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Original Article

Reproductive impact of Mishenland Polyherbal Mixture on steroid hormone-induced gonadotoxic effects in male Wistar rats

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Abstract

BACKGROUND AND AIM: Testosterone and its analogs have been used for anabolic and androgenic purposes for several decades. Initially restricted to professional bodybuilders, these substances have become increasingly popular among recreational athletes. The Mishenland polyherbal mixture (MPM) is a herbal preparation used locally for managing benign prostatic hyperplasia (BPH) and is claimed to reverse infertility of unknown cause in men. The aim of study was to investigate ability of MPM to mitigate the gonadotoxic effects of prolonged steroid hormone exposures in the adult male Wistar rats.

MATERIALS AND METHODS: This study used 25 adult male albino rats of age 9 weeks. The animals were divided into five groups of five rats each. After a two-week acclimatization period, all animals except the normal control group received combination doses of 4 mg/kg body weight (bw) testosterone (T) and 0.8 mg/kg bw estradiol (E2) subcutaneously on alternate days for 6 weeks. From the seventh week, experimental groups received 1 ml/kg, 2 ml/kg, and 4 ml/kg bw of MPM, while the control group received 2 ml/kg bw of distilled water. After 4 weeks of treatment, all animals were sacrificed, and their reproductive organs were removed for histological processing (H&E stain).

RESULTS: The testes showed evidence of spermatogenic arrest with partial recovery of the spermatogonia stem cells following MPM treatment. The medium dose-2ml/kg herbal treatment group exhibited greater recovery compared to the control. Priapism persisted in hormone-induced hypogonadal control animals. The induced hypogonadic group (IHPG) had the highest mean packed cell volume (PCV), followed by the normal dose MPM group.

CONCLUSION: MPM demonstrated a mild ability to restore impaired reproductive function in hormone induced gonadotoxic animals and prevented priapism development observed in untreated hormone-exposed animals.

Keywords:
Mishenland polyherbal mixture; hypogonadism; priapism; testosterone; testis

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INTRODUCTION

Exogenously supplied testosterone and its analogs have been reported to possess contraceptive potential (Patel *et al*., 2019). Several reports from the World Health Organization (WHO) on the search for male contraception using various doses of androgens, either alone or in combination with progestin and/or estrogen, support this finding (Grimes *et al*., 2012). Exogenously administered sex steroids to male animals can inhibit sperm production through effects on the pituitary and hypothalamus (Mahapokai *et al*., 2000; Page *et al*., 2022). However, this approach tends to disrupt testicular function such that after the withdrawal of treatment, the ability of the testes to produce testosterone may be altered, sometimes necessitating replacement therapy to restore normal testicular function (Nieschlag, 2015; Thirumalai and Page, 2022).

Mishenland Polyherbal Mixture (MPM) is a decoction made of garlic, spring onion (*Allium ascalonicum)* 7.5%, Aridan (Tetraptera tetrapleura) 0.5%, Garlic (*Allium sativum*) 1% and "Isirigun" *Mondia whitei* (root) 1%, MPD was obtained from Mishenland Glory Ventures, Nigeria, Limited. This product is claimed to alleviate benign prostatic hyperplasia (BPH) and resolve male infertility of unknown cause. Preliminary phytochemical studies of MPM revealed the presence of bioactive compounds such as tannins, saponins, cardiac glycosides, flavonoids, and zinc (Adefule *et al*., 2014). The two Allium species in MPM, *Allium ascalonicum* and *Allium sativum*, are rich in phytochemicals beneficial

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for controlling aging diseases such as prostatitis and nervous and cardiac conditions (Asemani *et al*., 2019; Tudu *et al*., 2021).

Allium sativum has been extensively studied for its medicinal properties, capable of inhibiting and killing bacteria and fungi, lowering blood pressure, cholesterol, and blood sugar, preventing blood clotting, and possessing anti-tumor properties. It also boosts the immune system (Cadmiel *et al*., 2022). *Allium ascalonicum*, another onion species, is valued as both a spice and medicinal plant, containing the highest concentration of total flavonols among onion species (Sun *et al*., 2019; Bhushan *et al*., 2021).

Mondia whitei, known as African ginger, is traditionally used to improve appetite and libido, as a galactagogue, fertility medication, and antidepressant (Oketch-Rabah, 2012; Pierre *et al*., 2019; Chokwe *et al*., 2021). *Tetrapleura tetraptera*, or "aridan" in Southwestern Nigeria, is used for various ailments such as fibroids, infertility, convulsions, and molluscidal activities (Adewunmi, 2004; Mensah *et al*., 2024).

Due to paucity of knowledge, the study aimed to investigate the effects of Mishenland polyherbal mixture (MPM) on steroid hormone-induced gonadotoxicity and hypogonadism in male Wistar rats, specifically assessing MPM's potential to reverse these conditions and restore normal reproductive function.

MATERIALS AND METHODS

Animals and Drug Administration:: This study was conducted in the Faculty of Basic Medical Sciences (FBMS) at Olabisi Onabanjo University, Ikenne, Nigeria. Twenty-five adult male Albino Wistar rats were obtained from the Department of Anatomy, FBMS, and divided into five groups: The animals were housed in plastic cages with wood shavings and allowed to acclimatize for two weeks before the experiment. They were fed with rat chow from Top Feed Nigeria Plc and given water ad libitum. The animals were weighed weekly. Hypogonadism was induced using 4mg/kgbw testosterone (T) and 0.8mg/kgbw estradiol (E2). The animals were injected subcutaneously at the inguinal region on every alternate day. Mishenland Polyherbal Mixture (MPM) is a decoction made of garlic, spring onion (*Allium ascalonicum)7*.5%, Aridan (Tetraptera tetrapleura) 0.5%, Garlic (*Allium sativum*) 1% and "Isirigun" *Mondia whitei* (root) 1%, MPM was obtained from Mishenland Glory Ventures, Nigeria, Limited. MPM was administered after inducing hypogonadism. Group 1: Normal Control (NC) was given - 0.2 ml distilled water (DW), Group 2: Induced Hypogonadism (IHPG) Control was given - 0.2 ml DW, while the MPM treated Group 3: IHPG and MPM Normal Dose (MPM-ND) -was given 2 ml/kgbw, Group 4: IHPG and MPM Low Dose (MPM-LD) - 1ml/kgbw and Group 5: IHPG and Mishenland High Dose (MPM-HD) - 4 ml/kgbw.

Ethics: Ethical clearance was obtained from the Departmental Ethical Committee, conforming to the Declaration of Helsinki and the Guiding Principles in the Use of Animals (American Physiological Society, 2002).

Sperm Count: Right epididymis and specimen of right testis were homogenized manually in 0.5ml of 0.9% NaCl solution. The homogenates were diluted with 1.5ml of saline. Spermatozoa were counted using Nuebauer haemocytometer at X400 magnification in five squares as WBC's. Five count /sample were averaged.

Histological Preparation: The testis of each group was excised and rinsed in 0.9% saline, and were blotted dry of saline and excess blood. They were fixed in 10% formalin for 24 hours. The tissues, after fixation were washed in water to remove excess fixative. Washed tissue were then dehydrated through graded series of ethyl alcohol, cleared with xylene and embedded in paraffin wax. Sections of 4microns were cut with a microtome blade, and mounted on clean glass slides. the sections were routinely stained with hematoxylin and eosin. The stained slides were observed in research microscope and photographed.

Statistical Analysis: Statistical analysis was performed using GraphPad Prism 8. One-way ANOVA. $P<0.05$ were considered statistically significant. Descriptive statistics were presented as mean ± standard deviation (Mean ± SD).

RESULTS

At the end of the research, the mean body weight of the animals in the control group was 185.8 ± 17.91 g, compared to their initial weight of 128.3 ± 17.61 g. The mean body weights of the treatment groups were as follows:- Normal dose: 158.56 ± 13.90 g compared to 138.31 ± 18.24 g initially - Medium dose: 165.50 ± 14.0 g compared to 127.57 ± 18.41 g initially - High dose: 156.82 ± 15.92 g compared to 125.17 ± 15.23 g initially. **Body Weight:** No significant difference ($P<0.05$) in body weight was observed between the control and high-dose MPM groups, suggesting no toxic impact of MPM on body weight.

The relative weight of the testis was significantly increased ($P<0.05$) in both the medium dose (MD) and high dose (HD) treated groups compared to the control groups A and B (Figure 2). **Relative Organ Weight:** MPM used caused significant reduction in the relative weight of the prostate at low, medium, and high doses. Testis weight increased significantly in medium and high dose groups compared to induced group-IHPG indicating a protective effect against hormone-induced hypogonadism.

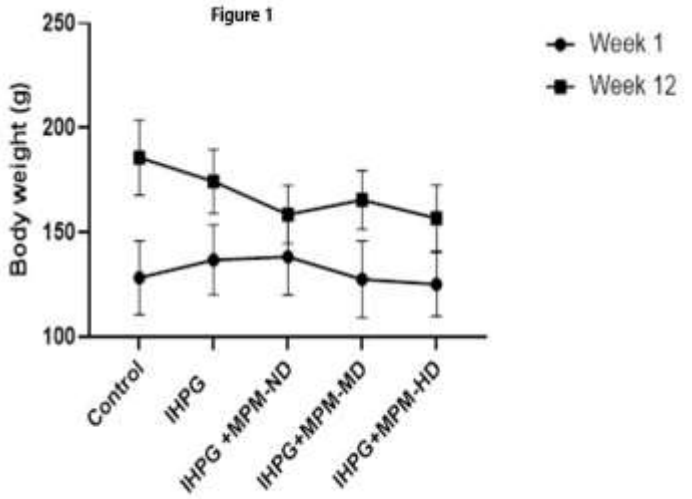
Sperm Count: Epididymal sperm count was significantly higher in the normal dose MPM group compared to the IHPG group. The normal dose MPM group showed a significant increase in sperm count with the highest recovery.

Histological Findings:

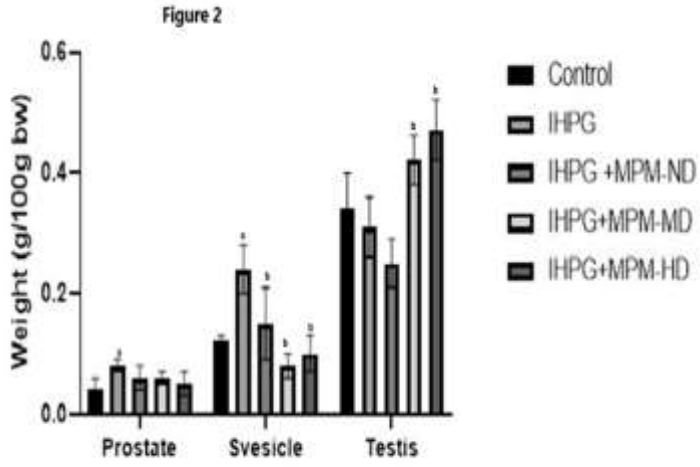
Plate 1: photomicrograph of control rat showing normal testicular architecture with different series of spermatogenic cells.

Plate 2: photomicrograph of induced control showing loss and degeneration of all types of spermatogenic cells within the seminiferous tubules and without sperms at the lumen,

Plates 3-5: Photomicrograph of MPM treated animals showing slight recovery of spermatogenic cells.

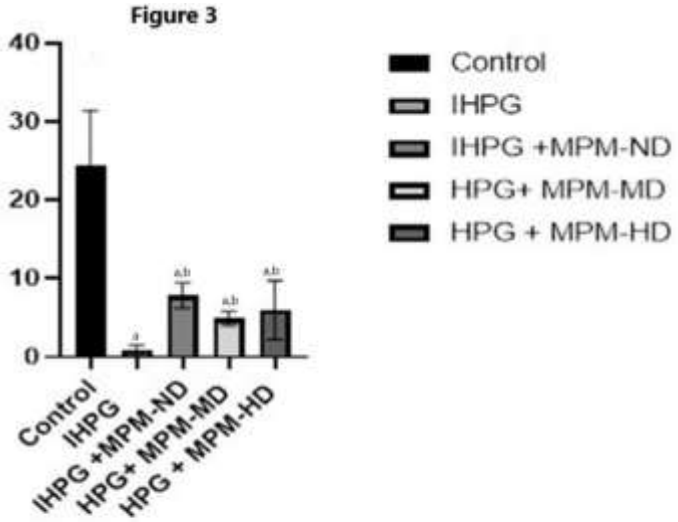


The Effects of MPM on Animal Body Weight



The effects of MPM on relative Organ Weight

Values are recorded as mean±SD. Superscript letters denote significant difference at (P<0.05)
a: Values are significantly different from control
b: Values are significantly different IHPG



The effects of MPM on Sperm Count (million/ml)

Vaues are recorded as mean ±SD. Superscript letters denote significant difference at (P<0.05)
a: Values are significantly different from control
b: Values are significantly different IHPG

Plate 1-6:

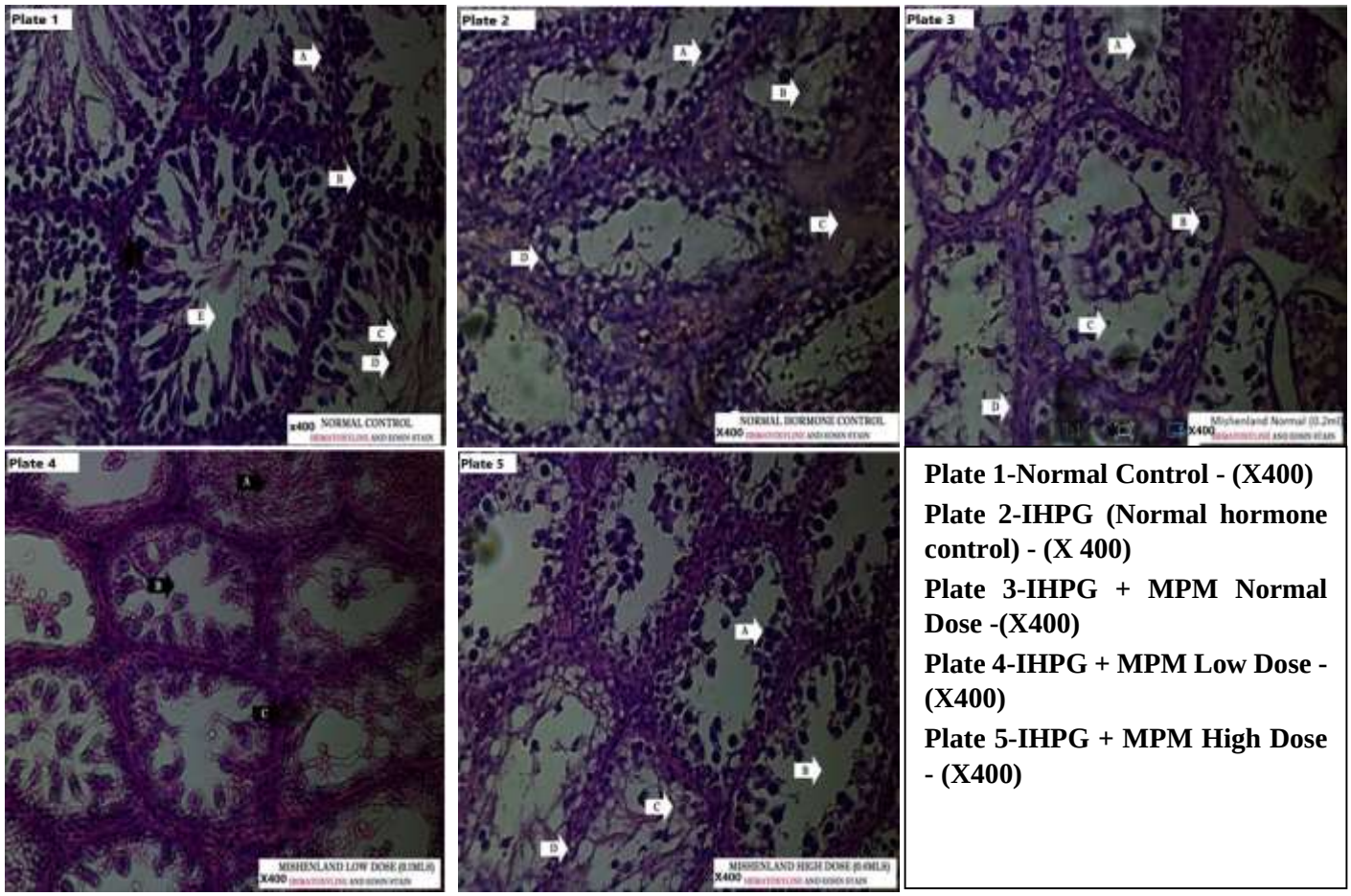


Plate 1-Normal Control - (X400)

Plate 2-IHPG (Normal hormone control) - (X 400)

Plate 3-IHPG + MPM Normal Dose -(X400)

Plate 4-IHPG + MPM Low Dose - (X400)

Plate 5-IHPG + MPM High Dose - (X400)

Plate 6- Showing priapism observed in 2/5 of the rats used as IHPG after prolonged hormone administration which persisted while the study lasted.

DISCUSSION

Mishenland polyherbal mixture (MPM) is a carefully formulated combination of medicinal plants known for their efficacy in managing reproductive health issues. The polyherbal approach leverages the synergistic effects observed when multiple herbs with complementary actions are used together, enhancing overall therapeutic outcomes (Karole *et al*., 2019, Ali *et al*. 2018). MPM's components, such as *Mondia whitei*, traditionally used as an aphrodisiac and for treating male infertility, contain bioactive compounds that support its efficacy in managing male reproductive health. Combining different herbs in traditional medicine can enhance their effects synergistically, increasing potency and mitigating side effects (Chokwe 2021).

In this study, MPM did not significantly alter the body weight of treated animals, consistent with previous research on its components, Allium ascalonicum and *Tetrapleura tetraptera*, which did not show significant impacts on body weight. Body weight changes can indicate responses to substance toxicity (Azu *et al.,* 2014), such as apoptosis or hypertrophy, but our findings

suggest that MPM does not exert such effects. Although, in some studies where *Allium sativum* (Chris *et al.,* 2016) *and Tetraptera tetrapleura* (Mensah *et al.,* 2024) administered alone caused increase in body weight, but this was not the case in the present study where they are in a mixed, perhaps due to the counteracting effects of the herbs in the mixture on one another.

Testosterone plays a critical role in spermatogenesis and testicular morphology. In normal controls, robust spermatogonia proliferation and mitotic activity were observed, resulting in nearly full lumens of sperm cells. However, exogenous testosterone administration disrupts natural testosterone production, leading to testicular shrinkage and altered histological architecture. The low dose of MPM proved the most potent and the recovery of testicular shape was evident and germ series are just starting to be repopulated. The normal testicular function is starting to pick up again due to the intervention (Thirumalai and Page, 2020).

At low doses, MPM infiltrated testicular connective tissues with cytoplasm partially filling lumens, inhibiting spermiogenesis. The herbs did not fully counteract the profound effects of exogenous testosterone on testicular structure and function, showing only minimal spermatogonia repopulation. However, the ability of the herb to intervene although in a subtle manner may be due to the effect of various mineral, nutritional substances and antioxidants present in some of the components of the product [MPM] (Adefule *et al*., 2014; Tung Nguyen-Thanh *et al*., 2024).

Conclusion: While MPM demonstrates potential in treating BPH and hypogonadism, its high-dose MPM administration may pose risks and side effects that could outweigh its benefits. High doses of MPM might compromise immunity and reduce body weight, exposing individuals to other diseases. Treatment with testosterone propionate significantly decreased testicular and seminal vesicle weights and disrupted normal testicular architecture, underscoring that exogenous androgens do not directly stimulate Leydig cell trophism and that stimulating Sertoli cell activity alone is insufficient to impact Leydig cell function effectively.

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Conflict of Interest: The authors declare no conflicts of interest.

Ethical Approval: Ethical approval for this study was obtained from the faculty ethics committee of Olabisi Onabanjo University.

Author Contributions: The conception and design of the study was by **OAA, APT** performed the animal experiments and **AAK, ABA and SLAJ** carried out the data analysis. **APT** and **SLAJ** wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

Adefule AK, Huthman AS, Adesanya OA, Otulana OJ, Adeyanju MM, Oyesiku OO (2014): Preliminary Phytochemical and Nutrient Evaluation of Mishenland polyherbal food supplement containing *Allium sativa*, *Allium ascalonicum*, *Tetrapleura tetraptera* and *Mondia whitei.* Int. J. Pharm. Res Vol 3(3):477-483.

Akintola OO, Bodede AI, Ogunbanjo OR (2015). Nutritional and medicinal importance of Tetrapleura tetraptera fruits (Aridan). African Journal of Science and Research.4 (6): 36-41.

Aladesanmi JA (2007). Tetraptera tetrapleura: Molluscidal activity and chemical constituents. Afr. J. Tradit. Complement. Altern. Med. 4(1): 23-36. DOI: 10.4314/ajtcam.v4i1.31210.

Alturkistani HA, Tashkandi FM, Mohammedsaleh ZM (2016). Histological stains: a literature review and case study. Int. J. Health Sci. 8(3): 72. DOI: 10.5539/gjhs.v8n3p72.

Amir Shahreza Pathel, Joon Yau Leong, Libert Ramos, Ranjith Ramasamy (2019): Testosterone Is a Contraceptive and Should Not Be Used in Men Who Desire Fertility World J Mens Health 37 (1):45-54.

Anderson DJ, Johnston DS (2023). A brief history and future prospects of contraception. Science 380(6641): 154-158. DOI: 10.1126/science.adf9341.

Arthi T, Stephanie TP (2020): Male Hormonal Contraception. Rev. Med. 71:17–31.

Asemani Y, Zamani N, Bayat M, Amirghofran Z (2019). Allium vegetables for possible future of cancer treatment. *Phytother Res* 33(5): 1159-1173. DOI: 10.1002/ptr.6490.

Azu OO, Naidu EC, Naidu JS, Masia TN, Nzemande NF, Singh SC (2014). Testicular histomorphologic and stereological alterations following short-term treatment with highly active antiretroviral drugs (HAART) in an experimental animal model. Andrology 2(6): 772-779. DOI: 10.1111/andr.12101.

Babalola IT, Adelakun EA (2018). Nutritional and medicinal importance of Tetrapleura tetraptera fruits (Aridan). J. Pharmacognosy Phytother. 7(3): 1983-1994.

Bhushan R (2021). Allium sativum, a potential phytopharmacological source of natural medicine for better health. GSCARR 6(3): 220-232. DOI: 10.30574/gscarr.2021.6.3.0018.

Bhushan R (2021). Allium sativum: A potential phytopharmacological source of natural medicine for better

health. N.a. J. Adv. Res. Rev. 6(3): 220-232. DOI: 10.30574/gscarr.2021.6.3.0018.

Chokwe RC, Dube S, Nindi MM (2021). Development of a Quantitative Method for Analysis of Compounds Found in Mondia whitei Using HPLC-DAD. Processes 9(11): 1864. DOI: 10.3390/pr9111864.

Chris-ozoko LE, Naiho AO, Okeke F (2016). Histological Effect of Aqueous Garlic (*Allium sativum)* Extract on the Testis and Ovary of an Adult Wistar Rat. IOSR-JDMS 15 (1): 38-45.

Grimes DA, Lopez LM, Gallo MF, Halpern V, Nanda K, Schulz KF (2012). Steroid hormones for contraception in men. Cochrane Database of Systematic Reviews. 3: CD004316. DOI: 10.1002/14651858.CD004316.pub4.

Karole S (2019). Polyherbal Formulation Concept for Synergic Action: A Review. J. Drug Deliv. Ther. 9(1-s): 453-466. DOI: 10.22270/jddt.v9i1-s.2339.

Mahapokai W, van Sluijs F, Schalken J (2000). Models for studying benign prostatic hyperplasia. Prostate Cancer Prostatic Dis 3: 28-33. DOI: 10.1038/sj.pcan.4500391.

Mensah RQ, Adusei S, Azupio S, Kwakye R (2024). Nutritive value, biological properties, health benefits, and applications of Tetrapleura tetraptera: An updated comprehensive review. Heliyon 10(6): e27834. DOI: 10.1016/j.heliyon.2024.e27834.

Nieschlag E, Vorona E (2015). Medical consequences of doping with anabolic androgenic steroids: effects on reproductive functions. Eur J Endocrinol 173: R47-R58. DOI: 10.1530/EJE-15-0080.

Oketch-Rabah HA (2012). Mondia whitei, a Medicinal Plant from Africa with Aphrodisiac and Antidepressant Properties: A Review. J. Diet. Suppl. 9(4): 272-284. DOI: 10.3109/19390211.2012.726704.

Page ST, Blithe D, Wang C (2022). Hormonal Male Contraception: Getting to Market. Front. Endocrinol. 13: 891589. DOI: 10.3389/fendo.2022.891589.

Radjabian T, Hosseinpur YZ, Naghizadeh MM (2022). Immunomodulatory Impacts of Bulbs Extracts From Five Allium Species on IFN-γ, IL-4, and IL-17 Cytokines. Immunoregulation 4(2): 91-100. DOI: 10.32598/Immunoregulation.4.2.4.

Richard Q, Mensah A, Stephen AB, Samuel AC, Richmond K (2024). Nutritional value, biological properties, health benefits and applications of *Tetrapleura tetraptera*: An updated comprehensive review. Heliyon 10: e27834.

Saddique HA, Hussain F, Shahid M (2015). Some Biological Perspectives of Allium sativum: A Review. Int. J. Chem. Biochem. Sci. 8: 35-39.

Thirumalai A, Page ST (2022). Androgens in male contraception. Best Pract Res Clin Endocrinol Metab 36(5): 101627. DOI: 10.1016/j.beem.2022.101627.

Tudu C.K, Dutta T, Ghora M, Biswas P, Samantha D (2022). "Traditional uses, phytochemistry, pharmacology, and toxicology of garlic (Allium sativum): A review of research from the last decade focusing on health and nutritional implications. Front. Nutr. 9:949554. DOI: 10.3389/fnut.2022.746944.

Tung Nguyen-Thanh, Phuc Dang-Ngoc, Manh-Hung Bui, Tam Le-Minh, Quoc- Huy Nguyen-Vu (2022): Effectiveness of Herbal medicines on male reproductive system: Evidence from meta-analysis. Pharmacol. Res. - Mod. Chin. Med *12 (2024):100462*

Wang C, Festin MPR, Swerdloff RS (2016). Male Hormonal Contraception: Where Are We Now? Curr Obstet Gynecol. 5: 38-47. DOI: 10.1007/s13669-016-0153-7.

Watcho P (2019). Sexual stimulant effects of the mixture of Mondia whitei, Dracaena arborea, and Bridelia ferruginea in normal and prediabetic male Wistar rats. J Basic Clin Physiol 30(4): 20180222.

Zhang Q, Zhao Q, Shen Y, Zhao F, Zhu Y (2022). Allium Vegetables, Garlic Supplements, and Risk of Cancer: A Systematic Review and Meta-analysis. Front. Nutr. 8: 746944. DOI: 10.3389/fnut.2021.746944.