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Effect of *Aloe vera* gel and mint tea on letrozole induced PCOS in rat model

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Abstract
The present study was conducted to investigate the effect of *Aloe vera* gel and Mint tea on Polycystic ovary syndrome in rat model. Adult female Wistar rat (150-200gm) rats treated orally with letrozole at the dose of 1mg/kg body wt. daily for 21 days for PCOS development, and were further treated with *Aloe vera* gel (1ml /rat p.o) and Mint tea (provided *ad-lib*) for 30 days. Parameters that were evaluated include b.wt, oestrus cyclicity, Ultrasonography, FSH and LH Level and Histopathology of ovary. Combination treatment of *Aloe vera* gel and Mint tea for 30 days in PCOS rats demonstrated most beneficial effects by restoring ovarian structure, hormonal level along with oestrus cyclicity. This preliminary study explores the effectiveness of *Aloe vera* gel and Mint tea in prevention and treatment of PCOS conditions.

Keywords: PCOS, *Aloe vera* gel, mint tea, letrozole

Introduction
Many new research and modern technologies are coming up to give a healthy and comfortable life for humans. As a result of this, human life has become effortless but on the other hand, stress is increasing day by day, leading to increases in various disorders. These lifestyle disorders are very common in young population especially the young generation in urban areas (Senapati *et al.* 2015) [13]. Among the various lifestyle disorder, PCOS is a common condition observed in 6 out of 10 women due to stressful and competitive lifestyles. (http://www.biospectrumindia.com /feature /21/10017/pcos-is-lifestyle-disorder html).
Polycystic ovary syndrome is a result of hormonal imbalance which is also known as endocrine disorder. PCOS causes anovulation in animals and women of reproductive age (Demirel *et al.* 2016). Irregular menstrual periods, hirsutism, acne, obesity, and excess male hormone levels are characteristic signs of PCOS.
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) [12]. *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) [10]. 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, antihelmintic, antidiabetic, anti-inflammatory, antiseptic, and antifungal activity (Sahu *et al.* 2013) [12].
Sethi *et al.* 2012 [14] 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) [15]. Some studies have been reported that the effect of *Aloe* barbadensis was similar to the effect estrogen on the reproductive system (Kosif *et al.* 2009) [7].
Another plant with numerous medicinal properties is Mint. Mint leaf is the part of the Indian food since ages. Mint is aromatic, perennial herbs belong to family Lamiaceae. (https://en.wikipedia.org/wiki/Mentha). It is locally known as Pudina. It is used as a household remedy for indigestion, common cold, fever, bronchitis, allergy, etc. It is also used as food seasoner and for industrial purposes (Thawkar *et al.* 2016) [16].
There are some studies reporting that herbal tea of *Mentha spicata* shows a significant decrease in free testosterone and an increase in the level of LH, FSH, and estradiol. Also in the same study, it is stated that it can be an alternative medicine for mild hirsutism (Akdogen *et al.* 2007) [1]. It is also reported that spearmint oil has a very good effect on PCOS by inhibition of testosterone and development of follicle in ovarian tissue (Ataabadi *et al*. 2017) [3].

In view of the various medicinal properties of *Aloe vera* and Mint, the present study has been selected to evaluate the effect of *Aloe vera* gel and Mint tea on PCOS, so as to obtain easy, effective and safe remedy which will be beneficial for humans and livestock.

Materials and Methods

1. Experimental animals

Female Wistar rats weighing around 150-200 gms were used in the present study. Rats were procured from CPCSEA recognized laboratory animal breeding centre (Nagpur Veterinary college, Nagpur).Animals were maintained in hygienic and standard environmental conditions with food and *ad-lib* water.

Selection and collection of plant Material

a. *Aloe vera*: Mature *Aloe vera* leaf freshly plucked was selected for the experiment **(**Mahajan R *et al*. 2013) [8]. The leaf selected was collected within the premises of Nagpur Veterinary College, Nagpur.

b. Mint: Fresh green Mint leaves were selected for the experiment. The plant was collected from a farmer in the periphery of Nagpur.

Processing of the plant material

a. 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) [8] and was used for the treatment the very same day.

b. Preparation of Mint tea

The collected plant leaves were washed and then air-dried under shed at normal room temp erature. The Mint tea was made by pouring 250 ml of boiling water over 10 gm of the air dried leaves and was let to steep for 5-10 min as suggested by Amoura *et al.* (2015) [2].

Aloe vera gel and Mint tea were prepared daily during the treatment period.

Induction of PCOS

Letrozole was dissolved in 1% CMC (Carboxymethylcellulose) and administered orally @ 1 mg/kg body wt. for 21 days to induce PCOS in rats. During this period, vaginal smears were collected daily and evaluated microscopically for oestrus cycle determination. 24 hours after the last dose of letrozole treatment blood samples were collected for hormone estimation. One animal from each group was sacrificed and ovaries were collected for histopathological examination as suggested by kafali *et al.* (2004) [6].

Design of experiment

PCOS was induced in thirty-five female Wistar rats. Five representative rats were sacrificed on the last 21st day of letrozole treatment for confirmation of PCOS in the rats. After confirmation, remaining thirty rats were divided into five groups i.e.T_1, T_2, T_3, T_4, T_5.

Group 'T_1' containing six PCOS positive rats were conducted as a Positive control group. Group 'T_2' received Clomiphene citrate @ 1mg /kg body wt. (p.o) as referral standard drug. Group 'T_3' received *Aloe vera* gel 1 ml daily (p.o). Group 'T_4' was provided with *ad-lib* Mint tea through water bottle and Group 'T_5' received a combination of *Aloe vera* gel and Mint tea i.e. 1 ml of *Aloe vera* gel with *ad-lib* Mint tea daily for a period of 30 days.

Body Weight

Body weight of each animal was measured on a weekly basis throughout the experiment.

Hormonal Estimations

Blood samples were collected in non-heparinized tubes and were kept undisturbed. The serum was then separated and stored at –20 ^{0}C for subsequent analysis. To biochemically estimate FSH and LH with commercial reagent using spectrophotometer, the blood samples were sent to pathological laboratory for further investigation.

Histopathological Examination

On the 22nd day and last day of the experiment, after the collection of blood, the animals were sacrificed for histopathological examinations. Ovaries and other organs from each rat were examined grossly. Ovaries of each rat were collected in 10% formalin for histopathological evaluation.

Vaginal Smear Examination

Sterile cotton swabs were slightly moistened by dipping into saline or distilled water. The tip of the cotton swab was gently rotated after inserting it into the vagina. After removing, the cotton swab tip was rolled gently onto a clean pre-labeled glass slide. After drying of vaginal smear they were examined under a microscope.

Ultrasonographical examination

Ultrasonography of rats was performed at Cattle Breeding Farm, Telankhedi, Nagpur Veterinary College, and Nagpur. Ultrasonographical examination was performed by using the Mindray Digital Ultrasound machine model Z5 vet.

The rats were anesthetised 30mins prior to the sonography examination and was placed on dorsal recumbency on to the platform. A water-filled balloon was then placed over the abdominal area to get a better sonography image. Gel was applied on the abdominal area as well as over the balloon then the Transducer or Probe was accordingly adjusted over the water-filled balloon to locate the ovary and the sonograph was viewed in the monitor and the best-obtained image was frozed and saved.

Later, the saved image was measured and examined to count the number of follicles present on the ovary. The data was recorded accordingly.

Result and Discussion

Body weight

All the groups showed gradual and constant increase in body weight during the induction of PCOS. Group T_1 was kept untreated and served as positive control showed increase in body weight from its initial 275.33±0.49 gm to 283.5±3.5gm at the end of 30 days of treatment period. Group T_2 treated with a standard drug i.e. Clomiphene citrate showed gradual weight loss from its initial 275±0.60 to 265.16±1.37grams. Group T_3 treated with *Aloe vera* gel showed decrease in body weight from 278.33±1.25 to 261.33±3.66 grams at the end of treatment period. Group T_4 provided with *ad lib* Mint tea through drinking water bottle showed a decrease in body weight from 277.16±0.60 to 258.8+1.37 grams. Whereas, combination treatment in group T_5 was found highly effective

with regards to weight loss as compared to other groups including standard drug treatment.

These results obtained in *Aloe vera* gel treated group coincides with an early finding of Mahajan *et al*., (2013) [8], who observed increase in body weight in PCOS rats and recorded that there was no further increase in body weight of PCOS rats treated with *Aloe vera* gel.

In the present study, results obtained in Mint tea treated group are in agreement with Amoura *et al*., (2015) [2] who observed a significant decrease in body and ovarian weights due to treatment of Mint tea. The treatment with combination of *Aloe vera* gel and Mint tea has not been observed in the literature searched.

Oestrus cycle

During the treatment period of 30 days in all the groups vaginal smears were observed regularly. It was found that group T_2 treated with Clomiphene citrate (standard group) and a group T_5 treated with *Aloe vera* gel in combination with Mint tea could restore the normal estrus cycle in the last week of the treatment period. Group treated with Mint tea restored the normal estrus cycle in 2/3rd animals in last week of treatment, whereas in *Aloe vera* treated group there was no remarkable changes towards restoring of the normal estrus cycle.

Mahajan *et al.*, 2013 [8] also recorded the alteration in the estrus cycle due to the administration of Letrozole are in agreement with the present study. They further studied effect of *Aloe vera* gel in PCOS rats and observed that the estrus cycle was normal. However, in the present study oestrus cycle was not normal after the treatment with Aloe vera. This might be because in the present study treatment was continued for 30 days as against 45 days in a study by Mahajan *et al*. 2013 [8].

Ultrasonography

Ultrasonography was carried out in rats under experiment to confirm the ovarian follicular number and size during the induction of PCOS and treatment thereafter. As shown in Table 3, groups T_1 (positive control), T_2 (Standard), T_3 (Aloe vera), T_4 (Mint tea) and T_5 (*Aloe vera* and Mint tea) showed mean follicles ranging between 1.40±0.87 to 3.40±1.21 at the beginning of the experiment i.e. before administration of Letrozole. On 21st day of Letrozole administration it was observed that in T_1, T_4, and T_5 groups there was significant increase in the follicle numbers whereas, in T_3 it was almost maintained and in T_2 groups the number was surprisingly reduced a little. (Plate 5)

After a treatment period of 30 days, it was observed that in all the treatment groups' follicle number was reduced except in the positive control group. The maximum reduction in follicular number was observed in Clomiphene citrate treated group. Groups treated with *Aloe vera* gel alone and in combination with Mint tea showed similar follicle numbers. Whereas, group treated with mint tea showed reduction in number of follicle to its zero day readings.

The results coincide with the observations obtained in the vaginal cytology carried out in the same study, which confirms the effects of treatments on induced PCOS in rats. The literature searched could not reveal the USG in rats.

Histopathology

In the present study, plate-6 showed the normal histological structure of the ovary. Development of cysts with a thin layer of granulosa cells observed in ovaries after the letrozole treatment (plate 7 and 8). The cysts remained as such in positive control animals (Plate 9). The treatment with Clomiphene citrate (1mg/kg) showed the normal architecture of ovary (plate 10). The section from the group treated with *Aloe vera* gel shows incomplete luteinization, no oocyte in present follicles and cyst as in positive control group (plate 11). The treatment with Mint tea showed improvement in ovarian structure, primary secondary follicle and developed corpus luteum observed in section (plate 12). The histological section has taken from the group treated with a combination of *Aloe vera* gel and Mint tea showed improvement of an ovarian structure as normal as standard drug treatment and showing the different stages of development of follicle (Plate 13).

The findings of the present study are in agreement with Kafali *et al.* (2004) [6] who observed inhibit growth follicle, large cystic follicle with thin granulosa cell layer, hyperplasia of thica cell, incomplete luteinization decrease number of corpus luteum in PCOS positive rat which as induced by using letrozole. Jadhav *et al.* (2013) [8] observed potential recovery in group of rats treated with Clomiphene citrate than group left for the natural recovery. Also, presence of cysts and atretic follicles were found in the group left for natural recovery. Amoura *et al.* (2015) [2] observed a decrease in an atretic cyst in the group supplemented with peppermint. While a histological section of ovarian tissue from the experimental group treated with letrozole in combination with peppermint showed improvement in its architecture.

In the present study histological findings of the group treated with *Aloe vera* gel is compliance with the findings of Mahajan *et al.* (2013) [8].

Table 1: Effect of Letrozole on mean Body weight (Gms) observed in different groups

Sr. No.	Group	0th day	7th day	14th day	21stday
1	T_1	226.17± 2.18	240.83 ± 2.94	257±1.42	267.17±0.83
2	T_2	227.50±2.28	242.50±2.81	256.83±2.26	265.83±1.62
3	T_3	226.67±5.43	237.83±5.51	261.83±1.58	268±1.18
4	T_4	219.67±2.60	228.00±2.92	254.17±2.39	265.33±1.31
5	T_5	224.67±1.67	228.00±2.92	254.50±1.67	265.33±1.31

Values are Mean + SE for 6 rats in each group.
C.D value = 3.278659

ANOVA: Mean Body weight (Gms) observed in different groups

Source of variation	SS	DF	MS	F
Treatment	776.7167	4	194.1792	4.740124
Days	31353.63	3	104521.21	255.1253
Interaction	529.0833	12	44.09028	1.076291
Error	4096.5	100	40.965	

Table 2: Effect of various treatment on Body weight (Gms) in PCOS positive rat during treatment period

Sr. No.	Group	Treatment	0th day	21st day	1st wk	2nd wk	3rd wk	4th day
1	T_1	Positive control	226.17± 2.18	267.17±0.83	275.33± 0.49	278± 0.36	281±0.57	283.5± 3.5
2	T_2	Std. Drug. Control @1mg/kg bd.wt	227.50±2.28	265.83±1.62	275±0.60	272.66±0.71	268.66±1.15	265.16±1.37
3	T_3	*Aloe vera* gel 1ml /rat p.o.	226.67±5.43	268±1.18	278.33±1.25	276±1.26	270.5±0.88	261.33±3.66
4	T_4	Mint tea provided ad lib through water bottle	219.67±2.60	265.33±1.31	277.16±0.60	271.5±0.718	265 +1.15	258.83+1.37

		p.o						
5	T_5	*Aloe vera* gel 1ml/rat p.o.+ Mint tea provided ad lib through water bottle p.o	224.67±1.67	265.33±1.31	274.66±0.76	269.5± 0.76	262.5±1.66	255.16±0.833

Each value is the Mean ±SE of six rats.
C.D Value for Treatment=2.08679
C.D Value for weeks=1.905051
C.D Value for interaction=4.66640

ANOVA: Mean Body weight (Gms) observed in different groups during treatment period

Source of variance	SS	DF	MS	F
Treatment	2230.278	4	557.56	33.32
Days	53593.89	5	10178.78	640.607
Interaction	2588.859	20	129.44	7.736
Error	2509.83	150	10.73	-
Total	60922.86	179	-	-

Table 3: Total No. of follicles observed in different treatment groups during experimental period

S. No.	Groups	Treatments	Total Ndo. of Follicles		
			Before Induction of PCOS	After Induction of PCOS	After Rx of 30days
1	T1	Positive Control	2.25±1.11	4.50±1.85	0.60±0.40
2	T2	Std. Drug Control @1ml/kg bd.wt	3.40±1.21	2.80±1.39	0.00
3.	T3	*Aloe vera* gel 1 ml / rat(p.o)	1.80±1.43	1.60±0.75	1.00±0.63
4.	T4	Min tea provided ad –lib through water bottle	2.80±1.32	5.00±2.20	2.40±1.17
5.	T5	*Aloe vera* gel 1 ml/ rat (p.o) + Mint tea provided ad –lib through water bottle	1.40±0.87	2.00±0.89	1.00±1.00

Table 4: FSH concentration observed in different treatment groups during experimental period

S. No.	Groups	Treatments	FSH concentration		
			Before Induction of PCOS	After Induction of PCOS	After Rx of 30days
1	T1	Positive Control	0.15	0	0
2	T2	Std. Drug Control @1ml/kg bd.wt	0.28	0	0.08
3.	T3	*Aloe vera* gel 1 ml / rat(p.o)	0.25	0	0.16
4.	T4	Min tea provided ad –lib through water bottle	0.33	0	0.019
5.	T5	*Aloe vera* gel 1 ml/ rat (p.o) + Mint tea provided ad –lib through water bottle	0.2	0	0

Table 5: LH concentration observed in different treatment group during experimental period

S. No.	Groups	Treatments	LH concentration		
			Before Induction of PCOS	After Induction of PCOS	After Rx of 30days
1	T1	Positive Control	0.03	0.01	0.06
2	T2	Std. Drug Control @1ml/kg bd.wt	0	0.06	0.003
3.	T3	*Aloe vera* gel 1 ml / rat(p.o)	0.03	0.15	0.02
4.	T4	Min tea provided ad –lib through water bottle	0.02	0.15	0.02
5.	T5	*Aloe vera* gel 1 ml/ rat (p.o) + Mint tea provided ad –lib through water bottle	0.03	0.15	0

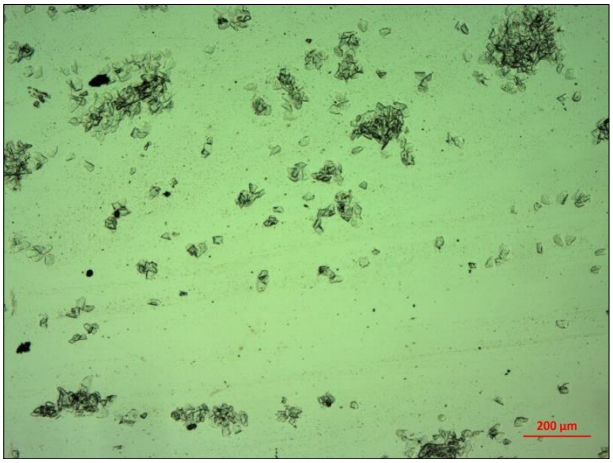

Plate 1: Microphotograph of unstained vaginal smear showing Proestrus stage constisting of nucleated round epithelium Maharashtra-440001

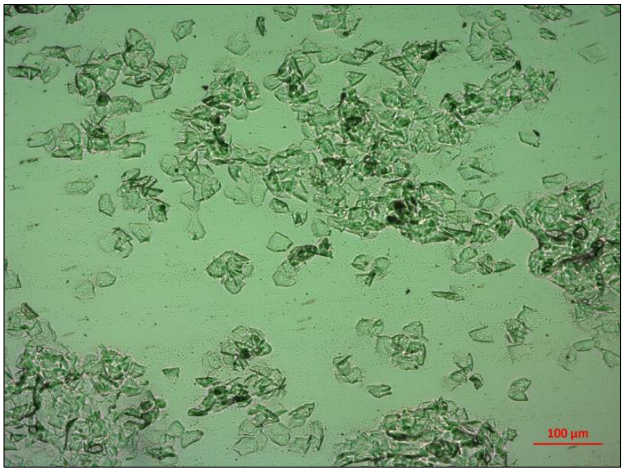

Plate 2: Microphotograph of unstained vaginal smear of rat showing estrus stage predominantly contain anucleated cornified cells

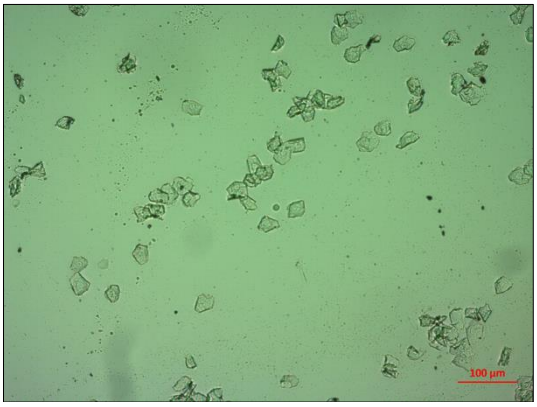

Plate 3: Microphotograph of unstained vaginal smear showing metestrus stage in which some cornified stages and leucocytes were observed

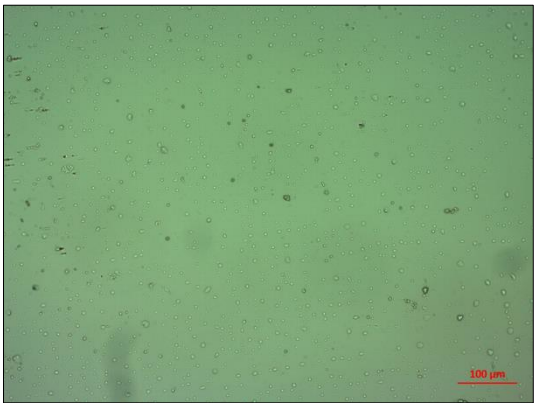

Plate 4: Microphotograph of unstained vaginal smear of rat showing diestrus stage in which predominantly leucocytes were observed

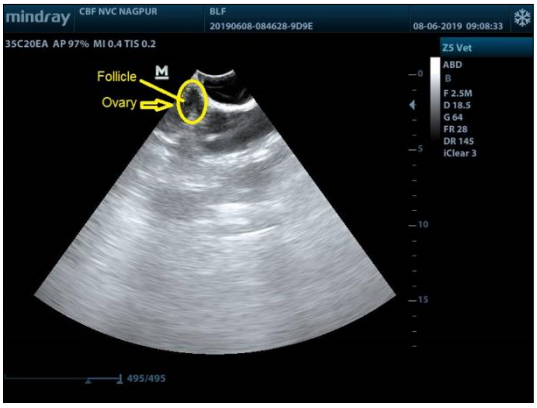

Plate 5: Ultrasonographical image of ovary and follicle of measurable size

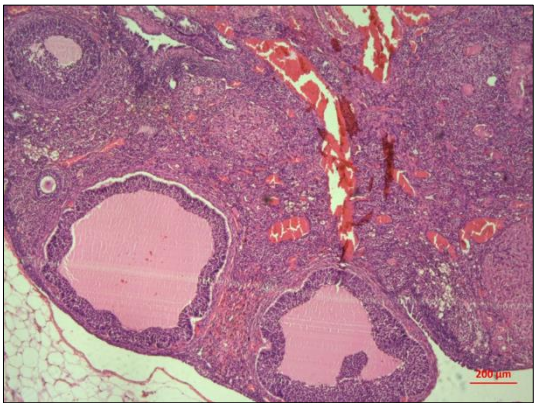

Plate 6: Microphotograph of rat ovary showing normal structure with presence of primary. Secondary and Graffian follicle. (H&E)

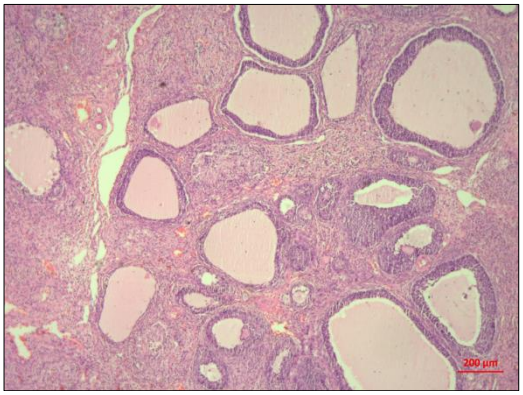

Plate 7: showing microphotograph of rat ovary showing development of cystic follicle with thin granulosa layer and numerous subscapular cyst after induction of PCOS by letrozole (H&E)

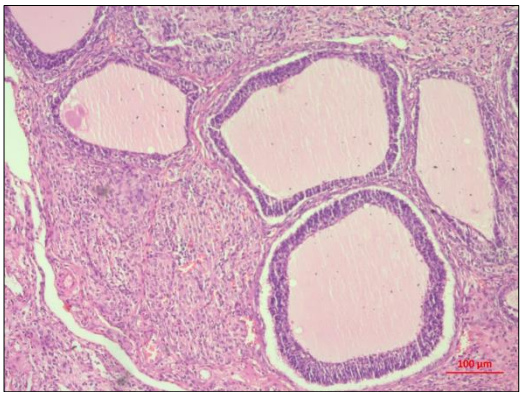

Plate 8: Microphotograph of rat ovary showing cyst with thin granulosa layer (H&E)

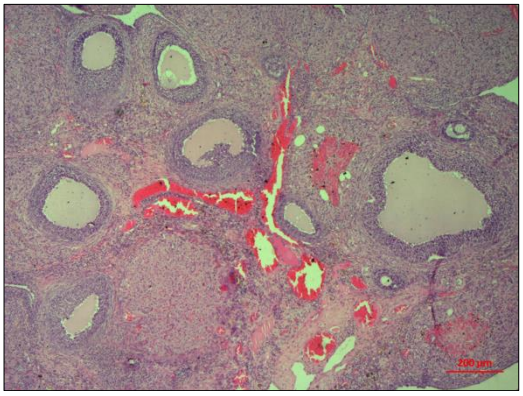

Plate 9: Microphotograph of rat ovary showing persistent cyst after 30 days of induction in letrozole induced PCOS rat.(groupT1)(H&E)

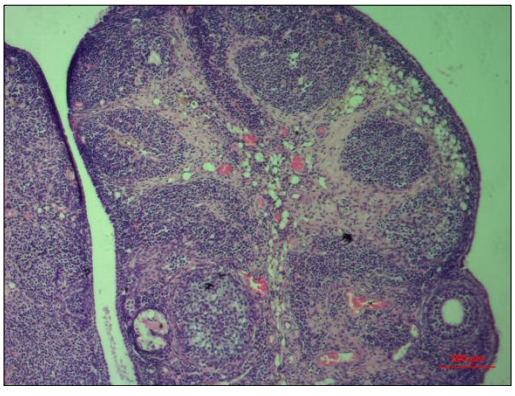

Plate 10: Microphotograph of rat ovary showing normal ovarian structure with presences of secondary follicle corpus luleum (group T2) (H&E)

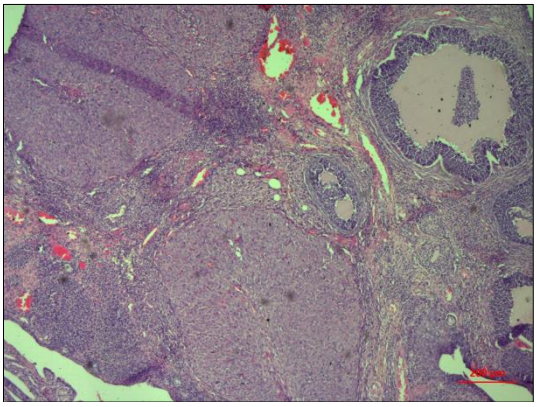

Plate 11: Microphotograph of rat ovary from the group treated with *Aloe vera* gel showing cystic follicle with thin granulosa layer (GroupT3) (H&E)

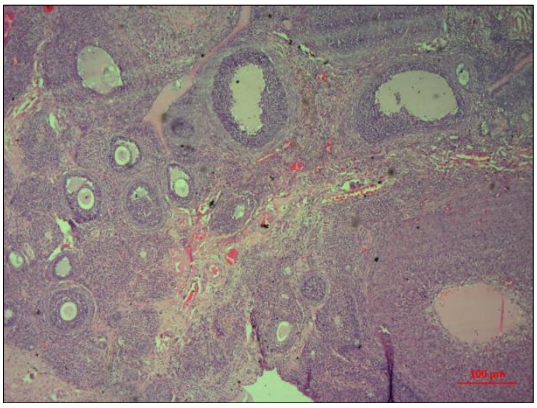

Plate 12: Microphotograph of rat ovary from the group treated with Mint tea showing Primary, secondary follicle and cystic follicle. (Group T4)(H&E)

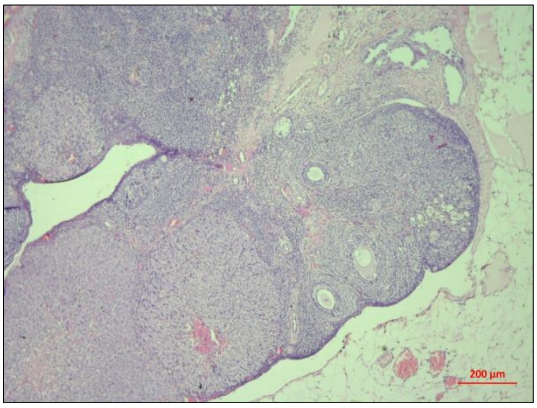

Plate 13: Microphotograph of rat ovary from the group treated with *Aloe vera* gel with Mint tea showing Primary follicle, secondary follicle and corpus luteum (Group T5) (H & E)

Conclusion

The present study suggested that combination treatment of *Aloe vera* gel and Mint tea has good efficacy for treatment of polycystic ovary syndrome comparable to the standard drug clomiphene citrate, followed by Mint tea and *Aloe vera* gel.

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