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Effects of Corchorus Olitorius Leaves and Stem Ethanolic Extract on Seminal Quality and Testicular Histology in Male Wistar Rats.

Elizabeth Eepho Krukru[1], Dagbota Dan-Jumbo[2], Progress Dakuro Victor[3], Elile Peace Okpara[4], Cedar Patrick Inanum[5], Progress Baridi[7], Buduka Justice Otto[8]

[1,2,4,8] Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Rivers State University, Nigeria.

[3,5,7] Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Rivers State University, Nigeria.

ABSTRACT

Corchorus Olitorius (Ewedu or Jute) plant leaf is a popularly used vegetable, especially, amongst the Yoruba tribe in Nigeria; it is also admired for its possible tradomedicinal potencies on several conditions including aphrodisiac effects. Thus, the present study investigated the effects of Corchorus olitorius leaves and stem ethanolic extract on seminal quality and testicular histology in male Wistar rats. Twenty four male Wistar rats weighing between 160g and 180g were procured for the study and housed in a standard animal house facility. The study models were selected into six different groups of 4 male rats per group: Group 1 (normal control) received 1ml normal saline daily, Group 2 (standard control group) received 5mg/kg body weight (b.w) of sildenafil citrate, Groups 3 (a) 3 (b) received 500mg/kg bw of the Corchorus Olitorius leaf and stem extracts respectively, Groups 4 (a) and 4 (b) received 1000mg/kg bw of the leaf and stem extracts of the plant. At the end of the separate treatments, semen and testicular tissues were harvested from the study models following standard methods. The result comparatively revealed increased abnormal sperm cells and reduced normal and viable sperm cells, as well as reduced sperm count and volume in the extracts treated models; these were marginal ($p<0.05$) in the Corchorus olitorius (Ewedu) stem extract treated models but significant ($p<0.05$) in the leaf extract treated models. The extracts were also noticed to have exerted mild interstitial tissue lesions (ISTL) with degeneration of Leydig cell as well as mild spermatic cells distortion when compared to those of the control and standard drug treated group. In conclusion, the findings of the current study points at seminal quality reductive potentials of the extracts in the models.

KEYWORDS: Corchorus Olitorius plant; seminal quality; ethanolic extracts; male reproductive system

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INTRODUCTION

Male fertility, as well as that of the female, is important and of significant impact on the general reproductive health and outcomes for couples (Turner et al., 2020). A 2017 meta-analysis of over one hundred and eighty studies, involving more than forty thousand men drawn from different countries, found that sperm count had decreased by over 50% in a period of about thirty years (Levine et al., 2017). Similarly, another study raised concerns about this reported trend, stating that the fact remains that sperm health is critical (Boulicault et al., 2022).

Considering the implication of the male factors of infertility; as to checkmate possible reproductive difficulty, the evaluation of semen quality as a reliable measure of male fertility, should be continuously explored alongside the procedures carried out on that of the females (Khatun et al., 2018; Tanga et al., 2021).

Aside from seeking healthcare guide, helpful lifestyle moderations and good diet; the use of safe helpful supplementations (which are mainly of plant origin), have been reported to impact on the quality of male fertility. On the other hand, there has been mounting levels of

disappointments in many populations with reliance on orthodox medications for male infertility; and this has led to the more and more popular use of complementary and alternative methods to treat male infertility (Brezina et al., 2012; Samplaski & Nangia, 2015; Fasanghari et al., 2024). A variety of herbs (for example Fumaria parviflora, zingiber officinale, cinnamomum zeylanicum, Phoenix dactylifera, etc.,) have been noted to be helpful in enhancing the functions of the male reproductive system (Boroujeni et al., 2022). This claim was attributed to the rich antioxidant potentials and low side effects of these plants (Boroujeni et al., 2022; CNY Fertility, 2025). But whether all of these presumed benefits of herbal supplements are safe on the system, is a question still begging for reliable answers.

The Corchorus olitorius (Ewedu or Jute) plant has been reported to have significant aphrodisiac potentials (libido/erectile dysfunction improving attributes) (Eshemokha, 2020). Therefore, in view of the popular and wide consumption of the Ewedu leaf in a local delicacy—Ewedu soup (Olugbuyi et al., 2023), investigating the possible semen quality enhancing potentials of the plant may produce beneficial outcomes.

Ewedu soup, prepared with the jute plant (Corchorus olitorius), is widely consumed by the Yorubas in Nigerian and other ethnic groups in the country, as a local dish (Okorejior et al., 2024). The plant belongs to the genus Olitorius, with a vast array of flowering plants in the Malvaceae family. The plant is indigenous to tropical/subtropical regions of the world (Akinwande et al., 2024; Olatunde, 2024). Consequently, the present study is aimed at investigating, the effects of Corchorus olitorius leaves and stem ethanolic extract on seminal quality and testicular histology in male Wistar rats.

MATERIALS AND METHODS

Plant collection

Fresh leaves and stems of Corchorus Olitorius (Ewedu plant) were obtained from Rivers State University farm located in Port Harcourt, Nigeria. A voucher sample was deposited in the herbarium located in the Department of Plant Science and Biotechnology of the Rivers State University for proper identification and authentication. The rest of the plant samples were sorted out, washed and air-dried for twenty two days. Thereafter, the dried leaves and stems were separately pulverized into fine powder using electric grinder.

Figure Ī: Ewedu leaf and Stem

Preparation of Plant Extract

The fine powders of the leaves and stems were then separately soaked in 80% ethanol solvent. The ratio of plant sample to solvent volume for the two different plant portions was 40g: 2500ml. The mixtures were periodically shaken at regular intervals to achieve maximum extraction. After 72 hours, the solution was filtered using Whatman No. 1 filter paper. And then, the filtrate was concentrated in water bath at 40^{o}C. The dried semi-solid extracts of the leaves and stems of the plant were then weighed and kept in the fridge at about 4^{O}C until when they were used.

With reference to the report of Egua et al., (2014), which stated that the LD50 of the ethanolic extract of the same plant was over 5000mg/kg, 500mg/kg (low dose) and 1000mg/kg (high dose) were adopted as effective doses for the present study.

The respective extracts (leaf and stem) of Corchorus Olitorius were orally administered; hence the extract was suspended in distilled water. Similarly, considering the appropriate dose

for the study models, sildenafil citrate and estradiol valerate were also suspended in distilled water for oral administration.

Study models and their handlings

Twenty four (24) adult male Wistar rats weighing between 160g and 180g were obtained for the study and housed in the Animal House unit of the Department of Human Physiology, Faculty of Basic Medical Sciences, Rivers State University, Nigeria. Standard cages were used and the models were maintained under the 12hr light/dark cycle with free access to feeds and water throughout the study. The route of all drug administration was oral using the oral gavage.

Experimental Protocol

The study models were randomly distributed into six different groups of 4 male rats each:

1. Group 1 served as normal control and received 1ml normal saline daily,
2. Group 2 served as standard control and received 5mg/kg body weight (b.w) of sildenafil citrate (at least an hour prior to the start of the experiment
3. Groups 3 (a) served as test group and received 500mg/kg bw of the Corchorus Olitorius leaf extract.
4. Groups 3 (b) served as test group and received 500mg/kg bw of the Corchorus Olitorius stem extract.
5. Groups 4 (a) served as test group and received 1000mg/kg bw of Corchorus Olitorius leaf extract.
6. Groups 4 (b) served as test group and received 1000mg/kg bw of Corchorus Olitorius stem extract.

Procedure for Semen Analysis: At the end of all treatments, the study models were exposed to inhalation anaesthetic (diethyl ether) in a desiccator for sedation and then they were sacrificed by cervical dislocation. Thereafter, the open castration method was used for orchidectomy via a midline scrotal incision (Kadir et al., 2018).

Procedure for Histological Analysis: Histology was done on the seminiferous tubules of the testes. As stated above, upon sacrificing of the animals, of the testes were carefully dissected and briefly fixed in 10% formaldehyde solution and thereafter, histological sections were well prepared and properly interpreted. The procedure adopted was as reported by Isaac et al., (2023).

Statistical analysis

Numerical data derived from the study were subjected to statistical analyses using the statistical package for social sciences software (SPSS) version 25.0. The analysis of variance (ANOVA) followed by LSD Post Hoc tests were used. The values were expressed as mean ± standard error of mean (Mean ± SEM). Statistical significance was determined at p-value less than 0.05 ($p < 0.05$).

RESULTS

Table 1: Effect of Corchorus olitorius stem on semen analysis

	Study Groups					
Semen Quality	**Control**	**Standard Drug (Sildenafil Citrate) Treated**	**500mg/kg b.w Treated**	**1000mg/kg b.w Treated**	**F**	**p-value**
Abnormal	17.5 ± 2.5	20.0 ± 10.0	26.7 ± 7.3	37.5 ± 2.5	1.48	0.38
Normal	82.5 ± 2.5	80.0 ± 10.0	73.3 ± 7.3	62.5 ± 2.5	1.48	0.33
Viability	87.5 ± 2.5	82.5 ± 7.5	75.0 ± 7.6	62.5 ± 2.5	2.44	0.18
Sperm Count	550.0 ± 50.0	550.0 ± 150.0	433.3 ± 120.0	200.0 ± 0.20	1.92	0.24
Volume	0.35 ± 0.05	0.30 ± 0.10	0.27 ± 0.09	0.10 ± 0.00	1.64	0.29

Values were expressed as mean ± standard error of mean (Mean ± SEM).

Note that the following for all groups are the same: appearance milky; pH=8.0; viscosity = normal

*Signifies significant difference ($P \leq 0.05$) in comparison with control group

[a]Signifies significant difference ($P \leq 0.05$) in comparison with sildenafil citrate group

Table 2: Effect of Corchorus olitorius leaves on semen analysis

Semen Quality	Study Groups					
	Control	Standard Drug (Sildenafil Citrate) Treated	500mg/kg b.w Treated	1000mg/kg b.w Treated	F	p-value
Abnormal	17.5 ± 2.5	20.0 ± 10.0	37.5 ± 2.5	27.5 ± 2.5	1.48	0.38
Normal	82.5 ± 2.5	80.0 ± 10.0	62.5 ± 2.5	60.0 ± 0.0*	1.48	0.04
Viability	87.5 ± 2.5	82.5 ± 7.5	65.0 ± 5.0*	60.0 ± 0.0*[a]	2.44	0.02
Sperm Count	550.0 ± 50.0	550.0 ± 150.0	175.0 ± 25.0*[a]	150.0 ± 0.0*[a]	1.92	0.03
Volume	0.35 ± 0.05	0.30 ± 0.10	0.15 ± 0.05	0.10 ± 0.00*	0.38	0.05

Values were expressed as mean ± standard error of mean (Mean ± SEM).
Note that the following for all groups are the same: appearance milky; pH=8.0; viscosity = normal
*Signifies significant difference (P ≤0.05) in comparison with control group
[a]Signifies significant difference (P≤0.05) in comparison with sildenafil citrate group

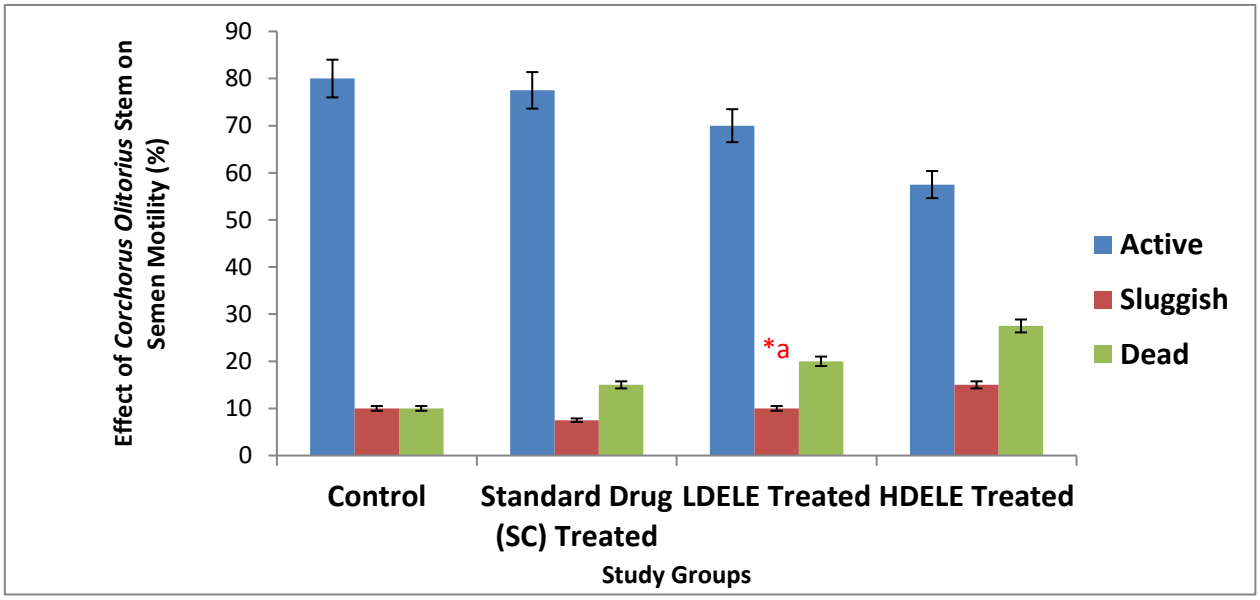

Figure 1: Effect of Corchorus olitorius Stem on Semen Motility

Note; The following under listed details apply for both Figures 1 and 2:
HDELE = High Dose (1000mg/kg b.w) Ewedu (Corchorus Olitorius) stem Extract.
LDELE = Low dose (500mg/kg b.w) Ewedu (Corchorus Olitorius) stem Extract.

*Signifies significant difference (P ≤0.05) in comparison with control group
[a]Signifies significant difference (P≤0.05) in comparison with sildenafil citrate group

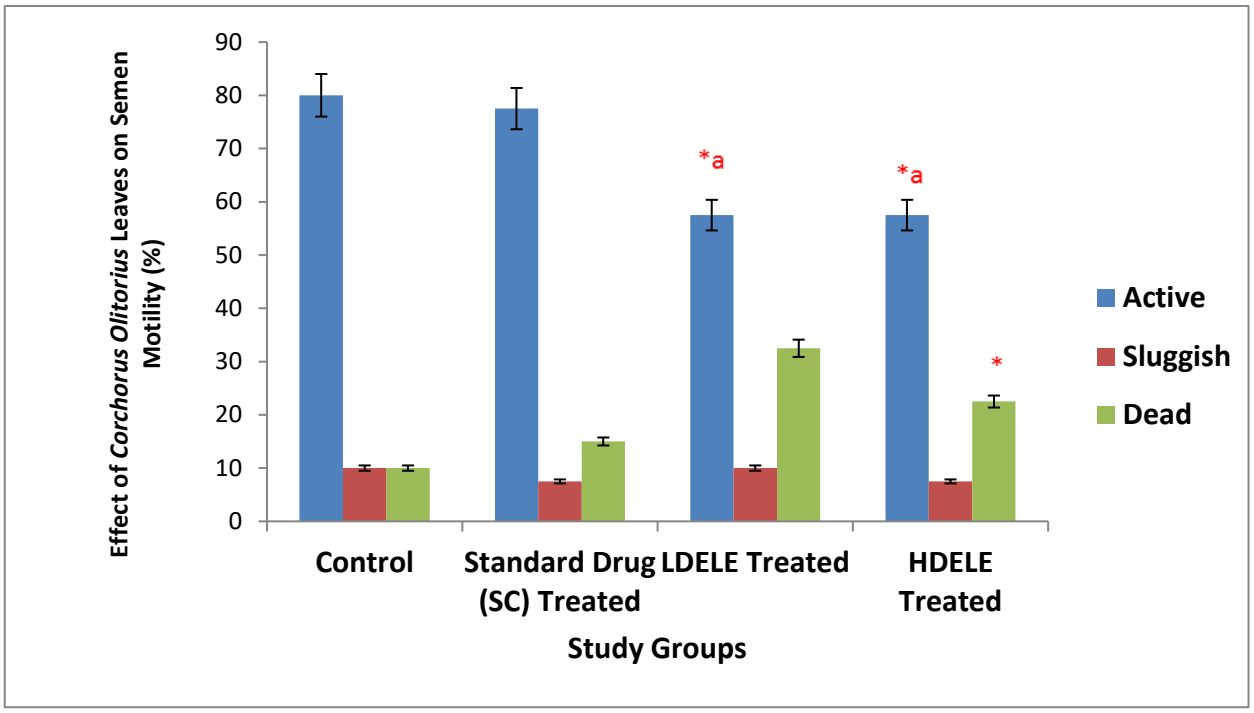

Figure 2: Effect of Corchorus olitorius Leaves on Semen Motility

Note; The following under listed details apply for both Figures 1 and 2:
HDELE = High Dose (1000mg/kg b.w) Ewedu (Corchorus Olitorius) Leaf Extract.
LDELE = Low dose (500mg/kg b.w) Ewedu (Corchorus Olitorius) Leaf Extract.

*Signifies significant difference (P ≤0.05) in comparison with control group
[a]Signifies significant difference (P≤0.05) in comparison with sildenafil citrate group

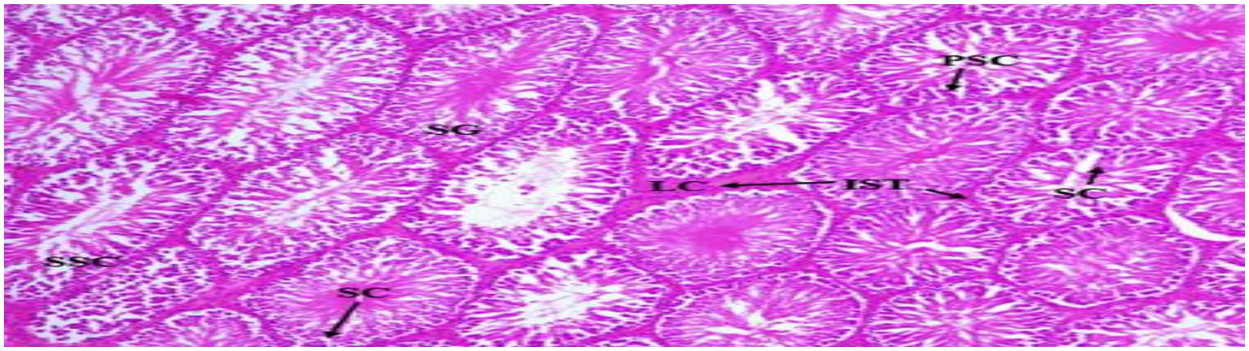

Plate I: Photomicrograph showing section of seminiferous tubules of the testis of male wistar rats Group 1 (Received distilled water). Section showed sertoli cells (SC) with germinal cells (SG), primary spermatid (PSC), secondary spermatids (SSC) and interstitial tissues with Leydig cells (LC). (H&E X100).

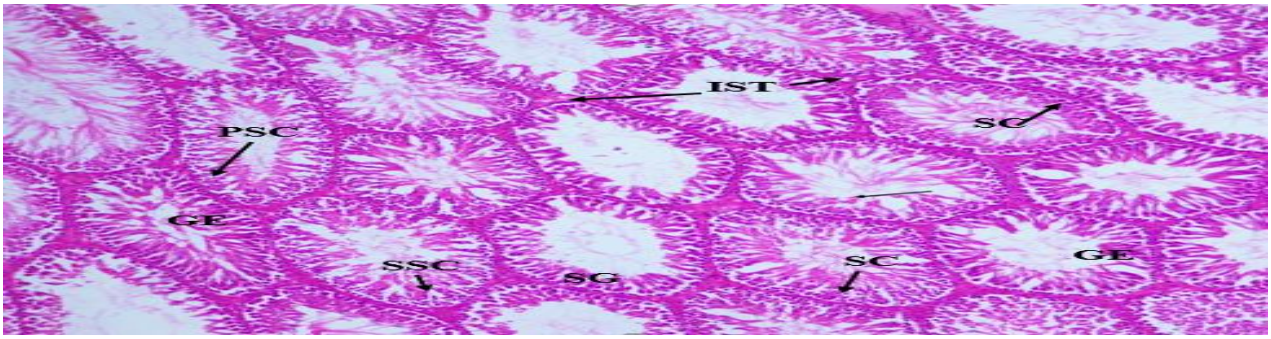

Plate II:Photomicrograph showing section of seminiferous tubules of testis Group II (Viagra). Section showed normal testicular sertoli cells (SC), germinal epithelium (GE), primary spermatids (PSC), and secondary spermatocytes (SSC) and mature spermatocytes. (H&E X100).

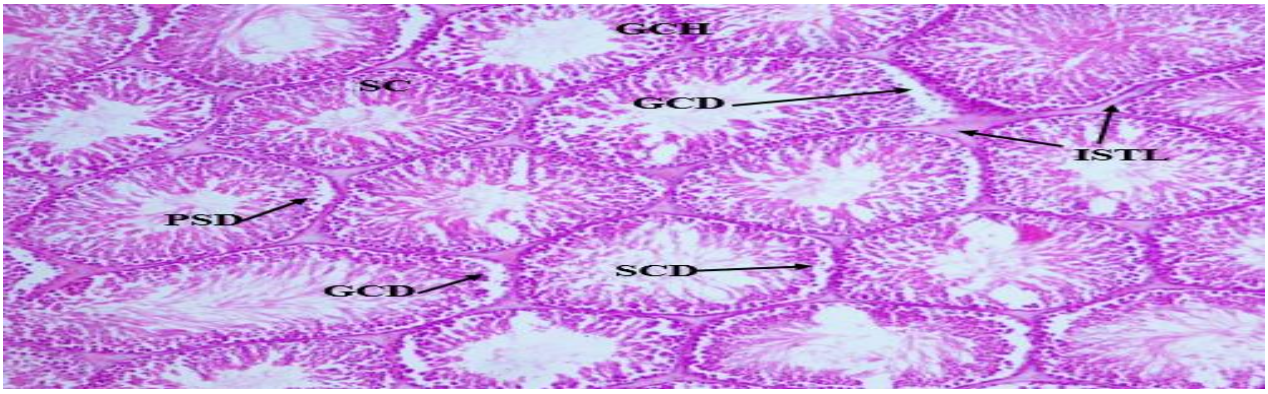

Plate III : Photomicrograph showing section of testicular tissue of testes. Group III (high dose Ewedu stem). Section showed mild interstitial tissue lesions (ISTL) with degeneration of Leydig cellS (LC). There is mild spermatic cells distortion and degeneration from the basement membrane. GCH= Germ cell hypoplasia. (H&E X100).

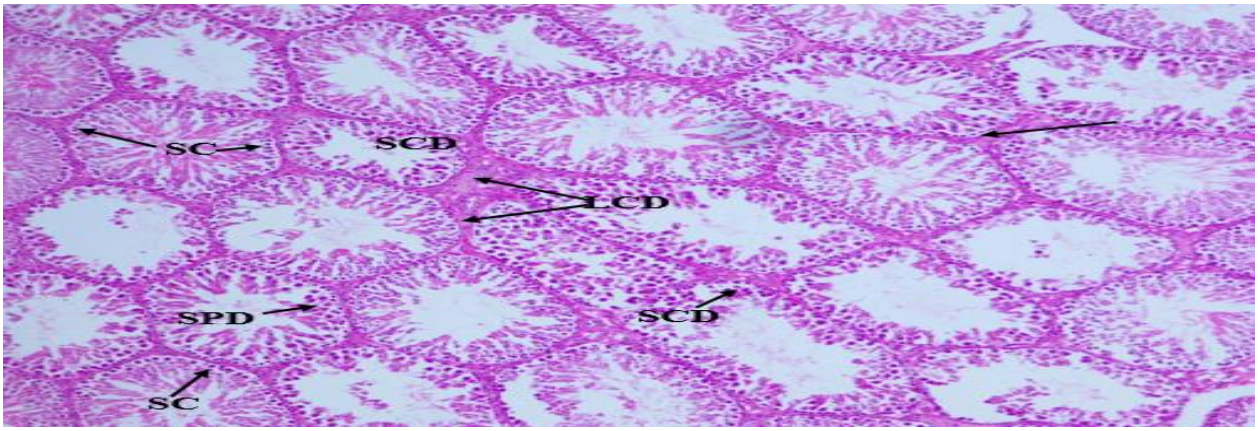

Plate IV: Photomicrograph showing section of testicular tissue of testis. Group IV (low dose Ewedu stem). Section showed mild interstitial tissue lesions (ISTL) with spermatic cells distortion and degeneration (SCD) and Leydig cells hypoplasia (LCD). There is spermatic cells distortion (SCD) and degeneration from the basement membrane. (H&E X100).

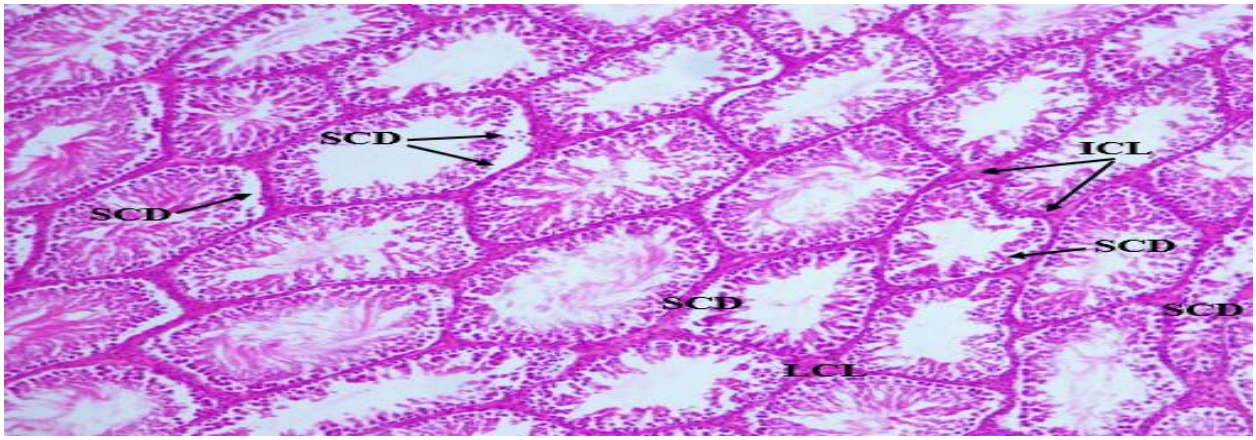

Plate V: Photomicrograph showing section of testicular tissue of male wistar rat of Group V (High dose Ewedu leave). Section showed interstitial cells and tissue lesions with degeneration of Leydig cells (LCL). There is a spermatic cell distorted from the basement membrane. (H&E X100).

RESULTS

The data on Table 1 is the outcome on the effect of Corchorus olitorius stem on semen analysis.

Considering outcome on the percentage of abnormal, normal and viable sperm cells, there were no significant ($p>0.05$) variations when the values of the extract treated groups were respectively compared to those of the control and standard drug treated groups. However, it was noted that marginal ($p>0.05$) increase in abnormal cells, decreases in normal and viable cells in all test groups. In fact, the seminal quality declined progressively from the standard drug treated to the extract treated (which was in a dose-dependent fashion). In a similar manner, the outcome on sperm count and volume, revealed that the standard drug treated and extract treated

groups of the study models had decreased values when compared to that of the control group (and it was in a dose-dependent manner for the extract treated groups).

Table 2 is showing the effect of Corchorus olitorius leaves on semen analysis. The results here followed the same trend as with that of the Corchorus olitorius stem treated. However, the decreases in the normal cells, viable cells, sperm cells and volume were virtually all significant ($p<0.05$) when the values of the extract treated models were compared to those of the control and standard drug respective models.

The outcome of the histological analyses on the testicular tissue of the study models showed normal sertoli cells (SC) with germinal cells (SG), primary spermatid (PSC), secondary spermatids (SSC) and interstitial tissues with Leydig cells (LC) for the control group and the standard drug treated group. But for the Corchorus olitorius stem and leaf extracts treated study models, the sections revealed mild interstitial tissue lesions (ISTL) with degeneration of Leydig cells (LC) as well as mild spermatic cells distortion and degeneration from the basement membrane.

DISCUSSIONS

It has been noted that herbal therapies have the potentials to be valuable and complementary in the treatment of male infertility (Nguyen-Thanh et al., 2024). However, there are yet unknown possible disadvantages of such herbal supplements (like their toxicity levels/side effects, interactions and regulations, etc.) (ElAmrawy et al. 2016; Pramodh, 2021). Thus, the present study conducted an investigation on the possible effects of Corchorus olitorius (Ewedu) leaves and stem ethanolic extracts on seminal quality and testicular histology in male Wistar rats. The outcomes are discussed in the following paragraphs.

The present study found increased abnormal and reduced normal/viable sperm cells in the extract treated groups compared to the control and standard drug treated groups and while these outcomes were marginal for the stem extract, it was significant for the leaf extract. The trend was similar for both sperm count and sperm volume outcomes.

Reports have it that the diagnosis of sperm abnormality following a comprehensive physical examination and endocrine/genetic screening may be due to structural defects, infections, exposure to toxins, drug-related impacts, etc., (Silber, 2000, Shaikh et al., 2011). Considering this submission in line with the above finding of the present study, it is obvious that certain constituents of the plant extracts may be potent toxicants to the sperm cells of the study models. Similar to this finding, Orieke et al., (2019) reported that the Corchorus olitorius leaf extract exerted remarkable azoospermia and reduced spermatid density per-tubule/prostatic degeneration.

Relating the above finding and the foregoing report with an earlier submission by Eshemokha, (2020), that noted significant aphrodisiac potentials of the same plant, calls for more in depth investigations on the plant. Beyond structural and endocrine evaluations, characterization and further study on the constituents of the plant would be helpfully revealing. In the meanwhile, caution must be exercised on the use of the plant extract, especially in infertility implicated scenarios.

In consideration of the histological analyses on the testicular tissue of the study models in the present study, the Corchorus olitorius (Ewedu) stem and leaf extracts was noticed to have exerted mild interstitial tissue lesions (ISTL) with degeneration of Leydig cell as well as mild spermatic cells distortion amongst other abnormalities.

Leydig cells are, of course, steroidogenic cells and are located in the interstitial compartment of testicular tissue; they play an important role in male reproductive functions. Consequently, their dysfunction can cause a wide range of testicular pathologies (Adamczewska et al., 2022). Furthermore, a mild spermatic cells distortion may result in lower **sperm count which** may distort fertility (Lavranos et al., 2012; Sakpa & Wilson, 2019). In view of these possibilities, it is suggestive to state that, the impact of the both extracts of Corchorus olitorius (Ewedu) on the testicular tissues is defective and may result in infertility of multiple origins with time. The above finding of the present study corroborates with the finding of this study on semen analyses.

CONCLUSION

The present study found increased abnormal sperm cells and reduced normal/viable sperm cells as well as reduced sperm count and sperm volume following the sub-chronic treatment of the study models with the Corchorus olitorius (Ewedu) stem and leaf extracts.

It was also noticed that, the extracts exerted mild interstitial tissue lesions (ISTL) with degeneration of Leydig cell as well as mild spermatic cells distortion amongst other abnormalities. In conclusion, the findings of the current study points at seminal quality reductive potentials of the extracts in the models. It is thus recommended that caution be taken on the use of the plant extract, especially in infertility implicated scenarios and further study should endeavor to characterize the possible constituents of the plant as to better understand these variable attributes of the plant.

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