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SCREENING OF URSOLIC ACID AS AN *IN VIVO* ANTIANDROGENIC ACTIVITY IN WISTAR MALE RATS

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ABSTRACT

This study investigates the antiandrogenic activity of ursolic acid in Wistar albino male rats. Ursolic acid solubility was optimized for uniform distribution in a carboxymethyl cellulose (CMC) vehicle. Dosages were derived from the LD50 of ursolic acid, with low and high doses selected as 100mg/kg and 250mg/kg, correspondingly. Rats were randomized to one of four groups: vehicle control, disease control, low dose and high dose. Ursolic acid was administered orally via gavage for consecutively twenty-one days, except cyclophosphamide in the disease control animals by intraperitoneal route (30 mg/kg. bwt.) to induce disease for only last five days of treatment period. Mortality, morbidity, clinical signs, body weight (BW), Testes absolute and relative weight, total sperm count, sperm viability, motility, and morphology, and histopathological parameters of testes were assessed. All animals were euthanized 22nd day of the study. No mortality or morbidity was observed. High-dose ursolic acid significantly reduced absolute and relative testes weight compared to controls. There were significant changes were observed in the sperm motility, viability and morphology in the high dose treated groups. Histopathological analysis showed germ cells, including spermatogonia (SG) primary spermatocytes (Pc), spermatids (St), and Sertoli cells (Sc), Interstitial tissue (IT) containing interstitial cells and Leydig cells (Lc), Spermatozoa (SZ) were not present in the lumen, vacuolation (V), and Germ cells with pyknotic nuclei (PN) in the cyclophosphamide and high dose treated groups. These findings suggest that ursolic

acid exhibits antiandrogenic activity in Wistar male rats under the conditions of this study.

KEYWORDS: Ursolic Acid, Antiandrogenic Activity, Male wistar rats.

INTRODUCTION

The pursuit of effective, reversible, and safe male contraceptive agents remains a major focus in reproductive health research. Currently, male contraceptive options are limited to condoms and vasectomy, both of which come with limitations in compliance and reversibility, respectively.[1] Unlike female contraception, male hormonal contraceptives have not yet reached widespread clinical acceptance, largely due to challenges in efficacy, side effects, and long-term safety concerns.[2] Hence, there is an increasing interest in identifying plant-derived compounds with potential antiandrogenic and antifertility properties that may serve as candidates for male contraceptive development.[3] Androgens, particularly testosterone and its potent derivative dihydrotestosterone (DHT), are central to the regulation of male reproductive function, including spermatogenesis, libido, and the maintenance of accessory sex organs.[4] Antiandrogenic compounds can disrupt these processes by reducing testosterone production, inhibiting 5α-reductase activity, or antagonizing androgen receptor binding, all of which can lead to reversible suppression of fertility.[5] Therefore, exploring safe antiandrogens that can modulate these pathways without inducing systemic toxicity is a promising strategy for male contraception.

Ursolic acid (UA) is a pentacyclic triterpenoid found abundantly in the peels of fruits and leaves of medicinal plants such as Ocimum sanctum, Rosmarinus officinalis, and Eriobotrya japonica.[6] UA has been extensively studied for its anti-inflammatory, anti-cancer, hepatoprotective, and metabolic benefits.[7,8] Recent research also suggests that UA possesses hormonal regulatory properties, including the ability to inhibit androgen receptor signaling and reduce steroidogenic enzyme expression in prostate cancer cells.[9,10] These effects highlight its potential as a natural antiandrogen with possible applications in male fertility control.

Despite these promising findings, there is limited in vivo evidence regarding the antiandrogenic and antifertility effects of UA in male animal models. The Wistar rat, due to its established use in reproductive toxicology and endocrine studies, provides a suitable model to explore these effects systematically.[11]

MATERIALS AND METHOD

Animal Subject

Twenty-four adult male Wistar albino rats were used in this study. Animals were housed in polypropylene cages under standard laboratory conditions (12:12-hour light-dark cycle, 22 ± 2°C temperature, and 55 ± 5% relative humidity) and provided with standard rodent feed and water **ad libitum**. The animals were acclimatized for five days prior to experimentation. All animal procedures were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee (IAEC).[12] The (IAEC Institutional Animal Ethical Committee) of Accuprec Research Labs Pvt. Ltd. in Ahmedabad, Gujarat, India, gave its approval to the research (Protocol No. ARL/PT/691/2023 dated 12-05-2023).

The rationale for Selection of Dose

Ursolic acid (UA) was administered at doses of 100 mg/kg and 250 mg/kg body weight based on previous studies demonstrating its pharmacological and endocrine-modulatory activities in rodents, including antiandrogenic, anti- inflammatory, and antifertility effects.[13,14] Cyclophosphamide, used as a positive control, was administered at 30 mg/kg body weight intraperitoneally from day 17 to day 21, as it is a well-established model compound for inducing reproductive toxicity and testicular damage.[15]

Rationale of vehicle selection

Due to the lipophilic nature of ursolic acid and its poor aqueous solubility, 0.5% carboxymethyl cellulose (CMC) was used as the suspending vehicle. CMC is widely used in oral drug delivery in preclinical models and is pharmacologically inert at low concentrations, making it an ideal vehicle for this study.[16]

Dose Formulation Preparation

The dose formulations were prepared daily for all dose groups before dosing. Ursolic acid was mixed with aqueous Carboxymethyl Cellulose (CMC) for high-dose and low-dose groups. Homogeneity of the ursolic acid in the vehicle was maintained by continuous stirring utilizing a magnetic stirrer.

Experimental Design and Dose Administration

The animals were randomly divided into four groups (n = 6 per group): Group 1 (Vehicle Control): Received 0.5% CMC orally for 21 days. Group 2 (Positive Control): Received cyclophosphamide at 30 mg/kg body weight intraperitoneally from day 17 to 21. Group 4 (UA High Dose): Received ursolic acid at 250 mg/kg body weight orally for 21 days. All oral administrations were performed using a calibrated oral gavage fitted with a stainless steel feeding needle. On day 22, all animals were euthanized using CO_2 asphyxiation followed by exsanguination. The testes and epididymides were immediately excised for further analyses.[17]

OBSERVATIONS

Clinical Signs and Body Weight Monitoring

All animals were observed daily for general clinical signs, morbidity, and mortality. Body weights were recorded on day 1 and subsequently at 3-day intervals throughout the study. Final body weights were recorded before euthanasia to calculate organ-to-body weight ratios.

Organ Weights

After euthanasia, both testes were excised, blotted free of blood, and weighed individually using a precision balance. Absolute testicular weights and relative weights (mg of testis per 100 g body weight) were calculated for comparison among the groups.[18]

Assessment of Epididymal Sperm Characteristics Total Sperm Count

A small segment of the right cauda epididymis was minced in 5 mL of 0.9% normal saline at room temperature and incubated for 2–5 minutes to allow sperm release (referred to as Suspension A). A 100 µL sample of Suspension A was diluted with 1.9 mL saline (20× dilution). A hemocytometer was charged with the diluted sample and sperm were counted under a 10× objective lens in four WBC counting squares.

Calculation

Sperm Count (million/mL)=N×20×1000×dilution factor/0.4\text{Sperm Count (million/mL)} = N \times 20\times 1000 \times \text{dilution factor} / 0.4Sperm Count (million/mL)=N×20×1000×dilution factor/0.4 Where N = total number of sperm counted in four WBC squares.[19]

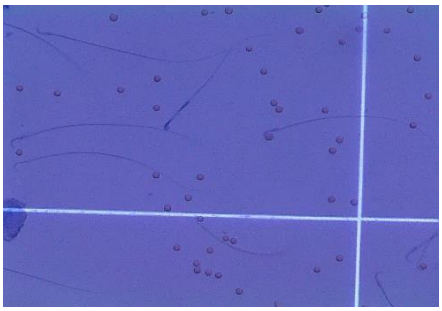

Fig: 1: Epidydimal Sperm Count.

Sperm Viability

A 10 µL sample from Suspension A was mixed with 10 µL normal saline and 10 µL of 0.4% trypan blue. The mixture was placed on a glass slide and covered with a coverslip. At least 200 sperm were observed under a 40×microscope objective. Viable sperm remained colorless; non-viable sperm absorbed the stain and appeared pink/red.

Viability (%) = (Viable sperm / Total sperm) × 100.[20]

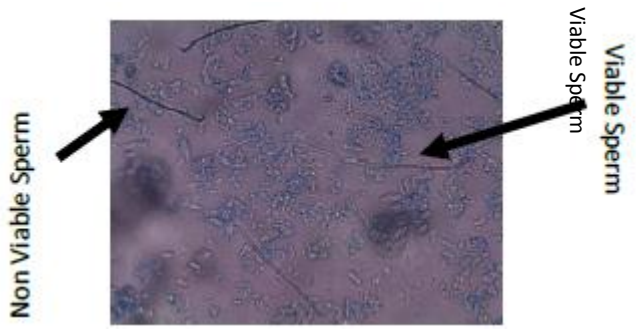

Fig: 2 – Sperm Viability.

Sperm Motility

A 10 µL sample from Suspension A was loaded onto a hemocytometer. Motile and non-motile sperm were counted under 40× magnification across four WBC squares.

Motility (%) = (Motile sperm / Total sperm) × 100.[21]

Sperm Morphology

A 100 µL sample from Suspension A was mixed with 1 mL of 10% neutral buffered formalin in a microcentrifuge tube. Two to three drops of 1% eosin stain were added, and the mixture was incubated at room temperature for 30–45 minutes. A few drops of the stained sample were mounted on a clean glass slide and observed under light microscopy. At least 100 sperm were analyzed for morphological abnormalities (e.g., tailless, headless, bent tail, coiled tail).

Abnormal Sperm (%) = (Abnormal sperm / Total sperm counted) × 100.[22]

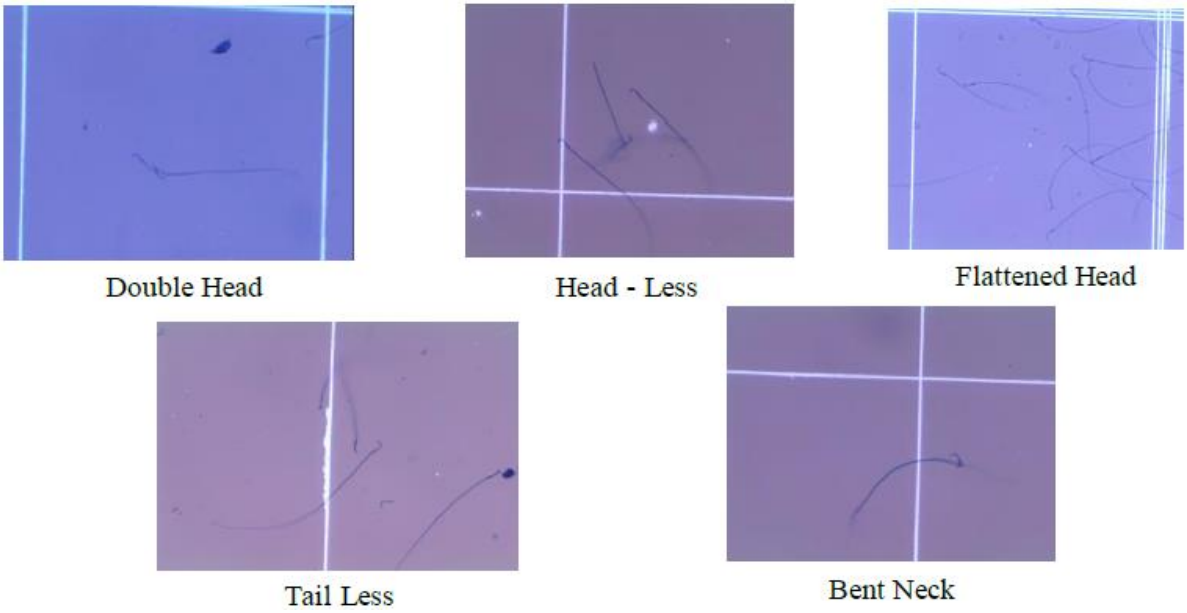

Fig: 3 – Sperm Morphology.

Gross pathology

At the time of termination, all animals were euthanized by the co_2 asphyxiation followed by the exsanguination except for group 4 animals. All animals were subjected to the excise the testes and cauda epididymis. Afterwards all the parameters were performed testicular morphometry, sperm quality & semen analysis and histopathology.

Statistical analysis

The impact of ursolic acid in comparison to the vehicle control was examined using ANOVA (one-way analysis of variance) and Tukey's test; a $P<0.05$, significance level was established.

RESULT

No mortality or morbidity was observed in any of the experimental groups throughout the study period (Table 1). All animals remained active, and no abnormal clinical signs were noted during daily observations (Table 1), indicating good tolerability of the treatments. Body weight measurements showed no statistically significant differences across the groups during the 21-day treatment period (Table 2), suggesting that neither ursolic acid nor cyclophosphamide adversely affected the general health or systemic growth of the animals. However, both absolute and relative testicular weights were significantly reduced ($P < 0.05$) in the disease control group (cyclophosphamide-treated) and the high-dose ursolic acid group (250 mg/kg) when compared to the vehicle control group (Table 3; Figures 4 and 5). In contrast, no significant changes in testicular weights were observed in the low-dose ursolic

acid group (100 mg/kg), which remained comparable to the vehicle control group. Analysis of epididymal sperm parameters revealed that the disease control group and the high-dose ursolic acid group exhibited a marked and statistically significant decrease ($P < 0.05$) in sperm count (Table 4, Figure 6), sperm viability (Table 4, Figure 7), and sperm motility (Table 5, Figure 8) when compared with the vehicle control group. Additionally, the percentage of morphologically abnormal sperm was significantly increased in both the disease control and high-dose groups ($P < 0.05$), indicating impaired spermatogenesis (Table 4, Figure 9). Importantly, animals in the low-dose ursolic acid group demonstrated a protective effect on reproductive parameters. Sperm count, viability, and motility in this group were significantly higher ($P < 0.05$) than those observed in the disease control group and were comparable to the values seen in the vehicle control group. Interestingly, even the high- dose ursolic acid group showed partial improvement in sperm parameters compared to the disease control group, although these improvements were not sufficient to fully restore normal function. These results suggest that while high doses of ursolic acid may exhibit mild antiandrogenic effects similar to cyclophosphamide, lower doses appear to preserve reproductive function and may even offer a protective effect against androgenic disruption.

Table No. 1: 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	No abnormal clinical sign
		20		

		21	during the treatment period	observed during the treatment period
		22		
		23		
		24		

Table No. 2: 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. 3: 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 (30mg/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

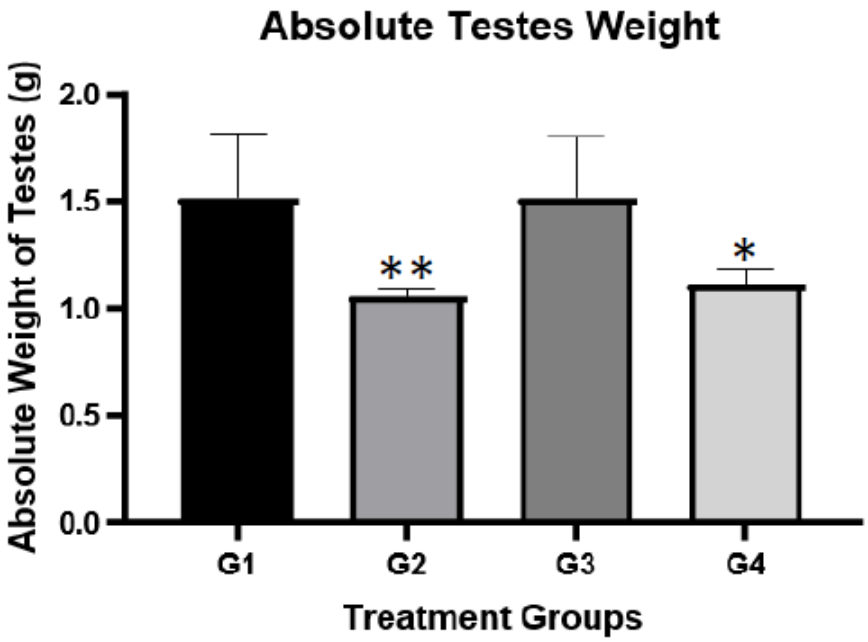

Fig: 4 – Comparison of absolute testes weight of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test.

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

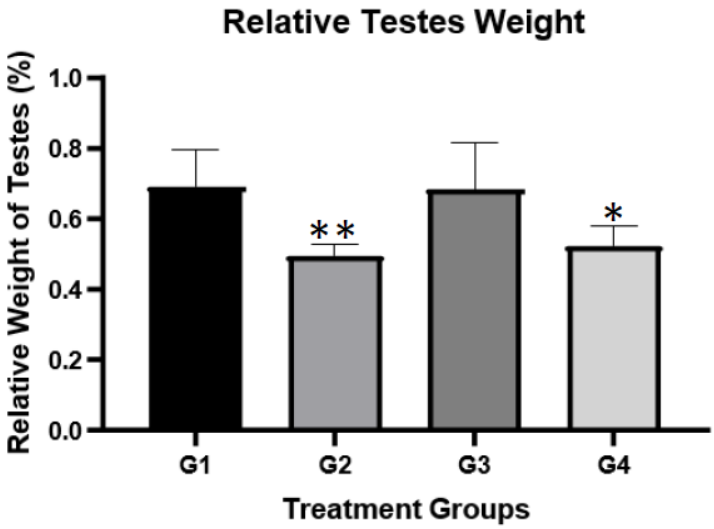

Fig: 5 – Comparison of relative testes weight of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test.

** & * 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 – 5).

Table No. 4: 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

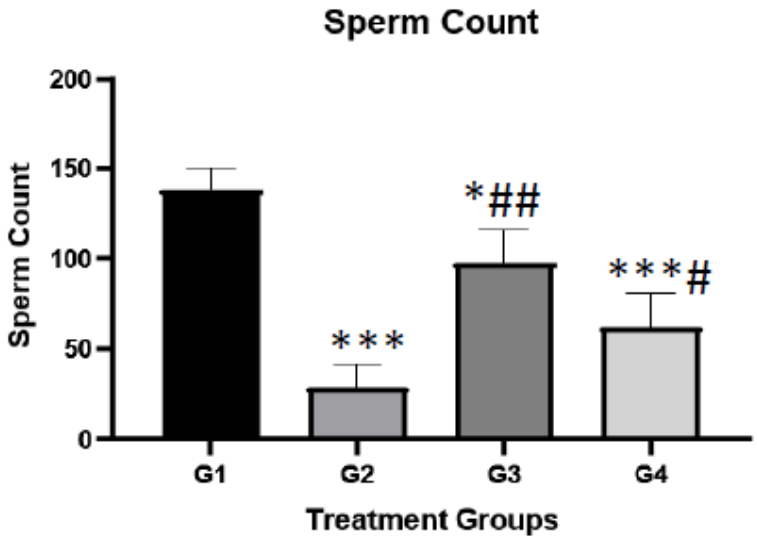

Fig: 6 - Comparison of sperm count of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test.

***, * & *** Indicates that the significant decrease (P<0.05) has been observed in the group 2, group 3 and group 4 animals in sperm count in comparison of control group animals, respectively (Fig – 6).

& # 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 – 6).

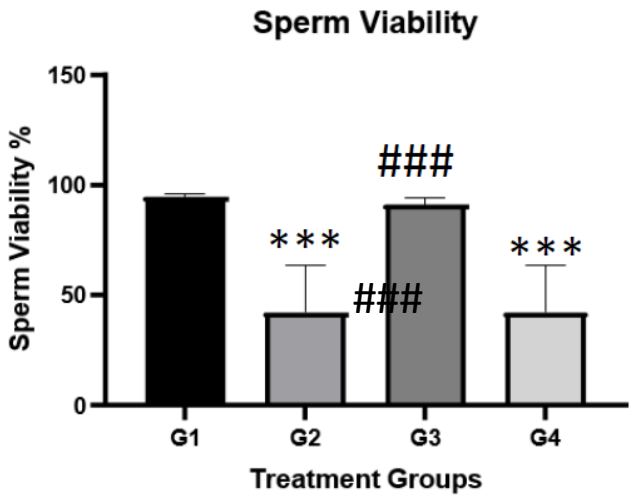

Fig: 7 - Comparison of sperm viability of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test.

***, & *** Indicates that the significant decrease (P<0.05) has been observed in the group 2, and group 4 animals in sperm viability in comparison of control group animals, respectively (Fig – 7).

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 – 7).

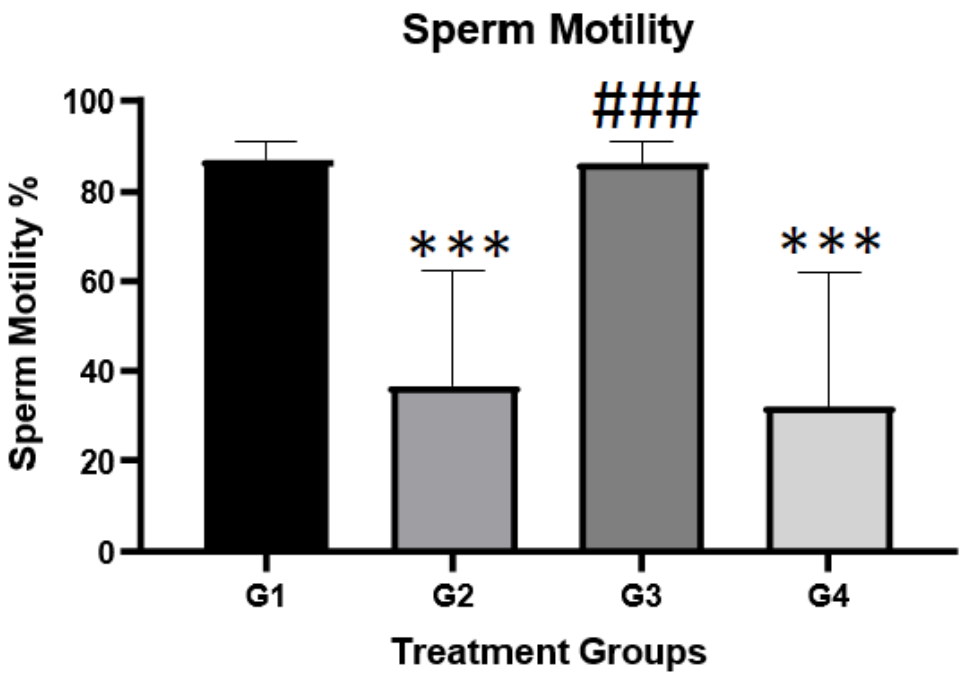

Fig: 8 - Comparison of sperm motility of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test.

***, & *** Indicates that the significant 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 – 8).

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 – 8).

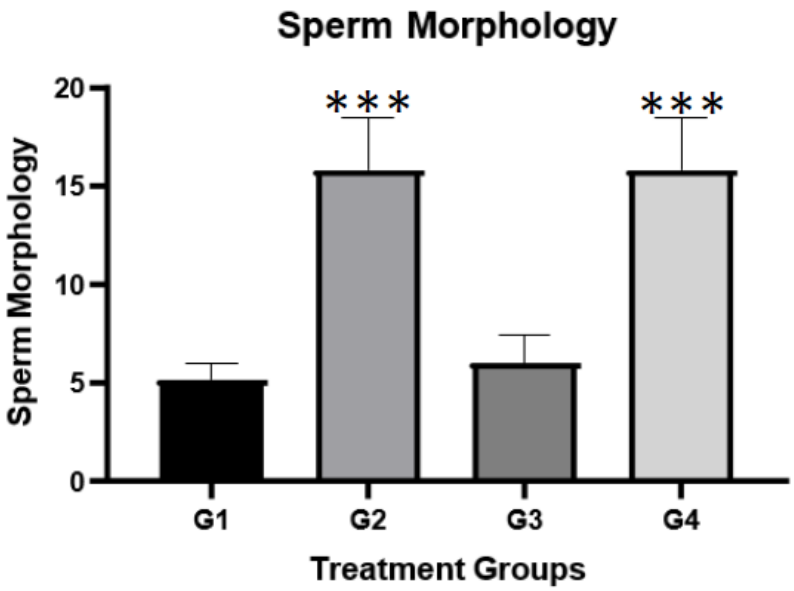

Fig: 9 - Comparison of sperm morphology of all four groups of animals using one way ANOVA followed by post – hoc tuckey's test.

***, & *** 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 – 9).

Histopathology

Histological examination of testicular tissue from the vehicle control group revealed normal architecture of seminiferous tubules with orderly arrangement of germ cell layers, including spermatogonia (SG), primary spermatocytes (Pc), spermatids (St), and Sertoli cells (Sc). The interstitial tissue (IT) was also intact, showing healthy Leydig cells (Lc) distributed between the tubules. The lumina of seminiferous tubules contained abundant spermatozoa (SZ), indicating active and functional spermatogenesis (Fig 10).

In contrast, marked histopathological alterations were observed in the disease control group treated with cyclophosphamide (30 mg/kg, i.p.), as well as in the high-dose ursolic acid group (250 mg/kg, oral). The seminiferous tubules in these groups exhibited disrupted architecture, with evident depletion of germ cells. Key findings included the absence or severe reduction of spermatozoa in the luminal space, increased vacuolization (V) within the seminiferous epithelium, and germ cells showing pyknotic nuclei (PN)—indicative of nuclear condensation and apoptosis. Disorganization of germinal epithelium and atrophy of seminiferous tubules were also evident. Additionally, the interstitial tissue appeared

edematous, with a reduction in Leydig cell population, suggesting impaired androgen production (Fig 11).

Notably, the low-dose ursolic acid group (100 mg/kg) displayed preserved testicular morphology comparable to that of the vehicle control. Seminiferous tubules appeared intact with all stages of spermatogenic cells present and normal luminal spermatozoa density. Minimal to no histopathological alterations were observed, indicating no adverse effects on testicular structure at this dose (Fig 12).

These histological findings correlate with the observed reductions in sperm count, motility, and viability in the high-dose and cyclophosphamide groups. Together, they support the hypothesis that ursolic acid, particularly at higher doses, exerts antiandrogenic effects in male Wistar rats, possibly through suppression of spermatogenesis and disruption of Leydig cell function.

Table No. 5: Histopathology.

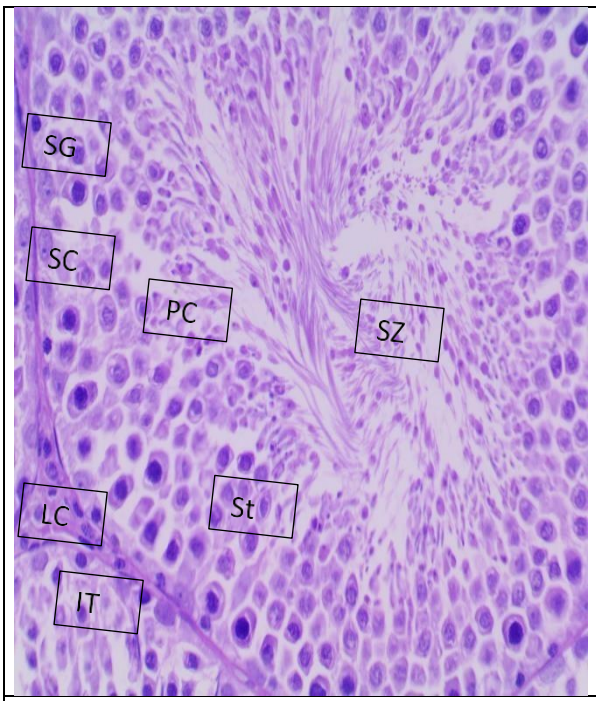

Fig: 10 - G1 (Vehicle Control)

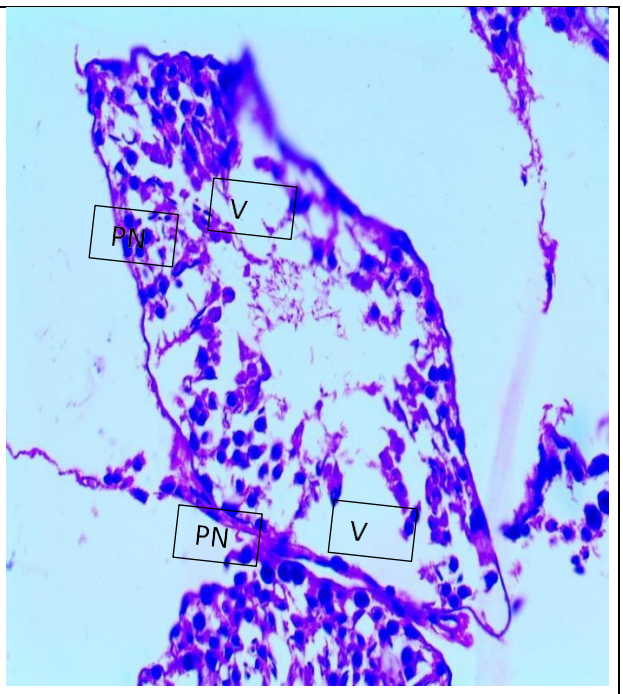

Fig: 11 - G2 (Disease Control)

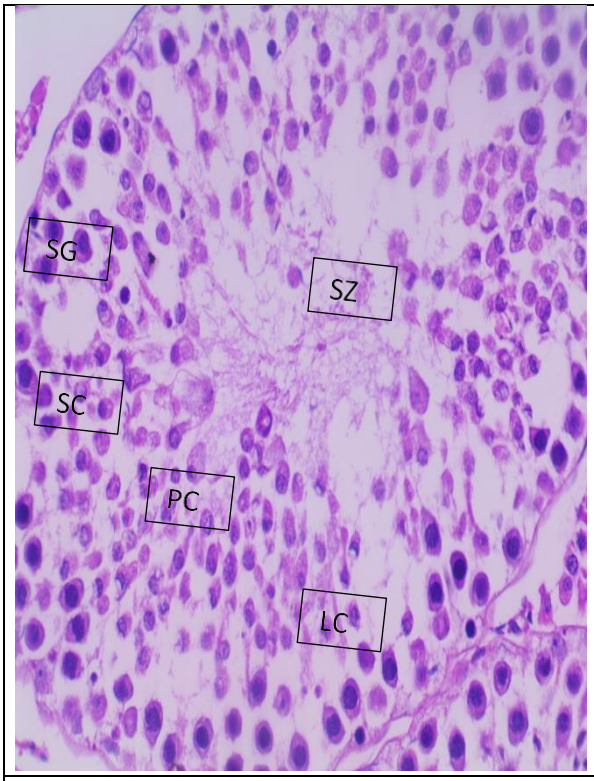

Fig: 12 - G3 (Low Dose)

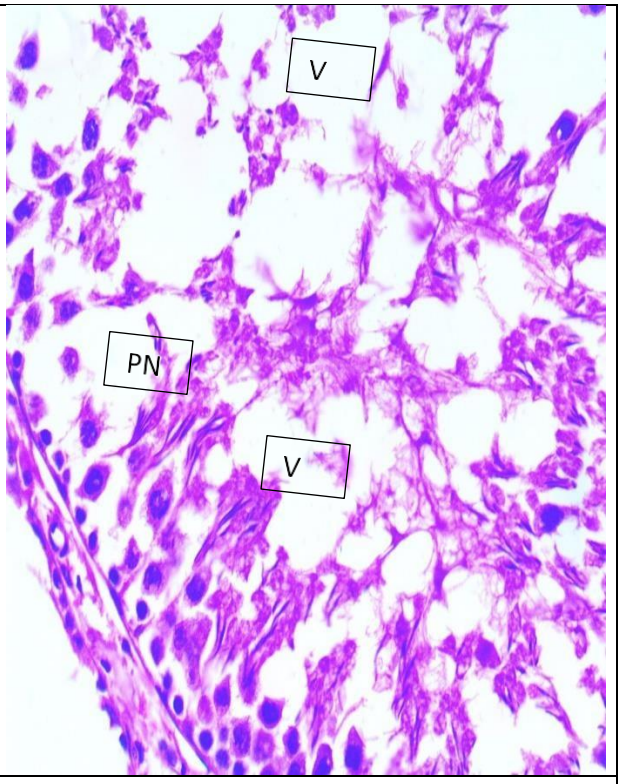

Fig: 13 - G4 (High Dose)

DISCUSSION

The present study evaluated the antiandrogenic and potential antifertility effects of ursolic acid (UA) in male Wistar rats, using multiple reproductive endpoints including testicular weight, sperm characteristics, and histopathological assessment. The data demonstrated that high-dose UA (250 mg/kg) and cyclophosphamide (30 mg/kg) induced comparable alterations in reproductive function, characterized by significant reductions in sperm count, motility, and viability, as well as degenerative changes in testicular architecture. These findings suggest that UA at higher doses exerts a suppressive effect on the male reproductive system, consistent with antiandrogenic activity.

The observed reduction in both absolute and relative testicular weights in the high-dose UA and cyclophosphamide groups is indicative of androgen suppression, as testicular weight is closely associated with spermatogenic activity and androgen status.[23] Cyclophosphamide is a well-documented gonadotoxic agent that impairs spermatogenesis through oxidative stress and germ cell apoptosis, serving as a positive control for testicular damage in this model.[24] Similarly, the high dose of UA resulted in depletion of germ cells and decreased sperm output, suggesting a possible inhibitory effect on steroidogenesis or direct cytotoxicity to germ cells.

Ursolic acid is a pentacyclic triterpenoid known for its wide pharmacological spectrum, including anti- inflammatory, antioxidant, and anticancer effects.[25,26] Importantly, several studies have reported its potential to modulate hormonal pathways. UA has been shown to inhibit androgen receptor expression in prostate cancer cells and reduce steroidogenic enzyme expression, such as 5α-reductase, which converts testosterone to the more potent dihydrotestosterone (DHT).[27,28] These mechanisms may underlie the antiandrogenic effects observed in our study, particularly at higher doses where testicular structure and sperm quality were significantly compromised.

Histopathological findings in the high-dose group further support this hypothesis. The presence of vacuolization, germ cell loss, and pyknotic nuclei in seminiferous tubules are hallmark features of testicular degeneration and impaired spermatogenesis.[29] Additionally, the reduction in spermatozoa within the tubular lumen and diminished Leydig cell density in the interstitial tissue suggest compromised androgen production, aligning with earlier reports linking UA to suppression of androgen-dependent processes.[28,30]

Interestingly, the low-dose UA group (100 mg/kg) did not show adverse effects on testicular morphology or sperm parameters. In fact, reproductive parameters in this group were comparable to controls and significantly better than the disease control group. These results suggest a potential threshold effect, where lower doses of UA may be biologically safe and could even exert protective effects against testicular damage. This finding aligns with previous research showing dose-dependent variations in the activity of plant-derived compounds, where low doses may exert antioxidant effects and higher doses become cytotoxic.[31]

From a contraceptive perspective, the reversibility and safety of an agent are crucial considerations. Although our study confirms that high-dose UA has antiandrogenic properties, further studies are needed to determine whether these effects are reversible upon cessation of treatment, as is required for an ideal male contraceptive.[32] Moreover, the exact molecular mechanisms—whether via androgen receptor antagonism, suppression of steroidogenesis, or induction of oxidative stress—remain to be elucidated.

CONCLUSION

The findings of this study demonstrate that ursolic acid exhibits dose-dependent antiandrogenic effects in male Wistar rats. High-dose ursolic acid (250 mg/kg) led to

significant reductions in testicular weight, sperm count, motility, and viability, accompanied by marked histopathological alterations in testicular architecture similar to those observed with cyclophosphamide, a known reproductive toxicant. These effects suggest suppression of spermatogenesis and impaired androgenic activity at higher doses. In contrast, the low dose of ursolic acid (100 mg/kg) did not adversely affect reproductive parameters or testicular morphology, and in fact showed a protective trend compared to the disease control group. Overall, these results indicate that ursolic acid, at appropriate doses, may have potential applications in the development of reversible, plant-derived male contraceptives. However, further studies are warranted to elucidate its underlying mechanisms, long-term safety, and reversibility of its antifertility effects.

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Declaration of Interest

None.

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