Journal of Biosciences and Medicines, 2025, 13(7), 105-120
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Manihot esculenta Leaf Extracts Disrupt Spermatogenesis in Wistar Rats

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How to cite this paper: Ganlaki Tomavo, H.T.R., Fiogbe, H.M.M., Segbo, J.A.G., Kougnimon, F.E.E., Mevi, P.P., Medehouenou, T.C.M., Senou, M., Agbangla, C. and Akpovi, C.D. (2025) *Manihot esculenta* Leaf Extracts Disrupt Spermatogenesis in Wistar Rats. *Journal of Biosciences and Medicines*, **13**, 105-120.
https://doi.org/10.4236/jbm.2025.137008

Received: December 30, 2024
Accepted: July 11, 2025
Published: July 14, 2025

Abstract

Manihot esculenta (*M. esculenta*) is widely used in traditional medicine and is known for its pharmacological properties. This study evaluated the effects of ethanolic leaf extracts of *M. esculenta* on biochemical markers of fertility, testicular histology, and epididymal sperm count in male Wistar rats. Ethanolic extraction and phytochemical screening were performed on powdered *M. esculenta* leaves using standard methods. Two groups of twenty adult male Wistar rats were included in the study. The control group received distilled water, while the treated group received ethanolic leaf extract of *M. esculenta* (200 mg/kg body weight) for 6 and 12 weeks. Body and testicular weights were recorded, and blood samples were collected to measure biochemical fertility markers. Testis and epididymis histology were analyzed, and sperm count was assessed. Phytochemical screening detected alkaloids, tannins, flavonoids, and saponins, which affect male fertility. A significant decrease in serum testosterone was observed in treated rats at 6 weeks ($p < 0.005$) and 12 weeks ($p < 0.0001$). Significant increases in glucose ($p = 0.015$) and LDL-cholesterol ($p = 0.001$) levels were recorded after 12 weeks of treatment. No significant changes were observed in body or testicular weights ($p > 0.05$). Epididymal sperm counts significantly decreased after 6 weeks ($p < 0.01$) and 12 weeks ($p < 0.005$) of treatment. This reduction is consistent with the histological analyses, which revealed seminiferous tubule damage and disrupted spermatogenesis in treated

rats. Ethanolic *M. esculenta* leaf extracts impair reproduction in male Wistar rats, highlighting potential contraceptive effects.

Keywords

Anti-Spermatogenic Effects, Epidydimal Sperm, *Manihot esculenta*, Testosterone, Wistar Rats, Benin

1. Introduction

Birth control represents a critical challenge of the 21st century, with significant implications for public health and sustainable development [1]. In a global context marked by rapid population growth and its socio-economic and environmental consequences, fertility management has become a priority [2]. However, access to modern contraceptive methods in Africa can be costly, and hesitation stemming from concerns about adverse effects is common. Some individuals favor natural methods due to distrust of "chemical" alternatives [3]. Among the strategies under consideration, the use of medicinal plants offers an intriguing alternative, deeply rooted in the cultural and medical practices of many populations worldwide.

Medicinal plants, rich in bioactive compounds, have been used for millennia for their therapeutic properties [4], including in the management of male reproduction [5]. Their bioactive components, particularly secondary metabolites, endow them with significant biological and medicinal properties [6] [7]. One such plant, *Manihot esculenta* (*M. esculenta*), commonly known as cassava, is widely recognized for its nutritional value but is also cited in traditional medicine for its effects on reproduction [5] [8] These pharmacological properties, which remain underexplored, pave the way for research aimed at better understanding and capitalizing on its potential for fertility control.

Reproductive disorders, such as disruptions in spermatogenesis, are often linked to hormonal imbalances, particularly deficiencies in testosterone, a hormone critical for sexual function and sperm production [9] [10]. Spermatogenesis, a complex process of cellular differentiation, results in the formation of functional spermatozoa, whose maturation in the epididymis is essential for their motility and fertilization capacity [11]. Any disruption of this process can contribute to fertility issues, highlighting the need to explore effective and accessible solutions that could potentially serve as the basis for future male contraception.

Previous studies have reported that *M. esculenta* may exert modulatory, or even deleterious, effects on reproductive organs [12] [13]. However, while its nutritional benefits are well documented, its pharmacological potential in fertility control remains largely unexplored.

In this context, the present study aims to investigate the effects of ethanolic extracts from the leaves of *M. esculenta* on reproduction in male Wistar rats by assessing their impact on serum testosterone levels, testicular histology, and epidid-

ymal sperm count. These findings could contribute to promoting innovative and sustainable solutions for birth control that are culturally relevant and rooted in ancestral medicinal practices.

2. Materials and Methods

2.1. Plant Material

The plant material used in this study comprised *Manihot esculenta* (*M. esculenta*) leaves, which were procured from a local vegetable market in the commune of Abomey-Calavi and authenticated at the National Herbarium of the University of Abomey-Calavi (Herbier national, UAC, Benin).

2.2. Animal Material

Sixty (60) adult male Wistar rats (*Rattus norvegicus*), weighing 150 - 200 g, were obtained from the Department of Animal Production and Health at the Institute of Applied Biomedical Sciences (ISBA), University of Abomey-Calavi (UAC, Benin), for the experiments. The animals were housed under standard laboratory conditions in well-ventilated cages with a 12-hour light/dark cycle. The rats were allowed to acclimatize to the laboratory environment for 3 weeks before the start of the experiments. During this period, they were provided with a standard rodent diet and water *ad libitum*. All procedures involving animals were conducted in compliance with the ethical guidelines for the care and use of laboratory animals.

2.3. Ethanolic Extraction

Ethanolic extraction was performed following the protocol of the Laboratory of Pharmacognosy and Essential Oils at the Faculty of Science and Technology (FAST, UAC, Benin). Young leaves were dried at room temperature (16˚C) in the open air, protected from light and heat, for approximately two to three weeks before being ground into powder. The ethanolic extract was prepared by macerating the powdered material in 96% ethanol for 72 hours. The mixture was stirred twice daily, and the resulting macerate was filtered using Whatman paper and evaporated under reduced pressure at 40˚C until a dry extract of constant weight was obtained. The extraction yield (r) was calculated using the formula:

$$r = \frac{\text{Mass of extract}}{\text{Mass of powder before extraction}} \times 100 .$$

2.4. Phytochemical Screening

Phytochemical screening was conducted based on differential reactions (coloration and precipitation) of major chemical groups present in plants, following the method described by Houghton and Raman [14], as adapted to the Laboratory of Pharmacognosy and Essential Oils' conditions. The presence of the following compounds was assessed: alkaloids, tannins, flavonoids, anthocyanins, sapono-

sides, triterpenoids, steroids, cardenolides, mucilage, coumarins, quinone derivatives, cyanogenic derivatives, reducing compounds, and anthraquinone derivatives.

2.5. Experimental Design

Male Wistar rats were divided into two groups of 30 rats each (Treated and Control groups) and were allowed three weeks to acclimate to the animal facility conditions at the Non-Communicable Diseases and Cancer Research Unit. This size was calculated based on group comparison of one-way ANOVA test using Degree of Freedom as reported by Charan and Kantharia [15]. The extracts were dosed based on the body weight of each rat at 200 mg per kg and administered to the treated group orally in 1 mL of distilled water daily according to the method described by Abu *et al.* [16].

Throughout the study, all male rats were provided with a free diet of pellets and water *ad libitum*. The effects of the extract were assessed at 6 and 12 weeks, focusing on body weight, testicular weight, serum testosterone levels, testicular histology, and sperm parameters in both the Treated and Control groups.

2.6. Body and Testicular Weight Measurements

Weekly body weight measurements were conducted using an electronic scale. At the start of the study (W0), the rats were weighed, and their body weight was monitored weekly up to the twelfth week (W12) of the experiment. After six weeks of extract administration, half of the representative rats were sacrificed, while the remaining half were sacrificed at the end of week 12. Testes were collected at the beginning (W0), after six weeks (W6), and at the end of the study (W12) through laparotomy in both control and treated groups.

Testicular weight was measured using Archimedes' principle, based on the volume (mL) of physiological saline solution (0.9% NaCl) displaced by each testis. The displaced volume was converted to mass (mg) to accurately estimate testicular weight. These measurements of body and testicular weight were further utilized to calculate the gonadosomatic index (GSI), defined as the ratio of the testicular weight to the total body weight, expressed as a percentage.

2.7. Laboratory Measurements

Blood samples were collected from rats through retro-orbital bleeding following an overnight fast. Plasma obtained from fluoride tubes was used for glucose testing, while serum was used for the measurement of other parameters. The following biomarkers were analyzed using an automated biochemical analyzer and reagent kits from ELITech (ELITech Group, France): fasting blood glucose, triglycerides, total cholesterol, and high-density lipoprotein cholesterol, considering their impact on spermatogenesis [17]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured to assess liver function, while creatinine and urea were determined to monitor the effect of treatments on kidney

function. Testosterone levels were measured using an ELISA method with a kit supplied by Bio-Techne (Bio-Techne, Abingdon, UK).

2.8. Epididymal Sperm Extraction and Counting

Male Wistar rats were anesthetized, and an abdominal incision was performed to expose the reproductive organs. The caudal epididymis was carefully excised using sterile scissors and placed in a Petri dish containing 10 mL of physiological saline solution (0.9% NaCl). The method of Akpovi *et al*., with slight modification, was used to determine the number of sperm in the epididymis [18]. The tissue was finely minced to release spermatozoa, and the suspension was incubated under orbital agitation at room temperature (approximately 24˚C) for 30 minutes to ensure optimal dispersion. Following incubation, the suspension was transferred to a sterile test tube and centrifuged at 250 g for 15 minutes. The spermatozoa formed a pellet at the bottom of the tube, while the supernatant was carefully discarded. The pellet was resuspended in 1 mL of 0.9% NaCl solution, creating the final suspension used for counting.

Spermatozoa counting was performed using a Malassez hemocytometer. A 10 µL aliquot of the suspension was placed into the hemocytometer chamber, covered with coverslip to ensure no air bubbles were present. After allowing the suspension to stabilize for 3 to 5 minutes, spermatozoa were observed under a light microscope at 40× magnification. The sperm concentration was calculated using standard World Health Organization protocols and expressed as 10^6 sperm/mm^3 [19].

2.9. Histology Analysis

Rats were sacrificed, and their testicles were immediately removed and fixed in a 10% formalin solution. After rinsing with water, the testicles were dehydrated through a graded series of ethanol concentrations (70%, 95%, and 100%) and embedded in paraffin wax. The paraffin-embedded testicles were carefully positioned in cassettes to ensure proper orientation. Tissue sections were prepared by slicing the paraffin blocks into 6-µm-thick sections. The histological sections were subsequently stained using hematoxylin and eosin (HE) for microscopic examination.

2.10. Statistical Analysis

Data were recorded using Microsoft Excel 2016, and statistical analyses were conducted using SigmaPlot version 14. Results are expressed as the mean ± standard error of the mean (SEM). Statistical tests included one-way analysis of variance (ANOVA) for parametric data and the Kruskal-Wallis ANOVA for non-parametric data. Post-hoc multiple comparison analyses, including Dunnett's test, were applied to compare the exposed groups with the control. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Ethanolic Extracts of *Manihot esculenta*

The ethanolic extract of *M. esculenta* leaves was obtained as a fractionable dark-green solid, weighing 29 g from an initial mass of 351 g prior to extraction. The extraction yield was calculated to be 8.26%.

3.2. Phytochemical Screening

The phytochemical screening of *M. esculenta* leaves showed the presence of alkaloids, tannins (catechin and gallic tannins), flavonoids, leuco-anthocyanins, quinone derivatives, saponosides, mucilage, reducing compounds, C-glycosides, and coumarins. In contrast, free and combined anthraquinones, O-glycosides, reduced O-glycosides, terpenoids, steroids, cyanogenic derivatives, and cardenolides were absent (Table 1).

Table 1. Phytochemical compounds in *Manihot esculenta*.

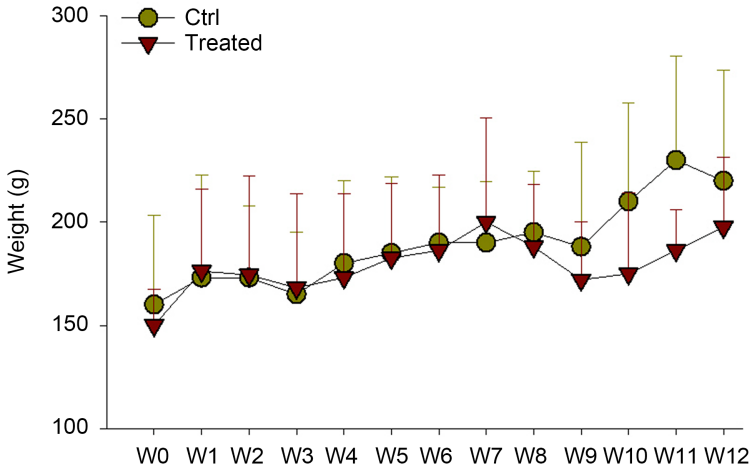
Compounds	Presence (+/−)	Compounds	Presence (+/−)
Alkaloids	+	Saponosides	+
Tannins	+	Mucilage	+
Catechin Tannins	+	Reducing Compounds	+
Gallic Tannins	+	Free Anthraquinones	−
Flavonoids (*Flav*)	+	Combined Anthraquinones	−
Anthocyanins	−	O-glycosides	−
Leuco-anthocyanins	+	Reduced O-glycosides	−
Quinone Derivatives	+	C-glycosides	+
Steroids	−	Cyanogenic Derivatives	−
Terpenoids	−	Coumarins	+
Cardenolides	−	Saponosides	+

The table summarizes the presence (+) or absence (−) of various phytochemical compounds identified in ethanolic leaf extracts of Manihot esculenta.

3.3. Body Weight, Testicular Weight and Testicular Biometry

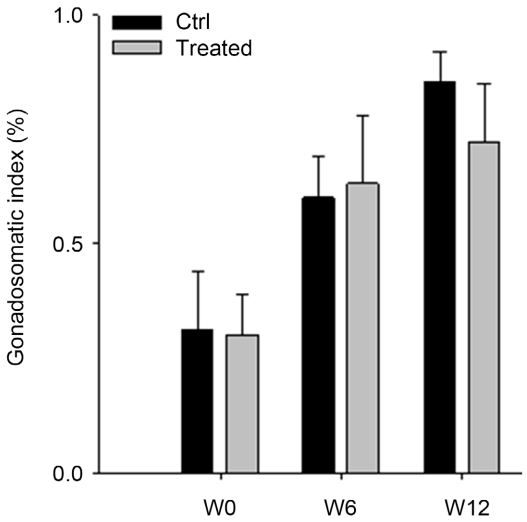
The average body weights of the rats in the treated group receiving *M. esculenta* extracts compared to the control group over time were monitored (Figure 1). Results showed no significant differences in the body weight of the treated rats compared to the control group throughout the study from week zero (W0) to week 12 (W12) ($p > 0.5$) (Figure 1).

Consistent with the body and testicular weights, the mean gonadosomatic index showed no significant differences in treated group compared to control at 6 weeks ($p = 0.87$) and 12 weeks ($p = 0.53$) (Figure 2).



Rats were weighed weekly from the start of the study (W0) to twelve weeks (W12). During the first six weeks, each group consisted of 20 rats, while in the last six weeks, the number was reduced to 8 rats per group. All rats in each group were weighed, and the results are presented as mean ± standard error of the mean (SEM). Weekly comparisons were made between the control group, which received distilled water, and the treated group, which received 200 mg/kg of extract.

Figure 1. Weight variation.



The gonadosomatic index, expressed as a percentage, was calculated for both control (Ctrl) and treated groups. Data are presented as mean ± standard error of the mean (SEM). Comparisons were made between the groups at each point to assess the effects of the treatment. Control rats received distilled water, while treated rats received 200 mg/kg of *M. esculenta* extract.

Figure 2. Gonadosomatic index.

3.4. Biochemical Parameters

Table 2 provides an overview of the biochemical parameters measured before and throughout the treatment period. Before the initiation of treatment, there were no significant differences observed between the groups across all measured biochem-

ical parameters, indicating baseline equivalence. However, after 12 weeks of treatment with *M. esculenta* extracts, significant increases were recorded in glucose levels ($p = 0.015$) and LDL cholesterol ($p = 0.001$) in the treated group compared to the control group (Table 2).

Other biochemical parameters, including urea, creatinine, ALAT, ASAT, total cholesterol, HDL cholesterol, triglycerides, and total proteins, remained unchanged throughout the 12-week treatment period, showing no statistically significant differences between the groups (Table 2).

Table 2. Biochemical parameter levels.

Groups	Control (n = 10)	Treated groups		p
		Week 6 (n = 10)	Week 12 (n = 8)	
Glucose (g/L)	0.34 ± 0.09	0.36 ± 0.18	0.67 ± 0.19^{+}	0.015
Urea (g/L)	0.51 ± 0.13	0.41 ± 0.08	0.68 ± 0.29	0.050
Creatinine (mg/L)	6.9 ± 0.14	6.46 ± 1.18	6.63 ± 1.42	0.240
ALAT (UI/L)	172.5 ± 33.91	193.03 ± 40.59	163 ± 38.04	0.400
ASAT (UI/L)	202.5 ± 75.24	177.75 ± 78.87	180.93 ± 46.52	0.062
Total Cholesterol (g/L)	1.54 ± 0.34	1.06 ± 0.17	0.95 ± 0.13	0.083
HDL Cholesterol (g/L)	0.94 ± 0.30	0.71 ± 0.13	0.74 ± 0.12	0.277
LDL Cholesterol (g/L)	0.66 ± 0.29	0.78 ± 0.24	1.12 ± 0.07**	0.001
Triglycerides (g/L)	0.89 ± 0.16	0.56 ± 0.15	0.66 ± 0.18	0.748
Proteins (g/L)	73.75 ± 41.75	115.55 ± 23.95	104 ± 6.38	0.263

Biochemical parameters are measured in control and treated rats at Week 6 and Week 12 of treatment. Values are expressed as mean ± standard deviation (SD). The number of rats per group (n) is indicated.

3.5. Testosterone Levels

Serum testosterone levels were comparable in both treated and control groups before treatment (Table 3). However, a significant decrease was observed in treated group after 6 weeks ($p < 0.04$) and 12 weeks ($p < 0.005$) of treatment compared to the control group (Table 3).

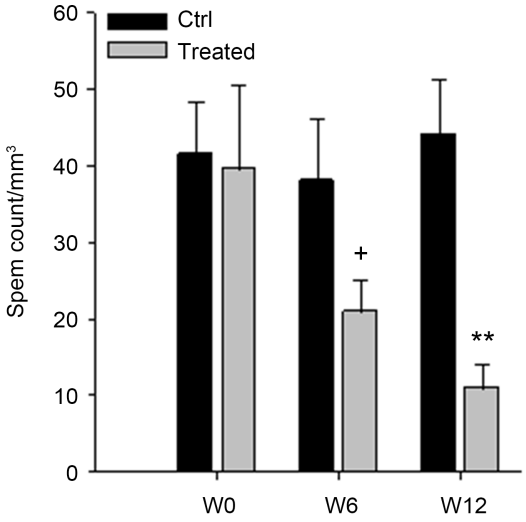
Table 3. Serum testosterone levels.

Schedule	Control	Treated groups	p
Week 0 (n = 10)	1.27 ± 0.41	1.32 ± 0.50	0.630
Week 6 (n = 10)	1.42 ± 0.31	0.73 ± 0.26	0.040
Week 12 (n = 8)	1.59 ± 0.21	0.53 ± 0.18	0.005

Values are presented as mean ± standard error of the mean (SEM) of serum testosterone levels expressed in ng/mL. The number of rats tested in each group (n) at the respective time points is indicated. Comparisons were made between the control group, which received distilled water, and the treated group, which received 200 mg/kg of M. esculenta extract. Significant reductions in testosterone levels were observed in the treated group at Week 6 ($p = 0.040$) and Week 12 ($p = 0.005$). No significant difference was noted at baseline (Week 0, $p = 0.630$).

3.6. Epididymal Spermatozoa

The epididymal spermatozoa level in the treated group was compared to the control group over the course of the study (Figure 3). Results showed a significant reduction in sperm count in treated groups at week 6 of treatment ($p < 0.005$) compared to the control group (Figure 3). The decrease became even more pronounced after 12 weeks ($p < 0.001$) (Figure 3). This progressive decline suggests a significant adverse effect of *M. esculenta* extracts on spermatogenesis, likely impacting sperm production and/or maturation in the epididymis.



Sperm counts were measured at baseline (W0), Week 6 (W6), and Week 12 (W12). Values are presented as mean ± standard error of the mean (SEM). Control rats received distilled water, while treated rats received 200 mg/kg of *M. esculenta* extract. ($p < 0.04$; **$p < 0.005$: treated group vs. control group).

Figure 3. Epididymal sperm count levels.

3.7. Histology Analysis

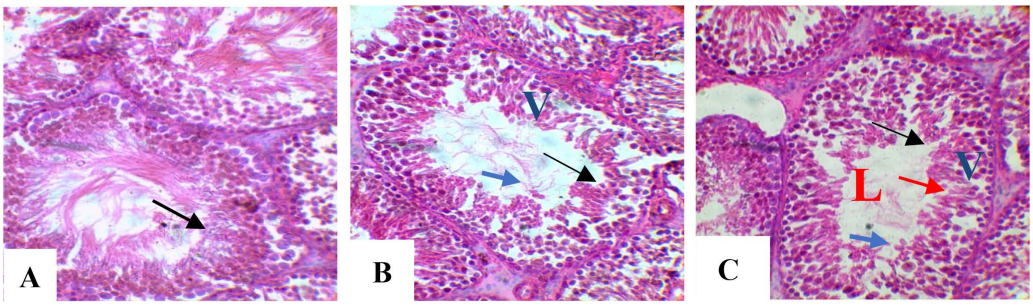
Representative pictures of histological sections of testis of control and treated rats at baseline (A), after 6 weeks (B), and after 12 weeks (C) are shown in Figure 4.

At baseline (Figure 4(A)), the seminiferous tubules of control rats displayed normal spermatogenesis, characterized by the presence of germinal cells at various stages of development, including spermatogonia, spermatocytes I and II, spermatids, and spermatozoa. The seminiferous epithelium was densely populated with spermatozoa, filling the lumen of the tubules.

At 6 weeks (Figure 4(B)), the treated group showed evidence of complete spermatogenesis. However, there was a notable reduction in the concentration of spermatozoa within the seminiferous tubule lumen compared to the control group. Additionally, partial deformation of some seminiferous tubules was observed in the treated rats (Figure 4(B)).

By 12 weeks (Figure 4(C)), the treated group exhibited a significant decline in the concentration of spermatozoa within the seminiferous tubule lumen. This re-

duction was more pronounced compared to both the control group and the 6-week treated group, indicating progressive disruption of spermatogenesis over the treatment period (Figure 4(C)).



(A) Seminiferous tubules from the control group exhibit a normal structure with a dense population of spermatozoa in the lumen (L) and germinal cells at various stages of spermatogenesis. (B) Testes from treated rats at 6 weeks show the presence of vacuoles (V) and a reduction in spermatozoa density in the seminiferous tubule lumen. (C) Testes from treated rats at 12 weeks reveal significant structural changes, including a pronounced reduction in spermatozoa within the lumen (L), the presence of vacuoles (V), and giant cells (red arrow), along with disruption of the seminiferous tubule architecture. Sections are representative of three independent experiments. L: Lumen of the seminiferous tubule, V: Vacuole, Black arrow: Sperm head, Blue arrow: Flagellum, Red arrow: Giant cell. Sections were stained with hematoxylin and eosin (Scale bar = 50 μm).

Figure 4. Testis histology.

4. Discussion

Birth control remains a critical public health challenge with significant socio-economic and environmental implications. *Manihot esculenta* (cassava), widely recognized for its nutritional value, also contains bioactive compounds that may interfere with spermatogenesis and induce oligospermia [5] [7]. This study investigated the effects of ethanolic leaf extracts of *M. esculenta* on male reproductive parameters, focusing on spermatogenesis and related biochemical outcomes.

The ethanolic extraction of *M. esculenta* leaves yielded a diverse range of bioactive compounds, including flavonoids, tannins, and alkaloids, which are well-known for their antioxidant, anti-inflammatory, and antimicrobial properties [6] [8] [20] [21]. These findings validate ethanol's effectiveness as a polar solvent for extracting phytochemicals and align with prior research highlighting the therapeutic potential of medicinal plants [22] [23]. The absence of cyanogenic derivatives in the extracts reduces the toxicity risk typically associated with raw *M. esculenta* [24] [25]. However, the presence of saponins and tannins, while exhibiting beneficial properties, suggests potential endocrine-disrupting effects. Saponins, for example, can modulate testosterone levels, potentially impairing spermatogenesis and overall reproductive health [26]-[28]. Similarly, tannins may interfere with nutrient absorption, particularly of iron and zinc, both critical for reproductive function [29] [30].

Despite no significant changes in body or testicular weights or testicular biometry, minor reproductive effects cannot be ruled out. The absence of significant

changes in these metrics suggests a lack of overt systemic toxicity at the administered doses, consistent with studies showing that plant extracts may not cause noticeable changes in body or organ weights over short durations or low doses [31] [32]. However, reproductive health may still be affected indirectly through mechanisms like hormonal disruption or impaired spermatogenesis [28] [33] [34]. Indeed, saponins and tannins in *M. esculenta* extracts are known to disrupt endocrine signaling, potentially altering testosterone synthesis and sperm quality [33]. Such disruptions may occur at a cellular or biochemical level rather than manifesting in gross anatomical changes.

Biochemical analyses revealed significant increases in glucose, ASAT, and LDL cholesterol levels, indicating potential systemic effects of *M. esculenta* extracts on metabolism. Elevated ASAT levels suggest hepatic stress, potentially caused by the metabolism of bioactive compounds in the extracts. Hepatic stress and associated oxidative damage may impair hormonal synthesis and testicular function, indirectly affecting reproductive health [35] [36]. These findings align with reports linking elevated liver enzymes, such as ASAT and ALT, to testicular dysfunction in pathological states [37]. Additionally, increased LDL cholesterol levels suggest disruptions in lipid metabolism, which could exacerbate testosterone reductions observed in this study [38]-[40]. Cholesterol dynamics are intricately linked to spermatogenic activity, as alterations in lipid profiles influence germ cell function and sperm maturation [18]. Similarly, elevated glucose levels may indicate insulin resistance or hepatic glucose dysregulation, both of which can adversely affect reproductive parameters. Glucose serves as a critical energy source for Sertoli and germ cells during spermatogenesis, and disruptions in glucose homeostasis could impair ATP production, further compromising germ cell development [17] [41] [42].

A significant reduction in serum testosterone levels observed at 6 and 12 weeks is a critical finding, indicating the potential endocrine-disrupting properties of *M. esculenta* extracts. Flavonoids and tannins may interfere with key enzymes involved in testosterone biosynthesis, such as 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD), contributing to reduced testosterone levels [43]. Saponins, which can induce oxidative stress, may further exacerbate these hormonal imbalances [44]. Oxidative stress is a known factor in Leydig cell dysfunction and diminished testosterone production, highlighting the role of bioactive compounds in mediating these effects [45].

Treated rats exhibited a significant decrease in epididymal sperm count, underscoring the potential contraceptive effects of M. esculenta extracts on male fertility. This reduction indicates impaired sperm maturation and viability, consistent with findings linking phytochemical exposure to reduced sperm motility and quality, often attributed to hormonal imbalances and oxidative damage [46]. Cholesterol homeostasis, critical for steroid hormone synthesis and germ cell membrane integrity, also plays a central role in this effect [18]. Furthermore, tannins and saponins in the extracts, known to modulate oxidative stress and endocrine

functions, likely contribute to these outcomes.

Histological analysis revealed progressive disruption of spermatogenesis in treated rats, characterized by reduced spermatozoa concentration and deformation of seminiferous tubules [30] [47]. These findings align with earlier results showing significant reductions in testosterone and metabolic alterations. Disruptions in steroidogenesis, potentially mediated by tannins and flavonoids, may have induced oxidative stress within the testicular environment, resulting in germ cell apoptosis and structural deformities in the seminiferous tubules [18] [48] [49]. Further emphasized that oxidative stress, driven by imbalanced cholesterol metabolism, exacerbates cellular damage and compromises the functionality of Sertoli and Leydig cells, essential for normal spermatogenesis.

The cumulative impact of *M. esculenta* extracts on reproductive parameters underscores the need for comprehensive evaluations integrating hormonal, histological, and metabolic endpoints. While this study provides valuable insights, its limitations include a relatively short experimental duration and doses that may not fully reflect human exposures. Future research should focus on extended treatment periods, varied dosing regimens, and detailed analyses of sperm quality and DNA integrity to better understand the broader implications of *M. esculenta* extracts on male reproductive health.

5. Conclusion

This study demonstrates that ethanolic leaf extracts of *Manihot esculenta* have significant anti-spermatogenic effects, as evidenced by decreased serum testosterone levels, disrupted testicular histology, and reduced epididymal sperm count in male Wistar rats. The observed alterations in metabolic markers, including increased glucose, ASAT, and LDL-cholesterol levels, further suggest systemic effects that may compound the plant's impact on male reproductive health. These findings highlight the potential of *M. esculenta* as a source of bioactive compounds with contraceptive properties, while also cautioning against its chronic consumption due to its detrimental effects on fertility. Further research is required to isolate and characterize the specific compounds responsible for these effects and to evaluate their safety and efficacy in broader contexts, including human applications.

Data Availability

Data are available from the corresponding author on request.

Ethics Statement

All animal procedures were conducted in strict accordance with the ethical guidelines for the use of laboratory animals established by the Institute of Applied Biomedical Sciences (ISBA), University of Abomey-Calavi, Cotonou, Benin.

Authors contributions

G.T.H.T.R., K.F.E.E., P.P.M., and A.D.C. were responsible for material prepara-

tion, data collection, and analysis. G.T.H.T.R. and H.M.M.F. drafted the initial version of the manuscript, which was subsequently reviewed by S.J.A.G., M.T.C.M., M.S., and A.D.C. All other authors, including K.F.E.E. and A.C., provided comments on earlier versions. All authors read and approved the final manuscript.

Acknowledgements

We sincerely thank Professor Yessoufou Akadiri (Laboratory of Cell Biology and Physiology, Institute of Applied Biomedical Sciences, Faculty of Sciences and Techniques, University of Abomey-Calavi, Benin) for his assistance in acquiring the testosterone kit. Finally, we extend our heartfelt thanks to Dr. Fiogbé E.M. Salomon (Laboratory of Applied Biology Research, University of Abomey-Calavi, Benin) for his technical support in conducting the laboratory tests.

Conflicts of Interest

The authors declare there are no conflicts of interest regarding the publication of this article.

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