World Journal of Pharmaceutical and Life Sciences

Impact Factor: 8.21 www.wjpls.org Coden USA: WJPLA7

ANTIFERTILITY AND HEPATIC EFFECTS OF *HYMENOCARDIA ACIDA* STEM-BARK EXTRACT AND FRACTIONS IN MALE WISTAR RATS

Wamanyi Naphtali Wanaunda[1*], Ambrose Teru Patrick[2,3], Abdullahi Usman Wurochekke[1]

[1]Department of Biochemistry, Faculty of Life Sciences, Modibbo Adama University, P.M.B, 2076, Yola, Adamawa State, Nigeria.
[2]Department of Ophthalmology, Julia Laboratory, Suwon, Republic of Korea.
[3]Department of Cellular and Molecular Medicine, School of Medicine, Chosun University, Dong-gu, Gwangju, Republic of Korea.

***Corresponding Author: Wamanyi Naphtali Wanaunda**

Department of Biochemistry, Faculty of Life Sciences, Modibbo Adama University, P.M.B, 2076, Yola, Adamawa State, Nigeria. **DOI:** https://doi.org/10.5281/zenodo.22159920

How to cite this Article: Wamanyi Naphtali Wanaunda[1*], Ambrose Teru Patrick[2,3], Abdullahi Usman Wurochekke[1] (2026). Antifertility And Hepatic Effects Of Hymenocardia Acida Stem-Bark Extract And Fractions In Male Wistar Rats. World Journal of Pharmaceutical and Life Sciences, 12(9), 93–102.

Article Received on 22/07/2026 **Article Revised on 12/08/2026** **Article Published on 01/09/2026**

ABSTRACT

Medicinal plants remain an important source of leads for the regulation of male fertility. This study evaluated the antifertility and hepatic effects of the methanol stem-bark extract (ME) and its aqueous (AF), hexane (HF) and dichloromethane (DCF) fractions of Hymenocardia acida in male Wistar rats. Phytochemical screening was performed on the methanol extract, acute toxicity (LD_{50}) was assessed over 1000–5000 mg/kg, and rats (n = 5 per group) were treated orally at 150, 300 and 450 mg/kg for 28 days before serum liver enzymes, reproductive hormones and epididymal sperm parameters were compared with a normal-diet control. Phytochemical analysis revealed phenols (12.47 ± 0.67%), tannins (7.10 ± 0.67%), saponins (3.28 ± 0.67%) and alkaloids (3.01 ± 0.33%) as the most abundant constituents. No mortality occurred up to 5000 mg/kg. All treatments significantly ($p < 0.05$) reduced epididymal sperm count (from 6.44 ± 0.74 to as low as 1.38 ± 0.37 $\times 10^6$ /mL), viability and progressive motility, and disrupted the reproductive hormones (reduced testosterone and luteinising hormone; elevated follicle-stimulating hormone and estrogen). Serum AST, ALT and ALP were significantly elevated, indicating dose-dependent hepatic stress. In conclusion, the stem-bark extract and fractions of H. acida exert a potent antifertility effect in male rats through disruption of the hormonal control of spermatogenesis, but with evidence of hepatotoxicity that must be weighed in any contraceptive application.

KEYWORDS: Hymenocardia acida; antifertility; spermatogenesis; reproductive hormones; Hepatotoxicity; medicinal plants.

INTRODUCTION

Rapid population growth remains a major public-health challenge, particularly in developing countries, where acceptable and reversible contraceptive options for men are still limited. A large proportion of the population in these regions relies on traditional plant-based medicines for primary health care, and medicinal plants continue to attract attention as sources of male antifertility agents because of their availability, lower cost and generally milder side-effect profiles compared with synthetic steroidal contraceptives.[1,2] An understanding of the normal male reproductive tract and of spermatogenesis (Supplementary Figures S1 and S2) is essential to appreciate how such agents act.

Male fertility depends on the tightly regulated hypothalamic–pituitary–gonadal (HPG) axis (Supplementary Figure S3). Gonadotropin-releasing hormone drives pituitary secretion of luteinising hormone (LH) and follicle-stimulating hormone (FSH); LH stimulates Leydig-cell synthesis of testosterone, which acts through the androgen receptor on Sertoli cells to sustain spermatogenesis, while FSH supports Sertoli-cell function.[3,4] Testosterone is essential for maintaining the blood–testis barrier and the ectoplasmic

specialisations that anchor developing spermatids; disruption of this hormonal balance, or direct spermicidal action, therefore provides a rational basis for antifertility intervention. Male infertility itself is multifactorial (Supplementary Figure S4).

Hymenocardia acida Tul. (Phyllanthaceae) is a savannah shrub or small tree widely used in African traditional medicine. Earlier reports indicated antifertility and anti-spermatogenic activity of its stem-bark extracts in rats,[5,6] but a systematic comparison of the methanol extract with its solvent fractions, combined with hormonal, sperm and hepatic assessment, is lacking. This study therefore determined the antifertility and hepatic effects of the methanol stem-bark extract and its aqueous, hexane and dichloromethane fractions of *H. acida* in male Wistar rats, with the specific aims of characterising the phytochemical composition, the acute toxicity (LD_{50}), and the effects on serum liver enzymes, epididymal sperm parameters and reproductive hormones.

MATERIALS AND METHODS

Plant material, extraction and fractionation

Fresh stem bark of H. acida was collected from Viniklang, Girei LGA, Adamawa State, Nigeria, and authenticated at the Department of Plant Science, Modibbo Adama University, Yola. The bark was air-dried, pulverised and extracted with methanol; the filtrate was concentrated and stored at 4 °C.[7] A portion of the methanol extract (10 g) was fractionated on a silica-gel column eluted with ethyl acetate: formic acid: water (5: 4: 1) to yield aqueous (AF), hexane (HF) and dichloromethane (DCF) fractions.[8]

Phytochemical analysis

Qualitative screening for alkaloids, tannins, saponins, phenols, flavonoids, terpenoids, steroids, glycosides, proteins, anthraquinones and phlobatannins followed standard methods.[9] Total phenols,[10] alkaloids,[11] tannins,[12] saponins[13] and flavonoids[14] were quantified spectrophotometrically.

Experimental animals and design

Male Wistar rats (100–150 g) obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria, were acclimatised for one week. For acute toxicity, groups received 1000–5000 mg/kg of the extract and were observed for 24 h.[15] For the main study, animals were assigned to a normal-diet control and to treatment groups receiving ME, AF, HF or DCF at 150, 300 or 450 mg/kg body weight orally for 28 days (n = 5 per group). Blood was collected by cardiac puncture under anaesthesia, and the epididymes were harvested for sperm analysis. All procedures complied with institutional guidelines for the care and use of laboratory animals.

Biochemical, sperm and hormonal assays

Serum aspartate and alanine aminotransferases (AST, ALT)[16] and alkaline phosphatase (ALP)[17] were assayed with commercial kits (Randox Laboratories Ltd., Crumlin, UK). Epididymal sperm count, viability (eosin–nigrosin), progressive/active motility, morphology and pH were determined by standard light microscopy (Olympus, Tokyo, Japan).[18,19] Serum testosterone, LH, FSH and estrogen were measured by microwell enzyme-linked immunosorbent assay (Cintron Bioresearch Inc.).[20]

Statistical analysis

Data are expressed as mean ± SEM (n = 5). Group means were compared with the control by Student's t-test using SPSS version 27 (IBM Corp., Armonk, NY, USA); $p < 0.05$ was considered statistically significant. Treated groups differing significantly from the control are marked with an asterisk (*).

RESULTS AND DISCUSSION

Phytochemical composition

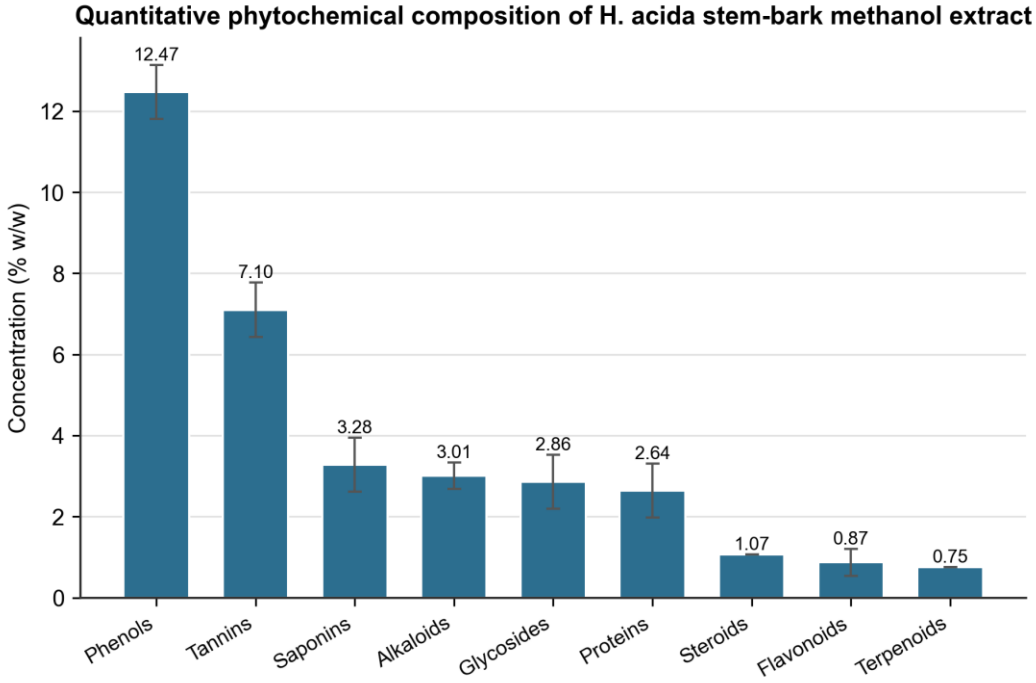
Qualitative screening detected alkaloids, tannins, saponins, phenols, flavonoids, terpenoids, steroids, glycosides and proteins in the methanol stem-bark extract, whereas anthraquinones and phlobatannins were absent (Table 1). Quantitatively, phenols were most abundant (12.47 ± 0.67%), followed by tannins, saponins and alkaloids, with terpenoids, flavonoids and steroids least abundant (Table 1; Figure 1). Phenolic compounds are recognised endocrine-disrupting chemicals that can interfere with hormone synthesis, transport and receptor action and impair sperm concentration, motility and morphology, while alkaloids and saponins have been associated with spermicidal and antispermatogenic activity in other plants; their abundance here provides a plausible chemical basis for the antifertility effects described below.[2,6]

Table 1: Qualitative and quantitative phytochemical composition of the methanol stem-bark extract of *H. acida*.

Phytochemical	Qualitative	Quantitative (% w/w)
Phenols	+	12.47 ± 0.67
Tannins	+	7.10 ± 0.67
Saponins	+	3.28 ± 0.67
Alkaloids	+	3.01 ± 0.33
Glycosides	+	2.86 ± 0.67
Proteins	+	2.64 ± 0.66
Steroids	+	1.07 ± 0.00
Flavonoids	+	0.87 ± 0.33

Terpenoids	+	0.75 ± 0.00
Anthraquinones	–	ND
Phlobatannins	–	ND

(Mean ± SEM, n = 5). + present; – absent; ND not detected.



(Mean ± SEM, n = 5).

Figure 1: Quantitative phytochemical composition of the *H. acida* methanol stem-bark extract.

Acute toxicity

No mortality or overt signs of toxicity were observed over the 1000–5000 mg/kg range within 24 h (Table 2), indicating that the oral LD_{50} exceeds 5000 mg/kg and that the extract is of low acute toxicity. This wide acute-safety margin is consistent with the traditional use of the plant and supports its further evaluation, although it does not preclude sub-chronic organ toxicity (below).

Table 2: Acute toxicity (LD_{50}) of the methanol stem-bark extract of *H. acida* over 24 h.

Group	Dose (mg/kg)	Mortality	Observation
G1	– (control)	0/5	No abnormal behaviour
G2	1000	0/5	No abnormal behaviour
G3	2000	0/5	No abnormal behaviour
G4	3000	0/5	No abnormal behaviour
G5	4000	0/5	No abnormal behaviour
G6	5000	0/5	No abnormal behaviour

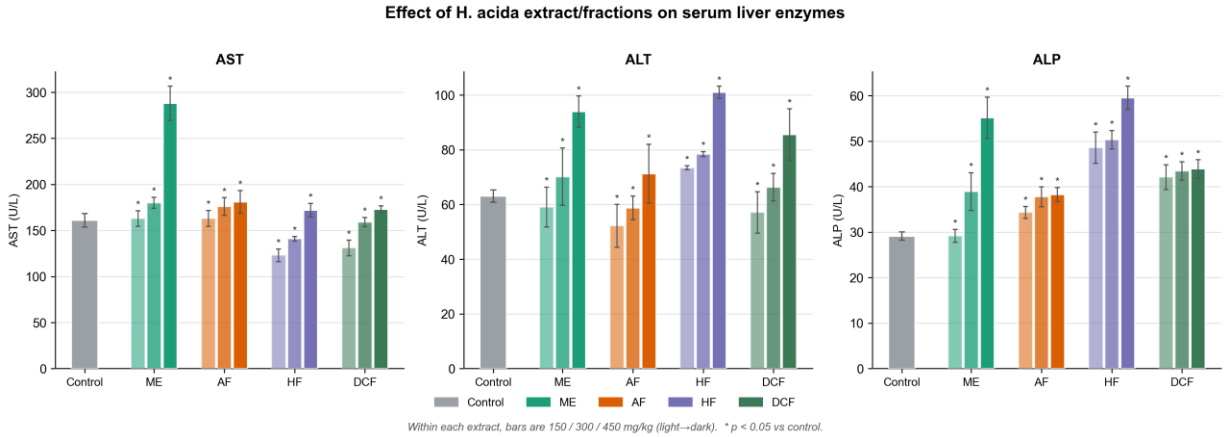
Effect on liver Enzymes

Relative to control, the extract and fractions produced dose-dependent, generally significant ($p < 0.05$) elevations in serum AST, ALT and ALP (Table 3; Figure 2). The largest effects were seen with the methanol extract at 450 mg/kg (AST 288.00 ± 18.56 U/L) and the hexane fraction (ALT 101.00 ± 2.23 U/L; ALP 59.54 ± 2.55 U/L). Because AST, ALT and ALP are released into serum when hepatocyte integrity is compromised, these increases indicate dose-related hepatic stress. This is an important safety signal: although the extract showed low acute lethality, its sub-chronic administration perturbs liver function, and any contraceptive application would need to dissociate the antifertility effect from hepatotoxicity.

Table 3: Effect of the methanol extract (ME) and aqueous (AF), hexane (HF) and dichloromethane (DCF) fractions of *H. acida* on serum liver enzymes.

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	161.00 ± 7.20	63.06 ± 2.26	29.14 ± 0.91
ME 150	163.00 ± 8.49*	59.04 ± 7.32*	29.19 ± 1.40*
ME 300	180.00 ± 5.92*	70.18 ± 10.49*	38.92 ± 4.13*
ME 450	288.00 ± 18.56*	93.94 ± 5.70*	55.16 ± 4.56*
AF 150	163.00 ± 8.74*	52.22 ± 7.80*	34.36 ± 1.31*
AF 300	176.00 ± 9.56*	58.74 ± 4.32*	37.78 ± 2.20*
AF 450	181.00 ± 12.21*	71.22 ± 10.77*	38.28 ± 1.56*
HF 150	123.00 ± 6.89*	73.40 ± 0.75*	48.60 ± 3.43*
HF 300	141.00 ± 2.47*	78.44 ± 0.87*	50.34 ± 2.00*
HF 450	172.00 ± 7.33*	101.00 ± 2.23*	59.54 ± 2.55*
DCF 150	131.00 ± 8.35*	57.10 ± 7.57*	42.10 ± 2.72*
DCF 300	159.00 ± 5.00*	66.34 ± 5.06*	43.46 ± 2.03*
DCF 450	173.00 ± 3.66*	85.58 ± 9.37*	43.90 ± 2.03*

(Mean ± SEM, n = 5). *p < 0.05 vs control.



(Mean ± SEM, n = 5). Within each treatment, bars are 150/300/450 mg/kg. *p < 0.05 vs control.

Figure 2: Serum AST, ALT and ALP in treated rats vs control.

Effect on sperm parameters

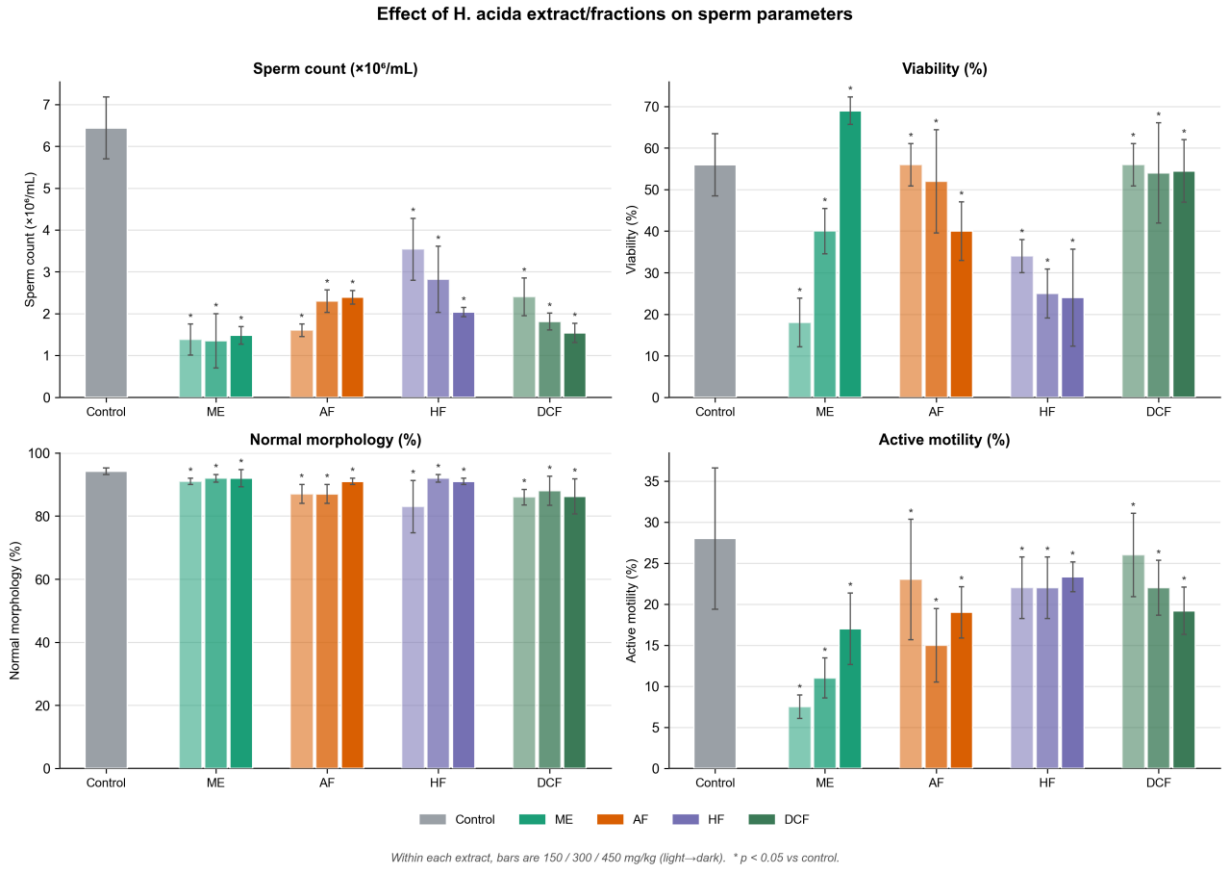
All treatments significantly ($p < 0.05$) reduced epididymal sperm count relative to control (6.44 ± 0.74 $\times 10^6$/mL), with the methanol extract producing the greatest reduction (to 1.38 ± 0.37 $\times 10^6$/mL) (Table 4; Figure 3). Sperm viability, progressive/active motility and the proportion of morphologically normal sperm were also significantly reduced, while sperm pH remained within the physiological range. This consistent suppression of the key determinants of male fertility—across the crude extract and all three fractions—demonstrates a robust antifertility effect and agrees with earlier reports of anti-spermatogenic activity of *H. acida*.[5,6] Because these sperm parameters are the accepted indices of fertility potential,[21] their marked decline points to a strong contraceptive potential for the plant.

Table 4: Effect of *H. acida* extract/fractions on epididymal sperm parameters.

Treatment	Active (%)	Slow (%)	Viability (%)	Morphology (%)	pH	Count ($\times 10^6$/mL)
Control	28.00 ± 8.60	28.00 ± 3.74	56.00 ± 7.48	94.20 ± 1.06	6.98 ± 0.20	6.44 ± 0.74
ME 150	7.50 ± 1.44*	15.00 ± 4.47*	18.00 ± 5.83*	91.00 ± 1.00*	7.60 ± 0.50*	1.38 ± 0.37*
ME 300	11.00 ± 2.44*	15.00 ± 4.47*	40.00 ± 5.47*	92.00 ± 1.22*	6.86 ± 0.35*	1.35 ± 0.65*
ME 450	17.00 ± 4.35*	15.00 ± 5.00*	69.00 ± 3.31*	92.00 ± 2.73*	7.00 ± 0.38*	1.48 ± 0.21*
AF 150	23.00 ± 7.34*	8.00 ± 1.22*	56.00 ± 5.09*	87.00 ± 3.00*	6.76 ± 0.19*	1.60 ± 0.15*
AF 300	15.00 ± 4.47*	15.00 ± 3.87*	52.00 ± 12.40*	87.00 ± 3.00*	6.16 ± 0.38*	2.30 ± 0.27*
AF 450	19.00 ± 3.14*	11.25 ± 3.14*	40.00 ± 7.07*	91.00 ± 1.00*	6.80 ± 0.12*	2.39 ± 0.16*
HF 150	22.00 ± 3.74*	28.00 ± 3.44*	34.00 ± 4.00*	83.00 ± 8.30*	6.70 ± 0.12*	3.54 ± 0.74*
HF 300	22.00 ± 3.74*	15.00 ± 4.47*	25.00 ± 5.91*	92.00 ± 1.22*	6.90 ± 0.10*	2.82 ± 0.79*
HF 450	23.33 ± 1.81*	11.00 ± 2.44*	24.00 ± 11.66*	91.00 ± 1.00*	6.96 ± 0.25*	2.04 ± 0.11*
DCF 150	26.00 ± 5.09*	15.00 ± 3.16*	56.00 ± 5.09*	86.00 ± 2.44*	6.30 ± 0.25*	2.40 ± 0.45*

DCF 300	22.00 ± 3.34*	21.00 ± 6.40*	54.00 ± 12.08*	88.00 ± 4.63*	6.58 ± 0.19*	1.81 ± 0.20*
DCF 450	19.20 ± 2.88*	22.50 ± 7.50*	54.50 ± 7.50*	86.25 ± 5.54*	6.82 ± 0.11*	1.54 ± 0.23*

(Mean ± SEM, n = 5). *p < 0.05 vs control.



(Mean ± SEM, n = 5). *p < 0.05 vs control.

Figure 3: Sperm count, viability, normal morphology and active motility vs control.

Effect on reproductive hormones

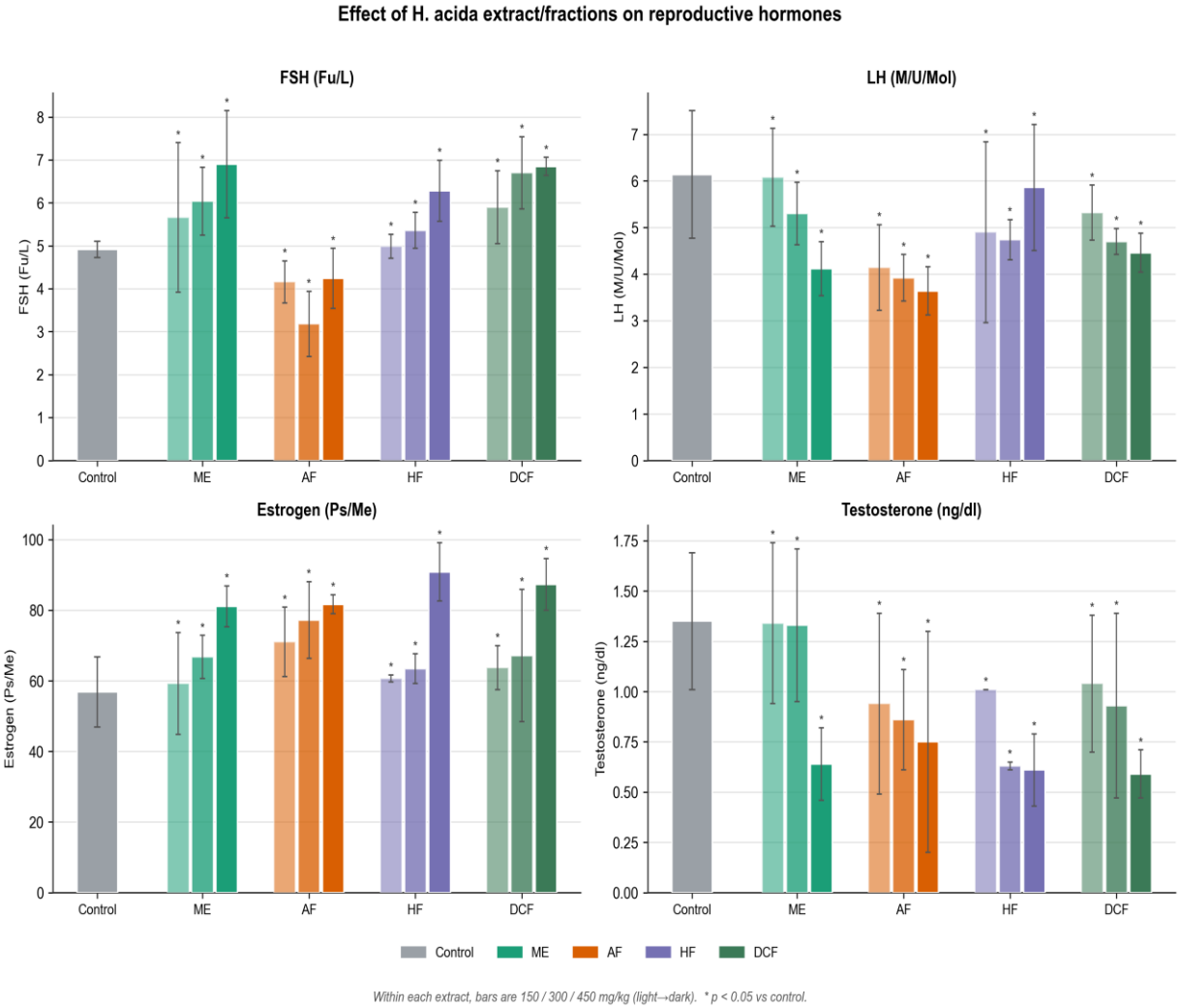
The extract and fractions significantly ($p < 0.05$) reduced serum testosterone (to 0.59–0.64 ng/dl at 450 mg/kg) and generally lowered LH, while serum estrogen was markedly increased and FSH tended to rise (Table 5; Figure 4). This pattern low testosterone and LH with elevated estrogen indicates disruption of the HPG axis. Reduced LH lowers Leydig-cell testosterone output, compromising the androgen support that Sertoli cells require to sustain spermatogenesis and the blood–testis barrier, and thereby precipitating the germ-cell loss reflected in the reduced sperm count and viability.[3,4] The elevated estrogen may further suppress the axis through negative feedback and directly impair spermatogenesis, whereas the compensatory rise in FSH is consistent with failing testicular function. Together, the hormonal, sperm and hepatic findings indicate that *H. acida* acts principally by disrupting the endocrine control of spermatogenesis, with a secondary hepatic cost.

Table 5: Effect of *H. acida* extract/fractions on serum reproductive hormones.

Treatment	FSH (Fu/L)	LH (M/U/Mol)	Estrogen (Ps/Me)	Testosterone (ng/dl)
Control	4.92 ± 0.19	6.14 ± 1.37	56.83 ± 9.96	1.35 ± 0.34
ME 150	5.66 ± 1.74*	6.08 ± 1.05*	59.26 ± 14.40*	1.34 ± 0.40*
ME 300	6.04 ± 0.79*	5.30 ± 0.67*	66.77 ± 6.08*	1.33 ± 0.38*
ME 450	6.90 ± 1.25*	4.12 ± 0.58*	81.06 ± 5.81*	0.64 ± 0.18*
AF 150	4.16 ± 0.49*	4.14 ± 0.92*	71.00 ± 9.83*	0.94 ± 0.45*
AF 300	3.18 ± 0.76*	3.92 ± 0.50*	77.19 ± 10.88*	0.86 ± 0.25*
AF 450	4.24 ± 0.70*	3.64 ± 0.52*	81.67 ± 2.71*	0.75 ± 0.55*
HF 150	4.99 ± 0.28*	4.90 ± 1.94*	60.68 ± 0.99*	1.01 ± 0.00*
HF 300	5.36 ± 0.42*	4.74 ± 0.43*	63.45 ± 4.25*	0.63 ± 0.02*
HF 450	6.28 ± 0.71*	5.86 ± 1.35*	90.84 ± 8.25*	0.61 ± 0.18*

DCF 150	5.90 ± 0.85*	5.32 ± 0.59*	63.70 ± 6.22*	1.04 ± 0.34*
DCF 300	6.70 ± 0.84*	4.70 ± 0.28*	67.13 ± 18.72*	0.93 ± 0.46*
DCF 450	6.85 ± 0.21*	4.46 ± 0.42*	87.31 ± 7.33*	0.59 ± 0.12*

(Mean ± SEM, n = 5). *p < 0.05 vs control.

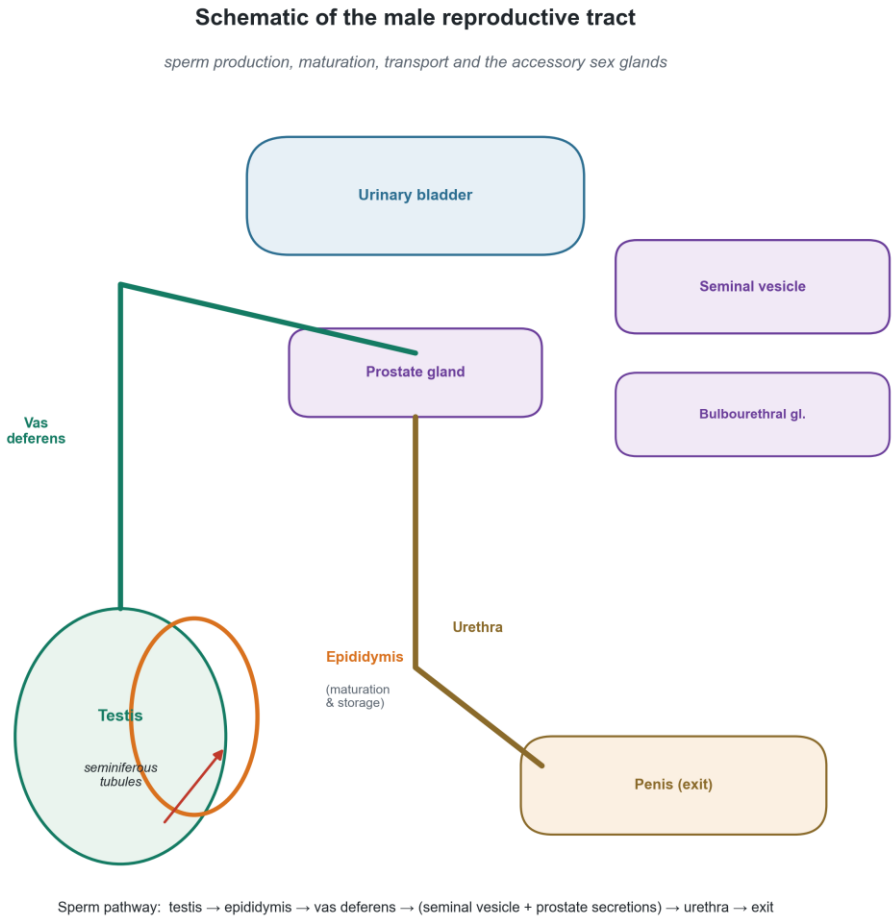


(Mean ± SEM, n = 5). *p < 0.05 vs control.

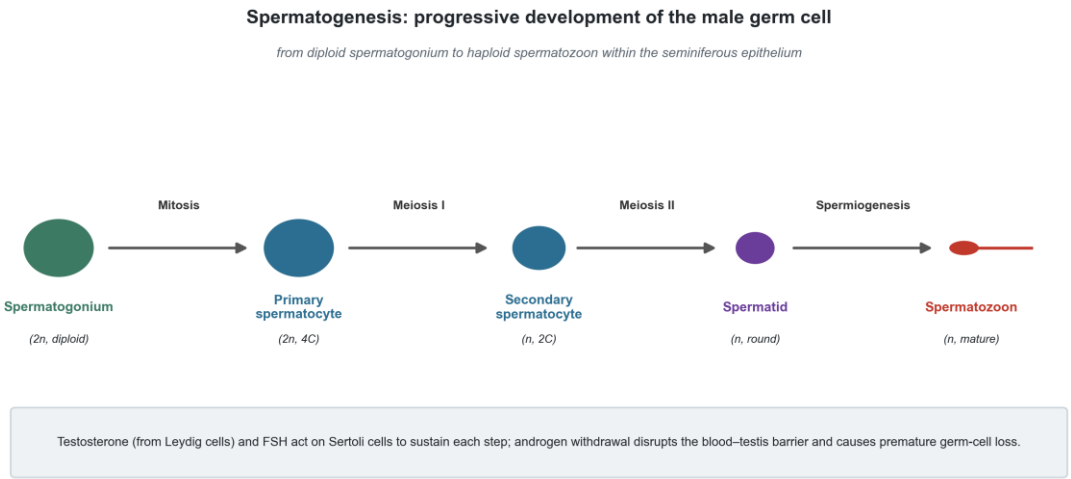
Figure 4: Serum FSH, LH, estrogen and testosterone vs control.

Supplementary Material

Antifertility and Hepatic Effects of Hymenocardia acida Stem-Bark Extract and Fractions in Male Wistar Rats.

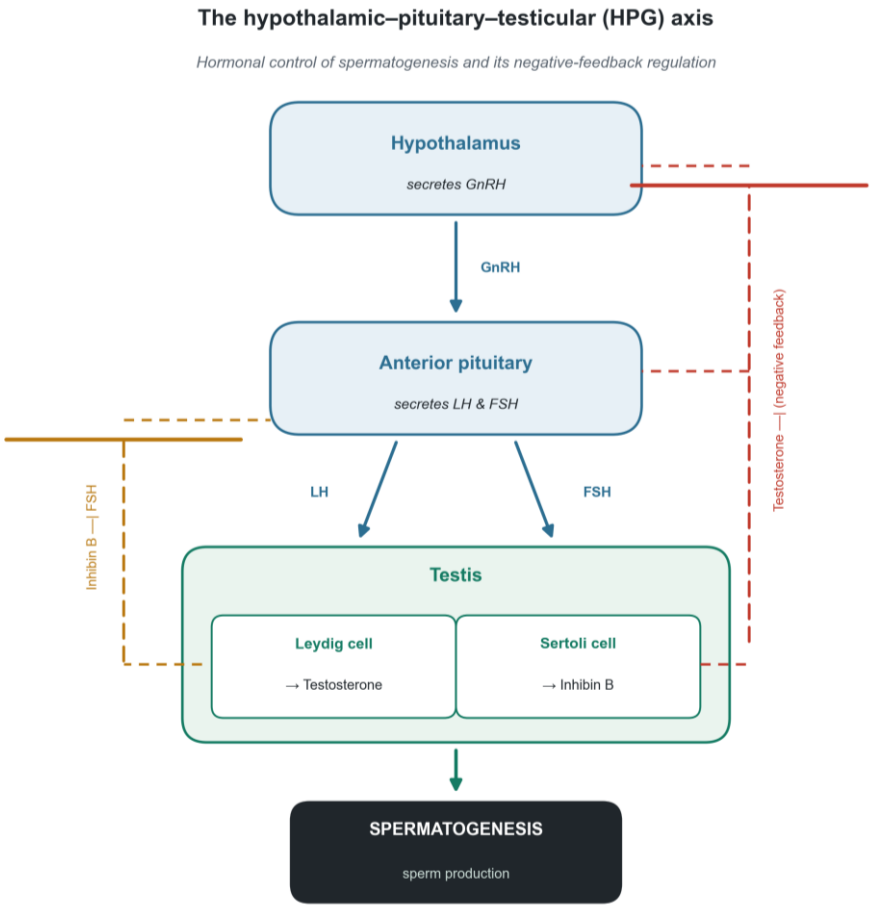


Supplementary Figure S1. Schematic of the male reproductive tract. The pathway of sperm production, maturation, storage and transport is shown: spermatozoa are produced in the seminiferous tubules of the testis, mature and are stored in the epididymis, and are transported through the vas deferens and urethra; the seminal vesicle, prostate and bulbourethral glands contribute secretions to the seminal fluid. (Original schematic prepared by the authors.)

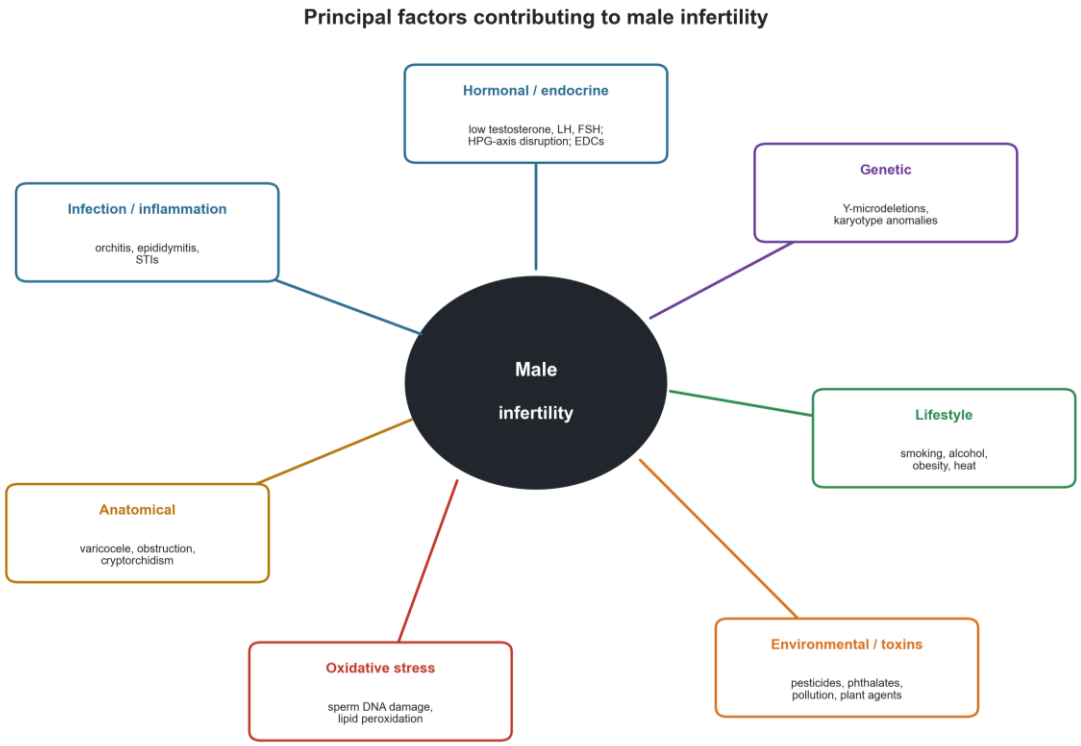


Supplementary Figure S2. Spermatogenesis. Progressive development of the male germ cell from the diploid spermatogonium through primary and secondary spermatocytes (meiosis I and II) and the spermatid stage to the haploid spermatozoon (spermiogenesis). Testosterone (from Leydig cells) and FSH act on Sertoli cells to sustain each

step; androgen withdrawal disrupts the blood–testis barrier and causes premature germ-cell loss. (Original schematic prepared by the authors.)



Supplementary Figure S3. The hypothalamic–pituitary–testicular (HPG) axis. Gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the anterior pituitary to secrete luteinising hormone (LH) and follicle-stimulating hormone (FSH); LH drives Leydig-cell testosterone synthesis and FSH supports Sertoli-cell function, together sustaining spermatogenesis. Testosterone and inhibin B exert negative feedback on the hypothalamus and pituitary (dashed lines). (Original schematic prepared by the authors.)



Supplementary Figure S4. Principal factors contributing to male infertility, including hormonal/endocrine, genetic, lifestyle, environmental/toxin, oxidative-stress, anatomical and infectious/inflammatory causes. Plant-derived antifertility agents such as those studied here act principally through the hormonal/endocrine axis. (Original schematic prepared by the authors.)

The parallel activity of the crude extract and all three fractions suggests that the responsible constituents span a range of polarities rather than residing in a single fraction. Some measures showed non-monotonic dose behaviour (for example, partial recovery of sperm viability at the highest methanol-extract dose), which may reflect biological variability, the multi-constituent nature of the fractions, or dose-dependent shifts in the balance of active compounds, and warrants confirmation with larger groups, additional time points and testicular histology.

CONCLUSION

The methanol stem-bark extract and the aqueous, hexane and dichloromethane fractions of *H. acida* possess a potent antifertility effect in male Wistar rats, evidenced by marked reductions in sperm count, viability, motility and normal morphology and by disruption of the testosterone/LH/FSH/estrogen balance that governs spermatogenesis. The extract showed low acute toxicity (LD_{50} > 5000 mg/kg) but produced dose-dependent elevations in liver enzymes. *H. acida* stem bark therefore represents a promising source of male antifertility leads, provided the antifertility action can be separated from hepatotoxicity; isolation of the active principle(s), reversibility and sub-chronic toxicity studies are recommended.

ACKNOWLEDGEMENTS

The authors thank the Department of Biochemistry, Modibbo Adama University, Yola, for laboratory support, and the Department of Plant Science for authentication of the plant material.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of interest

The authors declare no conflict of interest.

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