THE PHARMACOLOGICAL ACTIVITY OF LEAVES OF *ERYTHRINA VARIEGATA* LINN., ON METABOLIC SYMPTOMS OF EXPERIMENTALLY INDUCED POLYCYSTIC OVARIAN DISEASE

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ABSTRACT

To evaluate the beneficial effect of Ethanolic extract of *Erythrina variegata* Linn., in decreasing the major metabolic symptoms associated with Letrozole induced Polycystic Ovarian Disease (PCOD or PCOS) using female rat model. *Erythrina variegata* Linn., is commonly known as Indian coral tree belongs to the family of Fabaceae. Female wistar albino rats were grouped into five groups with six animals each. All rats were daily administered with letrozole (1mg/kg b.tw.) for 21 days except control, and during this period, changes in estrus cycle were observed. After letrozole treatment, group 2 was considered negative control, group 3 was considered standard, group 4 and 5 were treated orally with Ethanolic extract of *Erythrina variegata* Linn., 200 mg/kg b.wt. and 400 mg/kg b.wt., respectively, for 4 weeks (7 consecutive estrus cycles). Various parameters such as estrus cycle, blood sugar level, lipid profile and weights of reproductive system were determined. The characteristic of ovary and uterus were evaluated by histopathological studies.

KEYWORDS: PCOD, Letrozole, Histopathological, Fabaceae.

INTRODUCTION

The plant *Erythrina variegata* Linn., belongs to the Fabaceae family, is a tree with prickly stems. It is a wild growing forest plant in India. The common English names for *Erythrina variegata* Linn., include Indian coral tree, Moochy wood tree. *Erythrina variegata* Linn., also called as *Erythrina indica* is a thorny deciduous tree growing upto 60 feet tall. It is popular
in indigenous system of medicine like Ayurveda, Siddha, Unani and Homeopathy. A wide range of chemical compounds have been isolated from this plant mainly alkaloids, flavonoids, triterpenoids and lectin.[1-5]

Different parts of the plant have been used in traditional medicine as a nervinesedative, collyrium in ophthalmia, anti-asthmatic, antiepileptic, antiseptic, astringent, toothache, emmenagogue and galactagogue. Leaf juice said to cured long - standing dysmenorrhoea and also removed sterility in fatty women by gradually reducing fat and produce natural menstrual flow, if the medicine is being continued for two or three months Erythrina variegata is an adulterant to the ayurvedic drug Rohitaka.[6-10]

Polycystic ovarian syndrome or Polycystic ovarian disease (PCOS or PCOD) in humans is also known as the Stein-Leventhal syndrome. It is a hormonal endocrine disorder of child bearing age recognized as the primary cause of infertility. PCOS is characterized by multiple small ovarian cysts less than 1cm, LH is raised and LH/FSH ratio is ≥2. Endocrine and reproductive symptoms of PCOS are hyperandrogenism (Hirsutism, acne and alopecia), irregular menstrual cycles and subfertility.[11-15]

MATERIALS AND METHODS

Collection of Plant Material
The leaves of Erythrina variegata Linn., was collected from Karur district Pillapalayam village, Tamilnadu in August – 2016.

Identification and Authentication of Plant Material
The collected specimens was botanically identified and authenticated by Dr. R. Jayaraman Ph.D., Director, Institute of Herbal Botany Plant Anatomy Research Centre, West Tambaram, Chennai-45. It was identified as Erythrina variegata linn Fabaceae family.

Extraction procedure
The first step was the preparation of successive solvent extracts. The dried coarsely powdered sample of Erythrina variegata Linn., was first extracted with Petroleum ether (60-80°C) in Soxhlet apparatus and then with solvents of increasing polarity like Chloroform, Ethyl acetate and Ethanol at 60 - 70°C. They were then followed with maceration in aqueous solvent. Each extract was concentrated using rotary vacuum evaporator.
Selection of Active Extract

*In vitro* Anti Oxidant Activity \[^{16-18}\]*

**DPPH Assay:** 1, 2-Diphenyl-2-Picryl Hydrazyl Radical (DPPH)

Initial volume 0.1 ml of various concentrations of samples was mixed with 0.4 ml of 0.3M DPPH reagent prepared in ethanol. The mixture was shaken thoroughly and incubated in the darkness at room temperature for 30 min. The absorbance of the reaction was measured spectrophotometrically at 517nm, immediately after mixing and then after incubation as well. The scavenging effect of DPPH free radical was calculated by using the following equation.

\[
% \text{ scavenging activity} = \frac{\text{Abs (control)} - \text{Abs (standard)}}{\text{Abs (control)}} \times 100
\]

Where control is the absorbance of the blank (a reaction with all the reagents except the test extract), and absorbance of sample is the absorbance of the test extract. Tests were carried out in triplicates to obtain 50% inhibition (IC50). Using Butylated hydroxy Toulene.

**Hydrogen Peroxide Scavenging Assay (HPSA)**

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch. A solution of Hydrogen peroxide (2mmol/l) was prepared in phosphate buffer (PH 7.4). Various concentrations of extracts (10-100μg/ml) were added to hydrogen peroxide solution (0.6ml). Absorbance at 230nm was determined after 10min against a blank solution containing phosphate buffer without hydrogen peroxide.

\[
% \text{ Scavenging activity} = \frac{\text{Abs (control)} - \text{Abs (standard)}}{\text{Abs (control)}} \times 100
\]

**Acute Oral Toxicity Study (OECD 423 Guidelines)**

Literature survey showed that acute toxicity of the extracts was determined according to the OECD guideline No. 423. Female wistar albino rats weighing 150 – 200 g were used for ethanolic Extract of *Erythrina variegata Linn.*, was given to five groups (n = 5) of animals each at 5, 50, 300 and 2000 mg kg⁻¹ b.w. p. o. The treated animals were under observation for 14 days, for mortality and general behavior. No death was observed till the end of the study. The test sample was found to be safe up to the dose of 2000 mg/kg. So, 1/10th and 1/5th of the dose (200 and 400mg/kg) were selected for this study.\[^{21}\]

**In vivo** Evaluation Of Letrozole Induced Polycystic Ovarian Disease

The protocol for conducting the *In vivo* study in female adult albino wistar rats was approved by the Institutional Ethical Committee (ICE) of the Madras Medical College, Chennai – 600003 India.

Animal selection and procurement
Healthy young female wistar albino rats (weighing about 150 – 200 gm) were procured from the Madras Medical College animal house.

The procured animals were kept in a clean, dry polycarbonate cages and maintained in a well-ventilated animal house. The temperature of experimental animal room was maintained at 22ºC (± 3ºC) and the relative humidity was maintained from 50-60%. Lighting was artificially maintained for 12hrs dark and 12hrs light. All the animals were kept in the cages for at least 5days prior to dosing for acclimatization to the laboratory conditions. The animals were fed with standard pellet diet and water was given ad libitum. Before starting the dose, the animals were fasted overnight but allowed to access water.

PCOS induction
All the experimental animals except Control group, were orally administered with Letrozole at a dose of 1mg / kg dissolved in 0.5% Carboxy Methyl Cellulose (CMC) once daily for 21 days. Control group received vehicle only (0.5 % CMC). Vaginal Smears were collected daily and evaluated microscopically using Crystal violet stain to confirm the induction of PCOS.[22-24]

Study Design
The study consisted of 30 female wistar albino rats equally divided into five groups designated as Group I (served as control group), Group II (served as PCOD induced group), Group III (served as standard group), Group IV and V served as treatment groups. Following Letrozole administration, standard group was administered with Metformin at a dose of 1 mg/kg in per oral route and treatment groups IV and V were administered Ethanolic extract of Erythrina variegata Linn., with the dose of 200mg/kg (Group IV) and 400 mg/kg (Group V) body weight respectively in 0.5% CMC per oral for 22 to day 52.[22-24]

The animals received following treatment
A total of 30 Adult female wistar albino rats are divided into 5 groups of 6 animals each.

Table: 1 GROUPING OF ANIMALS

<table>
<thead>
<tr>
<th>S.NO</th>
<th>GROUP</th>
<th>INDUCTION</th>
<th>TREATMENT for 30 days</th>
<th>NO OF ANIMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I Control</td>
<td>Normal saline orally</td>
<td>Normal saline orally</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>II Disease control</td>
<td>III Standard</td>
<td>IV Test group (200 mg/kg)</td>
<td>V Test group (400 mg/kg)</td>
</tr>
<tr>
<td>---</td>
<td>-------------------</td>
<td>-------------</td>
<td>--------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td></td>
<td>1 mg/kg Letrozole OD for 21 days</td>
<td>1 mg/kg Letrozole OD for 21 days</td>
<td>1 mg/kg Letrozole OD for 21 days</td>
<td>1 mg/kg Letrozole OD for 21 days</td>
</tr>
<tr>
<td></td>
<td>Normal saline orally</td>
<td>Metformin 100 mg/kg orally</td>
<td>200 mg/kg ethanolic extract orally</td>
<td>400 mg/kg ethanolic extract orally</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**EVALUATION PARAMETERS**

- Body weight
- Vaginal exfoliative cytology
- Determination of blood glucose level
- Lipid profile
- Weight of Reproductive organ (Uterus and Ovary)
- Histopathological evaluation (Uterus and Ovary)

**Body weight**

Initial body weight of animals in all groups was determined on the first day. The body weight of different groups of rat was noted weekly for 2 months.

**Vaginal exfoliative cytology**

**Smear technique – Lavage or Washing with saline or Water from pipette**

A small amount (approximately 0.2ml) of saline or distilled water is drawn up into the pipette tip. The rat is held around the thorax, ventral surface uppermost, with one hand whilst the hand holding the pipette is used to restrain the tail, to provide additional support and help prevent the animal struggling.

The tip of the pipette is pushed gently into the entrance of the vagina to a depth of 2-5 mm and the fluid is flushed into the vagina and back up into the pipette two or three times by gently squeezing and releasing the bulb of the pipette. A small amount of the cell suspension is then expelled onto a labeled glass slide. Slides should be labeled with the female identification numbers; the data and study number may also be shown but as the smears are discarded immediately after reading this is not usually considered necessary as long as the tray holding the slides is identified appropriately.

Crystal violet stain was added to the slide to cover the smear. The slide was kept covered in petridish for 5 min. Distilled water was added to the crystal violet stain and gently rocked. A
violet scum appeared on top of the slide. The slide was stained for 10 min in dilute crystal violet stain. The stained slide was dried and then washed in tap water. The washed slide was air dried and observed under the microscope in 40× objective.[20]

**Stages of estrus cycle in rat**[19]
During the study, vaginal smears were observed microscopically using Crystal violet stain for determination of estrus cyclicity.

**4 stages of estrus cycle is seen in female rat**

**Proestrus**
It is a preparatory phase to the next estrus phase. Vaginal smear shows nucleated epithelial cells. This stage is last for about 12 hrs.

**Estrus**
The vaginal smear shows 100% cornified epithelial cells. This stage is last for 9-15 hrs.

**Metestrus**
It follow after estrus stage, the vaginal smear shows many leucocytes with a few cornified cells. This stage is last for 20 hrs.

**Diestrus**
It is the longest phase, consists of mainly leucocytes in vaginal smear. This stage is last for 57 hrs.

**Preparation of Blood Serum**
After 21 days, PCOS control group and after 52 days, animals from other groups were fasted overnight and anaesthetized with diethyl ether. Blood was collected by retinal orbital puncture then serum was separated by centrifugation and was used for estimation of blood glucose, lipid Profile.

**Determination of Blood Glucose Level**
The blood glucose levels of each group were monitored at the end of the study.
Lipid Profile
Lipid profile which includes total cholesterol (TC), triglycerides (TGs), low-density lipoprotein (LDL) and high density lipoprotein (HDL) were estimated by autoanalyzer microlab 200 using Ecoline-kits.

Weight of Reproductive organ
Uterus and ovaries were dissected out from each animal and weighed by a digital electronic weighing balance to evaluate the effect of the extract.

Histopathological evaluation
The isolated samples of ovaries and uterus in each group were selected for histopathological evaluation. The tissues placed in 10% buffered formalin. The fixed tissues were stained with Haematoxylin and Eosin. Slides were reviewed for the evaluation of histopathological changes like follicles, corpus luteum and cysts.

Statistical analysis
Results were expressed as Mean ± SEM. The data was analyzed using one way analysis of variance (ANOVA) followed by Dunnett’s test. P values < 0.01 were considered as Significant.

RESULTS AND DISCUSSION
Selection of Active Extract
In – vitro Anti – Oxidant Activity

Table: 2 DPPH ASSAY

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>100 g/ml</th>
<th>200 g/ml</th>
<th>400 g/ml</th>
<th>800 g/ml</th>
<th>1000 g/ml</th>
<th>IC_{50} (µ g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac Sodium</td>
<td>26.17±0.45</td>
<td>34.72±1.86</td>
<td>41.88±0.54</td>
<td>61.23±1.56</td>
<td>73.02±0.09</td>
<td>578</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>15.45±0.15</td>
<td>17.52±0.71</td>
<td>22.76±0.56</td>
<td>25.04±2.43</td>
<td>38.48±0.65</td>
<td>1549</td>
</tr>
<tr>
<td>Chloroform</td>
<td>4.64±0.52</td>
<td>7.68±1.06</td>
<td>19.53±0.67</td>
<td>26.48±1.46</td>
<td>39.99±1.84</td>
<td>1309</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>17.22±0.90</td>
<td>24.45±1.43</td>
<td>36.24±0.43</td>
<td>45.59±0.96</td>
<td>51.15±1.73</td>
<td>916</td>
</tr>
<tr>
<td>Ethanol</td>
<td>24.23±2.80</td>
<td>35.07±1.08</td>
<td>47.95±0.67</td>
<td>63.64±1.87</td>
<td>65.86±2.34</td>
<td>489</td>
</tr>
<tr>
<td>Aqueous</td>
<td>1.78±1.62</td>
<td>5.75±1.87</td>
<td>14.18±1.73</td>
<td>28.3±1.43</td>
<td>35.67±2.54</td>
<td>1388</td>
</tr>
</tbody>
</table>
Table 3: Hydrogen Peroxide Scavenging Activity

<table>
<thead>
<tr>
<th>EXTRACTS</th>
<th>% INHIBITION AT VARIOUS CONCENTRATION</th>
<th>IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 µg/ml</td>
<td>20 µg/ml</td>
</tr>
<tr>
<td>Standard (Ascorbic acid)</td>
<td>13.59</td>
<td>26.18</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>3.14</td>
<td>6.32</td>
</tr>
<tr>
<td>Chloroform</td>
<td>2.65</td>
<td>5.58</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>7.16</td>
<td>16.82</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12.48</td>
<td>24.63</td>
</tr>
<tr>
<td>Aqueous</td>
<td>3.54</td>
<td>5.72</td>
</tr>
</tbody>
</table>

The active extract was selected on the basis of in vitro anti oxidant activity. Accordingly the ethanolic extract with potential anti oxidant activity was selected for in vivo study.
Body weight

Table 4: Changes in the body weight (g)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial weight</th>
<th>14th day</th>
<th>28th day</th>
<th>42nd day</th>
<th>56th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Control</td>
<td>157±1.04</td>
<td>165.67±0.82</td>
<td>173.67±0.74</td>
<td>176±0.73</td>
<td>172.34±0.68</td>
</tr>
<tr>
<td>Group II Disease control</td>
<td>154±0.47</td>
<td>173±0.27</td>
<td>190.34±0.53</td>
<td>197.34±0.40</td>
<td>205.45±0.46</td>
</tr>
<tr>
<td>Group III Standard</td>
<td>158.64±0.72</td>
<td>174.67±0.5</td>
<td>185±0.62</td>
<td>177.34±0.89</td>
<td>176.67±0.91</td>
</tr>
<tr>
<td>Group IV 200 mg/kg</td>
<td>158.67±0.72</td>
<td>171.67±0.85</td>
<td>184.54±0.36</td>
<td>188.67±0.4</td>
<td>187.16±0.35</td>
</tr>
<tr>
<td>Group V 400 mg/kg</td>
<td>159.34±0.4</td>
<td>170.34±0.61</td>
<td>179±0.59</td>
<td>184.34±0.57</td>
<td>182.67±0.39</td>
</tr>
</tbody>
</table>

Fig. 3 Changes in body weight

The body weights of the different groups of rat were noted. The body weight of the Polycystic Ovarian Disease induced group shows an increase in body weight compared to the normal control group. After treatment, the body weight of the Group IV and Group V was found to decrease in body weight. This was compared to the standard group which showed change in body weight. This shows that the ethanolic extract is effective in normalizing the enhanced body weight.

Stages of Estrus Cycle

Table 5: Four stages of Estrus cycle

| Proestrus phase | Proestrus smears are characterized by rounded, nucleated, epithelial cells are present. |
Estrus phase

Estrus smears consist entirely of cornified cells and often non – nucleated.

Met estrus phase

Met estrus smears consists of large number of leucocytes and smaller numbers of mostly large, non – granular and non – nucleated epithelial cells.

Di – estrus phase

Di – estrus smears consists mainly of Leucocytes but with quite variable numbers of epithelial and small cornified cells.

In the present study, Letrazole – aromatase inhibitor, was used to induce Polycystic Ovarian syndrome in female Wister rats. The working of this model was confirmed by regular examination of vaginal smears and presence of persistent vaginal cornification.

Blood Glucose

Table 6: Blood glucose

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Control</td>
<td>110.34±0.64***</td>
</tr>
<tr>
<td>Group II Disease control</td>
<td>152.83±0.92</td>
</tr>
<tr>
<td>Group III Standard</td>
<td>118.83±0.94****</td>
</tr>
<tr>
<td>Group IV 200mg/kg</td>
<td>125.5±0.94****</td>
</tr>
<tr>
<td>Group V 400mg/kg</td>
<td>122.5±0.67****</td>
</tr>
</tbody>
</table>
Fig. 4: Blood Glucose Level

Values are means ±SEM, (n=6) ****p<0.0001 versus disease control, Group I – Control, Group II – Disease control, Group III – Standard, Group IV – Low dose (200mg/kg body weight of extract), Group V – High dose (400mg/kg body weight of extract), SEM – Standard error of mean.

Table: 7 Lipid Profile

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC</th>
<th>TG</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>53.34±0.43**</td>
<td>126.17±0.89**</td>
<td>46.25±0.56***</td>
<td>35.84±0.6***</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>61.83±0.92</td>
<td>142±0.78</td>
<td>43.91±0.49</td>
<td>20.67±0.36</td>
</tr>
<tr>
<td>Disease control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>29±0.35***</td>
<td>102.167±0.79***</td>
<td>27.34±0.67****</td>
<td>25.67±0.37*</td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>28.67±0.69***</td>
<td>100.67±0.62****</td>
<td>27.5±0.48****</td>
<td>26.34±0.43*</td>
</tr>
<tr>
<td>200mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group V</td>
<td>27.34±0.72***</td>
<td>105.84±0.9****</td>
<td>27.91±0.47****</td>
<td>28.5±0.31**</td>
</tr>
<tr>
<td>400mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ±SEM, (n=6) ****p<0.0001, ***p<0.001, **p<0.005 versus disease control, Group I – Control, Group II – Disease control, Group III – Standard, Group IV – Low dose (200mg/kg body weight of extract), Group V – High dose (400mg/kg body weight of extract), SEM – Standard error of mean.
In the present study, lipid profile, PCOS induced groups showed notable increase in TC, TGs, LDL and decrease in HDL levels. Ethanolic extract of Erythrina variegata Linn., displayed antihyperlipidemic action by considerably decreasing the enhanced serum TC, TGs, LDL while increasing HDL levels. This showed that the ethanolic extract is effective in normalizing the enhanced lipid levels.

Weight of Reproductive Organ

Table 8: Changes Of Ovary And Uterus Weight In Rat

<table>
<thead>
<tr>
<th>S.NO</th>
<th>GROUPS</th>
<th>OVARY WEIGHT</th>
<th>UTERUS WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I Control</td>
<td>50.45±4.02</td>
<td>67.54±2.48</td>
</tr>
<tr>
<td>2</td>
<td>Group II Disease control</td>
<td>68.58±2.44</td>
<td>115.31±9.57</td>
</tr>
<tr>
<td>3</td>
<td>Group III Standard</td>
<td>57.21±2.62</td>
<td>79.06±1.42</td>
</tr>
<tr>
<td>4</td>
<td>Group IV 200 mg/kg</td>
<td>60.12±1.7</td>
<td>71.8±1.18</td>
</tr>
<tr>
<td>5</td>
<td>Group V 400 mg/kg</td>
<td>62.45±2.81</td>
<td>84.04±1.36</td>
</tr>
</tbody>
</table>
The ovary and uterus weight of the different groups of rat were noted. The ovary and uterus weight of Polycystic Ovarian Disease group shows an increase in value when compared to the normal control group. After treatment, the Group IV and Group V were found to decrease in value. This was compared to the standard group which showed decrease in ovary and uterus weight due to the effect of standard drug. This shows that the ethanolic extract is effective in normalizing the enhanced ovary and uterus weight.

**Histopathological Examination Of Treated Animals**

**Histopathological Examination Of Group I: (Control)**

![Fig: 7 Section of Ovary](image1)
![Fig: 8 Section of Uterus](image2)

**Histopathological examination Of Group II (Disease Control)**

![Fig. 9 Section of Ovary](image3)
![Fig. 10 Section of Uterus](image4)
Histopathological Examination Of Group III (Standard)

Fig: 11 Section of Ovary

Fig: 12 Section of Uterus

Histopathological Examination Of Group IV (200 mg/kg)

Fig. 13 Section Uterus Showing of Secondary Follicles

Fig. 14 Section Ovary

Histopathological Examination Of Group V (400mg/kg)

Fig. 15 Section of uterus

Fig. 16: Section of Ovary
The histopathological results of control ovary show normal ovarian architecture with matured secondary follicles and oocyte. The fresh corpus luteum indicates the presence of previous ovulation.

Letrozole treated rats exhibited numerous subcapsular cysts, with a very thin or no granulose layer. Corpora lutea were completely absent indicating anovulation and irregular estrus cycle. Few follicles were observed at their early stages of development. In addition, they were accompanied with atretic follicles containing fluid filled antrum and higher incidence of pyknotic granulose cells.

Standard treatment led to disappearance of cysts and appearance of healthy follicles and corpora lutea.

Sections from Group IV of Ethanolic extract of Erythrina variegata Linn., (200mg/kg) group exhibited follicles large in size and many corpora lutea present. Also antral follicles with clearly differentiated oocyte, granulosa cell layer, corona radiate and theca cells were observed. Group V of Ethanolic extract of Erythrina variegata Linn., (400mg/kg) group showed secondary follicles with oocyte were visible in the histopathological results. It also showed a fresh and thick corpous luteum indicates ovulation.

In treated group decrease incidence of pkynotic granulose cells. Varying number of corpora lutea were seen suggesting ovulation and normal estrus cyclicity. Follicles at different stages of development with oocyte are clear, visible granulose cell layer were observed. Ovarian cortex appeared normal with many follicles.

**CONCLUSION**

Polycystic Ovarian disease was assessed by Letrozole induced Polycystic Ovarian disease method. The parameters studied were Body weight changes, Vaginal exfoliative cytology, Blood sugar level, Lipid profile (TC, TGs, LDL, HDL), Reproductive organ weight and Histopathological studies. Ethanolic extracts showed decrease in the elevated level of Body weight, Triglycerides, Total cholesterol, LDL and Reproductive Organ weight and increase in the HDL level. All the parameters revealed the effective role of ethanolic extract in Polycystic Ovarian disease activity which was comparable to the standard. In the study of Polycystic Ovarian disease activity the Ethanolic extract shows significant activity in both In – *vitro* and In – *vivo* models. Ethanolic extract of *Erythrina variegata* Linn., showed many
beneficial effects similar to standard drug in treating PCOS condition and inducing ovulation. These effects may be ascribed to its multiple pharmacological activities like estrogenic, antihyperlipidemic, antioxidant and hypoglycemic effects which could be useful in managing PCOS condition and prevent ovarian cell dysfunction, ovulation and thereby improving fertility. Together broad spectrum biological effects of Ethanolic extract of Erythrina variegata Linn., make it a promising drug for treating clinical and pathological abnormalities in PCOD condition.

REFERENCES
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