial thickening and thickening of cervical mucus, which may inhibit the sperm to enter cervix and further inhibit fertilization. Histological studies of ovarian section have also shown many primordial follicles in treated groups **{Figure 3.4 (b)}**, whereas in control mature secondary follicle were observed as shown in **Figure 3.4 (a)** which might be associated with elevated serum progesterone levels.

The effect of lipidine on follicular development, LH surge, and ovulation is dependent on time of treatment and dose given. Repeated administration of lepidine daily, during mid- to late follicular phase may delay the estrogen surge, which is required to trigger the LH surge and further ovulation **[22, 23]**.

5. CONCLUSION

On the basis findings we may consider that lepidine has role in up regulating the progesterone receptors and possibly suppressing the FSH, LH and estrogen receptors. However further work needs to be done to determine the precise effect of Lepidine on estrous cycle of rats with some different doses as it did not show any toxicity up till 100mg/kg of dose.

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