A Synopsis

on

Evaluation of Anti fertility Activity of *Saraca Indica* (Ashoka) in Experimental Animals

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1. TITLE OF THE THESIS AND ABSTRACT

TITLE: Evaluation of Anti fertility Activity of *Saraca Indica* (Ashoka) in Experimental Animals

ABSTRACT:

This thesis investigates the anti-fertility potential of Saraca indica (Ashoka), with a specific focus on its active compound, ursolic acid, extracted from the bark. The study addresses the need for safer, plant-based contraceptives by evaluating ursolic acid's effects through in vitro and in vivo experiments on experimental animals. Key methodologies include extraction and quantification of ursolic acid via maceration and HPLC (yielding 0.40% with 90% assay), in vitro oxytocic activity on rat uterine tissue, and in vivo assessments of anti-ovulatory, anti-implantation, anti-androgenic, and anti-estrogenic activities in Wistar rats and Swiss albino mice. Results demonstrate dose-dependent inhibition of ovulation, implantation loss (up to 63.47% pre-implantation and 57.66% post-implantation at 250 mg/kg.bwt.), reduced sperm parameters and testicular weight in males, and decreased estrogen levels and uterine weight in females. These findings support ursolic acid's multifaceted anti-fertility mechanisms, including LH surge inhibition, cervical mucus thickening, and endometrial alteration. The original contributions highlight ursolic acid's comparable efficacy to standards like oxytocin and cyclophosphamide, validating Saraca indica's traditional use and suggesting its potential for developing novel, reversible contraceptives to address population control and gender equity in birth control options. Future research could explore clinical applications and molecular pathways.

2. INTRODUCTION

Saraca indica, commonly known as Ashoka, is an ancient medicinal tree native to India, belonging to the Caesalpinaceae family. Its name, derived from Sanskrit, translates to "without sorrow," reflecting its historical use in alleviating gynecological ailments. Taxonomically classified under Kingdom: Plantae, Phylum: Magnoliophyta, Class: Magnoliopsida, Order: Fabales, Family: Caesalpinaceae, Genus: Saraca, Species: Asoca, the plant has been revered in Ayurvedic medicine for its uterotonic properties.

Brief Description on the State of the Art of the Research Topic:

The exploration of plant-derived compounds for anti-fertility applications has gained momentum due to the limitations of synthetic contraceptives, such as side effects and irreversibility. Saraca indica has been traditionally used for menstrual disorders and uterine health, with recent studies

confirming its pharmacological potential. For instance, research has identified crystalline glycosides in its bark that stimulate uterine contractions, suggesting applications in managing dysmenorrhea and potentially as abortifacients. Oxytocic activity has been observed in human uterine preparations, cautioning against its use during pregnancy due to risks of fetal harm.

Ursolic acid, a pentacyclic triterpenoid abundant in Saraca indica bark, mimics steroidal structures and exhibits anti-inflammatory and contractile effects via calcium channels and prostaglandin pathways. Recent investigations (2023-2024) have expanded on its anti-fertility roles, including attenuation of oligospermia in mice by improving sperm parameters and potential in treating polycystic ovary syndrome (PCOS) through ethanolic extracts. In-silico screenings have identified phytocompounds from Saraca asoca with anti-androgenic and anti-estrogenic properties for PCOS management. Comprehensive reviews highlight Saraca indica's larvicidal and gynecological efficacy, positioning it as a vulnerable yet promising species for modern therapeutics. Ursolic acid's antifertility potential is further supported by studies on related triterpenoids like oleanolic acid in other plants, showing sperm motility inhibition. This thesis builds on these advancements by isolating ursolic acid and rigorously testing its multi-faceted anti-fertility mechanisms in animal models, addressing gaps in dose-dependent efficacy and mechanistic insights.

3. DEFINITION OF THE PROBLEM

Current contraceptive options predominantly target women, perpetuating gender inequality in reproductive health despite societal advancements toward equality in power, finance, education, and opportunities. This disparity raises the question: why are male-oriented contraceptives underrepresented? Additionally, global population growth exacerbates scarcity of resources like food, water, and arable land, hindering improvements in education, housing, health, nutrition, and living standards.

Existing birth control methods have significant limitations:

- Implants: Require surgical procedures with uncertain reversibility.
- Short-acting injectables/pills: Cause harmful side effects.
- Combined hormone pills: Long-term harmful effects.
- Barrier methods: Discomfort during intercourse, stigma in acquisition due to low awareness, and increased STD risk.
- Spermicidal barriers: Higher STD risk and lower efficacy.
- Emergency pills: Low efficacy with more side effects.
- Sterilization (vasectomy/tubectomy): Surgical and irreversible.

These issues underscore the need for safe, effective, reversible, plant-based anti-fertility agents that address both genders and minimize side effects.

4. AIM AND OBJECTIVE OF RESEARCH WORK

To evaluate the anti-fertility activity of Saraca indica (Ashoka) in special context of Ursolic Acid.

Objectives

- To evaluate cupric acetate induced anti ovulatory activity in female wistar rats.
- To evaluate Anti-Implantation activity after determination of Post implantation loss and Pre implantation loss in female wistar rats.
- To evaluate anti estrogenic activity in swiss albino mice.
- To evaluate anti androgenic activity in male wistar rats.

Expanded Explanation of Aims and Objectives

The primary aim of this research is to systematically assess the potential of Saraca indica, commonly known as Ashoka, as a natural anti-fertility agent, with a particular emphasis on its bioactive compound, ursolic acid. This plant has been utilized in traditional Ayurvedic medicine for centuries, primarily for gynecological issues, but modern scientific validation is essential to bridge traditional knowledge with contemporary pharmacology. Ursolic acid, a pentacyclic triterpenoid, has shown promising properties in preliminary studies, including modulation of reproductive hormones and interference with fertility processes. By focusing on ursolic acid, this study seeks to isolate and quantify its role in anti-fertility mechanisms, potentially paving the way for developing safer, plant-based contraceptives.

The objectives are designed to cover both female and male reproductive systems, addressing a gap in current contraceptive research where options are disproportionately female-oriented. The first objective targets ovulation inhibition using a cupric acetate model, which mimics oxidative stress-induced ovulatory disruption. This is crucial as ovulation is a key step in female fertility. The second objective examines implantation, a critical post-fertilization event, by measuring pre- and post-implantation losses, providing insights into early pregnancy interruption. The third and fourth objectives extend to hormonal modulation, evaluating anti-estrogenic effects in mice and anti-androgenic effects in rats, respectively. These are vital for understanding broader reproductive impacts, including potential male contraceptives.

This structured approach ensures comprehensive evaluation, incorporating in vitro and in vivo models to validate efficacy and safety.

5. ORIGINAL CONTRIBUTION BY THE THESIS

- Why only women-oriented drug of contraceptives are available in today's era?

  In today's era men and women are sharing equality in terms of distribution of power and influence; have equal opportunities for financial independence through work or through setting up businesses; enjoy equal access to education and the opportunity to develop personal ambitions than why not for the contraceptives?

- To Control Population:
  Get rid from the scarcity of the natural resources like food, water and arable lands. We can improve our quality education, housing, health services, adequate nourishment and better standard of living for the humans.

- Limitation of the available birth control procedures:

  a. List of available birth control tools and its limitations
  b. Implants: Surgical procedure and its uncertainty of reversible fertility
  c. Short acting injectable/pills: harmful side effects
  d. Combined hormone pills: Harmful side effects for the longer period
  e. Barrier Methods: Discomfort during sexual intercourse, Shame to ask due to the less awareness and chances having STDs
  f. Spermicidal Barrier Tools: Having more chances to increase the STDs and less efficacious
  g. Emergency pills: Low efficacy and more side effects
  h. Sterilization: Vasectomy and tubectomy is a surgical procedure and irreversible fertility

Expanded Rationale for the Study

The need for this work stems from multiple societal, health, and scientific imperatives. First, the gender disparity in contraceptive options is stark. While women bear the majority of contraceptive burdens through hormonal methods or invasive procedures, male options remain limited to condoms or vasectomy, both with drawbacks. This inequality persists despite advancements in gender equity, highlighting a critical gap in reproductive health research. Developing plant-based agents like those from Saraca indica could offer reversible, non-hormonal alternatives for both genders.

Second, population control is a global challenge. With overpopulation leading to resource depletion, natural contraceptives could provide sustainable solutions without the environmental impact of synthetic drugs. Studies indicate that herbal remedies can improve quality of life by reducing unintended pregnancies.

Third, current contraceptives have significant limitations. Hormonal methods often cause side effects like weight gain, mood changes, and increased cancer risk. Surgical options are irreversible, and barrier methods have high failure rates due to user error. Plant extracts, such as Ashoka bark, offer potential for safer profiles with antioxidant and anti-inflammatory benefits. Recent research on Saraca asoca for PCOS and infertility underscores its relevance.

This study addresses these needs by exploring ursolic acid's role, potentially leading to novel therapeutics.

This thesis provides novel insights into ursolic acid's anti-fertility potential from Saraca indica:

First comprehensive evaluation combining in vitro oxytocic activity with in vivo models across ovulatory, implantation, androgenic, and estrogenic pathways.

Dose-dependent data showing high efficacy (e.g., 63.47% pre-implantation loss at 250 mg/kg), surpassing or comparable to standards like cyclophosphamide.

Mechanistic elucidation: Ursolic acid inhibits GnRH and LH surge, thickens cervical mucus, alters endometrium, and disrupts spermatogenesis via symplast formation—extending prior knowledge on calcium and prostaglandin modulation.

Validation of traditional uses through modern pharmacology, with quantified extraction (0.40% yield, 90% purity) and recovery studies.

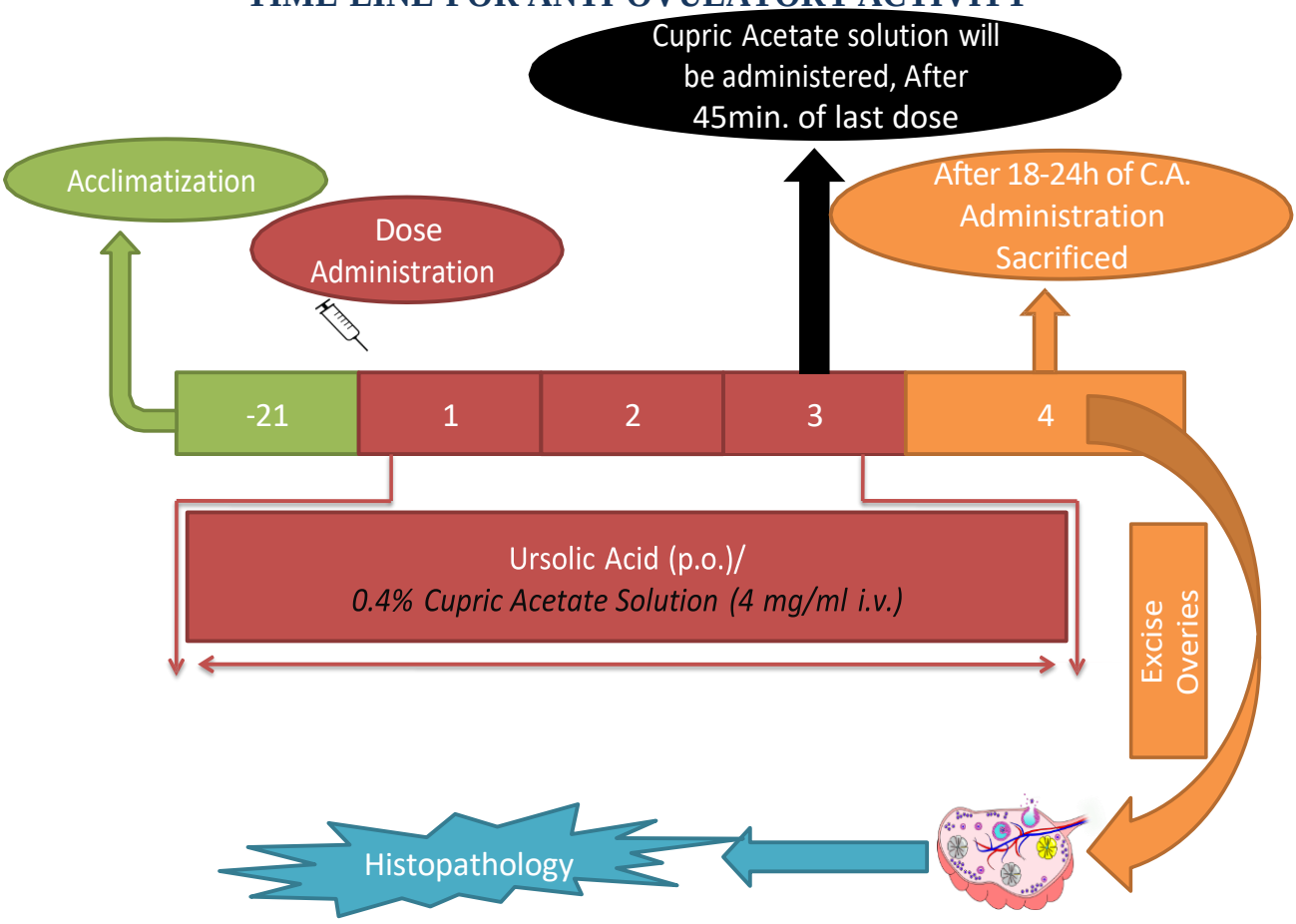
Potential for male contraceptives, addressing gender bias, with implications for PCOS and population control.

6. METHODOLOGY OF RESEARCH ALONG WITH RESULTS & DISCUSSION

Methodology: Bark of Saraca indica was procured from the domestic market and authenticated by CSIR-NIScPR, New Delhi. Ursolic acid was extracted using n-hexane via maceration, identified by TLC, and quantified by HPLC (yield: 0.40%, assay: 90%). Solubility was assessed in water and 0.5% CMC.

- **In Vitro Oxytocic Activity:** Isolated uterine tissue from female Wistar rats was exposed to ursolic acid (30 mg) in a kymograph setup, compared to oxytocin (IAEC:ARL/PT/975/2023).
- **Anti-Ovulatory Activity:** Female Wistar rats (n=24) in groups received ursolic acid (100/250 mg/kg p.o.) or vehicle, followed by cupric acetate (4 mg/kg i.v.). Ovaries were excised for weight and histopathology after 18-24 hours (IAEC: ARL/PT/691/2023).

TIME LINE FOR ANTI-OVULATORY ACTIVITY



Schematic Representation of Experimental Design for Anti- Ovulatory Activity

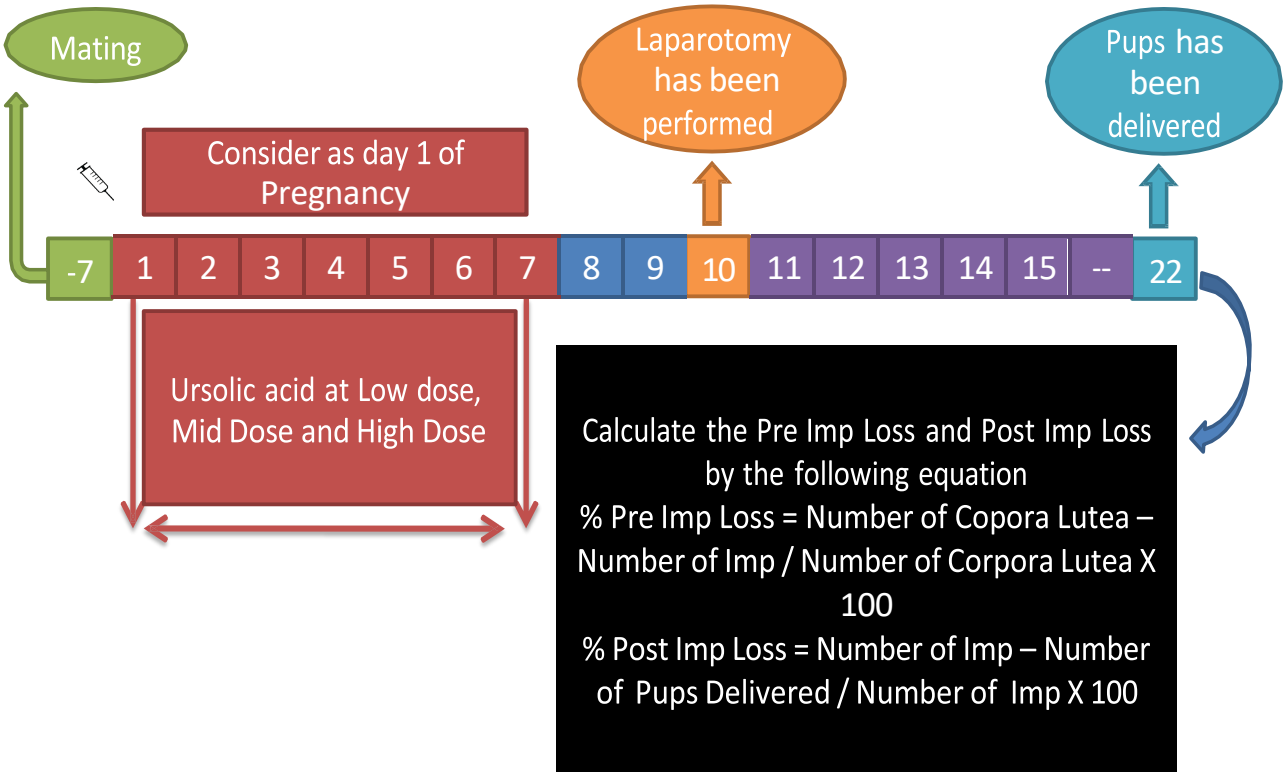
Experimental Design of Anti Ovulatory Activity:

GROUPS	TREATMENT	Dose & Route	Number of Female Rat
GROUP 1	Control + After 45 min. of last dose On the 3rd day 0.4% Cupric Acetate solution	Vehicle	1 - 6
GROUP 2	Ursolic Acid + After 45 min. of last dose On the 3rd day 0.4% Cupric Acetate solution	100 mg/kg b.wt. p.o. + (4 mg/kg i.v.)	7 - 12

GROUPS	TREATMENT	Dose & Route	Number of Female Rat
GROUP 3	Ursolic Acid + After 45 min. of last dose On the 3rd day 0.4% Cupric Acetate solution	250 mg/kg b.wt. p.o. + (4 mg/kg i.v.)	13 - 18
GROUP 4	Ursolic Acid + After 45 min. of last dose On the 3rd day 0.4% Cupric Acetate solution	250 mg/kg b.wt. p.o. + (4 mg/kg i.v.) (Recovery high dose)	19 - 24
Total number of animals			24

- **Anti-Implantation Activity:** Mated female Wistar rats (n=24 females, 8 males) received ursolic acid (100/125/250 mg/kg p.o.) from day 1-7 of pregnancy. Laparotomy on day 10 assessed implants; litters delivered on day 22 for loss calculation (IAEC: ARL/PT/691/2023).

TIME LINE FOR ANTI-IMPLANTATION ACTIVITY



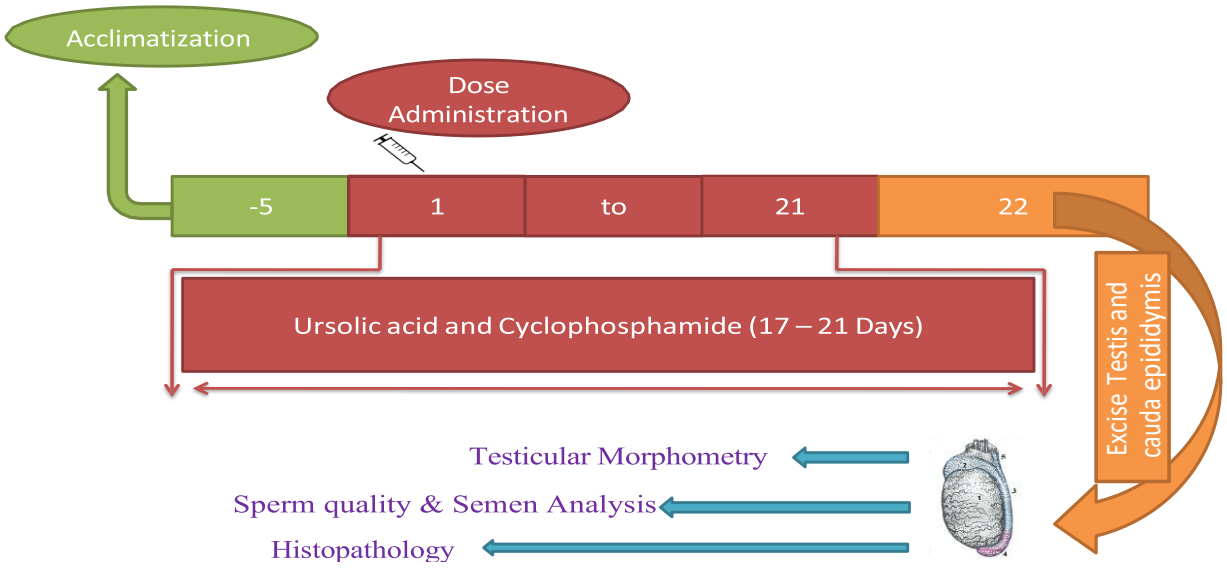
Schematic Representation of Experimental Design for Anti-Implantation Activity

Experimental Design of Anti Implantation Activity:

GROUPS	TREATMENT	Dose	Dose Volume & Route	Number of Rats (in each 6 females for two males)	
				Female	Male
GROUP 1	Vehicle Control	0.5 % CMC	10 ml/kg B.wt. P.O.	1 – 6	31 & 32
GROUP 2	Ursolic Acid	Low Dose (100 mg/kg b.wt.)	10 ml/kg B.wt. P.O.	7 – 12	33 & 34
GROUP 3	Ursolic Acid	Mid Dose (125 mg/kg b.wt.)	10 ml/kg B.wt. P.O.	13 – 18	35 & 36
GROUP 4	Ursolic Acid	High Dose (250 mg/kg b.wt.)	10 ml/kg B.wt. P.O.	19 - 24	37 & 38
Total number of animals				32	

- **Anti-Androgenic Activity:** Male Wistar rats (n=24) received ursolic acid (100/250 mg/kg p.o.) or cyclophosphamide (30 mg/kg i.p.) for 5 days after acclimatization. Assessed testicular weight, sperm count/viability/motility/morphology, and histopathology (IAEC: ARL/PT/691/2023).

TIME LINE FOR ANTI-ANDROGENIC & ANTI-SPERMATOGENIC ACTIVITY

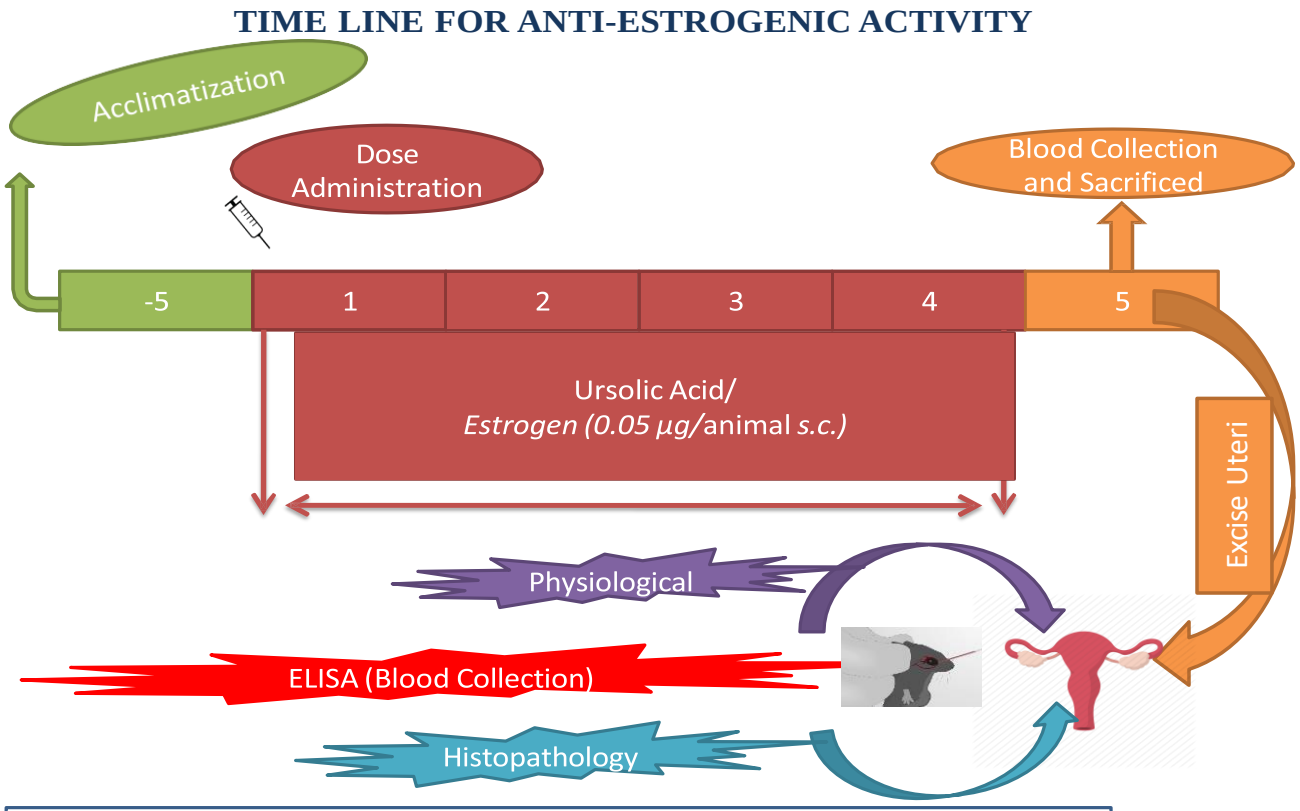


Schematic Representation of Experimental Design for ANTI-ANDROGENIC & ANTI-SPERMATOGENIC

Experimental Design of Anti Androgenic Activity:

GROUPS	TREATMENT	Dose	Dose Volume & Route	Number of Male Rat
Group 1	Control	Vehicle Control (0.5 % CMC)	10 mL/kg.b.wt., P.O.	1 - 6
Group 2	Cyclophosphamide (Archana M. Navale et al, 2023)	Standard (30 mg/kg b.wt.)	5 ml/kg.b.wt. I.P	7 - 12
Group 3	Ursolic Acid	Low Dose (100 mg/Kg b.wt.)	10 mL/kg.b.wt., P.O.	13 - 18
Group 4	Ursolic Acid	High Dose (250 mg/kg b.wt.)	10 mL/kg.b.wt., P.O.	19 - 24
Total No. of Animals				24

- **Anti-Estrogenic Activity:** Immature Swiss albino mice (n=36) in groups received ursolic acid (200/500 mg/kg p.o.) alone or with estrogen (0.05 µg s.c.). Evaluated uterine weight, estrogen levels (ELISA), and histopathology. Data analyzed by one-way ANOVA with Tukey's post-hoc test (IAEC: ARL/PT/691/2023).



Schematic Representation of Experimental Design for Anti-Estrogenic Activity

Experimental Design of Anti-Estrogenic Activity:

GROUPS	TREATMENT	Dose	Number of Immature Mice
GROUP 1	Control	Vehicle (0.5 % CMC, p.o.)	6
GROUP 2	Positive Control (Estrogen)	(0.05 µg/animal, s.c.)	6
GROUP 3	Ursolic Acid	Low Dose (200 mg/kg b.wt., p.o.)	6
GROUP 4	Ursolic Acid	High Dose (500 mg/kg b.wt., p.o.)	6
GROUP 5	Estrogen + Ursolic Acid	(0.05 µg/animal, s.c.) + Low Dose (200 mg/kg b.wt., p.o.)	6
GROUP 6	Estrogen + Ursolic Acid	(0.05 µg/animal, s.c.) + High Dose (500 mg/kg b.wt., p.o.)	6
Total number of animals			36

Results and Discussion:

- **Extraction and Quantification:** Ursolic acid was successfully isolated with high purity, confirming feasibility for pharmacological use. Solubility was insoluble in water but suspended in 0.5% CMC.
- **In Vitro Oxytocic:** Ursolic acid induced comparable contractions to oxytocin, suggesting uterotonic potential via calcium channels (Prissadova et al., 2015).
- **Anti-Ovulatory:** High dose (250 mg/kg) reduced absolute ovary weight (0.71 ± 0.17 g vs. 1.04 ± 0.06 g control, $P<0.001$) and relative weight (0.30 ± 0.07% vs. 0.43 ± 0.02%), with histopathology showing reduced corpora lutea, indicating ovulation inhibition. Recovery group normalized, suggesting reversibility.

Table No.1: Mortality and Morbidity Record

Group	Treatment	Animal No.	Observation
G1	Vehicle Control	1	No Mortality and Morbidity observed during the treatment period
		2	
		3	
		4	
		5	
		6	
G2	Low Dose	7	No Mortality and Morbidity observed during the treatment period
		8	
		9	
		10	
		11	
		12	
G3	High Dose	13	No Mortality and Morbidity observed during the treatment period
		14	
		15	
		16	
		17	
		18	
G4	Recovery High Dose	19	No Mortality and Morbidity observed during the treatment period
		20	
		21	
		22	
		23	
		24	

Table No.2: Clinical Sign Record

Group	Treatment	Animal No.	Observation
G1	Vehicle Control	1	No abnormal clinical sign observed during the treatment period
		2	
		3	
		4	
		5	
		6	
G2	Low Dose	7	No abnormal clinical sign observed during the treatment period
		8	
		9	
		10	
		11	
		12	
G3	High Dose	13	No abnormal clinical sign observed during the treatment period
		14	
		15	
		16	
		17	
		18	
G4	Recovery High Dose	19	No abnormal clinical sign observed during the treatment period
		20	
		21	
		22	
		23	
		24	

Table No.3: Individual Body Weight Record

Group	Treatment	Animal No.	Prior treatment Body weight (g)	Terminal treatment Body weight (g)
G1	Vehicle Control	1	228.40	232.54
		2	240.64	243.87
		3	240.10	245.41
		4	238.60	241.98
		5	242.65	246.78
		6	246.16	250.14
G2	Low Dose	7	230.61	234.15
		8	238.91	240.17
		9	237.92	241.74
		10	243.14	248.19
		11	244.66	246.72
		12	245.81	248.65
G3	High Dose	13	233.60	226.17
		14	230.49	226.47
		15	238.27	237.46
		16	240.61	232.67
		17	245.67	240.22
		18	240.64	233.87
G4	Recovery High Dose	19	230.01	244.47
		20	241.53	250.98
		21	238.24	246.78
		22	237.94	251.32
		23	238.91	254.14
		24	239.37	256.56

Group			Prior treatment Body weight (g)	Terminal treatment Body weight (g)
G1	Vehicle Control	Mean	239.43	243.45
		SD	6.00	6.02
		N	6	6
G2	Low Dose	Mean	240.18	243.27
		SD	5.64	5.66
		N	6	6
G3	High Dose	Mean	238.21	232.81 *
		SD	5.44	5.69
		N	6	6
G4	Recovery High Dose	Mean	237.67	250.71
		SD	3.96	4.49
		N	6	6

* Indicates that the significant decrease (P<0.05) has been observed in the group 3 animal body weight in comparison of group 1 animal body weight (Fig – 1).

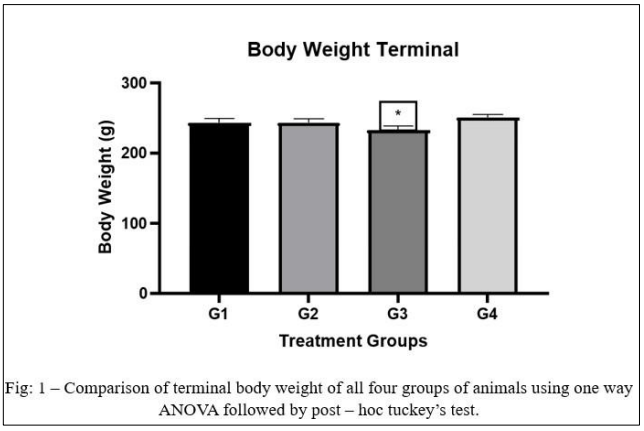


Fig: 1 – Comparison of terminal body weight of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test.

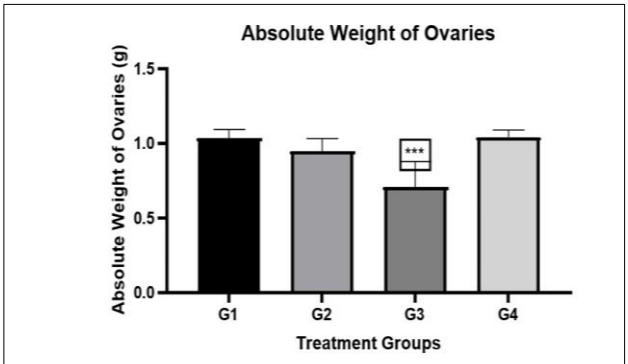


Fig: 2 – Comparison of absolute ovaries weight of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test

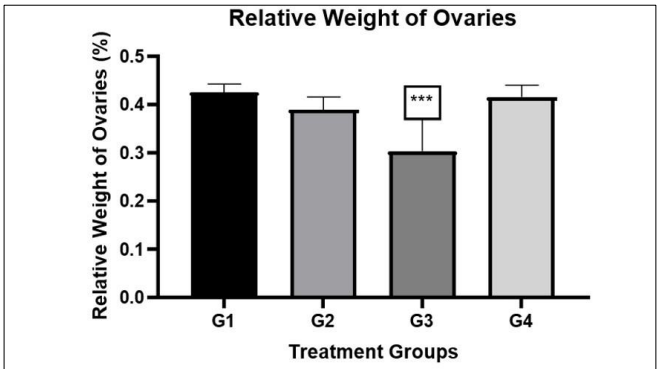


Fig: 3 – Comparison of relative ovaries weight of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test

Table No.4: Individual Absolute and Relative Ovary weight

Group	Treatment	Animal No.	Absolute Ovaries weight (g)	Terminal treatment Body weight (g)	Relative Ovaries weight (%)
G1	Vehicle Control	1	0.9737	232.54	0.4187
		2	0.9826	243.87	0.4029
		3	1.0892	245.41	0.4438
		4	0.9912	241.98	0.4096
		5	1.0986	246.78	0.4452
		6	1.0756	250.14	0.4300
G2	Low Dose	7	0.8272	234.15	0.3533
		8	0.8845	240.17	0.3683
		9	0.9426	241.74	0.3899
		10	0.9776	248.19	0.3939
		11	1.0621	246.72	0.4305
		12	0.9945	248.65	0.4000
G3	High Dose	13	0.6625	226.17	0.2929
		14	0.5746	226.47	0.2537
		15	0.7810	237.46	0.3289
		16	0.4654	232.67	0.2000
		17	0.8842	240.22	0.3681
		18	0.8801	233.87	0.3763
G4	Recovery High Dose	19	1.0612	244.47	0.4341
		20	0.9781	250.98	0.3897
		21	1.0889	246.78	0.4412
		22	1.0757	251.32	0.4280
		23	1.0627	254.14	0.4182
		24	0.9845	256.56	0.3837

Group			Absolute Ovaries weight (g)	Terminal treatment Body weight (g)	Relative Ovaries weight (%)
G1	Vehicle Control	Mean	1.04	243.45	0.43
		SD	0.06	6.02	0.02
		N	6	6	6
G2	Low Dose	Mean	0.95	243.27	0.39
		SD	0.08	5.66	0.03
		N	6	6	6
G3	High Dose	Mean	0.71***	232.81	0.30 ***
		SD	0.17	5.69	0.07
		N	6	6	6
G4	Recovery High Dose	Mean	1.04	250.71	0.42
		SD	0.05	4.49	0.02
		N	6	6	6

*** Indicates that the significant decrease (P<0.05) has been observed in the group 3 absolute weight of ovary in comparison of group 1 absolute weight of ovary (Fig – 2). *** Indicates that the significant decrease (P<0.05) has been observed in the group 3 relative weight of ovary in comparison of group 1 relative weight of ovary (Fig – 3).

Table No.5: Histopathology

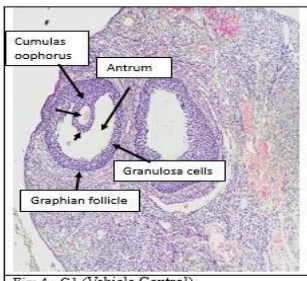


Fig: 4 - G1 (Vehicle Control)

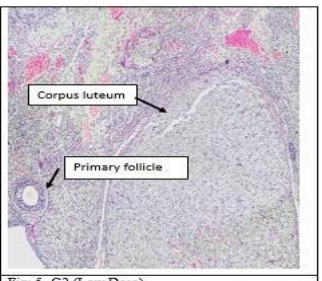


Fig: 5- G2 (Low Dose)

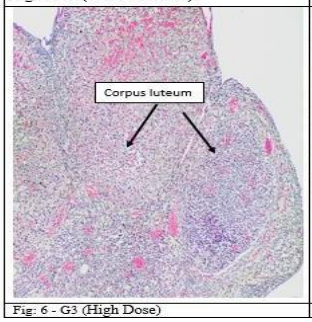


Fig: 6 - G3 (High Dose)

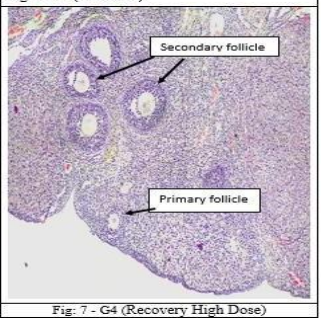


Fig: 7 - G4 (Recovery High Dose)

- **Anti-Implantation:** Dose-dependent losses: mid-dose 38.20% pre- and 32.49% post- (P<0.05/0.01); high-dose 63.47% pre- and 57.66% post- (P<0.001). Laparotomy photos confirmed fewer implants, linking to endometrial changes.

Table 6: Individual Animal Data of Anti-Implantation Activity

Group	Treatment	Animal No.	Body Weight (g)	No. of Corpora Lutea	No.of Implants	No. of Litters	% Pre Implantation Loss	% Post Implantation Loss
G1	Vehicle Control	1	182.45	14	12	10	14.29	16.67
		2	190.41	15	13	11	13.33	15.38
		3	188.74	11	10	9	9.09	10.00
		4	195.36	17	14	12	17.65	14.29
		5	180.21	10	8	7	20.00	12.50
		6	198.47	12	10	9	16.67	10.00
G2	Low Dose (100 mg/Kg. b.wt.)	7	194.45	11	10	9	9.09	10.00
		8	184.22	18	14	11	22.22	21.43
		9	191.54	17	12	10	29.41	16.67
		10	184.14	14	12	10	14.29	16.67
		11	196.24	16	14	11	12.50	21.43
		12	188.33	15	13	10	13.33	23.08
G3	Mid Dose (125 mg/Kg. b.wt.)	13	179.14	14	8	6	42.86	25.00
		14	180.02	12	10	7	16.67	30.00
		15	194.11	17	6	3	64.71	50.00
		16	189.41	14	11	7	21.43	36.36
		17	190.52	11	8	6	27.27	25.00
		18	184.41	16	7	5	56.25	28.57
G4	High Dose (250 mg/Kg. b.wt.)	19	186.54	17	6	2	64.71	66.67
		20	190.34	14	7	4	50.00	42.86
		21	187.14	18	5	3	72.22	40.00
		22	184.24	17	7	2	58.82	71.43
		23	192.32	11	4	2	63.64	50.00
		24	194.59	14	4	1	71.43	75.00
Group			Body Weight (g)	No. of Corpora Lutea	No.of Implants	No. of Litters	% Pre Implantation Loss	% Post Implantation Loss
G1 Vehicle Control		Mean	189.27	13.17	11.17	9.67	15.17	13.14
		SD	7.10	2.64	2.23	1.75	3.82	2.79
		N	6	6	6	6	6	6
G2 Low Dose (100 mg/Kg. b.wt.)		Mean	189.82	15.17	12.50	10.17	16.81	18.21
		SD	5.13	2.48	1.52	0.75	7.55	4.83
		N	6	6	6	6	6	6
G3 Mid Dose (125 mg/Kg. b.wt.)		Mean	186.27	14.00	8.33	5.67	38.20 *	32.49 **
		SD	6.05	2.28	1.86	1.51	19.57	9.54
		N	6	6	6	6	6	6
G4 High Dose (250 mg/Kg. b.wt.)		Mean	189.20	15.17	5.50	2.33	63.47 ***	57.66 ***
		SD	3.90	2.64	1.38	1.03	8.30	15.24
		N	6	6	6	6	6	6

* & *** indicates that the significant increase (P<0.05) has been observed in the group 3 and group animals % pre implantation loss in comparison of control group animals, respectively.

** & *** indicates that the significant increase (P<0.05) has been observed in the group 3 and group animals % post implantation loss in comparison of control group animals, respectively.

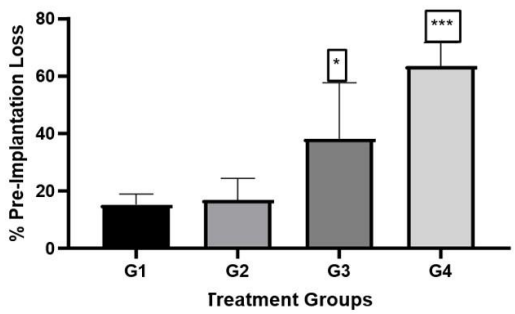


Fig: - 8 Comparison of % pre-implantation loss of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test

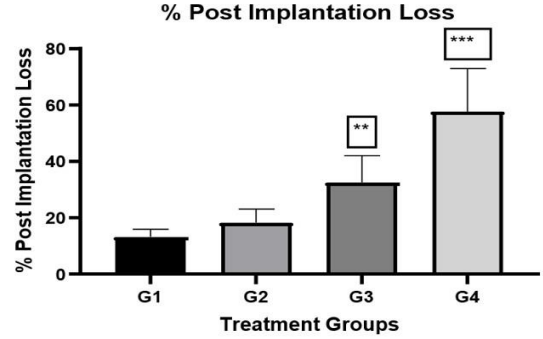


Fig: – 9 Comparison of % post-implantation loss of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test

- **Anti-Androgenic:** High dose reduced testicular weight (1.11 ± 0.08 g vs. 1.52 ± 0.30 g control), sperm count (61.67 ± 18.55 vs. 138.00 ± 12.31, $P<0.05$), viability/motility, with abnormalities (15.83 ± 2.64% vs. 5.17 ± 0.75%, $P<0.001$ vs. control). Histopathology showed disrupted spermatogenesis, akin to cyclophosphamide.

Table No.7: Individual Animal Mortality/Morbidity and Clinical Sign Record

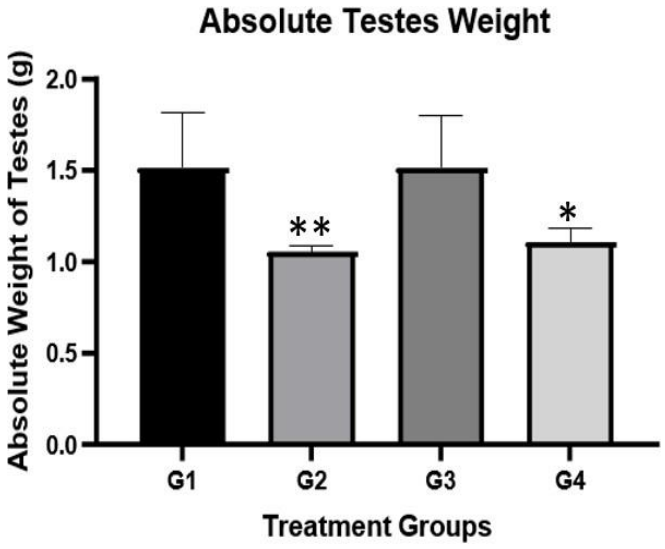
Group	Treatment	Animal No.	Mortality/Morbidity	Clinical Sign
G1	Vehicle Control	1	No Mortality and Morbidity observed during the treatment period	No abnormal clinical sign observed during the treatment period
		2		
		3		
		4		
		5		
		6		
G2	Disease Control-Cyclophophamide (30 mg/Kg b.wt. from Day 17 to Day 21)	7	No Mortality and Morbidity observed during the treatment period	No abnormal clinical sign observed during the treatment period
		8		
		9		
		10		
		11		
		12		
G3	Low Dose (100 mg/Kg. b.wt.)	13	No Mortality and Morbidity observed during the treatment period	No abnormal clinical sign observed during the treatment period
		14		
		15		
		16		
		17		
		18		
G4	High Dose (250 mg/Kg. b.wt.)	19	No Mortality and Morbidity observed during the treatment period	No abnormal clinical sign observed during the treatment period
		20		
		21		
		22		
		23		
		24		

Table No.8: Individual Animal Body Weight Record

Group	Treatment	Animal No.	Body Weight (g)			
			Day 1	Day 8	Day 15	Day 21
G1	Vehicle Control	1	201.24	214.54	224.47	232.54
		2	193.98	199.45	207.29	214.12
		3	184.24	192.41	200.73	214.21
		4	176.27	182.65	192.67	201.37
		5	197.87	202.41	216.24	220.18
		6	206.64	214.21	224.18	230.19
G2	Disease Control-Cyclophophamide (30 mg/Kg b.wt. from Day 17 to Day 21)	7	208.41	216.21	221.47	224.14
		8	198.46	204.47	215.35	220.54
		9	187.23	193.47	204.41	209.78
		10	191.65	198.24	206.21	210.44
		11	202.44	210.26	218.14	221.94
		12	182.01	188.65	198.17	201.11
G3	Low Dose (100 mg/Kg. b.wt.)	13	214.21	224.21	230.14	234.64
		14	201.22	209.12	219.57	226.41
		15	186.24	196.24	206.19	210.78
		16	197.65	206.19	213.14	220.28
		17	202.14	212.24	222.21	230.66
		18	184.75	194.27	206.74	209.41
G4	High Dose (250 mg/Kg. b.wt.)	19	179.24	186.51	192.47	192.97
		20	194.85	203.14	209.11	212.58
		21	193.47	204.25	207.54	210.71
		22	204.12	212.87	219.77	222.4
		23	200.14	212.95	216.32	220.55
		24	196.17	207.85	211.44	217.40
Body Weight Summary			**Day 1**	**Day 8**	**Day 15**	**Day 21**
G1	Vehicle Control	Mean	193.37	200.95	210.93	218.77
		SD	11.26	12.43	12.94	11.55
		N	6	6	6	6
G2	Disease Control-Cyclophophamide (30 mg/Kg b.wt. from Day 17 to Day 21)	Mean	195.03	201.88	210.63	214.66
		SD	9.87	10.40	9.05	8.97
		N	6	6	6	6
G3	Low Dose (100 mg/Kg. b.wt.)	Mean	197.70	207.05	216.33	222.03
		SD	10.99	11.01	9.39	10.41
		N	6	6	6	6
G4	High Dose (250 mg/Kg. b.wt.)	Mean	194.67	204.60	209.44	212.77
		SD	8.50	9.78	9.48	10.69
		N	6	6	6	6

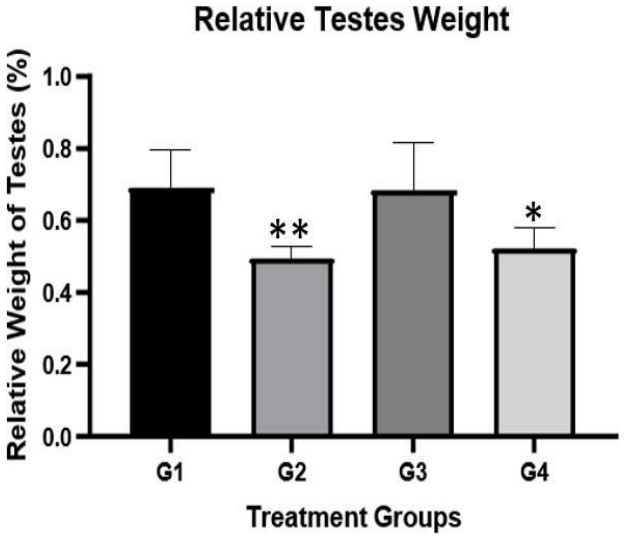
Table No.9: Individual Animal Absolute and Relative Testes weight

Group	Treatment	Animal No.	Absolute Testes weight (g)	Relative Testes weight (%)
G1	Vehicle Control	1	1.9794	0.85
		2	1.2456	0.58
		3	1.4785	0.69
		4	1.1597	0.58
		5	1.6890	0.77
		6	1.5457	0.67
G2	Disease Control-Cyclophophamide (30 mg/Kg b.wt. from Day 17 to Day 21)	7	1.0676	0.48
		8	1.0251	0.46
		9	1.0748	0.51
		10	1.0472	0.50
		11	1.0103	0.46
		12	1.0982	0.55
G3	Low Dose (100 mg/Kg. b.wt.)	13	1.9047	0.81
		14	1.2451	0.55
		15	1.2908	0.61
		16	1.4679	0.67
		17	1.3456	0.58
		18	1.8427	0.88
G4	High Dose (250 mg/Kg. b.wt.)	19	1.1571	0.60
		20	1.2482	0.59
		21	1.0497	0.50
		22	1.0513	0.47
		23	1.0482	0.48
		24	1.0892	0.50
Summary of Testes Weight			**Absolute Testes weight (g)**	**Relative Testes weight (%)**
G1	Vehicle Control	Mean	1.52	0.69
		SD	0.30	0.11
		N	6	6
G2	Disease Control-Cyclophophamide (30 mg/Kg b.wt. from Day 17 to Day 21)	Mean	1.05	0.49
		SD	0.03	0.03
		N	6	6
G3	Low Dose (100 mg/Kg. b.wt.)	Mean	1.52	0.68
		SD	0.29	0.13
		N	6	6
G4	High Dose (250 mg/Kg. b.wt.)	Mean	1.11	0.52
		SD	0.08	0.06
		N	6	6



** & * indicates that the significant decrease (P<0.05) has been observed in the group 2 and group 4 animals in absolute testes weight in comparison of control group animals, respectively (Fig – 10).

Fig: 10 – Comparison of absolute testes weight of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test

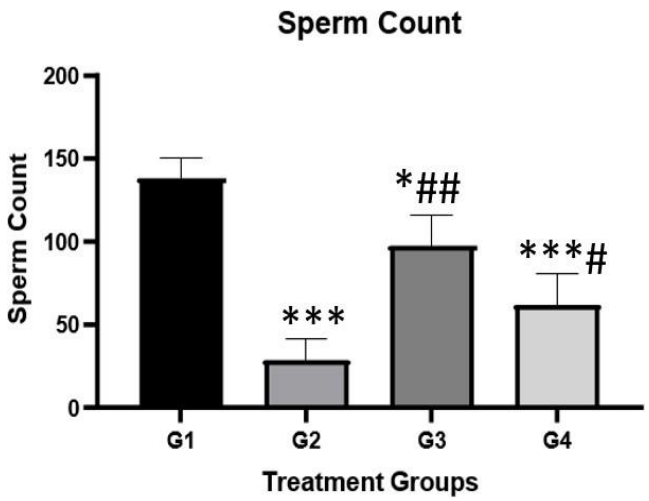


** & * indicates that the significant decrease (P<0.05) has been observed in the group 2 and group 4 animals in relative testes weight in comparison of control group animals, respectively (Fig – 11).

Fig: 11 – Comparison of relative testes weight of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test

Table No.10: Individual Animal Analysis of Epididymal Sperm Characteristics

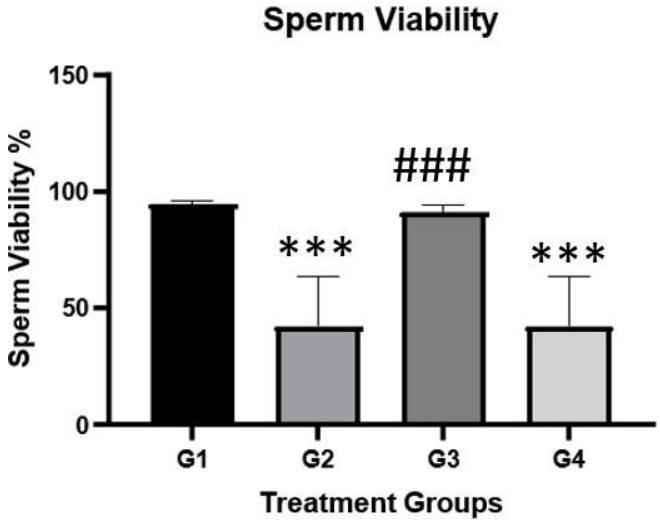
Group	Treatment	Animal No.	Analysis of Epididymal Sperm Characteristics			
			Sperm Count	Sperm Viability	Sperm Motility	Sperm Morphology
G1	Vehicle Control	1	150	93	84	6
		2	144	96	83	5
		3	144	96	93	6
		4	115	94	86	5
		5	135	95	87	4
		6	140	94	90	5
G2	Disease Control-Cyclophophamide (30 mg/Kg b.wt. from Day 17 to Day 21)	7	25	31	31	18
		8	45	32	20	17
		9	45	24	19	18
		10	14	84	88	11
		11	20	42	32	16
		12	21	40	30	15
G3	Low Dose (100 mg/Kg. b.wt.)	13	118	97	84	4
		14	75	88	83	5
		15	80	89	93	7
		16	109	91	86	8
		17	112	92	91	6
		18	92	89	81	6
G4	High Dose (250 mg/Kg. b.wt.)	19	57	31	31	18
		20	85	32	2	17
		21	84	24	9	18
		22	52	84	88	11
		23	40	42	32	16
		24	52	40	30	15
Body Weight Summary			**Sperm Count**	**Sperm Viability**	**Sperm Motility**	**Sperm Morphology**
G1	Vehicle Control	Mean	138.00	94.67	87.17	5.17
		SD	12.31	1.21	3.76	0.75
		N	6	6	6	6
G2	Disease Control-Cyclophophamide (30 mg/Kg b.wt. from Day 17 to Day 21)	Mean	28.33	42.17	36.67	15.83
		SD	13.38	21.51	25.78	2.64
		N	6	6	6	6
G3	Low Dose (100 mg/Kg. b.wt.)	Mean	97.67	91.00	86.33	6.00
		SD	17.92	3.29	4.72	1.41
		N	6	6	6	6
G4	High Dose (250 mg/Kg. b.wt.)	Mean	61.67	42.17	32.00	15.83
		SD	18.55	21.51	30.23	2.64
		N	6	6	6	6



***, * & *** indicates that the significant decrease (P<0.05) has been observed in the group 2, group 3 and group 4 animals in sperm count in comparison of control group animals, respectively (Fig – 12).

& # indicates that the significant increase (P<0.05) has been observed in the group 3 and group 4 animals in sperm count in comparison of disease control group animals, respectively (Fig – 12).

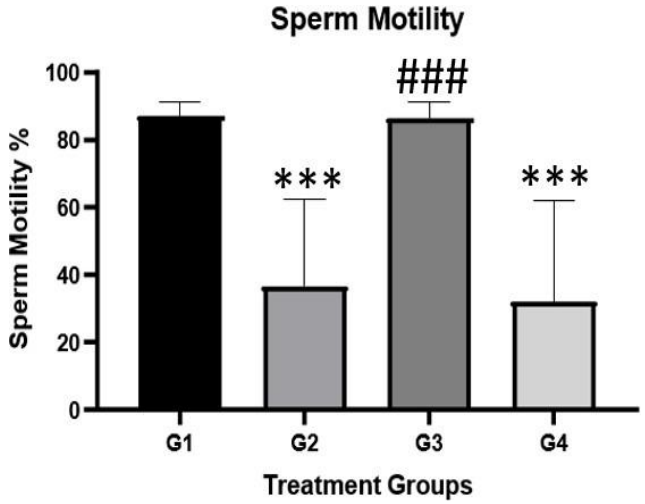
Fig: 12 - Comparison of sperm count of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test



***, & *** indicates that the significant decrease (P<0.05) has been observed in the group 2, and group 4 animals in sperm viability in comparison of control group animals, respectively (Fig – 13).

Indicates that the significant increase (P<0.05) has been observed in the group 3 animals in sperm viability in comparison of disease control group animals (Fig – 13).

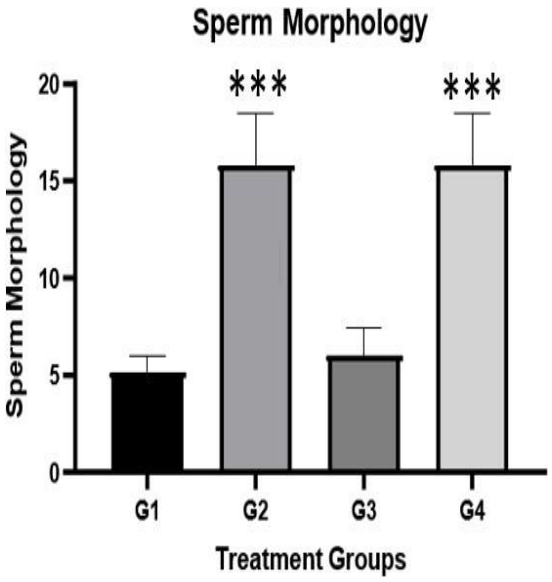
Fig: 13 - Comparison of sperm viability of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test



***, & *** indicates that the significant decrease (P<0.05) has been observed in the group 2, and group 4 animals in sperm motility in comparison of control group animals, respectively (Fig – 14).

Indicates that the significant increase (P<0.05) has been observed in the group 3 animals in sperm motility in comparison of disease control group animals (Fig – 14).

Fig: 14 - Comparison of sperm motility of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test



***, & *** indicates that the significant increase (P<0.05) has been observed in the group 2, and group 4 animals in sperm morphology in comparison of control group animals, respectively (Fig – 15).

Fig: 15 - Comparison of sperm morphology of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test

Table No.11: Histopathology of Testes

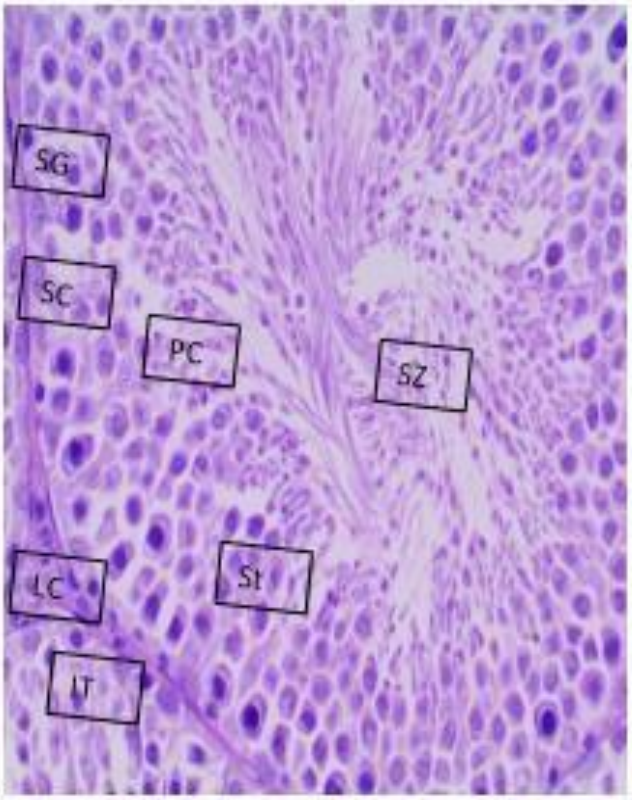


Fig: 16 - G1 (Vehicle Control)

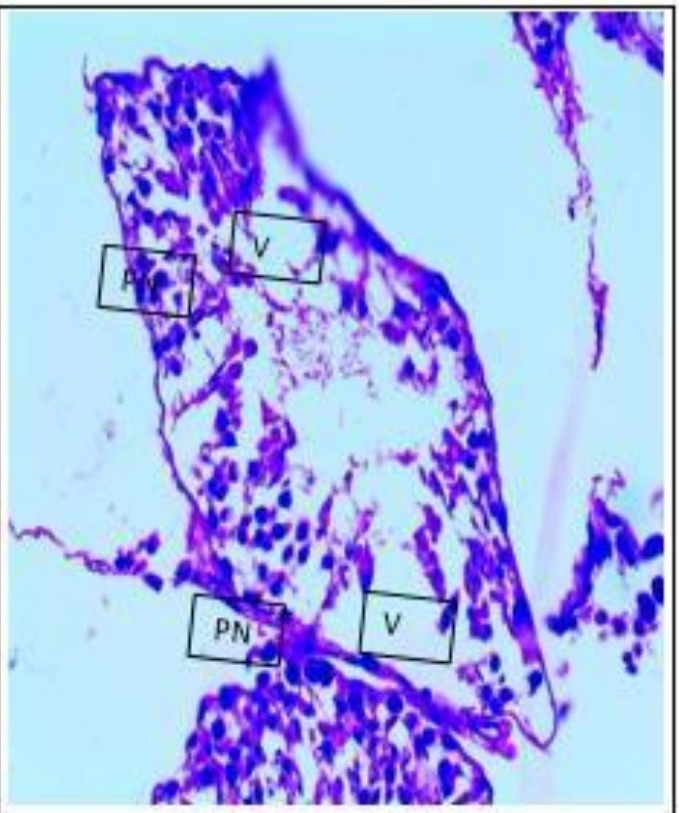


Fig: 17 - G2 (Disease Control)

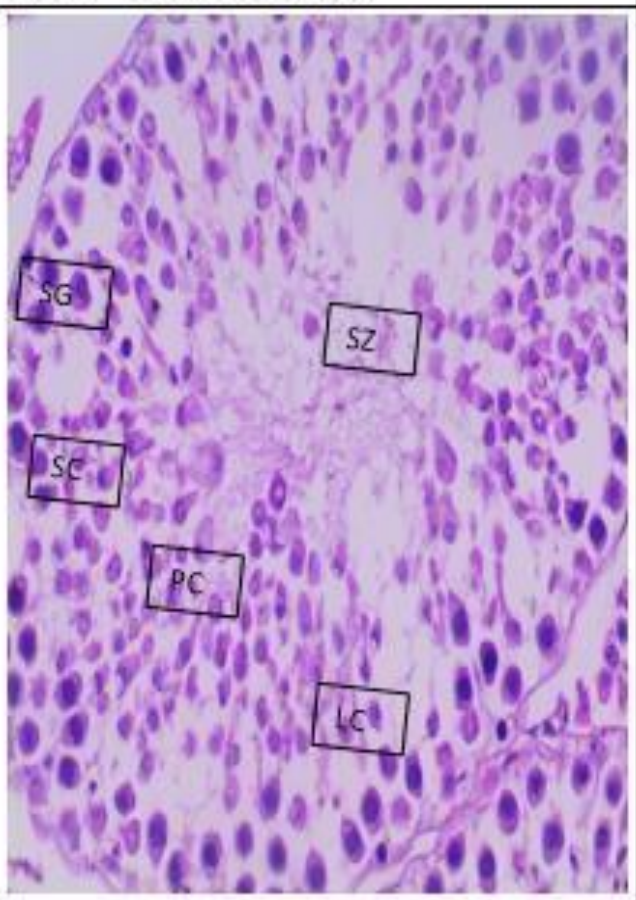


Fig: 18 - G3 (Low Dose)

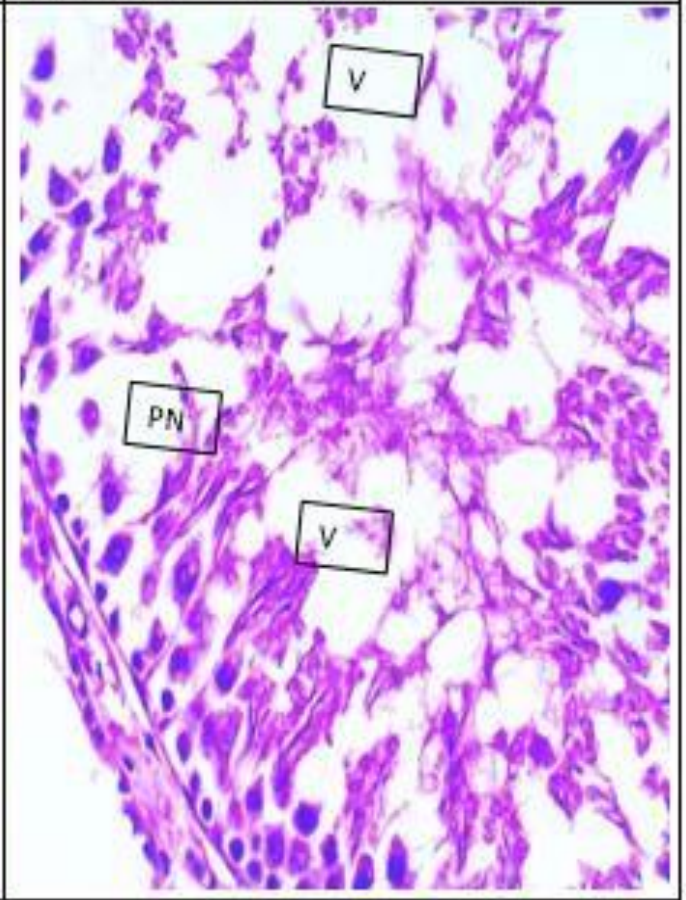


Fig: 19 - G4 (High Dose)

- **Anti-Estrogenic:** Combined with estrogen, high dose reduced uterine weight (23.67 ± 3.74 mg vs. 136.32 ± 1.70 mg estrogen alone, P<0.001) and estrogen levels, with thinner lining in histopathology, indicating antagonism.

Table No.11: Body Weight for Anti-Estrogenic Study

Group	Animal No.	Day 1	Day 5
		Animal Body Weight (g)	Animal Body Weight (g)
G1	1	9.12	11.21
	2	9.19	13.12
	3	10.01	13.24
	4	10.07	12.78
	5	10.74	13.95
	6	11.35	14.21
G2	7	9.32	13.95
	8	10.07	16.21
	9	10.65	15.67
	10	10.76	16.31
	11	11.66	15.62
	12	10.52	16.97
G3	13	9.37	10.12
	14	9.65	11.02
	15	10.11	12.01
	16	10.68	11.32
	17	10.77	11.24
	18	10.15	11.74
G4	19	9.33	9.50
	20	9.74	10.24
	21	10.12	10.18
	22	10.34	10.65
	23	10.99	11.34
	24	11.11	11.65
G5	25	9.76	11.32
	26	9.79	10.18
	27	10.54	11.12
	28	11.12	11.39
	29	11.13	11.98
	30	10.14	11.85
G6	31	9.33	10.02
	32	10.61	10.96
	33	11.16	11.36
	34	9.51	10.41
	35	11.46	11.65
	36	11.21	11.69
G1	Mean	10.08	13.09
	SD	0.87	1.06
	N	6	6
G2	Mean	10.50	15.79
	SD	0.78	1.03
	N	6	6
G3	Mean	10.12	11.24
	SD	0.55	0.66
	N	6	6
G4	Mean	10.27	10.59
	SD	0.70	0.80
	N	6	6
G5	Mean	10.41	11.31
	SD	0.62	0.64
	N	6	6
G6	Mean	10.55	11.02
	SD	0.92	0.68
	N	6	6

Table No.12: Uterus Weight for Anti-Estrogenic Study

Group	Animal No.	Uterus Weight (mg)
G1	1	15.1
	2	15.8
	3	15.3
	4	15.5
	5	14.2
	6	14.9
G2	7	135.9
	8	139.2
	9	134.3
	10	135.2
	11	136.2
	12	137.1
G3	13	12.2
	14	12.5
	15	12.6
	16	13.9
	17	12.3
	18	12.3
G4	19	6.7
	20	6.9
	21	7.2
	22	7.5
	23	7.6
	24	7.9
G5	25	52.5
	26	56.3
	27	65.4
	28	61.5
	29	51.9
	30	59.5
G6	31	19.3
	32	20.5
	33	22.9
	34	27.6
	35	28.6
	36	23.1
G1	Mean	15.13
	SD	0.55
	N	6
G2	Mean	136.32
	SD	1.70
	N	6
G3	Mean	12.63
	SD	0.64
	N	6
G4	Mean	7.30
	SD	0.45
	N	6
G5	Mean	57.85
	SD	5.28
	N	6
G6	Mean	23.67
	SD	3.74
	N	6

Table No.13: Estimated Conc of Estrogen (pg/mL)

Group	Animal No.	Estimated Conc of Estrogen pg/mL
G1	1	27.6
	2	18.6
	3	27.6
	4	27.6
	5	27.6
	6	36.7
G2	7	545.8
	8	545.8
	9	554.9
	10	545.8
	11	554.9
	12	545.8
G3	13	9.5
	14	82.2
	15	64.0
	16	18.6
	17	18.6
	18	18.6
G4	19	0.4
	20	0.4
	21	54.9
	22	27.6
	23	0.4
	24	9.5
G5	25	291.3
	26	300.4
	27	273.1
	28	291.3
	29	364.0
	30	273.1
G6	31	100.4
	32	118.6
	33	127.6
	34	300.4
	35	100.4
	36	164.0
G1	Mean	27.64
	SD	5.75
	N	6
G2	Mean	548.85
	SD	4.69
	N	6
G3	Mean	35.21
	SD	30.10
	N	6
G4	Mean	15.51
	SD	22.02
	N	6
G5	Mean	298.85
	SD	33.73
	N	6
G6	Mean	151.88
	SD	76.42
	N	6

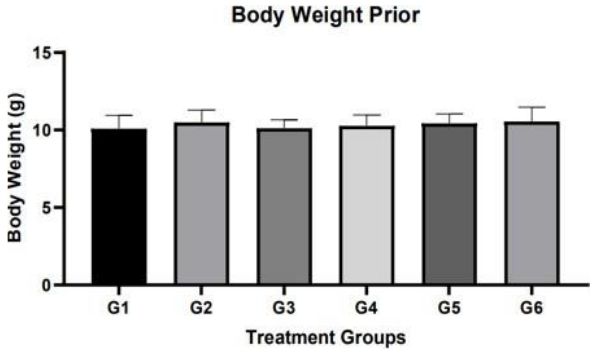


Fig: 20 - Each value is the mean ± SD of six animals in each group. All six groups data were analyzed by One-way ANOVA followed by tukey's test post-hoc analysis.

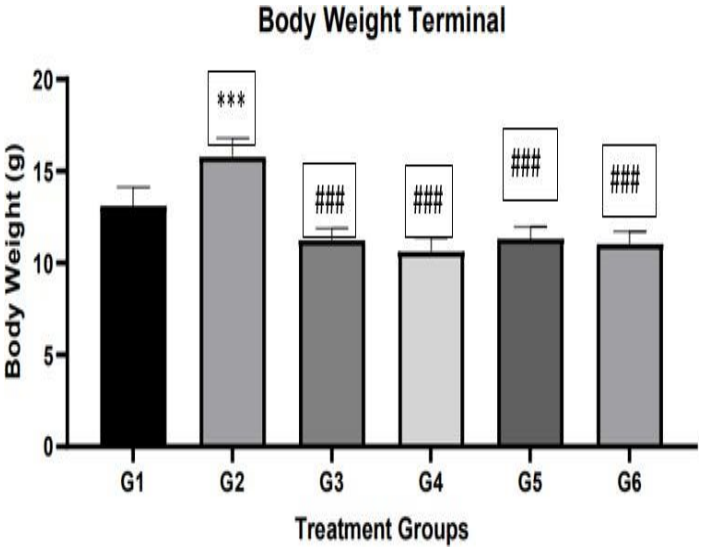


Fig: 21 - Each value is the mean ± SD of six animals in each group. All six groups data were analyzed by One-way ANOVA followed by tukey's test post-hoc analysis.

*** (P<0.001) Indicates that the significant decrease was observed in the group 2 animals in terminal body weight in comparison of control group animals.

(P<0.001), ### (P<0.001), ### (P<0.001) and ### (P<0.001) Indicates that the significant decrease was observed in the group 3, group 4, group 5 and group 6 animals in terminal body weight in comparison of positive control group animals, respectively.

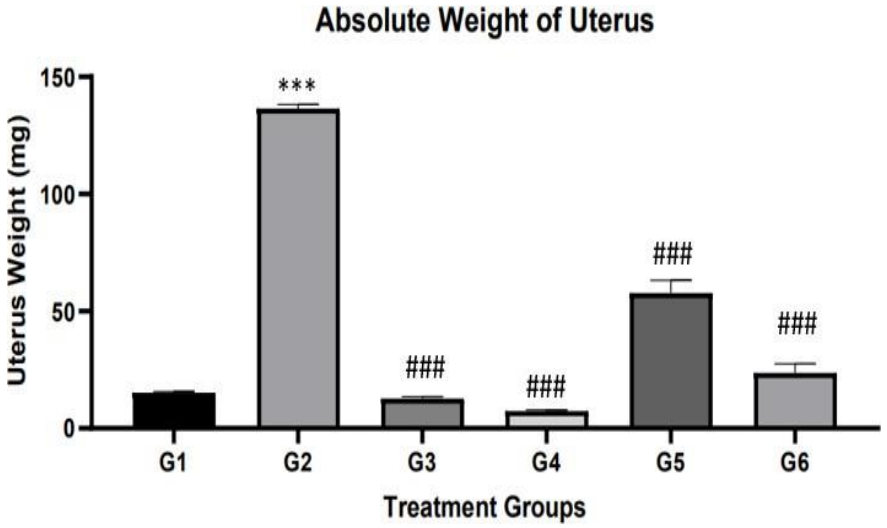


Fig: 22 - Each value is the mean ± SD of six animals in each group. All six groups data were analyzed by One-way ANOVA followed by tukey's test post-hoc analysis.

*** (P<0.001) Indicates that the significant changes was observed in the group 2 animals in absolute uterus weight in comparison of control group animals.

(P<0.001), ### (P<0.001), ### (P<0.001) and ### (P<0.001) Indicates that the significant decrease was observed in the group 3, group 4, group 5 and group 6 animals in absolute uterus weight in comparison of positive control group animals, respectively.

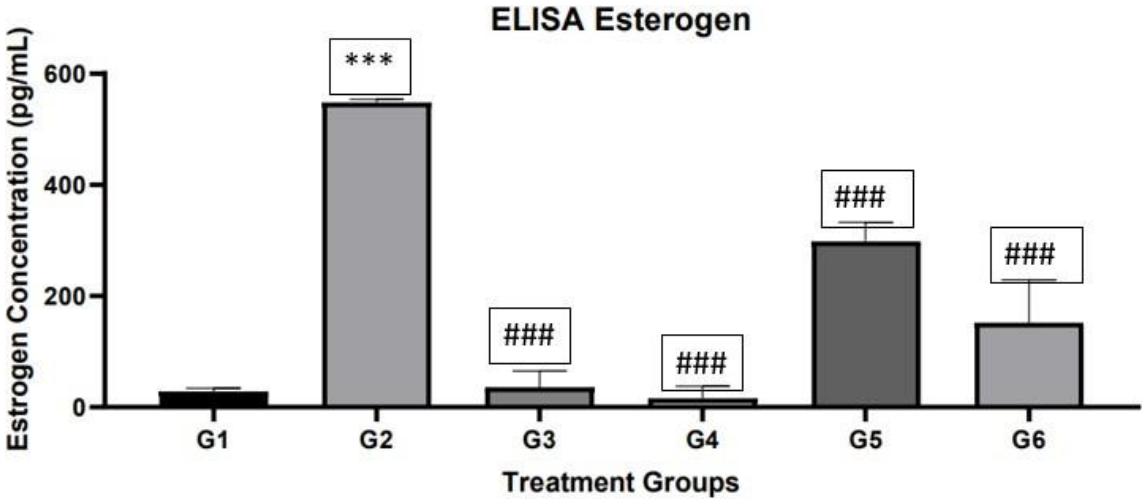
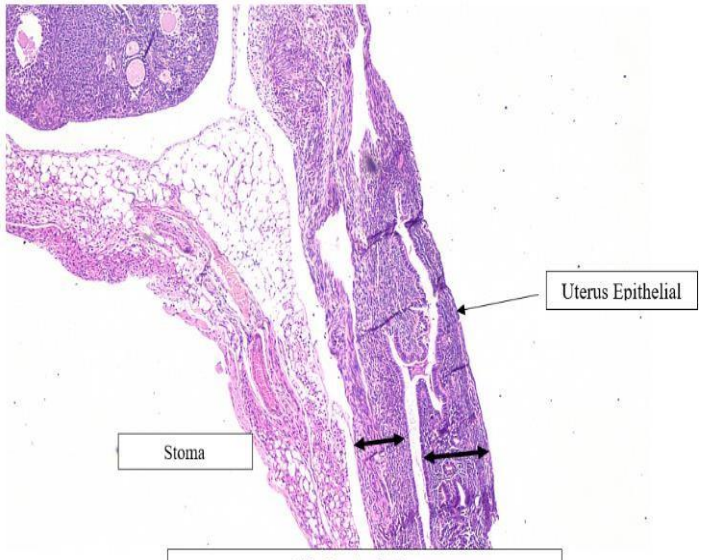


Fig: 23 - Each value is the mean ± SD of six animals in each group. All six groups data were analyzed by One-way ANOVA followed by tukey's test post-hoc analysis.

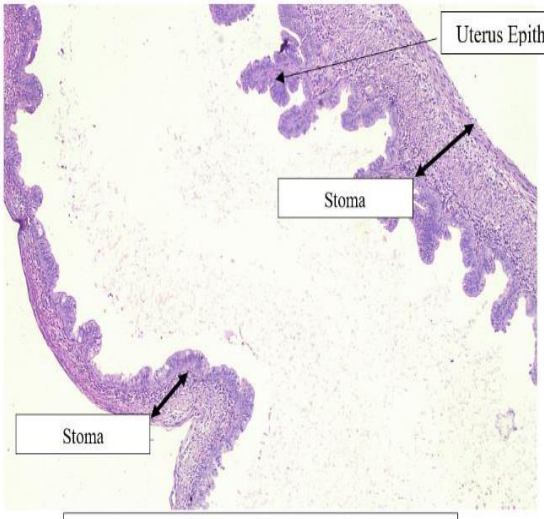
*** (P<0.001) indicates that the significant decrease was observed in the group 2 animals in blood estrogen in comparison of control group animals.

(P<0.001), ### (P<0.001), ### (P<0.001) and ### (P<0.001) Indicates that the significant decrease was observed in the group 3, group 4, group 5 and group 6 animals in blood estrogen in comparison of positive control group animals, respectively.

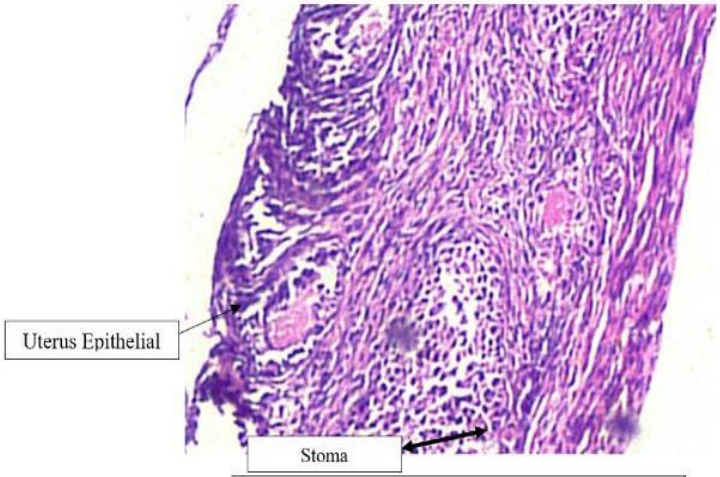
Table No.14: Histopathology of Uterus



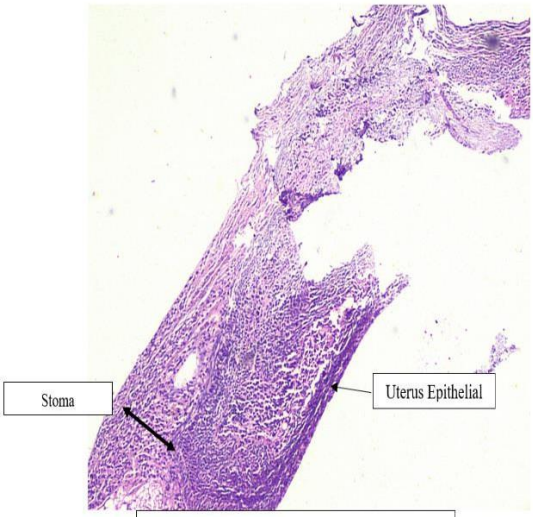
G1- Control Group



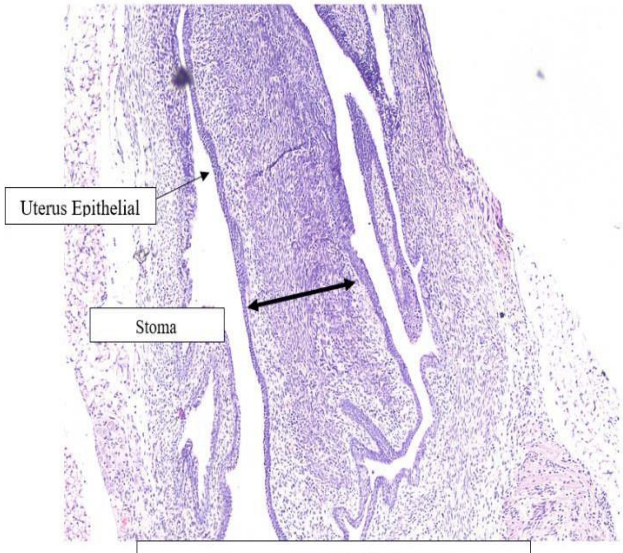
G2- Standard Group



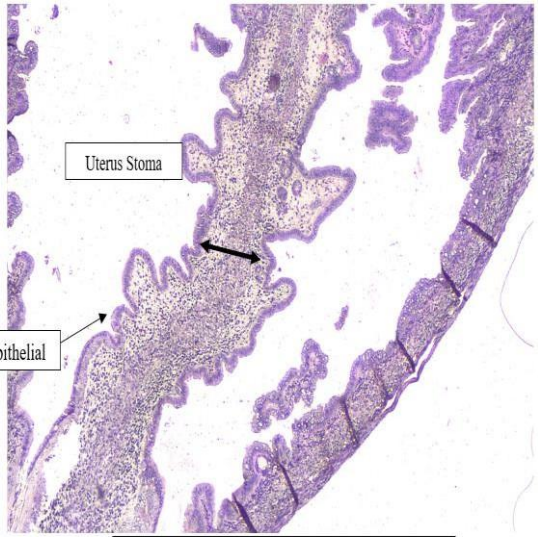
G3- Low Dose Group



G4- High Dose Group



G5- Low Dose + Estrogen Group



G6- High Dose + Estrogen Group

Discussion: The study evaluates ursolic acid (UA) from Saraca indica (Ashoka) bark for anti-fertility effects via in vitro and in vivo models, confirming oxytocic, anti-ovulatory, anti-implantation, anti-androgenic, and anti-estrogenic activities in a dose-dependent manner. With a 0.40% yield and 90% purity via HPLC, UA's poor solubility in water (suspended in 0.5% CMC) aligns with its lipophilic nature. In vitro, UA (30 mg) induced uterine contractions comparable to oxytocin, likely via calcium channels, supporting traditional uterotonic uses. Anti-ovulatory effects at 250 mg/kg reduced ovary weight and corpora lutea ($P<0.001$), suggesting LH suppression with reversibility. Anti-implantation losses peaked at 63.47% pre- and 57.66% post-implantation ($P<0.001$), likely due to endometrial changes. In males, 250 mg/kg decreased sperm count ($P<0.05$) and increased abnormalities ($P<0.001$) via symplast formation. Anti-estrogenic activity reduced uterine weight and estrogen levels ($P<0.001$) in mice. UA outperforms Moringa oleifera in potency across these parameters, likely due to steroidal mimicry and broader hormonal modulation.

These results align with UA's modulation of reproductive hormones and tissues, supporting S. indica's traditional anti-fertility role and offering potential for reversible contraceptives. Limitations include reliance on animal models, which may not fully predict human responses due to physiological differences. Short-term studies and cupric acetate artifacts limit generalizability, while bioavailability and toxicity at high doses require further investigation. Future research should explore human trials, solubility enhancements (e.g., nanoparticles), and molecular mechanisms to validate UA's contraceptive potential.

7. ACHIEVEMENTS WITH RESPECT TO OBJECTIVES

Mechanisms in Male Anti-Fertility (Anti-Androgenic Effects)

UA demonstrates anti-androgenic properties in male reproductive systems, primarily at higher doses, leading to reduced fertility through disruption of spermatogenesis and sperm function. Key mechanisms include:

- **Disruption of Spermatogenesis and Symplast Formation**: UA can interfere with sperm production by inducing the formation of symplasts (multinucleated giant cells) in spermatogenic cells, leading to arrested spermatogenesis without causing permanent damage to supporting cells like Leydig or Sertoli cells. In male Wistar rats, high doses (250 mg/kg orally for 21 days) significantly reduced sperm count (by ~55%), viability, and motility, while increasing morphological abnormalities (e.g., double heads, tailless sperm), mimicking the effects of anti-androgenic agents like cyclophosphamide. Histopathological analysis showed disrupted seminiferous tubules, germ cell depletion, vacuolation, and reduced spermatozoa in the lumen.

- **Hormonal Modulation and Anti-Estrogenic Effects**: UA's anti-estrogenic activity contributes to lowered androgen levels, such as testosterone, by potentially inhibiting enzymes like testosterone 5α-reductase. This leads to decreased spermatogenesis and sperm maturation due to hormonal imbalance. Studies in rats treated with UA from plants like Ocimum sanctum showed reduced sperm parameters linked to estrogen antagonism.
- **Testicular and Organ Effects**: High-dose UA decreases absolute and relative testicular weight, indicating suppression of testicular function and androgen production. This is supported by reduced Leydig cell density and interstitial tissue damage.

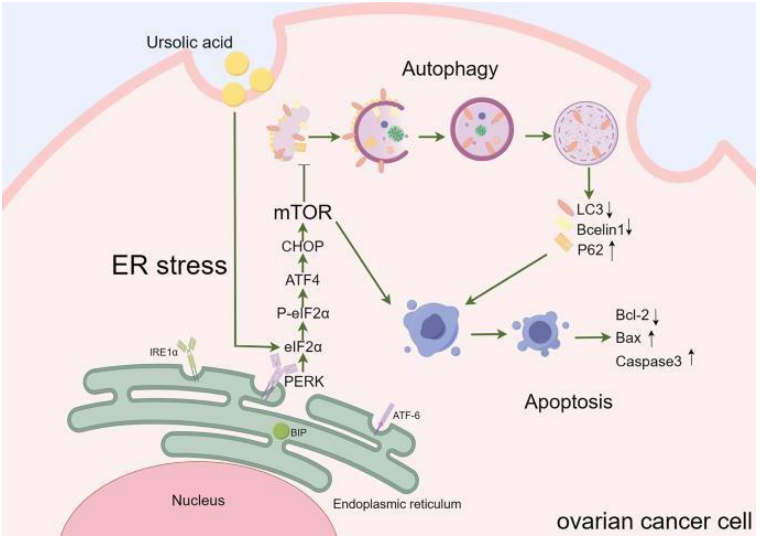
Dose-dependency is crucial: Low doses (e.g., 5-100 mg/kg) often have negligible or even protective effects on sperm parameters and hormones, as seen in studies where UA attenuated oligospermia (low sperm count) induced by toxins like busulfan by promoting motor proteins involved in sperm transport. Reversibility has been noted in related compounds like oleanolic acid, suggesting UA's effects may be temporary upon discontinuation.

Mechanisms in Female Anti-Fertility (Anti-Estrogenic and Uterotonic Effects)

In females, UA exerts anti-fertility effects through hormonal disruption, inhibition of ovulation and implantation, and induction of uterine contractions. These mechanisms are particularly evident in animal models like Wistar rats and Swiss albino mice.

- **Anti-Ovulatory and LH Surge Inhibition**: UA inhibits gonadotropin-releasing hormone (GnRH) release from the hypothalamus, suppressing the luteinizing hormone (LH) surge essential for ovulation. This prevents egg release, making fertilization impossible. In cupric acetate-induced models, high doses (250 mg/kg) reduced ovary weight and corpora lutea formation, indicating blocked ovulation.
- **Anti-Implantation and Endometrial Alteration**: UA alters the endometrial lining, making it less receptive to fertilized eggs, and may block progesterone action needed for maintaining pregnancy. This leads to pre- and post-implantation losses (up to 63% and 57% at 250 mg/kg in rats). It also thickens cervical mucus, impeding sperm-egg interaction.
- **Anti-Estrogenic Activity**: By antagonizing estrogen, UA reduces uterine weight, estrogen levels, and endometrial thickness. In immature mice, combined with estrogen, high doses (500 mg/kg) lowered uterine weight by ~83% and estrogen concentrations, disrupting estrogen-dependent processes like implantation.
- **Oxytocic and Uterotonic Effects**: UA induces uterine contractions via calcium channel activation and prostaglandin pathway modulation, similar to oxytocin. In vitro studies on rat uterine tissue showed contractions abolished by nifedipine (a calcium blocker), suggesting

mediation through L-type calcium channels. This can expel pregnancies or manage menstrual disorders but poses risks during gestation.



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The above diagram illustrates UA's induction of endoplasmic reticulum (ER) stress and autophagy inhibition in ovarian cells, which could indirectly contribute to anti-fertility by promoting apoptosis in reproductive tissues.

Dose-dependency applies here too: Low doses may alleviate oocyte abnormalities (e.g., in 3-NPA models by stabilizing meiotic processes), while high doses disrupt fertility.

Broader Implications and Dual Roles

While UA shows promise as a natural anti-fertility agent (e.g., for population control or contraceptives), its mechanisms overlap with protective effects in fertility disorders like oligospermia or metabolic syndrome-induced infertility. This duality underscores the need for dose optimization. In gynecological cancers, UA's apoptosis-inducing and pathway-inhibiting actions (e.g., PI3K/AKT) may indirectly affect fertility by targeting reproductive tissues. Future research should explore human applications, reversibility, and molecular targets for safe therapeutic use.

8. CONCLUSION

Anti-Ovulatory Activity: Depending on the outcomes, it could be decided that ursolic acid has been screened as a revisable anti-ovulatory agent in Wistar female rats, as it showed fewer corpora lutea in the ovaries.

Anti-Implantation Activity: Based on the results, it could be concluded that ursolic acid shows dose-dependent loss of the fetus during the delivery of litters.

Anti-Androgenic Activity: Based on the results, it could be concluded that high doses show a conservative effect on male anti-fertility, considering effects such as decreased weight of the testes, abnormalities in sperm morphology, reduced sperm count, sperm viability, and sperm motility. It seems to mimic the effects of a positive control.

Anti-Estrogenic Activity: Based on the results, it could be concluded that a dose-dependent estrogen-lowering effect was observed in the ursolic acid-treated groups. Animals treated with ursolic acid alongside estrogen showed decreased uterine lining thickness, indicating dose-dependent anti-estrogenic effects.

Cumulatively, it is concluded that ursolic acid possesses conservative anti-fertility effects in laboratory animals. It may suggest potential activity requiring further evaluation. Ursolic acid is abundantly present in *Saraca indica*, and these findings provide constructive evidence that *Saraca indica* acts as an anti-fertility agent in laboratory animals.

9. COPIES OF PUBLISHED RESEARCH PAPERS AND A LIST OF PUBLICATIONS ARISING FROM THE THESIS

Published research papers

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