International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 31, Issue 7, July 2025 **ijppr.humanjournals.com** ISSN: 2349-7203

Evaluation of Fertility Enhancing Activity of *Senna alata* (L.) Roxb. Leaves in Female Wistar Albino Rats

Agalya Udhayakumar[1*], Rani Shanmugam[2]

[1]Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai nagar-608 002, Tamilnadu, India.
[2]Associate Professor, Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai nagar-608 002, Tamilnadu, India.

Received: 2025-06-25 Revised: 2025-07-10 Accepted: 2025-07-25

ABSTRACT

Infertility occurs when a couple is unable to get pregnant after having sexual intercourse for one year or six months even after having regular, unprotected sex. It is also known as the inability to successfully carry a pregnancy to term and give birth to a live child. A lot of medicinal plants are used to treat female reproductive health disorders such as amenorrhea, uterine obstruction and female infertility like irregular menstrual cycle, hormonal imbalance and few have been tested for fertility activity but there is no scientific report for many plants. Our selected medicinal plant *Senna alata* (L.) Roxb. also have no scientific data regarding treatment of female infertility. The present study evaluated the fertility-enhancing effects of *Senna alata* (L.) Roxb. leaf extract (EESA) in female Wistar rats. Daily vaginal smear analysis over 28 days revealed that EESA (200 and 400 mg/kg, p.o.) regularized the estrous cycle and shortened the metestrus and diestrus phases. Hematological parameters remained within normal limits, indicating safety. EESA-treated groups showed significant increases in body, uterine, and ovarian weights compared to the ethinyl estradiol group. Histopathological examination confirmed regeneration of ovarian follicles and corpus luteum, suggesting enhanced folliculogenesis. This revealed that the above data strongly recommends that EESA could be used to treat the female infertility problems including immature ovum production, irregular estrous cycle.

Keywords: *Senna alata* (L.) Roxb., Clomiphene citrate, Ethinyl estradiol, Infertility.

1. INTRODUCTION

Infertility occurs when a couple is unable to get pregnant after having sexual intercourse for one year or six months even after having regular, unprotected sex. It is also known as the inability to successfully carry a pregnancy to term and give birth to a live child [1]. Pregnancy is one the most intimate and heart-warming experiences in the life of married couples worldwide. Not only are the events that lead very personal, but the actual incident represents a merging of essential features of both partners . Due to the inability to bear children, many couples are socially isolated from family and thus emotionally undergoing stress [2]. Motherliness is the only one of the available approaches for women to improve their position in the relationship and society [3]. The most significant drawbacks of allopathy are its adverse effects. In this new environment, it may be necessary to introduce new safe herbal drugs with minimal side effects for the treatment of fertility. Traditionally many plants have been used for the management of fertility. Senna alata (L.) Roxb. (=Cassia alata L.) belongs to the Fabaceae family. It is native to Central America and is mainly encountered in the Caribbean area but has also been introduced into many tropical countries and islands whatever the continent [4]. A detailed literature survey was carried out for the leaves of *Senna alata* (L.) Roxb. Literature review reveals that *Senna alata* (L.) Roxb. exhibit vital pharmacological properties such as antibacterial, antifungal, antiviral, antidiabetic and anti inflammatory activity [5]. *Senna alata* (L.) Roxb. leaves are traditionally used for the management of infertility. However, there is no scientific report for the treatment of infertility for the plant [6].

2. MATERIALS AND METHODS

2.1 Plant Collection

Fresh Senna alata (L.) Roxb. leaves were collected from Sirkazhi, Mayiladuthurai District, Tamil Nadu, India. The plant herbarium was prepared and authenticated by Prof. Dr. L. Mullainathan at the Department of Botany, Annamalai University,

Annamalai Nagar. A Specimen sample of the herbarium was preserved at Department of Botany, Annamalai University. The plant herbarium has an authenticated reference number : 618.

2.2 Plant extraction

The leaves of Senna alata (L.) Roxb. properly were cleaned, washed with distilled water, dried in a shaded area and then powdered using a mechanical mixer. With the help of a Soxhlet apparatus, 200 g of finely grounded leaf powders from Senna alata (L.) Roxb. were extracted for 48 hr using ethanol. The extracts were separated after extraction, condensed by distillation, and dried at room temperature until they formed a viscous solid mass. The obtained crude extracts were measured and maintained at 4 °C in sealed glass vials with labels and vacuum desiccators until use [7]. Preliminary phytochemical tests was carried out for ethanolic extract of Senna alata (L.) Roxb. leaves as per standard procedure [8].

2.3 Experimental animals:

Female Wistar albino rats weighing between 150 – 200 g were used. The experimental protocol was approved by the Institutional Animal Ethical Committee of Cuddalore Medical College and Hospital (IAEC PROPOSAL NO:GMCHC-IAEC/1400/4/25), Annamalai University, Annamalai Nagar, Cuddalore District of Tamil Nadu, India. Acclimatization, housing and feeding conditions were followed as per Committee for the Purpose of Control and Supervision of Experiments on Animals(CPCSEA) guidelines.

2.4 Acute toxicity study

Based on the literature review, the acute toxicity test was already done for the leaf of ethanolic extract of *Senna alata* (L.) Roxb. The study was performed as per OECD guidelines 423. Hence, the dose of EESA was fixed as 200 mg/kg and 400 mg/kg bw p.o. [9].

2.5 Estrous cycle monitoring

The stages of the estrous cycle were determined by preparing the vaginal smears. For this, the first thin cotton bud was taken which was dipped into normal saline (0.9 % w/v NaCl). While the vaginal margins were separated, the cotton bud rotated inside clockwise twice with an angle of 45°. The content obtained from the vagina was transferred to a microscope slide. The slide was stained with methylene blue (0.05% w/v) for 7 min. The slide was gently washed using plain water to remove excess stain and the stained slide was left at room temperature for 10 min. The slide was later examined for the various stages of the estrous cycle under the microscope. The stages of the estrous cycle were classified according to the cell type observed under the microscope [10].

2.6 Experimental design for fertility enhancing activity:

A total of 30 female Wistar albino rats having normal estrous cycle grouped into 5 having Six animals each was used for this study including three control groups and two test group. Group 1 served as normal control in which normal animals treated with vehicle, group 2 served as negative control in which ethinyl estradiol induced animals treated with vehicle and group 3 served as positive control in which ethinyl estradiol induced animals treated with standard clomiphene citrate 0.1mg/kg bw [11-13]. Group4 and 5 served as test in which ethinyl estradiol induced animals treated with low (200 mg/kg bw) and high (400 mg/kg bw) dose.

Table no 1: Experimental grouping of animals

Groups	Group specification	Treatment for 28 days
Group I	6	Animals received vehicle
Group II	6	Animals were treated with ethinyl estradiol (0.03mg/kg) for four days.
Group III	6	Animals were treated with ethinyl estradiol (0.03mg/kg) first four days and from the 5th day clomiphene citrate (0.1 mg/kg p.o) was administered.
Group IV	6	Animals were treated with ethinyl estradiol (0.03mg/kg) for first four days and from the 5th day low dose of EESA(200 mg/kg bw p.o) was administered.
Group V	6	Animals were treated with ethinyl estradiol (0.03mg/kg) for first four days and from the 5th day high dose of EESA (400 mg/kg bw p.o) was administered.

2.7 Inducing method for Anovulation

All groups other than group 1, with regular cycling female rats were treated orally twice daily for four consecutive days with ethinyl estradiol 0.03mg/kg, starting on the day of estrus after confirmation through smear test, to inhibit ovulation. On day 5 after the final dose, ethinyl estradiol blocked the ovulation [14,15].

2.8 Methodology

The treatment was given for 28 consecutive days. During the treatment period all the animals was examined for their food and water intake. The body weight of the animal was recorded. The length of the estrous cycle was monitored daily. On last day, all rats was weighed and euthanized as per the norms of CCSEA.

2.9 Haemotological Parameters

The blood were collected by intra cardiac puncture method. The collected blood were used to evaluate RBC , WBC and Hb level [16].

2.10 Estrogenic/Anti-estrogenic activity

Estrogenic/Anti-estrogenic activity was examined based on ovary and uterus weight. A significant increase in ovary and uterus weight shows estrogenic activity and decrease in ovary and uterus weight shows anti-estrogenic activity [17].

2.11 Histopathological analysis

Ovary tissues were excised, fixed and processed for histopathology. Sections were prepared, stained with H&E and examined microscopically for histopathological changes [18].

2.12 Statistical analysis

Data were expressed as mean ± SEM. Comparisons between different groups were performed using one-way ANOVA, followed by Dunnett's test multiple comparison tests (GraphPad Prism 10). $p < 0.05$ was considered to be statistically significant [19].

3. RESULTS

3.1 Weight and percentage yield of Ethanolic extract of *Senna alata* (L.) Roxb. leaves:

Table 2 : Percentage yield of EESA

Plant name and part used	Colour	Consistency	Weight of extract (g)	Percentage yield (%)
Senna alata (L.) Roxb. leaves	Greenish black	Semi-solid	31.5	15.75

The percentage yield of ethanolic extract of Senna alata (L.) Roxb. [EESA] was 15.75 % w/w.

3.2 Preliminary phytochemical analysis

Table 3 : Phytochemical analysis of EESA

Name of the compounds	Ethanolic extract of *Senna alata* (L.) Roxb. leaves
Alkaloids, Phytosterols, Tannin and phenolic compounds, Flavanoids, Terpenoids	Present(+)
Carbohydrates, Glycosides, Fixed oils and fats, Saponin, Proteins and amino acids	Absent(-)

International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 31, Issue 7, July 2025 ijppr.humanjournals.com ISSN: 2349-7203

3.3 Effect of EESA on estrous cycle

Table 4: Effect of EESA on phases of estrous cycle

A. No.	Control	EE	STD	EESA(Low dose)	EESA(High dose)
1	4.22±0.06	8.23±0.06	4.21± 0.4	5.44± 0.45	4.47±0.49
2	4.16±0.02	7.98±0.02	4.34±0.26	5.21±0.08	4.66±0.51
3	4.20±0.32	8.28±0.09	4.28±0.31	5.32±0.42	4.39±0.25
4	4.34±0.12	7.68±0.17	4.21±0.08	5.36±0.10	4.56±0.04
5	4.28±0.28	7.78±0.23	5.11±0.23	5.40±0.08	4.48±0.91
6	4.38±0.44	8.2±0.44	4.26±0.54	5.67±0.26	4.50±0.28
Average	4.26±0.20	8.02±0.17****	4.4±0.30^{ns}	5.40±0.23****	4.51±0.41****

n=6 animals per group; Values are expressed as mean ±SEM. One way ANOVA followed by Dunnett's test. ns = non-significant,*p < 0.05, **p < 0.01, ***p< 0.001 and ****p< 0.0001 denotes significant difference compared with control group.

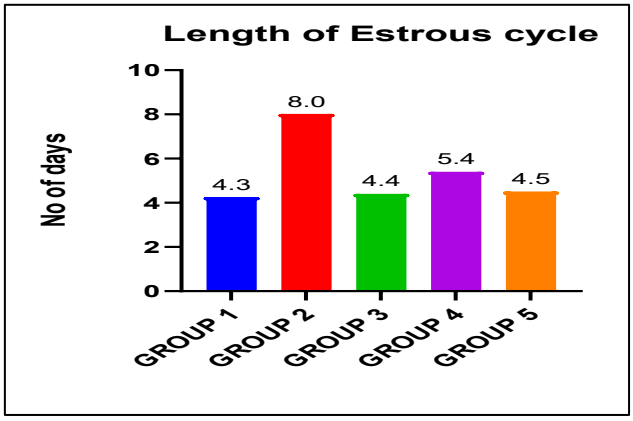

Figure 1: Effect of EESA on length of estrous cycle

Initially, an irregular estrous cycle was observed in all treated groups except group I. Treatment with EESA (200 and 400 mg/kg bw p.o) (P<0.0001) significantly regularized the estrous cycle length as that of standard treated group and normal control group, particularly shortened the metestrus and diestrus phase when compared to ethinyl estradiol group.

3.4 Effect of EESA on haematological parameters

Table 5: Effect of EESA on hematological parameters

GROUPS	TREATMENT	RBC (10^6/mm^3)	WBC (10^3 /mm^3)	Hb (g/dl)
GROUP-I	Normal control group	8.5±0.101	14±0.107	13.5±0.031
GROUP-II	Ethinyl estradiol treated group	5.86±0.047****	8.6±0.021****	9.5±0.013****
GROUP-III	Clomiphene citrate treated group	8.3±0.084^{ns}	11.2±0.135****	13.2±0.048***
GROUP-IV	EESA treated group (Low dose)	7.74±0.112***	10.26±0.08****	12.3±0.023****
GROUP-V	EESA treated group (High dose)	7.83±0.03****	11.15±0.037****	12.1±0.062****

n=6 animals per group; Values are expressed as mean ±SEM. One way ANOVA followed by Dunnett's test. ns = non-significant,*p < 0.05, **p < 0.01, ***p< 0.001 and ****p< 0.0001 denotes significant difference compared with control group.

International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 31, Issue 7, July 2025 ijppr.humanjournals.com ISSN: 2349-7203

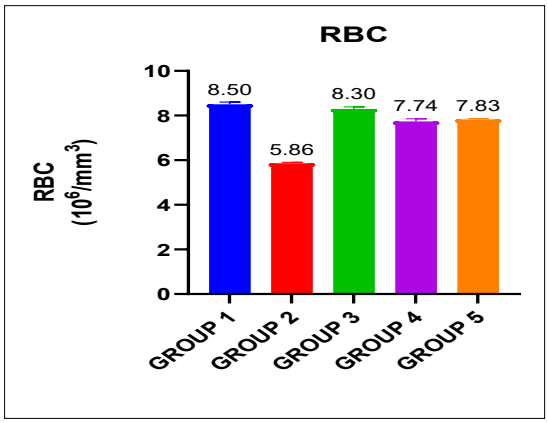

Figure 2: Effect of EESA on RBC Level

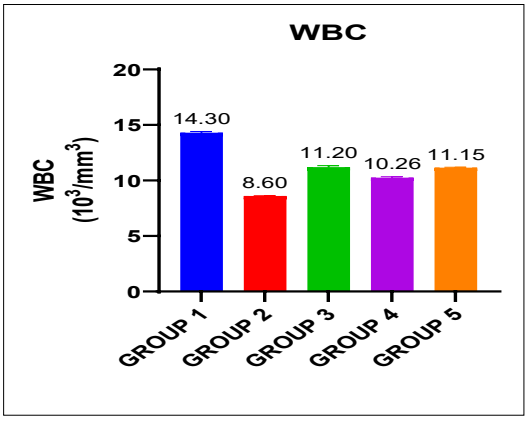

Figure 3: Effect of EESA on WBC Level

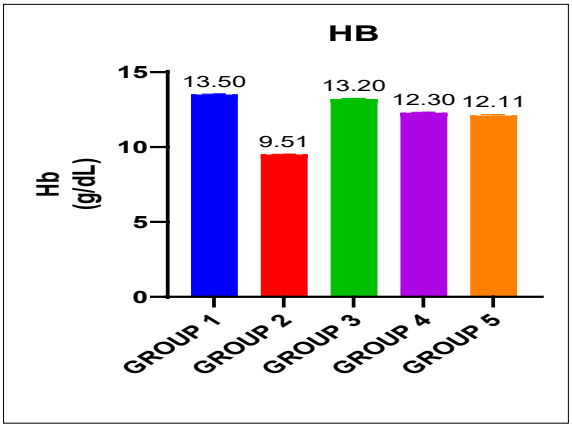

Figure 4: Effect of EESA on Hb Level

All of the treated groups haematological indicators, such as RBC, WBC and HB content, were within normal limits. The administered dosage levels of ethanolic extract of *Senna alata* (L.) Roxb. have no harmful effects on overall body metabolism. The normal range of haematological measurements reflects a normal physiological state with no haematological harmful effects.

International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 31, Issue 7, July 2025 ijppr.humanjournals.com ISSN: 2349-7203

3.5Effect of EESA on genital organ weight

Table 6: Effect of EESA on genital organs weight

S. No	Experimental group	Uterus weight (mg)	Ovarian weight (mg)
1	Control	285	47.31
2	EE (Ethinyl estradiol 0.03 mg/kg bw p.o)	190	23.56
3	STD (Clomiphene citrate 0.1 mg/kg bw p.o)	282	50.47
4	EESA (200 mg/kg bw p.o)	278	42.05
5	EESA (400 mg/kg bw p.o)	280	49.62

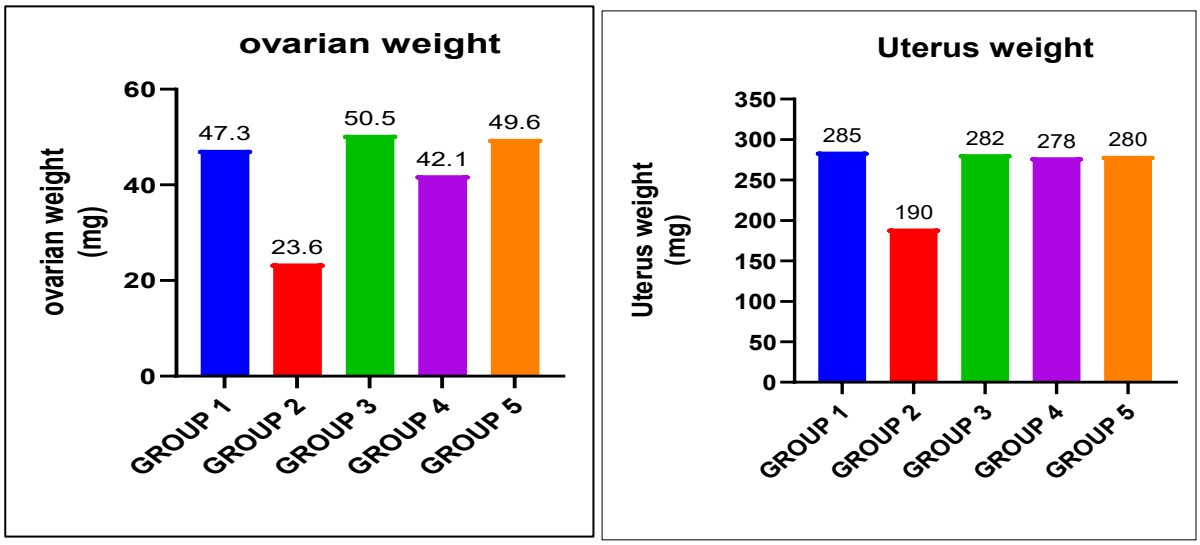

Figure 5: Effect of EESA on uterus and ovarian weight

The ethinyl estradiol group showed a decrease in uterus and Ovarian weight when compared to the control group. The standard group showed an increase in the uterus and ovarian weight when compared to the ethinyl estradiol group. *Senna alata* (L.) Roxb. leaf extracts may have estrogenic activity because the uterus and ovary weight are increased.

3.6 Effect of EESA on Ovary(Histopathology)

3.6.1 Section of Ovary in Normal Control(Group I)

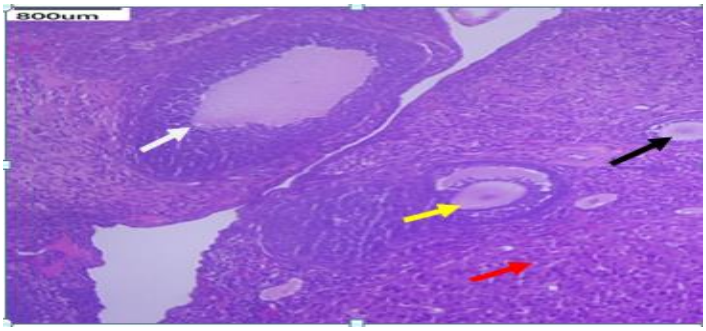

Figure 6: Section of ovary in normal control

Sections showed normal ovary with maturing stages of ovarian follicles, primary(black arrow), secondary(white arrow) and tertiary graffian follicle(yellow arrow) in the normal ovarian stroma(red arrow).

3.6.2 Section of Ovary in ethinyl estradiol treated group(Group II)

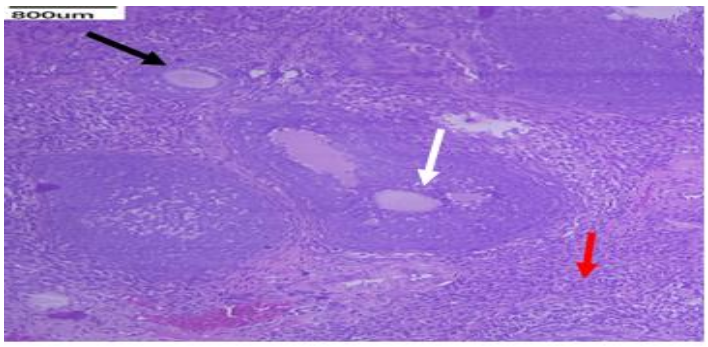

Figure 7: Section of ovary in negative control

Sections studied show ovarian tissue with reduced number of ovarian follicles, with predominantly primary follicles(black arrow) and a few secondary follicles(white arrow) in the ovarian stroma(red arrow).

3.6.3 Section of Ovary in clomiphene citrate treated group (Group III)

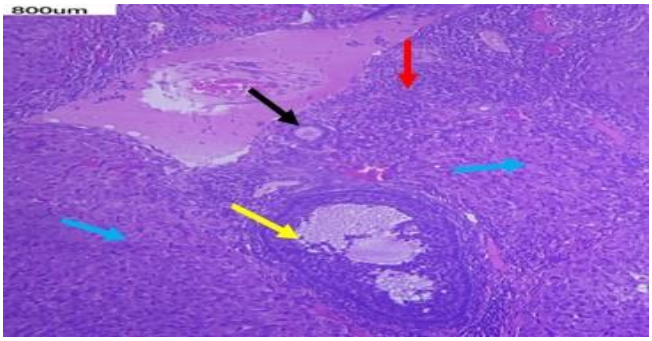

Figure 8: Section of ovary in standard treated group

Sections showed normal ovary with maturing stages of ovarian follicles, primary(black arrow), secondary(white arrow) and tertiary graffian follicle(yellow arrow) in the normal ovarian stroma(red arrow). Corpus luteum(blue arrow) is more evidently seen.

3.6.4 Section of Ovary in EESA treated group (200mg/kg) (Group IV)

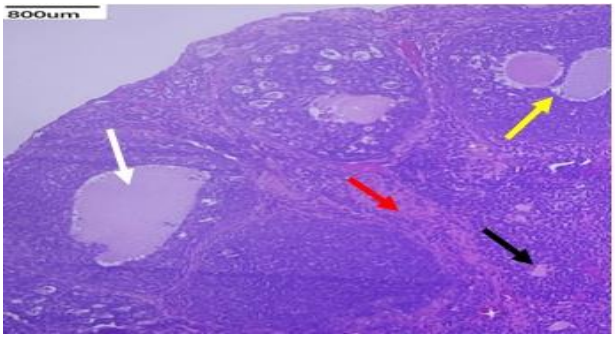

Figure 9: Section of ovary in EESA treated group (200mg/kg)

Sections showed normal ovary with maturing stages of ovarian follicles, primary(black arrow), secondary(white arrow) and tertiary graffian follicle(yellow arrow) in the normal ovarian stroma(red arrow).

3.6.5 Section of Ovary in EESA treated group (400mg/kg) (Group V)

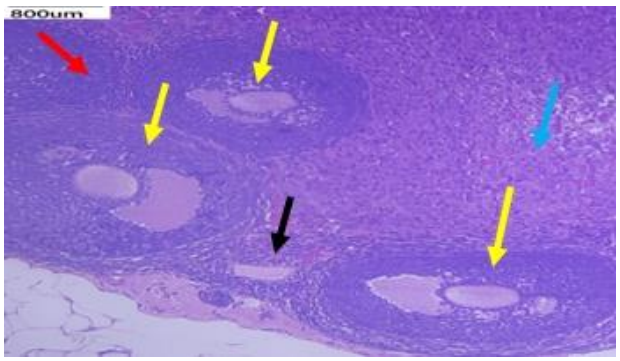

Figure 10: Section of ovary in EESA treated group (400mg/kg)

Sections showed normal ovary with maturing stages of ovarian follicles, primary(black arrow), secondary(white arrow) and more of tertiary graafian follicle(yellow arrow) in the normal ovarian stroma(red arrow). Corpus luteum are also seen(blue arrow).

The results showed that administration of EESA (200 and 400 mg/kg) has regeneration of ovary with maturing stages of ovarian follicles, primary, secondary and more of tertiary graafian follicle in the normal ovarian stroma and Corpus luteum. The myometrium and perimetrium shows normal morphology. The results revealed that EESA possess generating ability of follicles including PF, SF, and TF. Those are responsible for healthy ovum production.

4. Discussion

During the 28 days of treatment, the animals in all the groups were observed for daily vaginal smear cytology to evaluate the estrous cycle length. Initially, an irregular estrous cycle was observed in all treated groups except group I. Treatment with EESA (200 and 400 mg/kg bw p.o) regularized the estrous cycle length as that of standard treated group and normal control group, particularly shortened the metestrus and diestrus phase when compared to ethinyl estradiol group.Thus, it is proved that the EESA can normalize the estrous cycle irregularities.

All of the treated groups, the haematological indicators, such as HB, RBC, and WBC content, were within normal limits. This means that the administered dosage levels of EESA have no harmful effects on overall body metabolism.

The ethinyl estradiol group showed a decrease in uterus and Ovarian weight when compared to the control group. The standard group showed an increase in the uterus and ovarian weight when compared to the ethinyl estradiol group. EESA (200 and 400 mg/kg bw p.o) showed an increase in uterus and ovarian weight when compared to the ethinyl estradiol-induced group. Increase in ovarian weight was the direct effect of folliculogenesis responsible for mature ovaries. *Senna alata* (L.) Roxb. leaf extracts may have estrogenic activity because the uterus and ovary weight are increased.

Histopathological study showed degeneration (atretic) of primary and secondary follicles in Ethinyl estradiol treated rats(Negative control). EESA treated group (200 mg/kg) showed regeneration of primary (PF), secondary (SF) and tertiary follicles (TF). EESA treated group (400 mg/kg) showed regeneration of many PF, SF ,TF and corpus luteum as that of the standard treated group. These results suggested that female infertility issues such as the development of immature ovum could be treated with *Senna alata* (L.) Roxb. Immature ovum production is the major problem in infertility. Our selected plant *Senna alata* (L.) Roxb. help for healthy ovum production. It was confirmed by histopathological studies.

5. CONCLUSION

On the basis of the findings, it was concluded that the ethanolic extract of Senna alata (L.) Roxb. leaves possesses fertility enhancing activity. The above data strongly recommends that EESA could be used to treat the female infertility problems including immature ovum production, irregular estrous cycle. It has promising fertility activity. Further studies should be carried out to isolate and characterize the compounds responsible for fertility enhancing activity.

REFERENCES

1. Wasiu Eniola Olooto, Adebayo Adetola Amballi , Taiwo Abayomi Banjo (2012) A review of Female Infertility; important etiological factors and management volume 2 .Issues 3.pages 379-385.
2. Aarts JW, van Empel IW, Boivin J, Nelen WL, Kremer JA, Verhaak CM. Relationship between quality of life and distress in infertility: a validation study of the Dutch FertiQoL. Hum Reprod. 2011 .26(5):1112-8.
3. Cousineau TM, Domar AD. Psychological impact of infertility. Best Pract Res Clin Obstet Gynaecol. 2007 . 21(2):293-308.
4. Thierry Hennebelle , Bernard Weniger , Henry Joseph , Sevser Sahpaz , François Bailleul (2009). Senna alata. Fitoterapia 80 , 385–393.
5. Sri Fatmawati , Yuliana ,Adi Setyo Purnomo , Mohd Fadzelly Abu Bakar (2020). Chemical constituents, usage and pharmacological activity of Cassia alata.Heliyon.volume 6 (7). e04396.
6. Mike O. Soladoye, Emmanuel C. Chukwuma, Olatunji M. Sulaiman, and Roseline T. Feyisola (2014) Ethnobotanical Survey of Plants Used in the Traditional treatment of Female Infertility in Southwestern Nigeria.
7. Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A.,Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A.K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering, 117(4), 426-436.
8. Junaid R Shaikh and MK Patil(2020). Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies 2020; 8(2): 603-608.
9. L.Priyadarshini , P.B.Mazumder , M.D.Choudhury (2014). Acute toxicity and oral tolerance test of ethanol and methanol extracts of antihyperglycemic plant Cassia alata linn , 9(2),43-46.
10. Mary, F., Eswaramohan, T., Surendran, S. N., Murugananthan, A., & Romakeswaran, P. (2013). Mukkuddu Maathrai (triple tablet), an indigenous medicine elevates serum prolactin level of female rats (Rattus norvegicus). Journal of Pharmacy, 3(1), 38-42.
11. Jadhav M, Menon S, Shailajan S. In vivo evaluation of Mimosa pudica Linn. in the management of polycystic ovary using rat model. Int J Appl Biol Pharm. 2012; 4 (1): 285-292.
12. Jha U, Asad M, Asdaq SMB, Das AK, Prasad VS. Fertility inducing effect of aerial parts of Coccinia cordifolia L. in female rats. J Ethnopharmacol. 2010; 127: 561-564.
13. Ugwah-Oguejiofor CJ, Bello SO, Okolo RU, Etuk EU, Ugwah MO,Igbokwe VU. Ficus platyphylla promotes fertility in female Rattus norvegicus Wistar strain: a preliminary study. Reprod Biol Endocrinol. 2011; 9 (145): 1-6.
14. Bennink, C. H. J., Skouby, S., Bouchard, P., & Holinka, C. F. (2008). Ovulation inhibition by estetrol in an in vivo model. Contraception, 77(3).
15. Rahman, M. A. (2009). Phytochemical and experimental study of Aparmaga (Achyranthus aspera Linn) WSR to female anti-infertility activity. M.D (Ayurveda) [Thesis]. Rajiv Gandhi University of Health Sciences p. 61.
16. Amudha, M., & Rani, S. (2020). Fertility inducing activity of Cordia retusa in female albino rats. Asian Journal of Pharmaceutical Research, 10(3), 171.
17. Dinesh K. Dabhadkar, Varsha S. Zade, Pratibha H. Rohankar, Shital R. Pare and Manisha A. Wikhe (2012). Estrogenic and Anti-Estrogenic Potentials of Ethanolic Pod Extract of Plumeria rubra in Female Albino Rats. Global Journal of Pharmacology 6 (2): 142-147.
18. Mohamed Slaoui & Laurence Fiette.(2011).Histopathology Procedures: from Tissue Sampling to Histopathological Evaluation.Methods in Molecular Biology, vol. 691,69-82.
19. Dunnett CW(1955). A multiple comparison procedure for comparing several treatments with a control. Journal of the American Statistical Association. Dec 1;50(272):1096-121.

How to cite this article:
Agalya Udhayakumar et al. Ijppr.Human, 2025; Vol. 31 (7): 234-242.
Conflict of Interest Statement: All authors have nothing else to disclose.