Ethanolic seed extract of Artocarpus heterophyllus ameliorates histomorphological, morphometric and functional alterations in the rat testis induced by sildenafil citrate

John Juma Ochieng
Kampala International University
Jackline Namulema
jnamulema@uzimauniversity.ac.ke
Uzima University – Kisumu
Onanuga Ismail Olasile
Olabisi Onabanjo University
Victor Archibong
University of Rwanda
Ibrahim Ssengendo
Kampala International University - Western Campus

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Ethanolic seed extract of *Artocarpus heterophyllus* ameliorates histomorphological, morphometric and functional alterations in the rat testis induced by sildenafil citrate

Author details

John Juma Ochieng [1,5*], Jackline Namulema[2*], Onanuga Ismail Olasile [3], Victor Archibong [4], and Ibrahim Ssengendo [5]

Author affiliation

[1]Department of Human Anatomy, Faculty of Medicine and Pharmaceutical Sciences, Kampala International University – Dar es Salaam, Republic of Tanzania j.ochieng@kiut.ac.tz, https://orcid.org/0000-0003-2679-8949

[2] Department of Physiology, School of Medicine, Uzima University – Kisumu, Kenya jnamulema@uzimauniversity.ac.ke https://orcid.org/0000-0002-3600-5255

[3] Department of Human Anatomy Olabisi Onabanjo University, Shagamu, Ogun state, Nigeria onanuga.ismail@oouagoiwoye.edu.ng https://orcid.org/0000-0002-9552-7838

[4] Department of Human Anatomy, School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda victorarchibong3@gmail.com https://orcid.org/0000-0002-8160-381X

[5] Department of Human Anatomy Kampala International University – Western Campus P.O. Box 71 Ishaka Busheyi -Uganda ibrahim.ssengendo@kiu.ac.ug https://orcid.org/0009-0007-1796-5035

*Corresponding authors

Abstract

Background: One in every ten men is affected by ED regardless of their age. Sildenafil citrate is the first-line drug in the treatment of ED and has been associated with seminiferous tubules and testicular degeneration, translating into decreased spermatogenesis. The study evaluated the effect of ethanolic seed extract of *Artocarpus heterophyllus* on changes in testicular histology, seminal fluid and serum testosterone levels alone and in ameliorating testicular changes induced by Sildenafil Citrate.

Methods: Thirty-six adult male Wistar rats were randomly divided into six groups (n=6) and orally dosed daily with NS, seed extract of AH (500 or 1000 mg/kg), SC (50 mg/kg) or AH+SC (1000 mg/kg + 50 mg/kg or 1000 mg/kg + 100 mg/kg). Testicular histopathological and semen analyses were performed via H&E, PAS and Masson's trichome staining and microscopy. Differences in sperm parameters, testicular size, seminiferous tubule characteristics, Leydig cell volume, and serum testosterone levels were determined.

Results:

SC administration significantly decreased the sperm count (3.47±1.46), motility (23.414±6.63) and morphology (0.038±0.02) compared to the negative control (6.04±0.47, 27.888±0.81 and 0.058±0.02, respectively). The administration of AH 1000 to SC-positive rats improved these parameters in an SC dose-dependent manner, i.e., 10.54±1.48, 31.516±0.99 and 0.0781±0.02 and 9.36±0.36, 27.314±1.45 and 0.049±0.03 for 50 mg/kg and 100 mg/kg SC, respectively. AH improved the diameter of the seminiferous tubules but decreased the height in SC-positive rats [(297.2±21.64) and (289.0±8.60)] and [100.2±5.03 and 96.8±4.20] compared with SC alone (194.0±51.05 and 60.0±15.26, respectively). SC decreased the Leydig cell diameter (6696.05±2525.53), AH 1000 significantly reversed this (8218.53±1291.49) to above those of the negative control (7584.18±816.46)**.** Serum testosterone levels were lower in the SC group (2.53±1.88) than in the normal group (3.28±1.74), but these levels were improved by AH (4.596±0.73 and 5.46±2.55). Histological examination of the testes revealed increased spermatogenesis and Leydig cell proliferation with a higher dose of extract.

Conclusion: This study revealed that the ethanolic seed extract of *A. heterophyllus* affects male rat reproductive system function by improving sperm parameters, testicular volume, seminiferous tubule characteristics, Leydig cell volume and serum testosterone levels. AH also improved all these parameters in SC-positive rats in an SC dose-dependent manner.

Keywords: Jackfruit, *Artocarpus heterophyllus*, sildenafil citrate, male fertility, testicular morphology, serum testosterone, Leydig cells, seminiferous tubules

Introduction

Erectile dysfunction (ED) is the inability to obtain a sufficient erection or maintain an erection firm enough for satisfactory coitus. One in every ten men is affected by ED regardless of their age (1). The condition can be caused by vascular or neurogenic abnormalities, psychogenic and hormonal disorders, drugs, and birth defects, and certain lifestyle conditions, such as diabetes, may predispose an individual to ED (2).

Sildenafil citrate (Viagra, SC), which is commonly used for the treatment of erectile dysfunction, is a widely abused pharmaceutical drug. It is a water-soluble drug originally designed by Pfizer, a pharmaceutical company, to treat angina pectoris and hypertension (3). However, it was later discovered to cause long-lasting erections among volunteers, and as a result, it became the standard drug for the treatment of erectile dysfunction (4). As the first successful oral treatment for erectile dysfunction, SC appeals to patients who are seeking treatment for the first time. It increases the tolerance and frequency of erection, subsequently improving sexual satisfaction between partners (5). SC achieves this by inhibiting cGMP-specific phosphodiesterase type 5, an enzyme responsible for hydrolyzing cyclic guanosine monophosphate (cGMP) in the corpus cavernosum, the penile erectile tissue. Consequently, increased cGMP causes smooth muscle relaxation and inflow of blood to the corpus cavernosum, triggering penile erection (6). The revolutionary impact of Viagra has had drawbacks over the years. The long-term administration of sildenafil is associated with oxidative stress, inflammation, and structural changes in different parts of the body (7). In the testes, sildenafil has been reported to cause tubular and interstitial histological alterations of the seminiferous tubules and tubular degeneration (8), which could ultimately result in arrested spermatogenesis.

Artocarpus heterophyllus (AH) commonly known as Jackfruit, has been highly regarded for many ages due to its healing and nutritional properties (9). Its beneficial effects on the body, due to its numerous physicochemical components, have made it widely accepted as a preventive and restorative compound against various pathologies (10). In Uganda, Jackfruit is found throughout the country and is highly esteemed by the majority of the people (11). Seeds and pulp are the only edible parts, with an abundance of carbohydrates, proteins, vitamins, minerals, and bioactive compounds (10). Its various phytonutrients

such as carotenoids, phenolic compounds, flavonoids, and Vitamin C act as antioxidants. In Asia and some parts of Africa, the roasted seeds are considered aphrodisiacs. Several studies have been carried out to determine the effect of AH in rat models. For example, oral administration of 500 mg/kg of AH enhanced sexual desire, arousal, virility and performance in a rat model of sexual dysfunction. These effects persisted for about six hours before the offset of action was recorded (12). AH is known to increase testicular weight, sperm count and motility in a dose-dependent manner by decreasing DNA damage and increasing the total proteins. It also decreases oxidative stress by reducing levels of malondialdehyde and increasing those of catalase in rats (13). A combined extract studying the effects of *Annona muricata* and AH on reproductive parameters of type 2 diabetic Wistar albino rats improved testosterone levels (14). This was attributed to the antioxidant activity of the plant's leaves. AH has also been used in combination with standard drugs to protect against their side effects. For example, green AH flour reduces chemotherapy-induced leukopenia in cancer patients (15) However, studies on the East African variety of AH have focused only on its nutritional value and have not extensively addressed its effect on reproductive function and whether it could ameliorate the effects of SC on male reproductive function. Therefore, this study was guided by the following objectives**:**

1. To determine the effects of *A. heterophyllus* on the histomorphology, morphometry and function of the rat testis
2. To investigate the effects of *A. heterophyllus* on histomorphological, morphometric and functional alterations in the rat testis induced by sildenafil citrate

Materials and methods

Study Design

The study was carried out at the Institute of Biomedical Research Laboratory and Histology Laboratory of Kampala International University Western Campus.

Plant collection and identification

The AH fruit were cultivated in home garden belonging to Mzee Mgosi in the degraded coastal thickets of Dar es Salaam, Tanzania. Plant authentication was performed in the Herbarium (UDSM Herbarium) of the Department of Botany, University of Dar es Salaam by Frank Mgalla Mbago on 14th August 2023, under the following details;

Moraceae
Artocarpus heterophyllus Lam.
(Mfenesi- Swahili)
T6: Dar es Salaam, Kinondoni, Changanyikeni area
Altitude:83m GPS 37m 0520859/9251365
Cultivated in home garden belonging to Mzee Mgosi in
degraded coastal thickets.
Tree 7m tall. Stem slash produces white latex

Collectors: F. M. Mbago/ John Juma Ochieng
Collector number: FMM 4271 **Date: 14th August 2023**

Herbarium: UDSM Herbarium
Institution: UNIVERSITY OF DAR ES SALAAM
Department: Department of botany
Investigator: Frank Mgalla Mbago
mbagomgalla@gmail.com
mbago@udsm.ac.tz

The fruit was excised, the seeds cleaned and the seed coat peeled off. The seeds were dried for 14 days (at 60 °C) at room temperature, followed with slicing using with knife and dried. The dried seeds were blended using an electric blender (Olsemark OMSB2361-China) to produce a coarse powder. One hundred grams of the powder was dissolved in one liter of ethanol. This process was repeated to obtain an adequate stock solution for the experiment. The mixture was homogenized, kept on an orbital shaker for 72 hours, and later filtered through qualitative-grade Whatman No. 1 filter paper. The solvent was evaporated in an oven to recover the extract. The concentrated extract was kept in the freezer before use at 4 degrees Celsius (16).

Ethics Approval

Approval and authorization were sought and obtained from the Research Ethics Committee (REC) of Kampala International University- Western Campus, Uganda, and were registered as Nr. KIU-2021-20. The animals were cared for and handled in principle in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals (17).

147 **Experimental animals**

148 Thirty-six experimental animals (Wistar rats) were obtained from Kampala International University
149 Animal House. The animals were kept under standard laboratory conditions of a 12 h/12 h light/dark cycle
150 at room temperature with free access to food and water until they reached a mean body weight of 200 ±
151 2.51 g, upon which treatment was initiated per group. Before experimentation, the animals were allowed
152 to adapt to the new environment for a period of two weeks. The rats were allowed access to standard rat
153 pellets and water freely during the experiment.

154 **Experimental design**

155 Animals (N=36) were randomly distributed into six treatment groups (n=6), A (negative control) and B-
156 F (experimental), which were treated as follows (**Table 1**). Treatments were initiated on the day of the
157 grouping, which was marked as Day 1.

158 **AH and SC administration**

159 The seed extracts of AH and SC were administered orally via an orogastric tube (Kojak Selinge-India).
160 The dosage was adjusted to the animal's body weight. Individual drugs were weighed from each animal
161 and then mixed in distilled water, which served as a vehicle.

162 **Euthanasia and sample collection**

163 All animals were euthanized at the end of the different treatments (day 60). Before dissection, the animals
164 were exposed to a 4% halothane gas in oxygen anesthetic chamber until they were deeply anesthetized.
165 Loss of responsiveness to a toe pinch, loss of righting reflex and loss of intentional movements were used
166 to gauge the depth of anesthesia. A laparotomy was then performed to dissect the testes. One testis from
167 each animal was used for histological examination, and the other was used for testicular morphometry.
168 Blood samples were also collected via cardiac puncture for hormonal assays. Confirmation of death was
169 done by fixed and dilated pupils and absence of a heartbeat for two minutes.

170 **Epididymis preparation and seminal fluid collection and analysis**

171 The epididymis was carefully trimmed from the testis. A sperm sample was collected from the caudal part
172 of the epididymis by making several incisions of approximately 1 mm with a scalpel blade (Unilab Kenya
173 Ltd.) and then placed in 1 ml of Ham-F-10 solution. Estimates of motility and counts were carried out at
174 room temperature. The sperm count was determined via a hemocytometer (Marienfeld Germany), where
175 the epididymal sperm sample was diluted at a ratio of 1:1 with 10% neutral buffered formalin (Impact

chemicals-Nairobi, Kenya). The resulting mixture was then mixed with 1% trisodium citrate (Loba Chemie PVT Ltd-Nairobi, Kenya). I ml of the solution was drawn and placed on a hemocytometer (Marienfeld, Germany) for the determination of motile and nonmotile sperm via a light microscope (Carl Zeiss Microimaging GmbH AxioCam ERc 5 s SN: MKG 3383-Germany with a camera attached) at 400x magnification (18). Total motility was expressed as the percentage of progressively motile spermatozoa, immotile spermatozoa, and dead spermatozoa. This process was repeated before the average was calculated. Sperm morphology was determined by staining the sperm samples with Wells and Awa stains and then examination under a light microscope (Carl Zeiss Microimaging GmbH AxioCam ERc 5 s SN: MKG 3383-Germany with camera attached) at x400 to study the sizes and shapes of the samples by two independent individuals (19).

Histopathological examination of the testis

The testes were collected and fixed in a neutral buffered formaldehyde solution for microscopic examination. They were run in an automated tissue processor through which they were dehydrated in ascending grades of isopropyl alcohol 70, 80, 95% and absolute alcohol (Euro Chemical Industries). The tissues were subsequently cleared in xylene and embedded in paraffin wax. A rotary microtome (Leica RM2135) was used to slice the waxed tissues into 4 µm sections for light microscopy (above). The tissues were subjected to routine H&E and PAS and Masson's trichrome staining (20). The stained sections were observed under a compound light microscope (Zeiss Primo Star-Germany), and photomicrographs were captured via an Olympus CX41 light microscope with a camera attached for photomicrography (Carl Zeiss MicroImaging GmbH AxioCam ERc 5 s SN: MKG 3383-Germany) (21).

Morphometric analyses

Seven vertical sections were studied for each testis. By employing a systematic random scheme method, the following morphometric parameters were examined: tubular diameter (lumen) of the seminiferous tubules, height/thickness of the seminiferous epithelia, and seminiferous tubule basement membrane thickness as follows:

Testes size and volume

Testicular width and testicular length were measured with the aid of digital Vernier calipers (Bios) (22). Archimedes's principle was adopted for irregularly shaped objects and was used to measure the volume of testes that were not destined for processing (23). The testis was carefully placed in a cylinder that had

been graded and filled with a specific amount of distilled water. The volume of the testis was determined by subtracting the initial volume from the final volume of water via the equation below:

Testis volume (Vt) = final volume (Vf) – initial volume (Vi) (cm3)

Seminiferous tubule basement membrane (STBM) thickness

Serial sections of the testes were dyed with PAS and examined under a microscope with an image and video camera analyzer system (AxioCam ERc5s). The calculations were performed on thirty randomly selected samples from three randomly taken sections of STBM thickness and averaged (24).

Leydig cell morphometry

Leydig cell volume was approximated from the Leydig cell nuclear volume, which was estimated from the diameters of 30 spherical nuclei of each animal. The Leydig cell nuclear volume was expressed in cubic micrometer (μm3) units and calculated via the following formula:

Leydig V = (4/3) π R3

where R = nuclear diameter/2

Measurement of the serum testosterone concentration

Blood was drawn by cardiac puncture into heparinized tubes for hormone analysis. The blood samples were obtained in the morning because hormones, including testosterone, show high diurnal variability, with peak levels occurring in the morning [29]. The blood was centrifuged at 3000 rpm for 15 minutes via a SUNCOO benchtop centrifuge machine. Plasma was used for the testosterone assay via ELISA kits (Narcolab-Nairobi Kenya).

Statistical analysis and expected results

Microsoft Excel 2013 was used for data entry and analysis. Differences between study groups were determined via one-way ANOVA or Tukey's post hoc test methods, and P values < 0.05 were considered statistically significant. The data are presented as the means ± standard deviations.

Results

Changes in body weight

The final body weight of the rats in all the groups was greater than the initial weight (**Table 1**), with the greatest change in body mass observed in group B (87.68 ± 1.55 g) and the lowest change observed in

group D (60.32 ± 2.36 g). However, the differences in body weight changes among the groups were not significant.

Table 1: Experimental design and body weight changes

Group n=6	Treatment	Initial body mass (g)	Final body mass (g)	Body mass change (g)
A	**Normal saline solution**	199 ± 1.23	281.28 ± 3.21	82.28 ± 2.22
B	**AH 500**	197 ± 0.98	284.68 ± 2.12	87.68 ± 1.55
C	**AH 1000**	200 ± 1.73	285.16 ± 1.97	85.16 ± 1.85
D	**SC 50**	199 ± 2.34	259.32 ± 2.37	60.32 ± 2.36
E	**AH 1000 + SC 50**	200 ± 0.65	286.24 ± 3.11	86.24 ± 1.88
F	**AH 1000 + SC 100**	202 ± 0.31	278.16 ± 1.39	76.16 ± 0.85

A (negative control), C (positive control for part B), SC= sildenafil citrate, AH= *Artocarpus hetrophyllus* ethanoic seed extract. The data are presented as the means ± standard deviations.

A. Histological results

a. H&E Results

Sections from group A revealed rounded seminiferous tubules that had regular outlines of round oval shapes. The seminiferous tubules were lined by stratified germinal epithelium. The lumen of the seminiferous tubule, which is surrounded by a thick layer of cells, is packed with spermatozoa and their residual flagella. The interstitial spaces contained interstitial Leydig cells, fibrocytes, loose connective tissue, and blood vessel walls. The Sertoli cells and cells of the spermatogenic series form the germinal epithelium, which rests on the basement membrane. The cells adjacent to the basement membrane were spermatogonial cells. Large and rounded primary spermatocytes were prominent at the lower level of the tubule, whereas spermatids with darkly stained rounded nuclei faced the lumen of the tubules. Testicular sections from groups B and C revealed closely packed seminiferous tubules with seemingly thicker germ cell layers at different stages of spermatogenesis. The interstitial spaces exhibited pale staining due to the accumulation of lipid droplets in testosterone-producing Leydig cells. Certain spaces, however, revealed conspicuous Leydig cells with a distinct foamy appearance. Vascular elements were widely visible within

the interstitial spaces. Group D histological sections revealed seminiferous epithelium of varying thickness. The decreased thickness of certain epithelia was due to arrested spermatogenesis, which was demonstrated by the empty lumens. The cells were detached from the thickened basement membrane. The interstitial spaces were widened and appeared homogeneously translucent (hyalinization) with congested blood vessels. Group E microscopic examination revealed a reverse effect on changes in testicular structure. The interstitium appeared vascularized. Closely packed seminiferous tubules with regular outlines were evident. Slight epithelial detachment and increased spermatogenesis appeared in most seminiferous tubules. Group F presented with altered seminiferous tubules in the form of necrosis. Interstitial spaces appeared overly dilated and congested with blood. Signs of hyalinization were apparent, and the germinal epithelium in certain areas had undergone desquamation. (**Figure 1**).

b. PAS

The PAS results revealed a moderate distribution of PAS-positive material in the basement membrane of the seminiferous tubules and the germinal epithelium in group A. The PAS-stained sections in group B presented increased affinity for the basement membrane and seminiferous tubules (**Figure 2**). The affinity of the basement membrane for PAS-positive material was more intense in groups C and E. Groups D and F presented a dull irregular basement membrane, suggesting alterations in the basement membrane structure and therefore fewer spermatogenic cells.

c. Masson's Trichrome

Trichrome staining revealed fine collagen fibers in the connective tissue spaces, tunica vasculosa and seminiferous tubule basement membrane of the testis. In the groups given the extract, collagen was less preserved and completely removed in some of the groups. In Group D, the basement membrane within the stroma and testicular capsule of the testis was thickened the most (**Figure 3**).

B. Effects of *AH* on histomorphological, morphometric and functional alterations in the rat testis

To determine the potential ameliorative effect of the ethanoic seed extract of *A. heterophyllus* on the histomorphological, morphometric and functional alterations in the rat testis induced by SC, we sought to assess its effects on the same parameters.

a. Effects of AH on sperm count, motility and morphology

Compared with Group A rats, Group B and C rats presented increased sperm counts (Table 1). Compared with low concentrations, high concentrations of AH increased the sperm count (group C, 10.906±0.92) (group B, 9.22±0.83), but the difference was not statistically significant. However, the increase in the sperm count induced by high levels of AH compared with the normal control (group A, 6.048±0.47) was statistically significant ($P< 0.05$).

AH also improved sperm motility in Group B and C rats (31.348±1.05 and 32.606±1.02, respectively), and although the increase was not significant between the two groups, it was statistically significant for both groups compared with Group A (27.888±0.81).

Analysis of the morphological characteristics of the sperm induced by AH revealed an increased number of normally shaped sperm compared with those in group A (Table 1). Compared with those in group A (0.058±002), more normal-shaped sperm (0.0914±0.02 and 0.0722±0.02) were observed in groups B and C, respectively. Statistical significance was noted between group C and group A.

Table 2: Seminal fluid analysis

Group	Sperm Count	Sperm motility	Sperm Morphology
A	6.04±0.47	27.888±0.81	0.058±0.02
B	9.22±0.83	**31.348±1.05α**	0.0722±0.02
C	**10.91±0.92α**	**32.606±1.02α**	**0.0914±0.02α**
D	**5.47±1.46α**	**23.414±6.63α**	0.031±0.02
E	**10.54±1.48αδ**	**31.516±0.99**αδ	**0.0781±0.02δ**
F	**9.36±0.36δ**	**27.314±1.45δ**	0.049±0.03

The data are presented as the means; α and δ indicate significant differences compared with groups A and D, respectively ($P < 0.05$) (Tukey–Kramer multiple range post hoc test).

b. Effects of AH on testicular morphometric indices in rats (Table 3)

Testicular gonadosomatic index studies revealed alterations in the morphometric indices (**Table 3**) in the groups that were treated compared with those in the normal control group. For example, AH administration (B and C) increased both testicular size (2213.33±308.51 and 22254.59±356.33,

respectively) and volume (3.57±0.10 and 3.71±0.18, respectively) compared with those of the normal control group (2194.69±275.20 and 3.54±0.11, respectively). However, these changes were not statistically significant. There was a nonsignificant increase in the testicular length of groups B (20.804±0.93) and C (21.002±1.30) compared with that of group A (20.662±0.69). However, the testicular width was greater and significantly greater in group C (15.804±0.88) than in groups B (14.72±0.66) and A (13.098±0.72).

The seminiferous tubule diameter also showed a similar pattern, where an increase was observed in both AH-administered groups, with a statistically significant increase observed between groups A (291.0±7.00) and C (297.8±13.09) at ($P>0.05$) (Table 3). Additionally, there was an increase in the number of seminiferous tubules and basement membrane heights in groups B (99.7±4.17 and 1.64±0.22, respectively) and C (101.3±7.09 and 1.67±0.22, respectively) compared with those in group A (97.2±5.47 and 1.52±0.29, respectively). The difference in seminiferous tubule height between groups A and C was statistically significant.

Administration of AH also increased the Leydig cell nucleus diameter (LCND) and volume (LCNV). For example, there was an increase in LCND between groups A (24.04±0.77) and B (25.17±0.91), which was not significant (**Table 3**). However, the increase between A and D (28.09±0.92α) was statistically significant ($P<0.05$). The changes in the LCNV followed the same pattern, i.e., statistically significant differences between A (7584.18±816.46) and C (8401.68±919.92α).

c. Effect of AH on serum testosterone levels

Like other parameters, the administration of AH led to a dose-dependent increase in the serum testosterone level. However, the increase was not significant between groups A (3.28±1.74) and B (4.12±1.89), and that between groups A and C (5.98±1.61α) was statistically significant.

Table 3: Testicular gonadosomatic indices, morphometric indices and serum testosterone

GP	TS	TV	TL	TWd	STD	SEH	STBM	LCV	ST
A	2194.69±275.20	3.54±0.11	20.66±0.69	13.09±0.72	291.0±7.00	97.2±5.47	1.52±0.29	7584.18±816.46	3.28±1.74
B	2213.33±308.51	3.57±0.10	20.80±0.93	14.72±0.66	294.2±6.62	99.7±4.17	1.64±0.22	7617.9±831.23	4.12±1.89
C	2254.59±356.33	3.71±0.18	21.00±1.30	**15.80±0.88 α**	**297.8±13.09 α**	**101.3±7.09 α**	1.67±0.22	**8401.68±919.92 α**	**5.98±1.61 α**
D	2168.82±606.35	2.63±0.70	18.53±4.77	11.86±3.00	194.0±51.05	**60.0±15.26α**	2.24±0.64	6696.05±2525.53	2.53±1.88
E	2214.48±243.12	3.68±0.11	20.75±0.55	13.93±0.75	**297.2±21.64 Aδ**	**100.2±5.03δ**	1.46±0.24	**8218.53±1291.49 αδ**	**5.46±2.55 αδ**
F	2195.8±307.60	3.55±0.17	18.80±0.98	13.80±0.83	**289.0±8.60δ**	**96.8±4.20 δ**	1.24±0.09	76226.66±1178.98	4.596±0.73

The data are presented as the means ± standard deviations; α and δ indicate significant differences compared with groups A and D, respectively ($P < 0.05$) (Tukey–Kramer multiple range post hoc test); GP = group; TS = testicular size; TV = testicular volume; TL = testicular length; TWd = testis width; STD = seminiferous tubule diameter; SHE = seminiferous epithelium height; STBM = seminiferous tubule basement membrane; LCV = Leydig cell volume; and ST = serum testosterone.

C. The effects of *AH* on histomorphological, morphometric and functional alterations in the rat testis induced by SC

Having confirmed the improvement in seminiferous tubules, Leydig cells and serum testosterone levels induced by AH 1000, we used AH 1000 as a positive control to investigate whether it could ameliorate the histomorphology, morphometric and functional deficits induced by long-term administration of SC. The treated subgroups included rats that received 500 mg/kg AH or 1000 mg/kg AH simultaneously with 50 mg/kg SC orally daily for 60 days. We obtained the following results:

a. Effects of AH on sperm count, motility and morphological changes induced by SC

Compared with those in the negative control group, the sperm count (3.47±1.46), motility (23.414±6.63) and number of normally shaped sperm (0.038±0.02) **in group D were significantly lower (Table 2)**. However, the administration of AH 1000 after SC administration improved all the parameters. For example, there was a statistically significant increase in the sperm count in groups E (**10.54±1.48)** and F (9.36±0.36) compared with that in group D. This increase depended on the dose of SC given, with a greater increase noted for lower doses. The improvement in sperm motility in both groups (E and F) also surpassed that of the negative control (group A, 6.04±0.47), and the increase was statistically significant for group E.

There was a statistically significant decrease in sperm motility in group D (23.414±6.63) compared with that in group A (27.888±0.81). AH combined with SC improved sperm motility in groups E (**31.516±0.99**) and F (27.314±1.45), and this improvement was statistically significant in both groups (Table 2) compared with that in group D (23.414±6.63). The improvement in group F was, however, still lower than that in group A.

With respect to sperm morphology, group D (0.031±0.02) had the lowest number of sperm with normal shapes compared with group A (0.058±0.02). There was improvement in sperm morphology in both groups E (**0.0781±0.02**) and F (0.049±0.03), with the greatest and statistically significant improvements occurring at lower concentrations of SC (group E). However, the improvement in group F was still lower than that in group A (0.058±0.02).

b. Effects of AH and SC coadministration on testis morphometric indices in rats

SC administration alone (group D) decreased all the testicular morphology parameters (**Table 3**), although the decrease was not statistically significant compared with that in all the other groups. However, the administration of AH to SC rats (groups E and F) slightly improved all the parameters in a manner dependent on the concentration of SC administered, with values closer to or slightly greater than those of the negative control (group A).

AH improved the STD when co-administered with SC in both groups E (297.2±21.64), and F (289.0±8.60) increased the seminiferous tubule diameter compared with that in group D (194.0±51.05), and this difference was statistically significant for both groups. In addition, the improvement in group E was also statistically significant compared with that in group A (291.0±7.00). There was a statistically significant decrease in the seminiferous epithelial height between group D (60.0±15.26) and the negative control group (A, 97.2±5.47). However, the administration of AH in groups E and F significantly improved the SEH to 100.2±5.03 and 96.8±4.20, respectively. The seminiferous tubule basement membrane was the largest in group D (2.24±0.64), although it was not significantly different from that in any of the other groups.

The diameter of the Leydig cell nucleus was lower in group D (22.76±5.72) than in group A (24.04±0.77). This, however, improved with the administration of AH to 23.24±0.88 and 27.64±0.95 for groups F and E, respectively, with the latter being statistically significant compared with the D values of the other groups. A similar pattern was also observed for the Leydig cell nucleus volume, with significant differences noted only between groups D (6696.05±2525.53) and E (8218.53±1291.49) and between group E and the negative control (group A, 7584.18±816.46).

c. Effect of the coadministration of AH and SC on serum testosterone levels

SC decreased the serum testosterone level to 2.53±1.88 (group D) instead of 3.28±1.74 (group A). However, the administration of AH to SC-positive rats increased the ST to 4.596±0.73 (group F) and 5.46±2.55 (group E), with the improvement being statistically significant in group E rats compared with that in group D. The increase in the ST levels in the SC + AH groups was greater than that in the negative control (group A, 3.28±1.74), although the difference was not statistically significant.

Discussion

Erectile dysfunction is a common condition, with a majority of those affected not seeking medical care (25). Lifestyle changes such as regular physical exercise and a healthy diet are good treatment options (26). However, many ED patients prefer oral medications because they are fast-acting and readily available (27). Many resort to the use of SC, which greatly improves their sexual lives. This, however, has several side effects, including headache, dizziness and diarrhea; vision changes; tinnitus; respiratory failure; and sexual dysfunction, including a reduced sperm count and testosterone levels and priapism (28). Therefore, novel ED treatment options with fewer side effects or combination therapies of SC and other components that reduce its side effects are highly desirable.

In the present study, compared with the negative control, AH alone increased the sperm count, motility, and shape in a dose-dependent manner. AH at a dose of 1000 mg/kg significantly increased testicular width, seminiferous tubule diameter, epithelial height, tubule basement membrane diameter, Leydig cell volume and serum testosterone compared with those of the normal control. These findings are consistent with those of Ranasinghe et al., 2019, who reported that the ingestion of AH has several nutritional and medical benefits (9). According to Ratnasooriya and Jayakody (2002), nontoxic doses of AH transiently decrease libido, sexual arousal, vigor and performance, coupled with mild erectile dysfunction, but do not inhibit male fertility in rats (12). The increase in body weight and testicular weight at high doses of the extract suggests possible effects of the drug on metabolic activities because of increased testosterone levels, as confirmed in this study. Testosterone leads to tissue regeneration and muscle building, which leads to a general increase in body mass index (29). Indeed, the results of the present study confirmed that this was due to the dose-dependent increase in body weight and testicular weight in the AH and AH + SC groups compared with the normal control and SC alone groups, respectively. Additionally, the increased testicular weight noted during this study could be due to an increase in the number and volume of Leydig cells, spermatozoa and Sertoli cells. Our study confirmed an increase in both Leydig cell and sperm counts in all AH-treated groups compared with the controls. Physiologically, the increased number and volume of Leydig cells translates into increased levels of testosterone, which stimulates spermatogenesis. This subsequently leads to a high sperm count, as observed in our study. However, these findings contrast with those of Rizk et al. (2023), who reported no correlation between the number of Leydig cells and testicular weight in postmortem human testicular samples. Other studies strongly correlate testicular volume and function (30) and therefore support the findings of the current study. The AH dose-dependent increase in

sperm count, motility and morphology noted in this study could also be attributed to its stimulation of an increase in both Leydig cell volume and the synthesis and secretion of testosterone. The secretion of gonadotrophin releasing hormone from the hypothalamus and the follicle stimulating and luteinizing hormone from the anterior pituitary gland significantly improves male fertility (31) and could be a point of action of AH in increasing all the abovementioned parameters, including its effect on the seminiferous tubules. In addition, morphometric data demonstrating the thickness of the germinal epithelium also confirmed a significant increase in the height and diameter of the seminiferous epithelia and lumen, respectively, supporting the increased sperm concentration. The findings from the semen parameters observed in this study corroborate those of a previous study that established that a polyherbal formulation could produce synergistic efficacy, leading to an increase in the major semen parameters in males with oligospermia (30).

SC has negative effects on testicular tissue by suppressing spermatogenic cells, lowering sperm motility, and distorting the shape of spermatozoa (32), which this study confirms. For example, compared with the normal control, the administration of 50 mg/kg SC alone decreased testicular size, volume and width. This could be due to decreases in the seminiferous tubule diameter, epithelial height and Leydig cell volume in this study. In a similar study, Zaghlol et al. (2020) reported impaired spermatogenesis, seminiferous tubule necrosis and a thicker seminiferous tubule basement membrane after long-term SC administration (33). This then translates into a reduction in the sperm count and the number of motile sperm and a decrease in the number of normally shaped sperm in our study. Furthermore, the histopathology results revealed a dull irregular basement membrane in the rats that were administered SC. This means that the carbohydrate content, mucopolysaccharides, was possibly affected by changes in either the basement membrane or the germinal epithelium. Glycogen remains the primary source of energy in the testis (34), and it is a vital element in maintaining the structure of tissue components as well as in the contraction of tubular walls via myoid peritubular cells. Additionally, glycogen is strongly expressed at the proximal end of elongated spermatids for acrosome formation (35). The SC disrupts seminiferous tubule nutrition by leading to intertubular edema, which subsequently causes lysis of spermatogenic cells (36). Additionally, with the decrease in the Leydig cell volume induced by SC, there is limited secretion of testosterone and thus decreased spermatogenesis (37).

Co-current administration of AH with SC ameliorated SC-induced testicular morphometric and functional alterations in a dose-dependent manner. For example, the administration of 1000 mg/kg AH after 50 mg/kg

SC improved all aspects of testicular morphometry and function to levels slightly above normal, with greater changes observed at lower levels of AH administration. The increase in Leydig cell volume induced by AH administration in SC-treated rats translated into increased serum testosterone levels, an AH dose-dependent increase in seminiferous tubule diameter and epithelial height and decreased basement membrane thickness compared with those of both controls, highlighting the ameliorative effect of AH in SC-induced testicular dysfunction. These effects of AH are similar to those of selenium when AH is co-administered with SC. Selenium, an essential micronutrient, improves the germinal epithelium and decreases the thickness of the seminiferous tubule basement membrane when it is co-administered with SC because of its antioxidative and antiapoptotic effects (33). Our results are also in agreement with those of Fawsia et al., 2021, who showed that simultaneous ingestion of sesame seeds and SC protected against the testicular histomorphometric changes induced by the administration of SC alone (38). These include an increase in the length of the spermatogenic cell layers, which translates to increased sperm count, and good spermatogonial architecture attainment. These changes were due to increased levels of testosterone resulting from the flourishing Leydig cells in the sesame seed-treated groups, which was attributed to the reactive oxygen species and free radical scavenging abilities of sesame lignans in addition to the many micronutrients that could promote spermiogenesis (38). AH also contains several phytochemicals, such as artocarpertin, betulinic acid, isoartocarpin, artocarpin and heterophylol, which contribute to its anti-inflammatory, antidiabetic, antioxidant and immunomodulatory properties (39). These effects could also be responsible for its ability to ameliorate SC-induced testicular damage.

Conclusion

The results of this study confirm the effects of SC on histomorphometry, morphometry and function by decreasing testicular volume and width, seminiferous tubule diameter, epithelial height and Leydig cell volume. This then translates into a decrease in the sperm count, motility, and number of normally shaped sperm due to a decrease in the serum testosterone level. However, AH was confirmed by this study to improve all these aspects when used alone and to reverse the testicular effects of SC in an SC dose-dependent manner. Thus, when SC is used as a drug to treat ED, coupling it with a testicular histomorphometric and functional protective substance such as AH is recommended to preserve testicular histology and function. This could be the basis for translational research on the use of AH as a testicular protective substance during the treatment of ED via SC in humans.

Declarations

Data availability statement

The authors confirm that the data supporting this study's findings are available within the article, and Figures 1- 3 are available as attachments. Raw data will be provided upon request from the corresponding author.

Competing interests

The authors report no conflicts of interest in this work.

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Author contributions

Conceived and designed the experiments: John Juma Ochieng and Ssegendo Ibrahim; Performed the experiments: John Juma Ochieng; Analyzed the data: Jackline Namulema, Onanuga Ismail Olasile, Ssengendo Ibrahim; Contributed reagents/materials/analysis tools: John Juma Ochieng; Wrote the manuscript: John Juma Ochieng, Jackline Namulema, Victor Archibong, Ssengendo Ibrahim; Final edit of paper: Jackline Namulema; John Juma Ochieng

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Figures

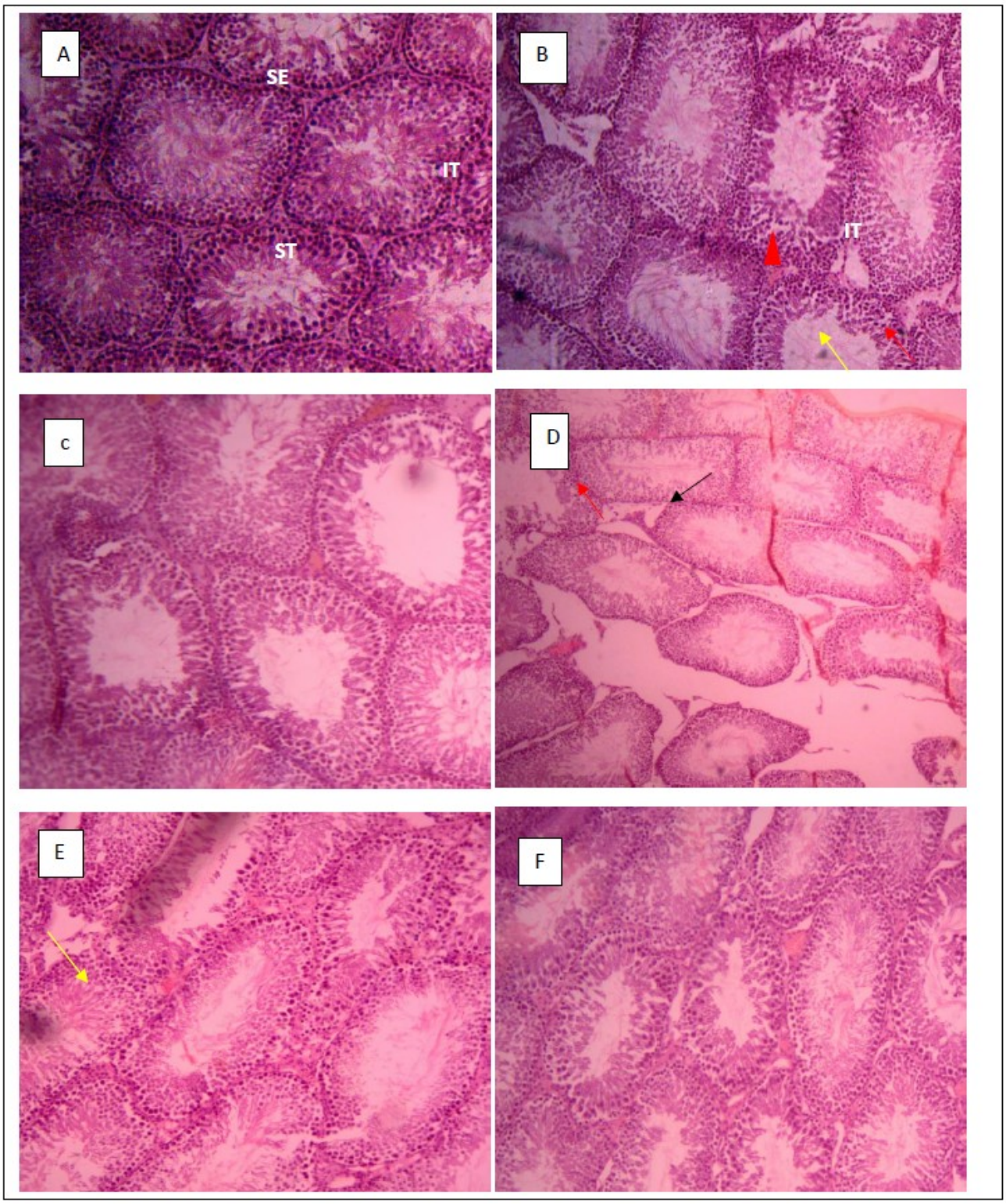

Figure 1

Hematoxylin and eosin stain. Magnification X100: Seminiferous tubules (ST) lined inside by seminiferous epithelium (SE) supported by an intact basement membrane. SE is formed of cells arranged in many layers: Sertoli cells and cells of spermatogenic series: spermatogonia, primary spermatocyte, secondary

spermatocyte and spermatids (round and elongated). Interstitial tissue (IT) of the testis contains blood vessels, connective tissue and Leydig cells

A. Round-oval-shaped ST are densely packed in the testicular parenchyma. Elongated spermatids and spermatozoa fill the lumen of the ST

B. The germinal epithelium showed increased thickness. IT appeared pale staining due to the accumulation of lipid droplets in testosterone-producing Leydig cells. Blood vessels (red arrow) could be seen centered in the IT

C. Closely packed seminiferous tubules with thick SE. The IT is filled with cells of a foamy appearance. Increased vascular elements in the IT

D. Empty ST with thin germinal epithelium and wider lumen. Hyalinized tissue due to increased fibroblast cells was conspicuous, along with congested and dilated blood vessels (yellow arrow). Detached germ cells lined some tubules. Arrested spermatogenesis in some tubules

E. Congested and dilated blood vessels with detached ST. The IT appears full and indistinct with signs of hyalinization. Spermatozoa occupy irregular-shaped ST.

F. Vascularized interstitium with closely packed ST having a regular-shaped outline. Slight epithelial detachment and increased spermatogenesis

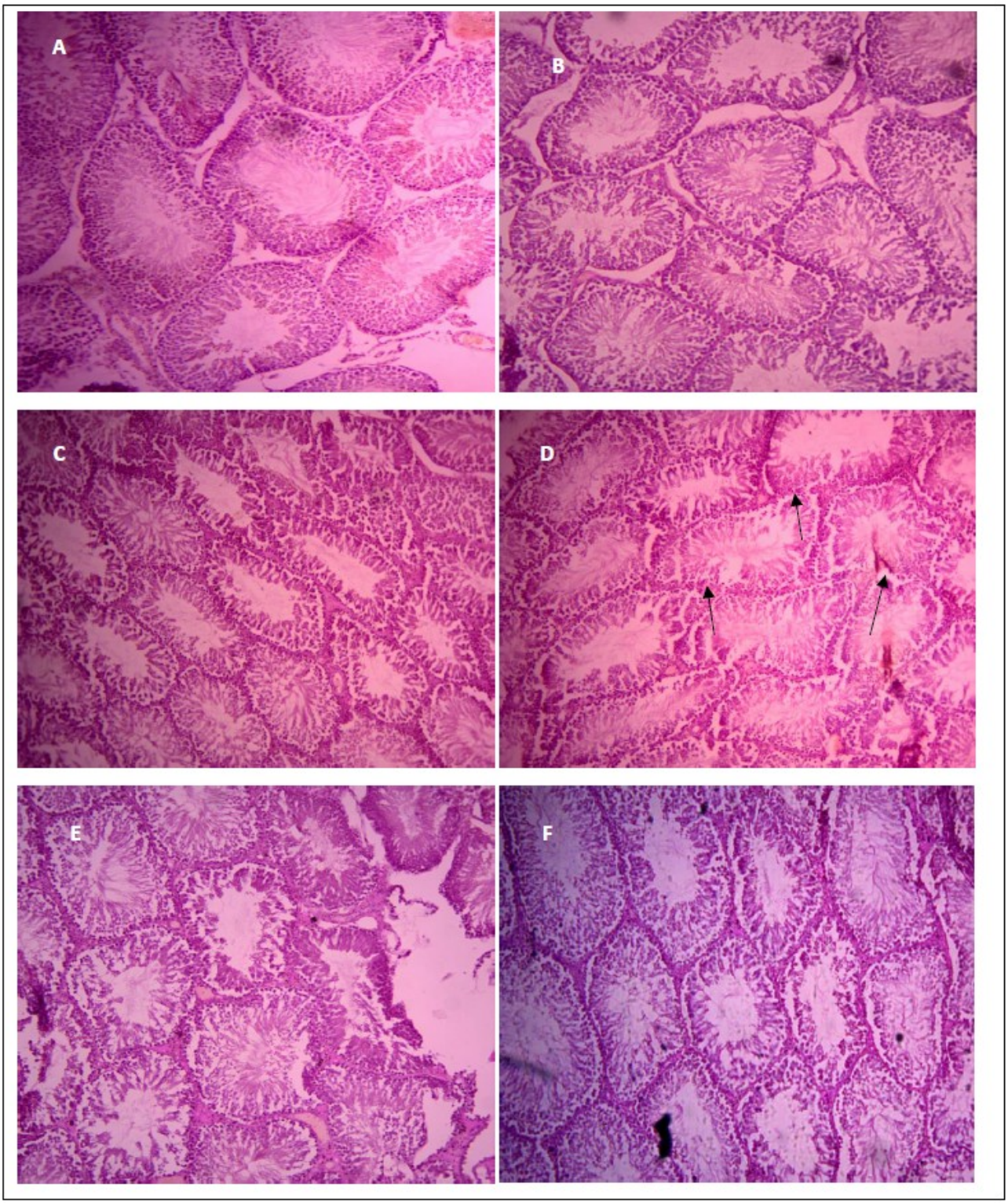

Figure 2

PAS histochemical staining. Magnification X100: A, B, C, E, F showing an apparent strong PAS positive reaction of the basement membrane with Groups C and F being intensely strong. Group D showing weak PAS-positive reaction in the basement membrane (arrows).

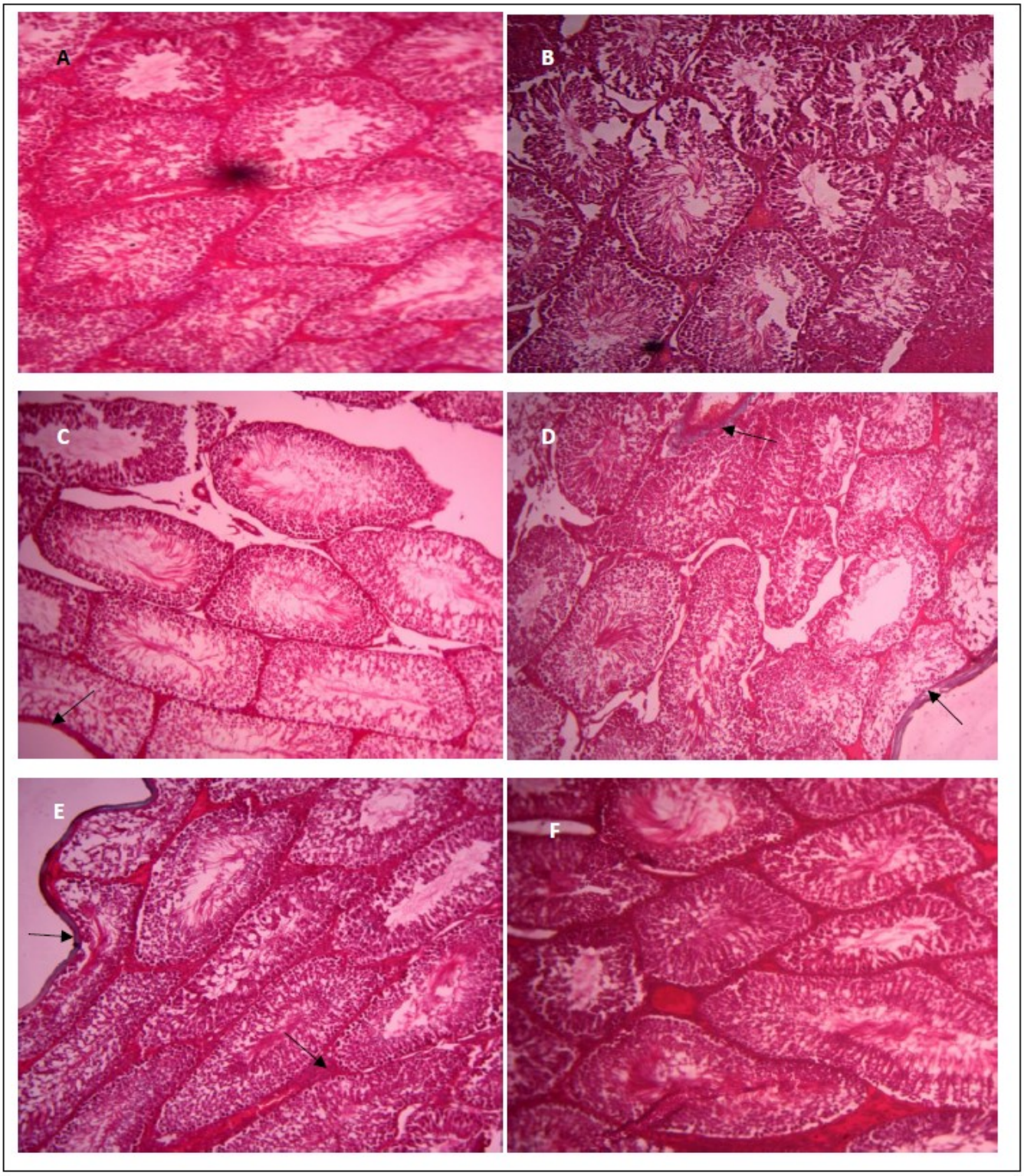

Figure 3

Masson's Trichrome staining of testis. Magnification X100:

A, B and C showing minimal collagen fiber deposition in the tunica albuginea (capsule surrounding testis), the IT and ST basement membrane and blood vessels. The deposition (blue) was intensive in

groups D and E. F showed the least deposition of collagen fibers in the tunica albuginea, the IT and ST basement membrane, and blood vessels.