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Original Research Article

Investigation of effect of ellagic acid on premature ovarian insufficiency

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ABSTRACT

Background: One common medical condition affecting the reproductive system in older women is premature ovarian failure (POF). POF can result from radiation therapy, chemotherapy, immune system problems, infection, inflammation, and genetic mutation.
Aim: The current study examined the effect of Ellagic acid on Premature Ovarian Insufficiency.
Materials and Methods: The study utilized, 8-12 months old 150 ± 20 gm, 42 female wistar rats. For the induction of Premature Ovarian Insufficiency, Tripterygium Glycoside (TG) was administered at a dosage of 50 mg/ml dissolved in water for injection once daily. Clomiphene Citrate, 1 mg/kg was administered orally once daily for 21 days. Ellagic acid was given orally once daily at concentration of 10 mg/kg, 20 mg/kg, 30 mg/kg. The statistic of the data was determined by using ANOVA test.
Results: The results showed that effect of Ellagic acid on premature ovarian insufficiency as evidenced by decreased levels of cholesterol, triglycerides, follicle stimulating hormone and Luteinizing hormone and increased levels of estrogen.
Conclusions: According to the current study, female wistar rats with Tripterygium Glycoside induced Premature Ovarian Insufficiency can benefit from treatment with an Ellagic acid.

1. Introduction

There are other terms used to describe early Ovarian Insufficiency (POI), such as Hypergonadotropic Amenorrhea, Primary Ovarian Failure (POF), and early menopause. It means that the ovaries stop working before the menopause is often expected to occur.[1]

It's an enigmatic disorder. The condition is identified by the co-occurrence of amenorrhea, insufficient sex hormones, and increased (menopausal) gonadotropin levels in the serum prior to reaching 40 years of age. It is expected to affect 1 in 100 people by the age of 40 and 1 in 1000 people by the age of 20, indicating that it is not an uncommon condition.[2]

The syndrome is typically characterized by the following:

1. Primary or secondary amenorrhea.
2. At least intermittent hypo-oestrogenism.
3. Hypergonadotropinism in postmenopausal range.
4. Patient's age under 40 years at the time of onset.[1]

Menopausal symptoms including vaginal dryness, hot flashes, and nocturnal sweats are experienced by women with post-partum illness (POI).[3] The loss of fertility, which is also brought on by the lack of follicles, is linked to the development of POI. In the alternative, the residual follicles incapacity to react to stimulation.[4]

Folliculogenesis, or the maturation of ovarian follicles, is a highly structured and intricate process that occurs in female humans. The gradual development of tiny

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primordial follicles into giant ovulatory follicles is known as folliculogenesis. The gamete or oocyte itself makes up the follicle, which is encircled by thecal cells, granulosa, and supportive somatic cells that are critical to the follicles' growth and development. When follicles reach a certain maturity, the oocytes are discharged from the ovary's surface, gathered by the uterine tube, and either fertilized and inserted into the uterus, or they are lost.

Follicle maturation is a continuous process that can take up to a year to complete from the point at which a primordial follicle begins to mature into an ovulatory follicle. Comprehending the folliculogenesis process is necessary to determine the etiology of POI.[5]

The occurrence of POI is 0.9-1.2% in women with 40 years or younger. There are ethnic differences ranging from 1.4% in women of African-American, 0.5% in Chinese and 0.1% in Japanese women. In women with primary amenorrhea the occurrence of POI is 10-28% whereas 4-18% with secondary amenorrhea.[6,7] Approximately 20–30% of women with POI will have other affected female family members. Before the age of 20, POI may be defined as early POI. In these young women, Turner syndrome and gonadal dysgenesis are the best known causes of early POI. Nevertheless, in normal 46XX karyotype females presenting with early POI, the etiology is most often unknown.[8,9]

2. Ellagic Acid

IUPAC Name: 2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione.[10]

2.1. Structure

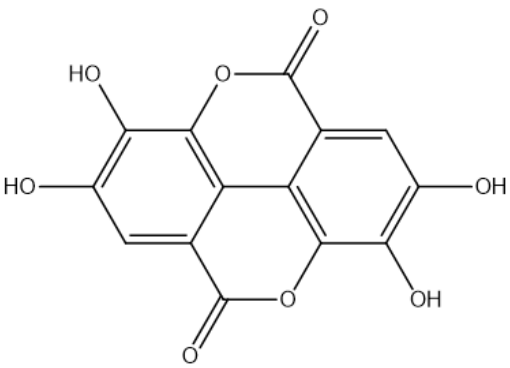

Figure 1: Structure of ellagic acid

2.2. Source

1. Ellagic acid is a natural phenol antioxidant.[11]
2. Ellagic acid is the dilactone of hexahydroxydiphenic acid.
3. The macrophyte Myriophyllum spicatum produces Ellagic acid.
4. Ellagic acid can be found in the medicinal mushroom Phellinus linteus.[12]

2.3. Uses of ellagic acid

1. Ellagic acid has anti-proliferative, anti-mutagenic and antioxidant activity in a number of in vitro and small animal models.[13]
2. Ellagic acid is used to prevent cancer and treat viral and bacterial infections.[14]
3. Ellagic acid shows various activities like anti-androgenic, anti-diabetic, anti-inflammatory, hypolipidemic, anti-helminthic, antiulcer, hepatoprotective and anti-angiogenic etc.[15,16]
4. Ellagic acid also have radical scavenging, chemo preventive, anti-apoptotic action and estrogen receptor modulator properties.[17]

3. Ellagic Acid Mechanism

Ellagic acid contain four hydroxyl groups and two lactone groups in which hydroxyl group is known to increase antioxidant activity in lipid peroxidation and protect cells from oxidative damage.[18]
Reactive oxygen species (ROS) like hydroxyl radical (OH), hydrogen peroxide (H_2O_2), superoxide anion (O_2) and molecules affect both male and female gametes.[19]

Ellagic acid inhibits generation of ROS and OH in both enzymatic and non-enzymatic system by its metal chelating property, thus providing protection against lipid peroxidation.[20]

The increase of free radicals in cells can induce the lipid peroxidation by oxidative breakdown of polyunsaturated fatty acids in membranes of cells.[21] When ROS begin to accumulate, testes exhibit a defensive mechanism using various antioxidant enzymes. The first enzymatic reaction in the reduction pathway of oxygen occurs during the dismutation of two molecules of O_2 when they are converted to H_2O_2 and diatomic oxygen by the superoxide dismutase enzyme.[22]

4. Materials and Methods

4.1. Procurement of experimental animal

The Female Albino rats (Wistar strain) of age 8-12 months, weighing between 150 ± 30g, were procured from the animal house of Institute of Pharmaceutical Education and Research (I.P.E.R.), Wardha. The animals were housed in the polypropylene cage at a temperature of 24º ± 2ºC with the relative humidity of 40-60% and 12 hour light / dark cycle. Animals were fed with balanced diet and water. The

experimental protocol was approved by the Institutional Animal Ethics Committee of Institute of Pharmaceutical Education and Research, Wardha (Registration Number 535/PO/Re/02/CPCSEA/Jan2002) on dated 01.12.2018 with Protocol No. IAEC/2018-19/08.

4.2. Preparation of doses

1. The doses of Tripterygium Glycoside (TG) (50 mg/kg, s.c.) and Clomiphene Citrate (1 mg/kg, p.o.) were selected on basis of literature survey.
2. The dose of Tripterygium Glycoside (50 mg/kg, s.c.) was calculated and weighed quantity of it was dissolved in water for injection and administered, according to the body weight, to all groups of animals subcutaneously (s.c.) except normal group animals.
3. The dose of Ellagic Acid (10 mg/kg, p.o.), (20 mg/kg, p.o.), and (30 mg/kg, p.o.), was calculated and weighed quantity of extract was dissolved in distilled water and administered, according to the body weight, to animals of all treatment groups per orally (p.o.).
4. The dose of Clomiphene Citrate (CC) (1 mg/kg, p.o.), was calculated and weighed quantity of it was dissolved in distilled water and administered, according to the body weight, to animals of standard group per orally (p.o.).
5. All the drug solutions were prepared freshly.

5. Experimental Design

The present study was conducted using female albino Wistar rats divided into seven groups (containing 6 rats in each group). The groups are as follows

1. ***Group-I :*** Normal animals group
2. ***Group-II :*** Control group
3. ***Group-III :*** Standard Drug receiving animal group
4. ***Group-IV :*** Test Animal group
5. ***Group-V :*** Test Animal group
6. ***Group-VI :*** Test Animal group
7. ***Group-VII :*** Standard drug and Test drug receiving animal group

1. All groups except Normal group animals were administered with Tripterygium Glycoside at concentration of 50mg/kg dissolved in water for injection once daily subcutaneously for 35 days to induce rat model of Premature Ovarian Insufficiency.
2. The Control group was left for natural recovery for 21 days post Tripterygium glycoside treatment.
3. The Standard group animals, were administered with Clomiphene Citrate at concentration of 1 mg/kg once daily per orally for 21 days.
4. Group-IV, Group-V and Group-VI animals, were administered with Ellagic Acid respectively at concentration of 10 mg/kg, 20 mg/kg, 30mg/kg once daily per orally for 21 days.
5. Group-VII animals, received sub-effective doses Ellagic Acid (10 mg/kg) & Clomiphene Citrate (1 mg/kg) in combination respectively once daily per orally for 21 days.

Table 1: Different groups of experimental animals

Sr. No.	Groups	Doses and Route
1.	**Group-I** (Normal, Saline Solution)	Saline Solution, p. o.
2.	**Group-II** (Control, Tripterygium Glycoside)	50mg/kg, s. c.
3.	**Group-III** (Standard, Clomiphene Citrate)	1mg/kg, p. o.
4.	**Group-IV** (Test 1, Ellagic acid)	10mg/kg, p. o.
5.	**Group-V**(Test 2, Ellagic acid)	20mg/kg, p. o.
6.	**Group-VI**(Test 3, Ellagic acid)	30mg/kg, p. o.
7.	**Group-VII**(Test + Standard)	10mg/kg+1mg/kg, p. o.

After induction period of 35 days i.e. on 36^{th}day and after treatment period of 21 days i.e. on 57^{th}day the initial and final weight were taken respectively.

Blood was withdrawal by retro-orbital plexus method and biochemical parameters were performed on 36^{th} and 58^{th} day.

Two rats from each group were sacrificedon 36^{th} dayand 58^{th}day and ovaries of rats were removed and sent for histopathological studies.

6. Results

6.1. Determination of physiological parameters

6.1.1. Measurement of body weight

The body weight of animals of each group was measured on 1^{st} day i.e. initial weight & on 36^{th} day i.e. intermediate weight & on 57^{th} day i.e. final weight after treatment.

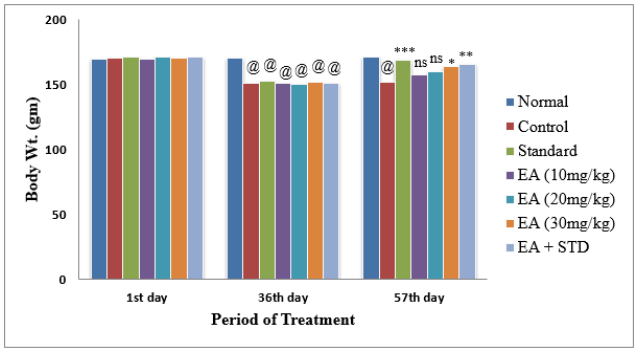

Figure 2: Result of body weight

Observation and results sows that the body weight of animal slightly decreased after induction of POI by TG on

Table 2: Results of body weight

Sr. No.	Groups	Body wt. (g) at 1^{st} Day	Body wt. (g) at 36^{th} Day	Body wt. (g) at 57^{th} Day
1.	Group-I (Normal)	169.910±1.520	170.552±1.775	171.022±2.087
2.	Group-II (Control)	170.055±2.018	150.668±0.978$^{@}$	151.486±1.739$^{@}$
3.	Group-III (Standard)	171.545±2.257	152.612±2.198$^{@}$	168.462±1.489***
4.	Group-IV (Test 1)	169.987±2.023	151.170±2.042$^{@}$	157.222±2.232^{ns}
5.	Group-IV (Test 2)	171.095±1.518	150.148±1.991$^{@}$	159.688±2.284^{ns}
6.	Group-VII (Test 3)	170.527±1.807	151.718±2.290$^{@}$	164.194±1.984*
7.	Group-VII (Test+STD)	171.148±2.102	151.078±2.929$^{@}$	165.380±1.420**

Values expressed as mean ± S.E.M., (n = 6, 6 & 5 respectively) Two-way analysis of variance (ANOVA) followed by Bonferroni's test $^{@}$P< 0.0001 compared to normal and *P<0.05, **P<0.01, ***P<0.001 compared to control.

Day 36^{th} than the body weight of Day 1^{st} of study. After treatment with Standard drug, Ellagic acid and Ellagic acid in combination with standard, body weight was measured on 57^{th} day. The body weight of animal was increased but not more than normal body weight.

6.2. Determination of biochemical parameters

6.2.1. Determination of total cholesterol (TC)

Quantification of TC allows the detection of hypercholesterolemia. High cholesterol concentrations are associated with a high risk of developing cardiovascular disease. For the determination of cholesterol level after induction of POI and after treatment, the blood samples were collected and were sent ot Amey Pathology Laboratory, Socialist Square, Wardha.

Table 3: Observation of total cholesterol level

Sr. No.	Groups	Cholesterol at 36^{th} Day (mg%)	Cholesterol at 56^{th} Day (mg%)
1.	Group-I (Normal)	54.308±1.352	54.378±1.216
2.	Group-II (Control)	85.866±1.182$^{@}$	78.624±1.034$^{@}$
3.	Group-III (Standard)	84.871±1.100$^{@}$	56.14±0.708***
4.	Group-IV (Test 1)	85.845±0.696$^{@}$	69.822±0.991^{ns}
5.	Group-IV (Test 2)	83.283±0.863$^{@}$	66.096±0.843^{ns}
6.	Group-VII (Test 3)	84.353±0.947$^{@}$	63.58±0.675*
7.	Group-VII (Test+STD)	84.948±1.172$^{@}$	62.958±0.936**

Values expressed as mean ± S.E.M., (n = 6 & 5 respectively $^{@}$P< 0.0001 compared to normal and *P<0.05, **P<0.01, ***P<0.001 compared to control.

Observation and results shows that level of cholesterol increases after induction on 36^{th} day. After treatment with Standard drug, Ellagic acid and Ellagic acid in combination with standard, shows the significant decrease in cholesterol level in animals on 58^{th} day.

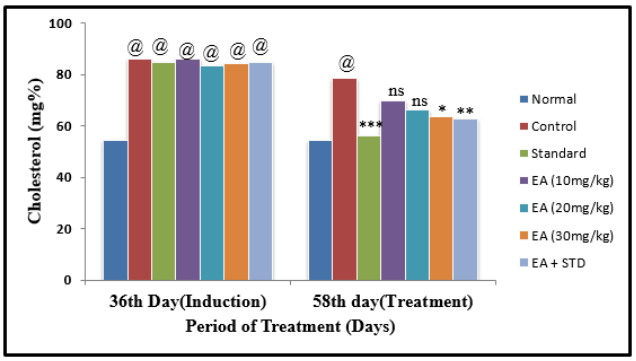

Figure 3: Result of cholesterol level

6.2.2. Determination of Triglycerides (TG)

High serum triglyceride level is associated with important risk of atherosclerosis. They can be due to diseases like hyperlipoproteinemia, apolipoprotein C-II deficiency also due to diabetes and other endocrine disorders. The collected blood samples after induction and treatment were sent to Amey Pathology Laboratory, Socialist Square, Wardha, for triglyceride level determination.

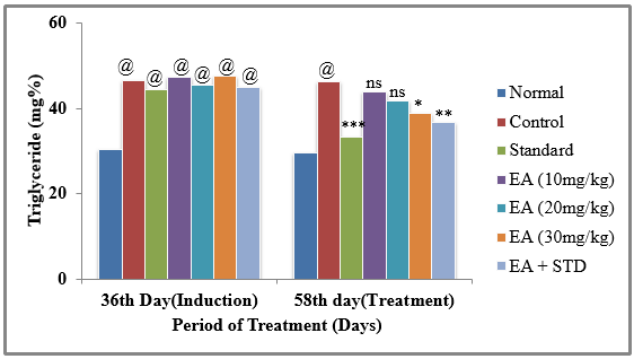

Figure 4: Result of triglyceride level

Observation and results shows that level of triglyceride increases after induction of POI on 36^{th} day. After treatment with Standard drug, Ellagic acid, Ellagic acid in combination with standard, the triglyceride level

Table 4: Observation of triglyceride level

Sr. No.	Groups	Triglyceride at 36^{th} Day (mg%)	Triglyceride at 56^{th} Day (mg%)
1.	Group-I (Normal)	30.461±0.338	29.574±0.295
2.	Group-II (Control)	46.618±0.648$^{@}$	46.206±0.672$^{@}$
3.	Group-III (Standard)	44.356±0.632$^{@}$	33.31±0.580***
4.	Group-IV (Test 1)	47.318±0.618$^{@}$	43.91±0.743^{ns}
5.	Group-IV (Test 2)	45.36±0.600$^{@}$	41.844±0.55^{ns}
6.	Group-VII (Test 3)	47.506±0.711$^{@}$	38.754±0.450*
7.	Group-VII (Test+STD)	44.84±0.721$^{@}$	36.67±0.480**

Values expressed as mean ± S.E.M., (n = 6 & 5 respectively $^{@}$P< 0.0001 compared to normal and *P<0.05, **P<0.01, ***P<0.001 compared to control.

significantly decreases. The result also shows that the level of triglyceride decreases as the dose of Ellagic acid increases.

6.3. Determination of Hormonal Parameters

6.3.1. Estrogen

In POI the level of estrogen generally decreases. To determine the level of estrogen, the collected blood samples from animals were sent to Amey Pathology Laboratory, Socialist Square, Wardha. The serum estrogen level was estimated by fully automated bidirectionally interfaced Chemi Luminscent Immuno Assay (CLIA).

Table 5: Observation of estrogen level

Sr. No.	Groups	Estrogen at 36^{th} Day (pg/ml)	Estrogen at 56^{th} Day (pg/ml)
1.	Group-I (Normal)	184.34±2.308	183.404±2.290
2.	Group-II (Control)	98.276±2.525$^{@}$	97.314±2.551$^{@}$
3.	Group-III (Standard)	99.448±2.611$^{@}$	175.864±2.297***
4.	Group-IV (Test 1)	97.491±2.432$^{@}$	143.032±2.980^{ns}
5.	Group-IV (Test 2)	99.615±3.146$^{@}$	157.454±2.603^{ns}
6.	Group-VII (Test 3)	100.53±3.105$^{@}$	163.48±3.086*
7.	Group-VII (Test+STD)	99.448±2.624$^{@}$	170.622±2.235**

Values expressed as mean ± S.E.M., (n = 6 & 5 respectively $^{@}$P< 0.0001 compared to normal and *P<0.05, **P<0.01, ***P<0.001 compared to control.

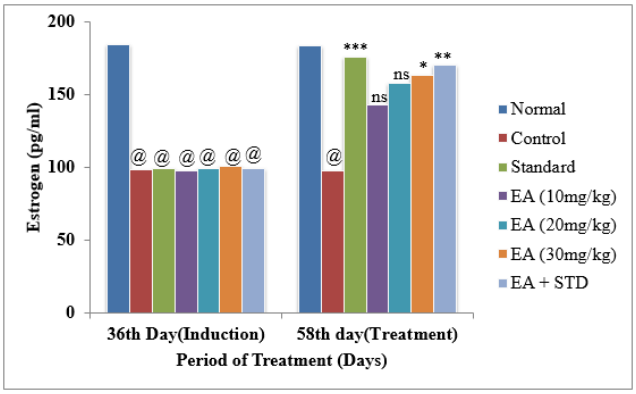

Figure 5: Result of estrogen level

Observation and results shows that level of estrogen in the animals decrease after induction of POI on 36^{th} day. After treatment with Standard, Ellagic acid and Ellagic acid in combination with standard, estrogen levels significantly increases as the dose of Ellagic acid increases.

6.3.2. Follicle stimulating hormone (FSH)

In POI shots of Hypergonadotropin observed which indicates elevated level of FSH. The collected blood samples were sent to Amey Pathology Laboratory, Socialist Square, Wardha, for determination of FSH level. The FSH level was estimated by Enzyme Linked Fluorescence Assay (ELFA).

Table 6: Observation of FSH level

Sr. No.	Groups	FSH at 36^{th} Day (mIU/ml)	FSH at 56^{th} Day (mIU/ml)
1.	Group-I (Normal)	10.551±0.301	9.963±0.275
2.	Group-II (Control)	21.765±0.301$^{@}$	21.536±0.329$^{@}$
3.	Group-III (Standard)	22.47±0.303$^{@}$	12.63±0.222***
4.	Group-IV (Test 1)	21.751±0.270$^{@}$	16.411±0.424^{ns}
5.	Group-IV (Test 2)	21.976±0.353$^{@}$	15.661±0.408^{ns}
6.	Group-VII (Test 3)	22.255±0.326$^{@}$	14.398±0.244*
7.	Group-VII (Test+STD)	21.226±0.235$^{@}$	13.443±0.281**

Values expressed as mean ± S.E.M., (n = 6 & 5 respectively $^{@}$P< 0.0001 compared to normal and *P<0.05, **P<0.01, ***P<0.001 compared to control.

Observation and results shows that level of Follicle Stimulating Hormone (FSH) in the animals increases after induction of POI on 36^{th} day. After treatment with Standard, Ellagic acid and Ellagic acid in combination with standard, shows the significant decrease in FSH level.

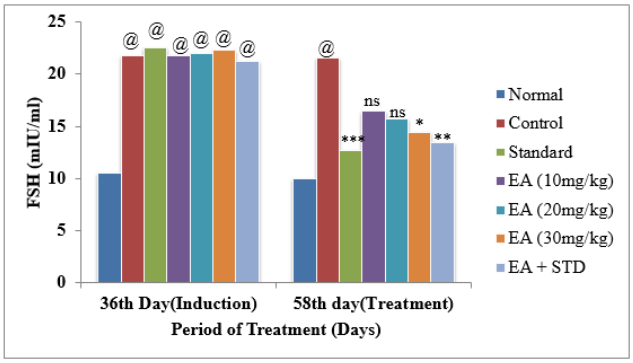

Figure 6: Result of FSH level

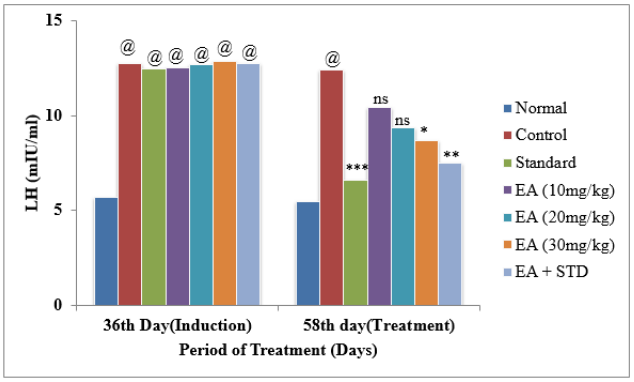

Figure 7: Result of LH level

6.3.3. Luteinizing hormone (LH)

In POI there is elevation of Luteinizing Hormone level by the excess secretion of gonadotropin releasing hormone (GnRH). The collected blood samples were sent to Amey Pathology Laboratory, Socialist Square, Wardha, for determination of LH level. The LH level was estimated by Enzyme Linked Fluorescence Assay (ELFA).

Table 7: Observation of LH Level

Sr. No.	Groups	LH level at 36^{th} Day (mIU/ml)	LH level at 56^{th} Day (mIU/ml)
1.	Group-I (Normal)	5.688±0.142	5.488±0142
2.	Group-II (Control)	12.731±0.131$^{@}$	12.43±0.132$^{@}$
3.	Group-III (Standard)	12.465±0.145$^{@}$	6.575±0.124***
4.	Group-IV (Test 1)	12.531±0.131$^{@}$	10.458±0164^{ns}
5.	Group-IV (Test 2)	12.666±0.140$^{@}$	9.355±0.155^{ns}
6.	Group-VII (Test 3)	12.835±0.127$^{@}$	8.698±0.159*
7.	Group-VII (Test+STD)	12.755±0.195$^{@}$	7.486±0.201**

Values expressed as mean ± S.E.M., (n = 6 & 5 respectively $^{@}$P< 0.0001 compared to normal and *P<0.05, **P<0.01, ***P<0.001 compared to control.

Observation and results shows that level of Luteinizing Hormone (LH) in the animals increases after induction of POI on 36^{th} day. After treatment with Standard, Ellagic acid and Ellagic acid in combination with standard, the LH level significantly decreases.

7. Histopathological Studies

For histopathological studies two rats from each group were sacrificed and ovaries were removed, cleaned to remove extra tissues and fats. The clean ovaries were stored in 10% formalin solution. The samples of ovary were send to Histopathology Department of Javaharlal Nehru Medical College, Sawangi (Meghe), Wardha. The slides of section of ovaries and observations of histological studies are given below:

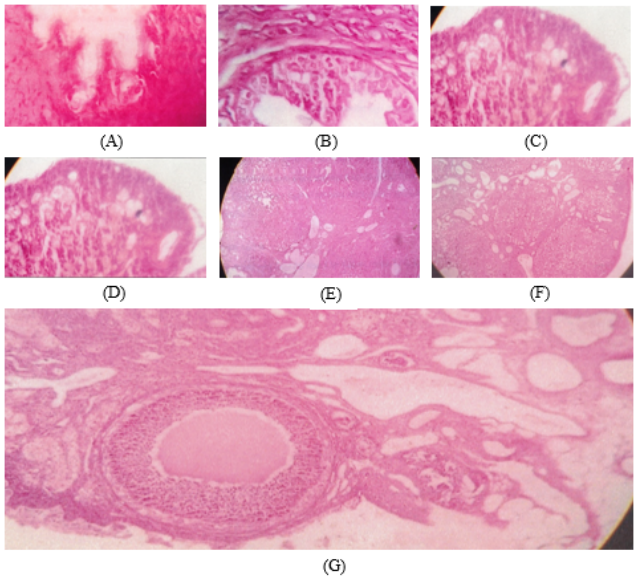

Figure 8: Histological section of ovary of (A) Normal Rat, **(B)** Control Group (i.e. after induction of POI), **(C)** Rats treated with Standard drug, **(D)** Rats treated with Ellagic acid (10mg/kg), **(E)** Rats treated with Ellagic acid (20mg/kg), **(F)** Rats treated with Ellagic acid (30mg/kg), **(G)** Rats treated with Ellagic acid and Standard drug.

Histological section of normal ovary shows healthy as well as primordial follicles, rare Graafian follicular change and rare secondary follicles.

Histological section of ovary after induction of POI shows occasional primordial follicles and also shows the areas of cell hyperplasia and spindle theca cell hyperplasia. Corpus luteum is completely absent indicating anovulation.

Histological section of ovary treated with standard drug shows marked recovery with appearance of healthy follicles,

rare Graafian follicles and edematous separation of stroma at follicle place. Along with presence of corpus luteum which shows that ovulation has occurred.

Histological section of ovary treated with dose 10mg/kg Ellagic acid shows rare Graafian follicles along with cell nodules and congested blood vessles also shows developing healthy follicle with the corpus luteum at various stages of maturation.

Histological section of ovary treated with dose 20mg/ml Ellagic acid shows maturating healthy Graafian follicles with corpus luteum at various stages of maturation.

Histological section of ovary treated with dose 30mg/kg Ellagic acid shows Graafian follicles and rare primordial follicles at various stages of maturation.

Histological section of ovary treated with sub-effective dose 10mg/kg Ellagic acid and 1mg/kg standard drug shows stromal congestion along with Graafian follicles and primordial follicles at various stages of maturation which indicates restoration of healthy follicles.

8. Statistical Analysis

Graph Pad Prism 8 for Windows (version 8.1.0) was used to examine the data. Mean ± Standard Error of Mean were used to express the results. The significance of the difference between the variables in different groups was examined using two-way analysis of variance (ANOVA), Bonferroni's, and Tukey's post-test. Less than 0.05 of P values were regarded as statistically significant.

9. Discussion

Premature Ovarian Insufficiency (POI) usually caused by the cessation in the functioning of ovaries before the expected age of menopause.[1] The menopausal symptoms possessed by POI are hot flushes, night sweats, vaginal dryness, infertility.[3]

POI is characterized by amenorrhea, sex steroid hormone deficiency and elevated levels of serum gonadotropins before the age of 40 years.[1] The loss of fertility is the important aspect for development of POI because the follicles are absent or lose their ability to respond to the stimulation.

The process of maturation of follicles is a highly organized and very complex process called as Folliculogenesis. It is a progressive maturation of small primordial follicles that progress to become large ovulatory follicles. As the follicles get matured the oocytes are released from ovary surface, gets collected in uterine tube which are fertilized, implanted or lost.

Currently there are some therapies available for treatment of POI but generally drugs like Clomiphene Citrate, tamoxifen, etc. are mostly used. The HRT and IVF are also the treatment of the POI but they may cause the loss of fertility, pain during intercourse and embarrassment among the women. All of these treatments shows its associated mild or serious side effects. So it is important to develop and to laid on medcines from natural sources which should be potent and show minimal or no side effects.

Numerous medicinal plants have proven to improve the ovary size and improve endocrine disorders associated with POI. The plants also show the effect on various hormonal changes and biochemical parameters of POI.

As per the literature survey it observed that various chemical constituents contribute to the treatment of various endocrine disorders. Ellagic acid is one of the constituent can work against POI by their radical scavenging property.

Ellagic acid contain four hydroxyl groups and two lactone groups in which hydroxyl group is known to increase antioxidant activity in lipid peroxidation and protect cells from oxidative damage. Ellagic acid inhibits the generation of Reactive oxygen species (ROS) such as hydroxyl radical (OH) and superoxide (O_2) by its metal chelating property which affect both male and female gamete.

The present study was conducted using female albino wistar rats which are divided into seven groups (Table 1), conducting six rats in each group.

Cyclophosphamide, Cisplatin and galactose are some of the POI inducers but they produce highest mortality and other side effects. Thus in present study Tripterygium Glycoside (TG) is used as POI inducer as it shows fewer side effects than other inducers.[21] Clomiphene Citrate is used as Standard drug for the treatment of POI, which is mostly used in various endocrine disorders. Whereas the Ellagic acid is used as the test drug.

After the induction of POI by TG and after the treatment with standard drug and various doses of Ellagic acid, the various physiological, biochemical parameters and hormones level were determined from blood samples.

The body weight, as the important factor in POI, is decreases slightly by the inducer TG which may be due to absence of corpus luteum as seen in histological section of ovary or by shrinkage of ovary [Figure 8 (B)]. TG induces apoptosis and necrosis within the ovary to initiate POI. After the treatment with standard drug, various doses of ellagic acid alone and in combination shows the increase in the body weight may be due to marked recovery with appearance of healthy follicles along with presence of corpus luteum which observed in histological sections. [Figure 8 (C-G)]

Cholesterol and triglyceride level increases after induction (Tables 3 and 4) may be due to lack of endogenous ovarian function. Treatment with standard drug, 30mg/kg ellagic acid with standard drug has significantly decreased the cholesterol and triglyceride level with P value ***P<0.001, *P<0.05and **P<0.01 respectively (Figures 3 and 4). The ellagic acid decreases cholesterol and triglyceride level may be due to its

antioxidant activity. It may inhibit the lipid peroxidation and thus restores healthy follicles for proper functioning of ovaries.

Estrogen level decreases (Table 5) whereas follicle stimulating and luteinizing hormone increases (Tables 5 and 6) after TG induced POI. TG induceds ovarian dysfunction and failure. The secretion of estrogen decreased caused by increased FSH and luteinizing hormone. Continued treatment of TG caused ovarian tissue damage, granuloma cell swelling and necrosis.[22] Treatment with standard drug, 30mg/kg ellagic acid and ellagic acid with standard drug has significantly increased estrogen level and decreased the FSH and LH level with P value ***P<0.001, *P<0.05 and **P<0.01 respectively (Figures 5, 6 and 7).

The standard drug Clomiphene citrate is capable of interacting with estrogen receptor containing tissues, including pituitary and ovary and may delay replenishment of intracellular estrogen receptors. It initiates a series of endocrine events ending in a preovulatory gonadotropin surge and subsequent follicular rupture which results in an increase in the release of pituitary gonadotropins. This initiates steroidogenesis and folliculogenesis, resulting in growth of the ovarian follicle and an increase in the circulating level of estradiol. For this evidence shown in histological section [Figure 8 (C)]

Ellagic acid inhibits the generation of reactive oxygen species such as hydroxyl radical (OH) and superoxide anion (O_2) by its radical scavenging activity. Histological section after treatment shows recovery of healthy follicles and restoration of Corpus luteum. [Figure 8 (D-G)]

10. Conclusion

Based on observations of physiological, biochemical, and hormonal parameters, the current study demonstrated that Ellagic acid can be used to treat female Albino Wistar rats with Tripterygium Glycoside (TG)-induced premature ovarian insufficiency (POI). This treatment can help the rats lose weight, normalize their lipid profile, and balance their hormone profile.

Additionally, the histological sections of the ovaries ensures the POI condition and ellagic acid treatment, which normalizes the ovarian cycle by maturing the follicles and restoring the number of healthy follicles.

Above all, the data indicates that ellagic acid has a major impact on treating POI.

11. Source of Funding

None.

12. Conflict of Interest

None.

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