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Effect of *Mentha piperita* and *Aloe vera* in Estrous cycle on Estradiol valerate induced Polycystic ovarian syndrome in Mice (*Mus musculus*)

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ABSTRACT

Background:- Infertility due to polycystic ovarian syndrome (PCOS) is a worldwide problem that is increasing at alarming rates. It is characterized by polycystic ovaries, chronic anovulation and hyper-androgenism leading to symptoms of irregular menstrual cycles, hirsutism, acne and infertility. Insulin resistance and elevated levels of male hormones (androgens) are associated with PCOS. The sedentary lifestyle, dietary variations, lack of exercise and stress etc. are also the contributory factors. The present study was conducted to investigate the effect of *Aloe vera* gel and peppermint on polycystic ovary syndrome in Swiss albino.

Materials and Methods:- Adult female albino mice (25-30gm) treated by one intramuscular injection of 2mg/kg body wt. with Estradiol valerate solvated in olive oil at the estrous stage for PCOS development, and were further treated with *Aloe vera* gel (0.5ml /mice) and peppermint(0.5ml\mice) for 30 days. Parameters that were evaluated include, estrus cyclicity. This preliminary study explores the effectiveness of *Aloe vera* gel and peppermint in prevention and treatment of PCOS condition.

Result and conclusions:-The combination of *Aloe vera* gel (AVG) and peppermint extract is of interest because of its ability to regulate estrous cyclicity in PCOS-induced mice. Both herbal plants have unique features that may help restore estrous cycle regularity and improve ovarian function.

Keywords:- Estradiol valerate, Clomiphene citrate, PCOS, *Aloe vera* gel, anovulation, anti-hyperlipidemic, estrus cycle, insulin resistance, hyperandrogenism, infertility.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a multifactorial, heterogeneous, complex genetic, endocrine and metabolic disorder, diagnostically characterized by chronic anovulation, polycystic ovaries, biochemical and clinical manifestations of hyperandrogenism (Homburg, 2009). The symptoms of PCOS include clinical ones (menstrual disorders, hirsutism, acne, baldness, and infertility), changes in endocrine hormones (increased levels of androgen, estrogen, and prolactin and decreased level of progesterone), and

metabolic disorders (insulin resistance, diabetes, dyslipidemia, and type 2 diabetes). However, in some cases, estradiol level does not change (Jelodar and Askari, 2012). In vivo and in vitro studies on theca cells suggested that ovarian theca cells are much more active to convert androgenic precursors into testosterone in women with PCOS than in healthy women. Indeed, Theca cells produce androgen in response to luteinizing hormone (LH); therefore, androgen level in blood increase in people with PCOS (Rosenfield, and Ehrmann, 2016). The high level of androgen especially testosterone in PCOS, cause lack of ovulation and disrupted synthesis of sex hormones, result into clinical symptoms and dysfunction of genital tract in the patients. These are the main reasons for infertility in reproductive-age women (Kirilovas et. al. 2016, Brenner et. al. 2002). PCOS is detected in approximately 4–10% of women of reproductive age (Strowitzki et. al. 2010; Teede et. al. 2010).

The best known treatment for PCOS at present is by using allopathic medicines such as clomiphene citrate, metformin, letrozole, tamoxifene and troglitazone. All these allopathic medicines have mild to severe side effects such as hot flushes, arthritis, joint or muscle pain and psychological side effects such as irritability, mood swings, depression and bloating (Jadhav and Bhutani, 2005; Sasikala and Shamila, 2009). In spite of tremendous progress in the development of modern medicine, plants continue to be an important source of drug for the treatment of several diseases and hence demands for plant drug have increased (Pandey et. al. 2003). Herbal plant drug form main source of health care due to more effectiveness, lower cost and well tolerated by the patient having fewer unintended consequences and fewer side effects than traditional medicine and may be safer to use. Herbal plants have been used since centuries to correct disorders caused by the hormonal imbalance related to female reproductive system (Nadkarni, 1982; Shearman, 1985; Khare, 2004). To a large worldly population, medicinal plants are the only source to prevent and treat various diseases. Herbal product such as Ashwagandha, Mimosa, *Aloe vera* etc. has been used by some researchers (Craig, 1999).

Due to the increasing prevalence of PCOS and associated physical and mental problems as well as the effects of changes in sex hormones in development of this disease, our aim is to study the pathophysiology and etiology of this disease along with the effects of different herbal extracts on changes in the serum levels of sex hormones and ovarian tissue.

The plant *Aloe vera* is used in various streams of medicine. Numerous vitamins, minerals, enzymes, natural sugars, amino acids and bioactive compounds present in *Aloe vera* leaf (Sahu et. al. 2013). *Aloe vera* is a short-stemmed succulent perennial herb belongs to family Liliaceae. It is common plant cultivated throughout India and has many varieties (Palkhade, 2003). This plant is mostly used externally in cosmetic related problems like acne, burn and minor cut and it is an extremely powerful laxative. The plant also possess antioxidant, anti-helminthic, antidiabetic, anti-inflammatory, antiseptic, and antifungal activity (Sahu et. al. 2013).

Sethiet. al. (2012) showed that *Aloe vera* has good antioxidant and antidiabetic effect. It has been reported that aqueous extract of *Aloe vera* might contain estrogen. In the same study, it was determined that *Aloe vera* extract increases ovarian steroidogenesis and significantly increases the production of estradiol and progesterone (Telefo et. al. 2004). Some studies have been reported that the effect of Aloe barbadensis was similar to the effect of estrogen on the reproductive system (Kosif et. al. 2009).

The entire peppermint plant has therapeutic properties. Dried peppermint leaves are used to make tea, which can help alleviate coughs and bronchitis, as well as oral mucosal and throat irritation. Traditionally, the plant was used to treat flatulence, diarrhoea, nausea, vomiting, indigestion, anorexia, and morning

sickness. The medicine also serves as a spasmolytic agent, reducing gas and stomach cramps. Menthol oil is used in the manufacture of toothpaste and can help ease menstruation cramps in women. Species like M. piperita are being utilized to treat Crohn's disease, irritable bowel syndrome, gall bladder, and biliary tract illnesses (Rita et al., 2011; Tyler, 1992).

Aside from therapeutic applications, it is used to flavour medications, dental preparations, mouthwashes, cough drops, soaps, chewing gum, sweets, confectionery, and alcoholic beverages. The green plant that remains after oil extraction may be dried into hay and fed to cattle. Protein (12.7%), digestible protein (8.5%), and total digestible elements (49.4%) make up the hay's nutritive ratio of 4.8. (The Wealth of India, 1962).

MATERIALS AND METHODS

Experimental animals:

Experimental animals i.e. Mus musculus will be reared and maintained in polypropylene cage in departmental animal house as per ethical rule and regulations. Animals were maintained in hygienic and standard environmental conditions with food and ad-lib water. Female Swiss albino mice weighing around 25-30gms were used in the present study.

Selection and Collection of Plant Material

***Mentha piperita* (Peppermint):-** Among the several plants, *Mentha piperita* Linn. commonly known as peppermint, is an important medicinal herb. It belongs to family Laminaceae. Fresh mature peppermint leaves are collected from Bhagalpur market and grown it in own garden

***Aloe vera*:**Mature leaf freshly plucked was selected for the experiment (Maharjan R. et. al. 2013). It belongs to family asphodelaceae (Liliaceae). About 3-3.5 year old *Aloe vera* leaves will be collected from different garden.

Estradiol Valerate: - For an experimental induction of PCOS, a long-acting Estradiol valerate (EV) has been used (Brawer et. al. 1978, 1986). The morphological changes include atretic antral follicles, follicular cysts with a well-developed theca cell layer, a diminished granulosa cell compartment and luteinized cysts (Brawer et. al. 1978, 1986).

Preparations of peppermint herbal tea:-Fresh mature peppermint leaves were taken and washed with tap water then leaves were shed dried at normal room temperature. The herbal tea of peppermint was prepared daily by pouring 250 ml (1 cup) of boiling water over approximately 10gm (40gm/l) of the dried leaves and left for steeping for 5–10 min. Then the peppermint herbal tea was filtered and preserved for the further use (Akdogan et. al., 2007).

Preparation of *Aloe vera* gel-A fresh mature leaf of *Aloe vera* was washed and cleaned with water then was cut transversely with a sterilized knife. The gel was then scoped with the help of a spoon and homogenized in a blender as suggested by Mahajan et. al. (2013) and was used for the treatment the very same day.

EXPERIMENTAL SETUP

Induction of PCOS:-Animals will be checked 15 days for regular cycles by smear test (Marcondes and Bianchi et. al., 2002). PCOS will be induced by one intramuscular injection of 2mg/kg Estradiol Valerate solvated in olive oil at the estrous stage after (Brawer, 1986). The most important sign of PCOS is irregular estrous cycles and persistent vaginal cornification (PVC) which will be confirmed by vaginal smear. 24

hours after the administration of Estradiol valerate blood samples were collected for hormone estimation. One mice was sacrificed and ovaries were collected for histopathological examination as suggested by (Kafali et. al., 2004).

Design of Experiment:-PCOS was induced in thirty three (33) female albino mice. Five (5) representive mice will be sacrificed on the last 40th day of Estradiol valerate (2mg/kg body wt.) treatment for confirmation of PCOS in the albino mice. After confirmation, remaining 28 albino mice will be divided into 5 group i.e.T0, T1, T2, T3, T4and T5.Mice will be treated with dose used by (Kashinath, 2019).

Group-T0 Normal control**,**without E.V. treated

Group-T1 PCOS positive mice (E.V Treated) were conducted as a Positive control group. **Group-T2** received Clomiphene citrate @ 1mg /kg body wt. (p.o.) as referral standard drug. **Group-T3** received *Aloe vera* gel 0.5 ml daily (p.o).

Group-T4 received peppermint 0.5 ml/mice.

Group-T5 received a combination of *Aloe vera* gel and peppermint.

Estrous cycle (vaginal smear examination):- Vaginal smear of all mice will be collected daily before treatment by using dropper filled with normal saline (0.9% NaCl). Different stages will be evaluated microscopically (Shorr, 1941).

RESULT AND DISCUSSION

Estrous cycle:-

When the mice reached adolescence, daily vaginal smears were studied using the standard way to see if they were cycling regularly (Marcondes et. al., 2002). Smears from vaginal washing were analysed under a microscope to determine the relative quantity of nucleated epithelial cells, cornified cells, and leukocytes. Vaginal smears were taken every day at 10:00 a.m. from 5 or 6 weeks of age till the completion of the investigation. Cycles lasting 4-5 days were considered regular. Persistent vaginal cornification PVC) was defined as the presence of cornified cells in smears for at least ten consecutive days and was thought to indicate follicular cystic development (Salvetti et. al. 2004).

The predominant clinical signs of polycystic ovarian syndrome (PCOS) include irregular menstrual cycles and chronic anovulation, which has been associated with nearly 80% of PCOS women. (Dunaif 1999). Hence, estrus cyclicity in PCOS albino mice was monitored. Wherein PCOS albino mice (EV treated @2mg/kg bd. wt.) exhibited arrested cyclicity in late diestrus phase of cycle as compared to normal control group of mice. After treatment of CC, AVG, Peppermint extract and AVG + Peppermint at various doses (1mg/kg bd. wt., 0.5ml/mice, 0.5 ml/mice, 0.5 ml + 0.5ml respectively) and various time period (10 days, 20 days and 30 days), estrus cyclicity was evaluated (Table- 1).

Wherein administration of std. drug CC (1mg/kg bd. wt.), AVG (0.5 ml/mice), Peppermint extract (0.5 ml/mice) and AVG + Peppermint extract for 10 days treated group of mice exhibited reversion to normal cycle in 80% of PCOS mice. But at the same dose of CC, AVG, Peppermint and AVG + Peppermint extract for longer period of time (20 days and 30 days), all mice showed improved cyclicity and reverted back to normal cycle.

Table- 1: Comparison of estrous cycle of different treatment groups of albino mice.

DAYS	GROUP-T0	GROUP-T1	GROUP-T2	GROUP-T3	GROUP-T4	GROUP-T5

	Normal control (without any treatment)	Positive control (EV treated)	Std. Drug CC @ 1mg/kg bd. wt.	*Aloe vera* gel 0.5ml/mice	Peppermint 0.5ml/mice	*Aloe vera* gel +Peppermint
01	Estrus	Diestrus	Diestrus	Metestrus	Estrus	Metestrus
02	Estrus	Diestrus	Metestrus	Metestrus	Estrus	Metestrus
03	Estrus	Diestrus	Diestrus	Metestrus	Metestrus	Diestrus
04	Metestrus	Proestrus	Diestrus	Metestrus-Diestrus	Diestrus	Diestrus
05	Metestrus	Early Proestrus	Proestrus	Metestrus-Diestrus	Diestrus	Diestrus
06	Diestrus	Proestrus	Proestrus	Metestrus-Diestrus	Diestrus	Proestrus
07	Diestrus	Proestrus	Proestrus	Diestrus	Proestrus	Proestrus
08	Diestrus	Proestrus	Metestrus	Diestrus	Proestrus	Proestrus
09	Proestrus	Metestrus	Metestrus	Diestrus	Proestrus	Proestrus
10	Proestrus	Metaestous	Metestrus-Diestrus	Proestrus	Proestrus	Estrus
11	Estrus	Metestrus-Diestrus	Metestrus-Diestrus	Proestrus	Estrus	Estrus
12	Estrus	Metestrus-Diestrus	Diestrus	Metestrus	Estrus	Metestrus
13	Metestrus	Metestrus-Diestrus	Diestrus	Metestrus	Metestrus	Diestrus
14	Metestrus	Metaestous	Diestrus	Metestrus	Diestrus	Diestrus
15	Diestrus	Diestrus	Diestrus	Metestrus-Diestrus	Diestrus	Diestrus
16	Diestrus	Diestrus	Diestrus	Diestrus	Diestrus	Proestrus
17	Diestrus	Diestrus	Proestrus	Diestrus	Proestrus	Proestrus
18	Proestrus	Diestrus	Proestrus	Diestrus	Proestrus	Proestrus
19	Proestrus	Diestrus	Proestrus	Diestrus	Proestrus	Proestrus
20	Estrus	Diestrus	Metestrus-Diestrus	Metestrus-Diestrus	Estrus	Estrus
21	Estrus	Diestrus-Proestrus	Proestrus-	Metestrus-	Estrus	Estrus
22	Metaestous	Diestrus-Proestrus	Proestrus	Metestrus-Diestrus	Metestrus	Metestrus
23	Metestrus	Diestrus-Proestrus	Proestrus	Proestrus	Metestrus	Metestrus
24	Diestrus	Proestrus	Metestrus	Proestrus	Diestrus	Diestrus
25	Diestrus	Proestrus	Metestrus	Proestrus	Diestrus	Diestrus

26	Diestrus	Proestrus	Metestrus	Metestrus	Diestrus	Diestrus
27	Proestrus	Metestrus	Diestrus	Metestrus	Diestrus	Proestrus
28	Proestrus	Metestrus	Diestrus	Diestrus	Proestrus	Proestrus
29	Proestrus	Metestrus	Diestrus	Diestrus	Proestrus	Estrus
30	Proestrus	Metestrus	Proestrus	Diestrus	Proestrus	Estrus

Table- 2: Appearance of the vagina at different phases of the estrus cycle:-

Estrus Phase	Appearance
Proestrus	Vagina is gaping and the tissues are moist and reddish pink. There are numerous longitudinal folds or striations visible on the dorsal and ventral lips.
Estrus	Vaginal appears similar to that seen at proestrus, but the tissues are lighter pink and less moist. The striations are more prominent.
Metaestrus	Vagina tissues are pale and dry. The dorsal lip is not as edematous as in the estrus. Whitish cellular debris may line the inner walls or partially fill the vagina.
Diestrus	Vagina is moist and has a small opening and the tissues are bluish-purple in colour.

Vaginal Smear Cytology:-

Vaginal cytology, like visual assessments, is universally accepted. It appears to be the most popular method for determining the phases of the estrus cycle. It's non-invasive and relatively affordable. Although microscopic analysis of vaginal discharge cells needs some skill, it is precise and dependable. However, this method has also been reported to be tedious and time-consuming (Sahoo et. al., 2014).

During the assessment, the animal's forepaws are restrained. The tail is raised to reveal the vagina. To flush the vaginal cells, carefully introduce 100µl of distilled water or saline using a pipette or sterile latex bulb. Alternative fluids include phosphate-buffered saline. Slowly release the liquid into the vagina and bring it back into the tip; repeat 4 to 5 times with the same sterile latex bulb. It is critical that the pipette or sterile latex bulb is inserted at the entrance of the vaginal canal and does not enter the vaginal opening. The solution containing a few drops of cell suspension is then placed on a glass slide, air-dried, and dyed suitably. The 0.1% crystal violet stain (0.1 g of crystal violet powder in 100ml of double distilled water) can be used (Auta et. al., 2016). The slide will then be covered with a coverslip and viewed immediately under a light microscope at 200X magnification.

The vaginal secretion contains three types of cells: leucocytes, cornified epithelial cells, and nucleated epithelial cells. The percentage of these cells in the secretion is used to estimate the phases of the estrus cycle (Auta et. al., 2016) (Table- 1 to 3, Fig; 1).

The proestrus phase is distinguished by the presence of many spherical nucleated cells of uniform size and appearance. They appear in groups or alone. There are also a few enucleated, cornified epithelial cells. Some white blood cells may be present in the female during early proestrus (Table- 2, Plate-1; Fig; 2).

During the estrus phase, there are a lot of enucleated cornified epithelial cells with irregular shapes and granular cytoplasm. There are also a lot of bacteria and, on rare occasions, nucleated epithelial cells (Plate-2, Fig; 3).

At metestrus, there are many leucocytes and a few big, non-granular, and enucleated cornified epithelial cells. The cornified epithelial cells and polymorphonuclear leukocytes found in vaginal swabs are caused by the production of corpus luteum, which fails to fully luteinize due to a lack of progesterone and results in the shedding of the uterine lining. Some nucleated epithelial cells may be found in late metastrus (Plate-3, Fig; 4).

Diestrus phase showed numerous polymorphonuclear leukocytes, as well as a few epithelial and cornified cells. Leukocytes remain the major cell type after removing cellular debris (Plate-4, Fig; 5).

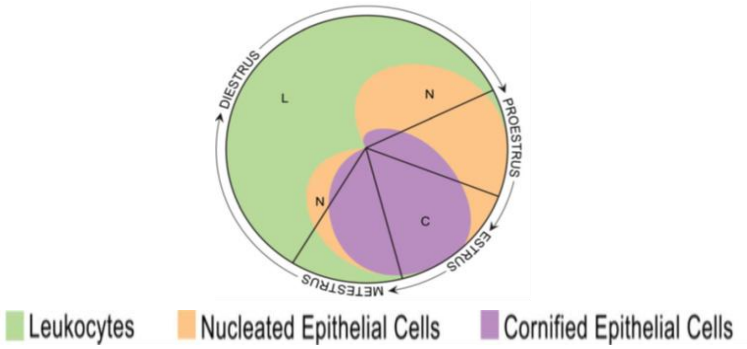

FIG:-1

Table- 3: Classification of the phases of estrus cycle based on the cell types and relative number of these cells in vaginal smears (Cora et al.):-

Estrus phase	Neutrophils	Small nucleated epithelial cells	Large nucleated epithelial cells	Anucleated keratinized epithelial cells	Relative cell density
Proestrus	0 to +	++ to +++	0 to +	0 to +	Low to moderate
Estrus	0 to +	0 to +	0 to +	++ to +++	Low to moderate
Metaestrus	++ to ++	0 to +	0 to +	++ to +++	Moderate to high
Diestrus	+ to ++	+ to ++	+ to ++	0 to +	Low to moderate

0 = none; + = few; ++ = moderate; +++ = high

Numerous round nucleated cells which are uniform in size and appearance characterize the proestrus phase. They appear in clusters or individually. There are also few anucleated cornified epithelial cells. Some white blood cells may be present in the female in early proestrus (Fig. 2).

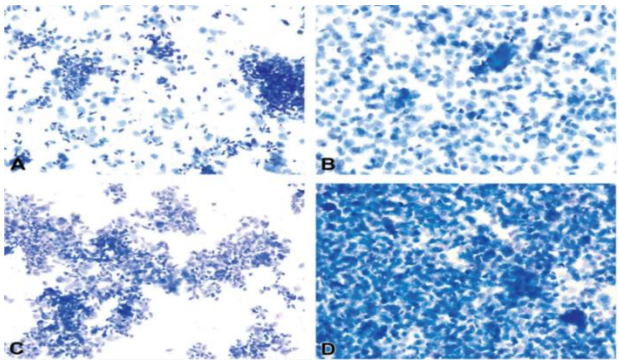

Plate:1; Fig: 2; Vaginal smears at proestrus in Albino mice

The estrus phase shows abundant anucleated cornified epithelial cells. The cytoplasm is granular, and the cells are irregular in shape. Also observed are numerous bacteria and occasionally, nucleated epithelial cells (Fig; 3).

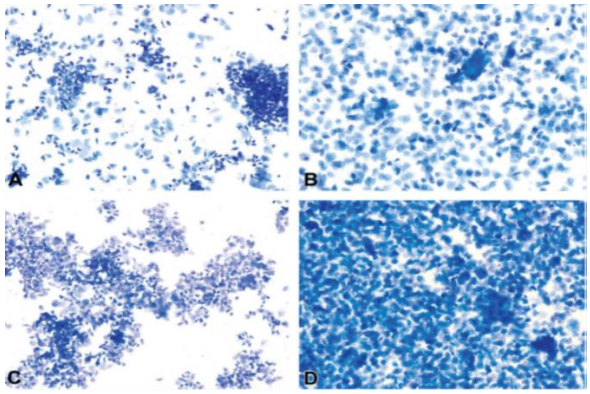

PLATE-2;Fig- 3: Vaginal smears at estrus stage in mice

At metestrus, a large number of leucocytes and a small number of large, non-granular and anucleated cornified epithelial cells are seen. The formation of corpus luteum which fails to fully luteinize due to lack of progesterone and results in sloughing off of the uterine lining accounts for the cornified epithelial cells and polymorphonuclear leukocytes present in vaginal swabs. Some nucleated epithelial cells may also be present in late metastrus (Fig; 4).

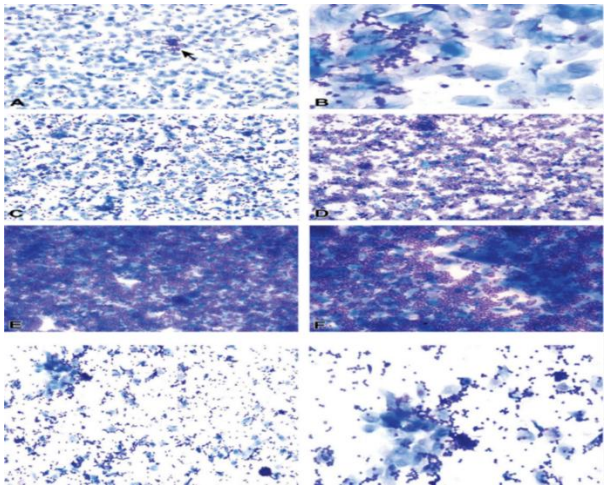

PLATE-3;Fig- 4: Vaginal smears at metaestrus stage in Albino mice

Diestrus shows prominent polymorphonuclear leukocytes and a few epithelial and cornified cells. Leukocytes remain the predominant cell type having removed cellular debris (Fig. 5).

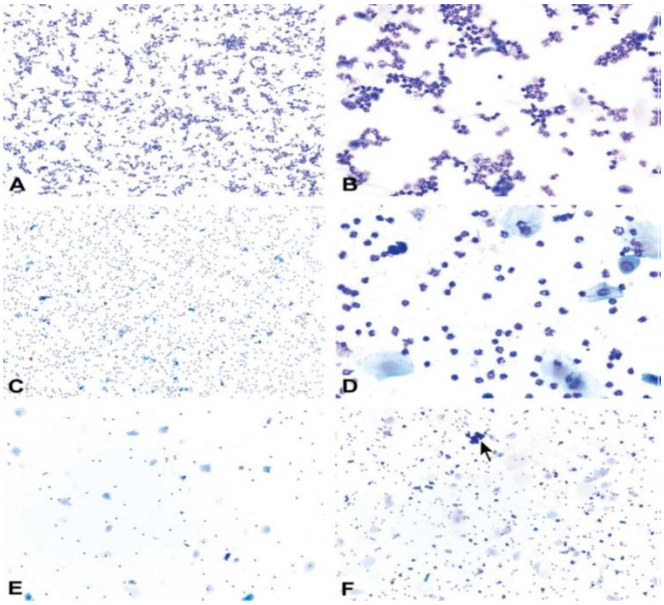

PLATE-4, Fig: 5; Vaginal smears at diestrus in mice (A-F).

CONCLUSION

Clomiphene citrate (CC) is a selective estrogen receptor modulator (SERM) that is frequently used as a fertility medication to stimulate ovulation, particularly in women with polycystic ovary syndrome. Clomiphene citrate has been tested in mice with PCOS to see how it affects estrou scyclicity and reproductive results.

Peppermint extract's effect on estrous cyclicity in PCOS-induced mice has piqued researchers' interest because of its anti-androgenic, antioxidant, and anti-inflammatory qualities. While research is still ongoing, several studies indicate that peppermint extract may help restore estrous cyclicity in PCOS models by modifying hormone levels and enhancing ovarian function.

AVG has been proven to restore estrous cyclicity in PCOS-induced mice by increasing ovarian function and managing the hormonal milieu required for a normal estrous cycle. AVG's effects may be mediated via antioxidant, anti-inflammatory, and hormonal balancing capabilities (Hanmman, 2008; Shilve et. al., 2014), which assist restore the disturbed hypothalamic-pituitary-ovarian (HPO) axis in PCOS

The combination of *Aloe vera* gel (AVG) and peppermint extract is of interest because of its ability to regulate estrous cyclicity in PCOS-induced mice. Both herbs have unique features that may help restore estrous cycle regularity and improve ovarian function.

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REFERENCES

1. Brawer J. R., Munoz M., Farookh R., (1986): Development of the polycystic ovarian condition

(PCO) in the estradiol valerate-treated rat: Biol. Reprod; 35: 647–655.
2. Brawer J.R., Munoz M., Farookh R. (1986): Development of the polycystic ovarian condition (PCO) in the estradiol valerate-treated rat: Biol. Reprod. 35: pp- 647–655.
3. Dunaif A., Segal K. R., Futterweit W., et. al., (1989): Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes; 38: 1165.
4. Hamman J. H., (2008): Composition and applications of *Aloe vera* leaf gel. Molecules; 13: 1599-1616.
5. Homburg R. (2009): Androgen circle of polycystic ovary syndrome: Human Reproduction: 24(7): pp-1548-1555.
6. Jadhav A.N., Bhutani K.K. (2005): Ayurveda and Gynaecological disorders: Journal of Ethnopharmacology: 97: pp-151-159.
7. Jelodar G., Askari K. (2012): Effect of Vitexagnus-castus fruits hydroalcoholic extract on sex hormones in rat with induced polycystic ovary syndrome (PCOS): Physiol. Pharmacol: 16: pp-62-69.
8. Kafali H., Iriadam M., Ozardali I., Demir N. (2004): Letrozole induced polycystic ovaries in the Rat: A new model for Cystic Ovarian Disease. Arch Med Res; 35: pp-103-108.
9. Kashinath B.S. (2019): Effect of *Aloe vera* gel and Mint tea on Letrozole Induced Polycystic Ovary Syndrome in Wistar rats: Maharashtra animal and fishery sciences University, Nagpur: 11: pp-156-196.
10. Kirilovas D., Chaika A., Bergstrom M., Prtterman E.B., Carlstrom K., Nosenko J., Korniyenko S., Yakovets A., Mogilevkina I., Naessen T. (2006): Granulosa cell aromatase enzyme activity: effects of follicular fluid from patients with polycystic ovary syndrome, using aromatase conversion and [11c] vorozole-binding assays, Gynecol. Endocrinol. 22: pp-685-691
11. Kosif R., Aktas R.G. (2009): Investigation of the Effects of *Aloe barbadensis* on Rat Ovaries: A Preliminary Study. J Med. Food; 12(6): pp- 1393-1397.
12. Maharjan R., Nagar P.S., Nampoothiri L. (2010): Effect of *Aloe barbadensis* Mill.formulation on Letrozole induced polycystic ovarian syndrome rat model: J. of Ayurveda & Integrative Medi. 1: pp-273-279.
13. Maharjan R., Nagar P.S., Nampoothiri L. (2013): Effect of *Aloe vera* extract on letrozole induced g Polycystic Ovarian Syndrome (PCOS) Rat Model. J Ayurveda Integr Med; 4: pp-273-279.
14. Marcondes F.K., Bianchi F.J., Tanno A.P. (2002,): Determination of the estrous cycle phases of rats: some helpful considerations: Braz J Biol: 62(4A): pp- 609–614.
15. Nadkarni A.K. (1982): Indian MeteriaMedica. Popular Prakashan: Private limited, Bombay: 3(1): pp-561-562.
16. Palkhade R.R. (2003): Pharmacological Investigation of *Aloe vera* Linn. With Reference to Analgesic, Antipyretic and Anti-inflammatory Activities. Master Thesis of Veterinary Science in Veterinary Pharmacology. Maharashtra Animal and Fishery Science University.
17. Pandey S.N., Singh M.P., Srivastava J.L. (2003): Indigenous medicinal plants, Social Forestry and Tribal, First edition, Daya publishing house, Delhi.
18. Rosenfield R.L., Ehrmann D.A. (2016): The pathogenesis of polycystic ovary syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism revisited, Endocrine: 37: pp-467-520.
19. Sahu P.K., Deendayalgiri R., Sing P., Pandey S., Gupta A.K., Shrivastava A. (2013): Therapeutic and Medicinal Uses of *Aloe vera*: A Review. Pharmacology and Pharmacy, 4.

20. Sethi J., Gupta A., Sood S., Dahiya K., Singh G., Gupta R. (2012): Antioxidant effect of *Aloe vera* in experimentally induced diabetes mellitus. Inter. J of Pharma. Sci. and Res. 3(8): pp-2522-2526.
21. Shorr E. A., (1941): New technic for staining vaginal smears III, a single differential stain: Science: 94: 545-546.
22. Silve M. A., Trevisan G., Hoffmeister C., et. al. (2014): Anti-inflammatory and antioxidant effects of Aloe saponaria Haw in a model of UVB-induced paw sunburn in rats. J PhotochemPhotobiol B.; 133: 47-54.
23. Strowitzki T., Capp E., Corleta H. (2010): The degree of cycle irregularity correlates with the grade of endocrine and metabolic disorders in PCOS patients: Eur. J. Obstet. Gynecol. Reprod. Bio: 149: pp-178-181.
24. Teede H., Deeks A., Moran L., (2010): Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med; 8(41):1-10.
25. Telefo P.B., Moundipa P.F., Tchouanguep F.M. (2004): Inductive effect of the leaf mixture extract of Aloe buettneri, Justiciainsularis, Diclipteraverticillata and Hibiscus macranthus on in vitro production of estradiol. J. of Ethnopharmacology; 91: pp-225-230.