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Comparative Effects of Phytochemical Constituents (Alkaloids, Tannins, and Flavonoids) of *Uvaria ovata* on the Male Reproductive Organs of Albino Rats

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ABSTRACT

Background: Uvaria ovata is a medicinal plant widely used in traditional medicine, but its individual phytochemical effects on male reproduction remain unclear.
Methods: Twenty-four adult male albino rats were divided into four groups (n = 6): control, alkaloid-, tannin-, and flavonoid-treated groups. Extracts (100 mg/kg) were administered orally for 28 days. Body and organ weights, sperm parameters, serum testosterone, and testicular histology were evaluated.
Results: Alkaloid fractions significantly reduced sperm count, motility, and testosterone levels, with marked testicular degeneration. Tannin fractions caused moderate suppression of reproductive indices. Flavonoid fractions significantly improved sperm parameters and testosterone levels while preserving testicular architecture.
Conclusion: Alkaloids and tannins exhibit anti-fertility effects, whereas flavonoids enhance reproductive function, suggesting potential therapeutic applications.

Keywords: Uvaria ovata; Alkaloids; Tannins; Flavonoids; Male reproductive organs

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INTRODUCTION

Medicinal plants remain a cornerstone of traditional and complementary medicine, particularly in developing regions where access to orthodox healthcare is limited. It is estimated that over 80% of the world's population relies partly or wholly on plant-based remedies for primary healthcare needs. These plants are rich sources of secondary metabolites such as alkaloids, tannins, flavonoids, saponins, and phenolic compounds, which exert diverse physiological and pharmacological effects in humans and animals.[1]

In recent years, increasing scientific attention has been directed toward understanding how these bioactive phytochemicals influence reproductive health. Male infertility, which contributes to nearly 50% of infertility cases globally, has been linked to hormonal imbalance, oxidative stress, environmental toxins, and indiscriminate use of herbal medications.[2] According to Chike[3], reproductive dysfunction arising from uncontrolled exposure to phytochemicals is an emerging public health concern, especially in regions where herbal medicine is widely practiced without standardized dosage or toxicity evaluation.

The male reproductive system is particularly vulnerable to xenobiotics and plant-derived compounds due to the high rate of cell division during spermatogenesis and the sensitivity of Leydig and Sertoli cells to oxidative and hormonal disturbances. Any alteration in this delicate system may manifest as changes in sperm quality, testosterone production, and testicular architecture.[4] Consequently, systematic evaluation of medicinal plants and their isolated constituents is critical for validating their safety and therapeutic relevance.

Uvaria ovata, a member of the family Annonaceae, is a tropical plant widely distributed in parts of Africa and traditionally used for the treatment of infections, inflammation, gastrointestinal disorders, and fertility-related conditions. Phytochemical investigations have revealed that *U. ovata* contains appreciable amounts of alkaloids, tannins, flavonoids, saponins, and phenolic compounds.[5] Despite its widespread use, scientific data on the reproductive safety and fertility implications of its individual phytochemical constituents remain limited.

Idam and Ebisintei[6] demonstrated that extracts of *Uvaria ovata* possess significant gastroprotective and anti-inflammatory properties, supporting its ethnomedicinal relevance. However, they emphasized that the presence of potent bioactive compounds raises concerns regarding possible organ-specific toxicity when consumed chronically or at high doses. Similarly, Ebisintei and Okaba[7] reported that plant-derived bioactive substances can exert either protective or deleterious effects on male reproductive organs depending on their phytochemical composition and mode of administration.

Alkaloids, for instance, have been associated with cytotoxic and endocrine-disrupting effects that may impair spermatogenesis and testosterone synthesis (8). Tannins, while beneficial for their antimicrobial and antioxidant activities, have been reported to suppress reproductive function by interfering with nutrient absorption and enzyme activity essential for germ cell development.[9] In contrast, flavonoids are widely recognized for their antioxidant, anti-inflammatory, and androgen-supportive properties, which may enhance sperm quality and protect testicular tissue from oxidative damage.[10, 11]

CPR Chike emphasized that the therapeutic paradox of medicinal plants lies in their complex phytochemical makeup, where beneficial and harmful constituents may coexist within the same extract. This underscores the importance of fractionation studies that isolate individual phytochemical classes to determine their specific biological effects. Such an approach not only enhances drug safety but also facilitates the identification of lead compounds for pharmaceutical development.[3]

Although *Uvaria ovata* is traditionally regarded as beneficial for fertility, there is insufficient empirical evidence distinguishing the reproductive effects of its individual phytochemical fractions. Most existing studies focus on crude extracts, which may mask the specific actions of individual constituents. Ebisintei P. and Idam B. U. have both advocated for phytochemical-specific investigations as a necessary step toward safe medicinal plant utilization and evidence-based ethnopharmacology [6]

Therefore, this study was designed to isolate the major phytochemical constituents' alkaloids, tannins, and flavonoids from *Uvaria ovata* and evaluate their comparative effects on body and reproductive organ weights, sperm quality parameters, serum testosterone levels, and testicular histopathology in male albino rats.

MATERIALS AND METHODS

Study Area and Experimental Design: This experimental laboratory study was designed to evaluate the comparative effects of alkaloid, tannin, and flavonoid fractions of *Uvaria ovata* on male reproductive parameters in albino rats. The study followed a

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completely randomized design and was conducted in accordance with standard laboratory animal handling guidelines.

Collection, Identification, and Preparation of Plant Material: Fresh leaves of *Uvaria ovata* were collected from forested areas of Bolou-Orua community, Bayelsa State, Nigeria. The plant was authenticated by a qualified botanist in the Department of Biological Sciences, University of Africa, Toru-Orua. A voucher specimen was deposited in the departmental herbarium for future reference.

The leaves were thoroughly washed with clean water to remove debris and air-dried at room temperature (25–28 °C) for two weeks until a constant weight was achieved. The dried leaves were pulverized into fine powder using an electric grinder and stored in airtight containers until extraction.

Extraction of Crude Methanolic Extract: Two hundred grams (200 g) of the powdered plant material were extracted using 80% methanol in a Soxhlet extractor for 8 hours. Methanol was selected due to its efficiency in extracting both polar and moderately non-polar phytochemicals. The resulting extract was concentrated under reduced pressure using a rotary evaporator and further dried to obtain a semi-solid crude extract. The extract was stored at 4 °C until fractionation.[17]

Phytochemical Screening: Preliminary qualitative phytochemical screening of the crude extract was carried out using standard methods to confirm the presence of alkaloids, tannins, and flavonoids. Tests such as Dragendorff's test (alkaloids), Ferric chloride test (tannins), and Shinoda test (flavonoids) were employed following established protocols.

Fractionation of Phytochemical Constituents: The crude methanolic extract was subjected to solvent–solvent partitioning to obtain alkaloid, tannin, and flavonoid fractions. The extract was suspended in distilled water and sequentially partitioned using chloroform, ethyl acetate, and aqueous solvents based on polarity differences.

- **Alkaloid fraction** was obtained following acid-base extraction procedures.
- **Flavonoid fraction** was concentrated from the ethyl acetate layer.
- **Tannin fraction** was recovered from the aqueous phase.

Each fraction was concentrated using a rotary evaporator, dried, weighed, and stored in labeled containers at 4 °C until administration

Experimental Animals: Twenty-four (n =24) healthy adult male albino rats (*Rattus norvegicus*), weighing between 180–200 g, were obtained from a certified animal breeding facility. The animals were housed in standard laboratory cages under controlled environmental conditions (12-hour light/dark cycle, temperature 25 ± 2 °C) and allowed free access to standard rat feed and clean drinking water.

The animals were acclimatized for two weeks prior to the commencement of the experiment.

Ethical Consideration: All experimental procedures were conducted in accordance with international guidelines for the care and use of laboratory animals. Ethical approval was obtained from the Institutional Animal Ethics Committee of the University of Africa, Toru-Orua

Experimental Design: Twenty-four adult male albino rats (180–200 g) were randomly divided into four groups (n = 6):

Grp	Treatment	Dose	Duration
1	Control (distilled water)		28 days
2	Alkaloid fraction of Uvaria ovata	100mg/kg	28 days
3	Tannin fraction of Uvaria ovata	100mg/kg	28 days
4	Flavonoid fraction of Uvaria ovata	100mg/kg	28 days

Determination of Body and Organ Weights: Body weights of the animals were recorded at the beginning of the experiment and weekly thereafter. At the end of the 28-day treatment period, the rats were sacrificed under mild anesthesia. The testes and epididymides were carefully excised, cleared of adherent tissues, and weighed using a digital analytical balance. Relative organ weights were calculated.

Evaluation of Sperm Parameters: The cauda epididymis was excised and minced in normal saline to release spermatozoa.

- **Sperm count** was determined using a Neubauer hemocytometer.
- **Sperm motility** was assessed microscopically and expressed as a percentage of progressively motile sperm.
- **Sperm morphology** was evaluated using stained smears, and the percentage of normal sperm cells was recorded.

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All analyses were performed according to standard reproductive toxicology protocols.

Serum Testosterone Assay: Blood samples were collected via cardiac puncture and allowed to clot at room temperature. Serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum testosterone levels were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's instructions.

Histopathological Examination: Testes were fixed in 10% neutral buffered formalin for 48 hours, dehydrated in graded ethanol concentrations, cleared in xylene, and embedded in paraffin wax. Sections of 5 μm thickness were cut using a microtome and stained with hematoxylin and eosin (H&E). Slides were examined under a light microscope for histological alterations in seminiferous tubules, germinal epithelium, and interstitial cells.

Statistical Analysis: Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

Parameters Measured

- **Body and Organ Weights**
- **Sperm Parameters:** Count, motility, viability, morphology
- **Serum Testosterone:** Measured using ELISA
- **Histopathological Examination:** Testes fixed in 10% formalin, stained with H&E

Organ Weights

Alkaloid and tannin fractions caused slight reductions in testicular and epididymal weights compared to control, while the flavonoid group showed mild increases.

RESULTS

Table 1: Sperm Characteristics

Group	Sperm Count ($\times 10^6$/mL)	Motility (%)	Morphology (normal %)
Control	75 ± 3.2	81 ± 2.5	88 ± 3.1
Alkaloid	48 ± 2.1*	55 ± 3.4*	72 ± 2.7*
Tannin	56 ± 2.8*	61 ± 2.5*	78 ± 2.4*
Flavonoid	84 ± 3.5	89 ± 2.6	91 ± 2.9

(*p<0.05 vs control)

Table 2: Serum Testosterone

Group	Testosterone (ng/mL)
Control	3.25 ± 0.15
Alkaloid	1.98 ± 0.12*
Tannin	2.30 ± 0.18*
Flavonoid	3.95 ± 0.20

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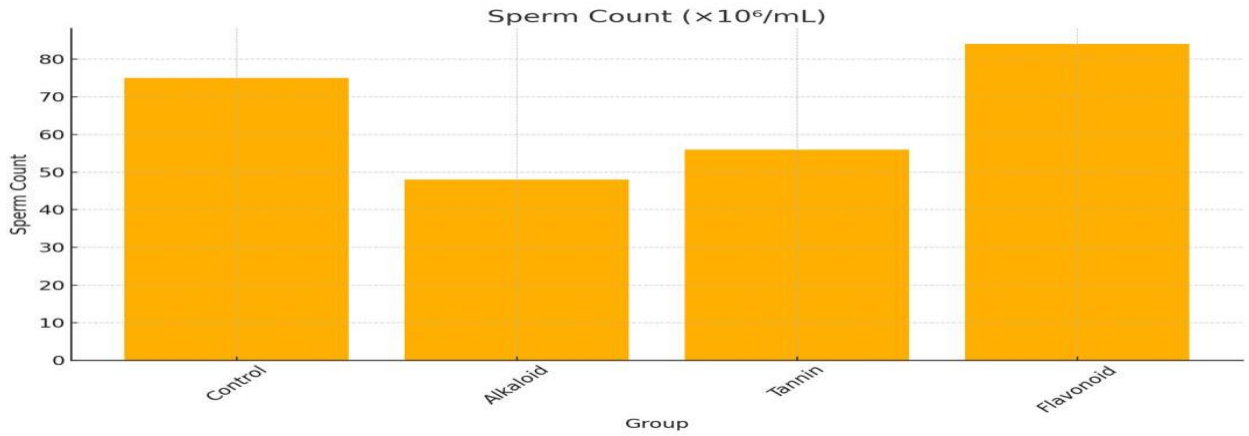

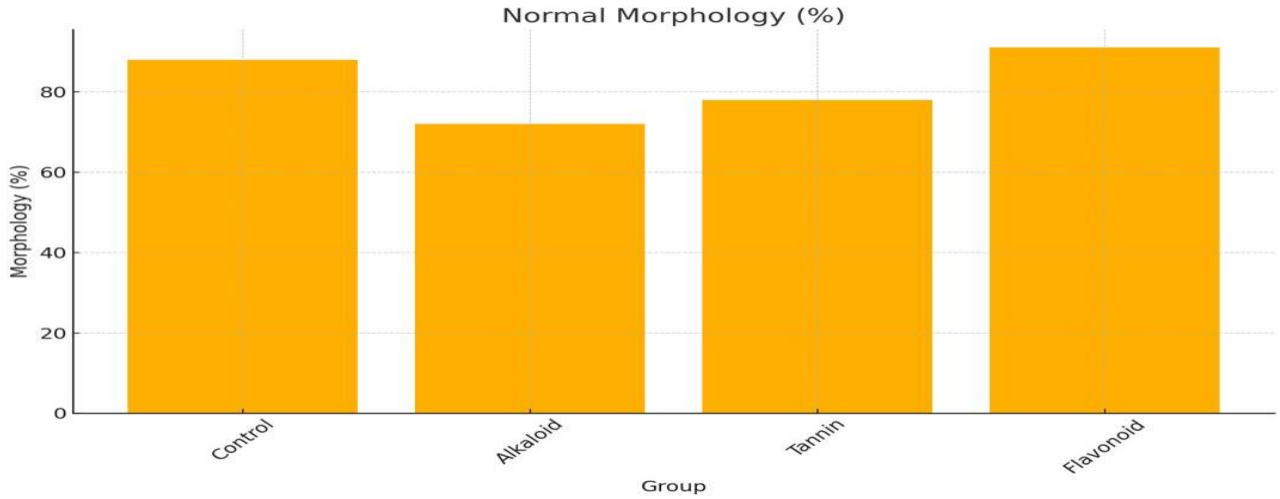

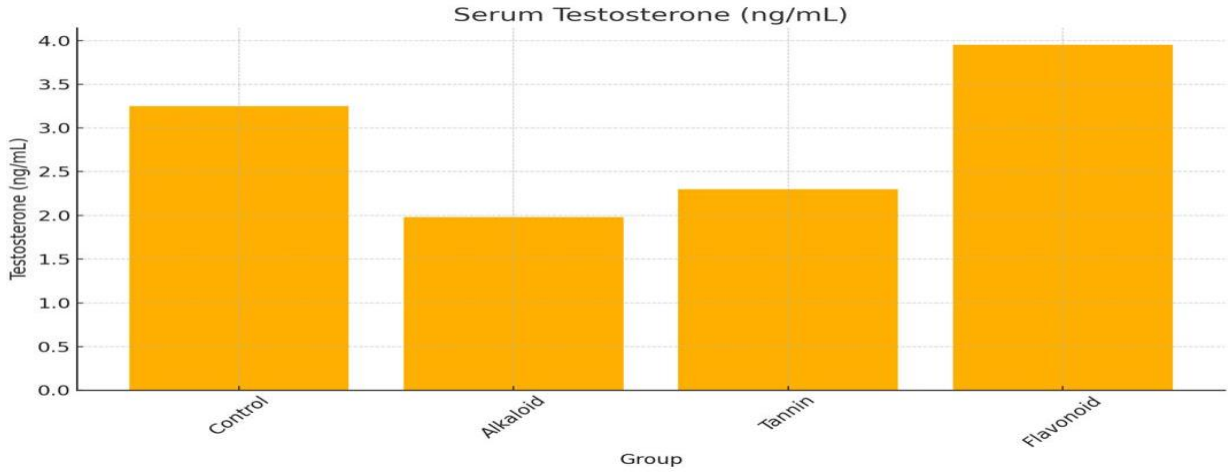

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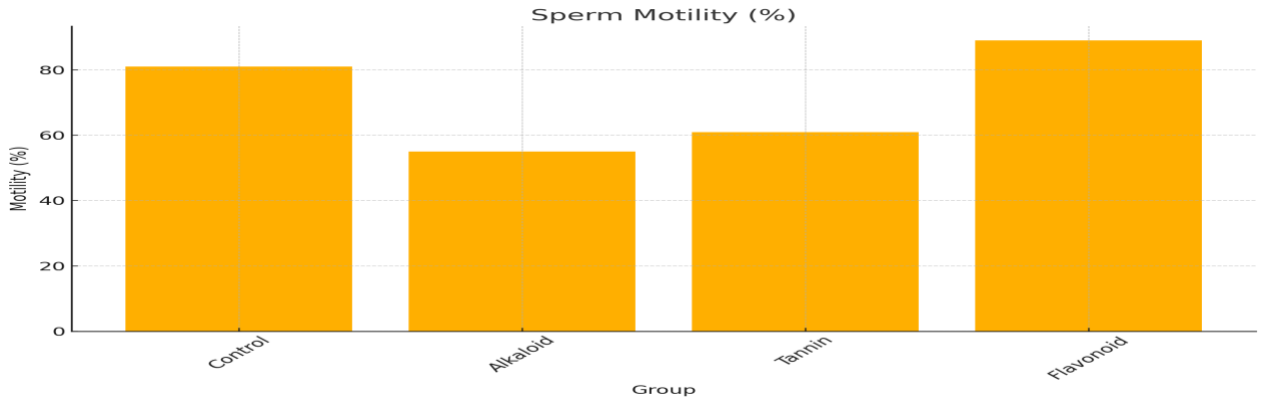
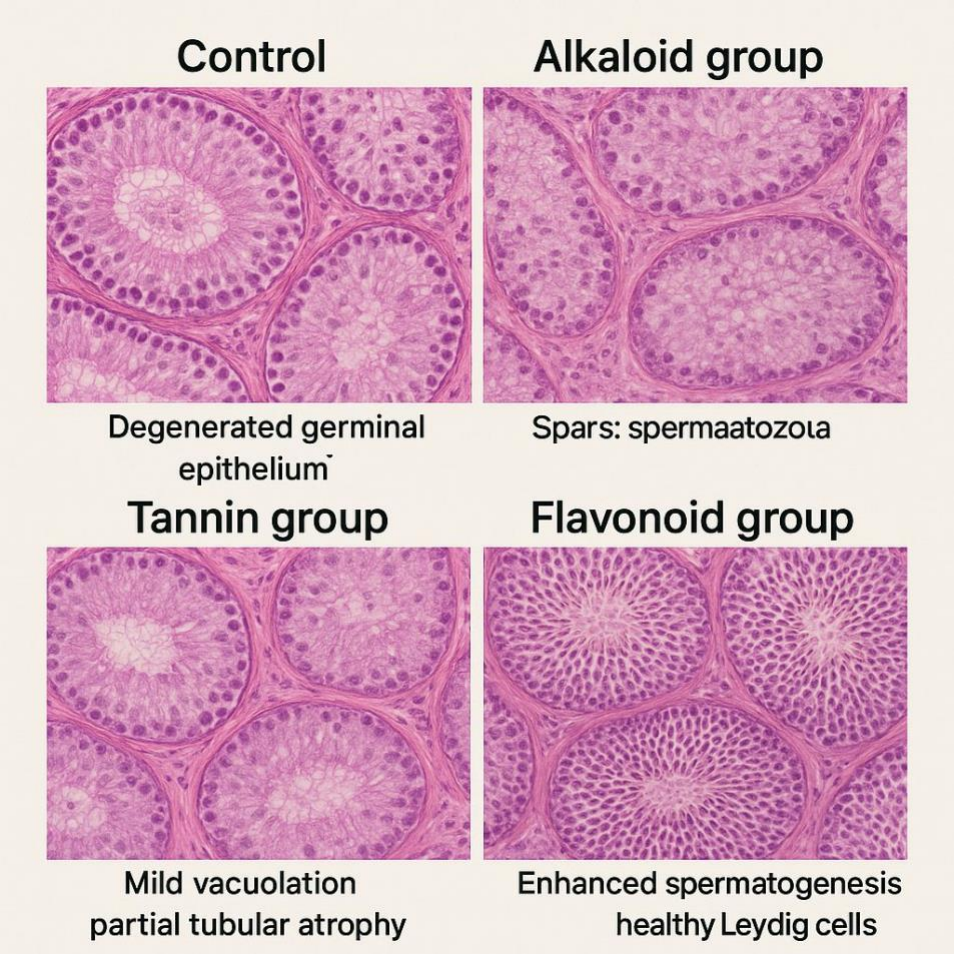


Fig 1. "Effects of Alkaloid, Tannin, and Flavonoid Fractions of Uvaria ovata on Male Reproductive Parameters"

Histopathological Findings



- Control: Normal spermatogenic layers and intact seminiferous tubules.
- Alkaloid group: Degenerated germinal epithelium and sparse spermatozoa.
- Tannin group: Mild vacuolation and partial tubular atrophy.
- Flavonoid group: Enhanced spermatogenesis, abundant spermatozoa, and healthy Leydig cells.

Figure 2. Histopathological Changes in Testicular Tissue Following Administration of Alkaloid, Tannin, and Flavonoid Fractions of Uvaria ovata

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DISCUSSION

The present study investigated the differential effects of alkaloid, tannin, and flavonoid fractions of *Uvaria ovata* on male reproductive function in albino rats. The observed variations in body and organ weights, sperm parameters, serum testosterone concentration, and testicular histoarchitecture confirm that individual phytochemical constituents of medicinal plants can exert divergent and sometimes opposing physiological effects. These findings provide experimental evidence supporting ethnomedicinal claims while also emphasizing the need for caution in the indiscriminate use of unrefined plant preparations.

Effects on Body and Organ Weight

Body weight changes are commonly used as preliminary indicators of systemic toxicity and general health status in experimental animals. In this study, the absence of significant body weight loss across all treated groups suggests that the phytochemical fractions of *Uvaria ovata* at 100 mg/kg were well tolerated and did not induce overt toxicity. This observation aligns with reports by Yakubu et al., who noted that moderate doses of plant secondary metabolites may selectively affect reproductive tissues without causing generalized systemic toxicity.[8]

Reproductive organ weights, particularly those of the testes and epididymis, are closely associated with androgen availability and spermatogenic activity. The slight reduction in testicular and epididymal weights observed in the alkaloid- and tannin-treated groups may indicate suppressed spermatogenesis or impaired androgenic support. Brennan and Capel, reported that reductions in seminiferous tubule mass and germ cell population directly translate into decreased testicular weight.[13] Alkaloids have been shown to disrupt cellular metabolism and induce oxidative stress within reproductive tissues, leading to reduced organ mass (14). Conversely, the flavonoid-treated group showed a mild increase in reproductive organ weights, suggesting enhanced spermatogenic activity and improved endocrine function. This finding corroborates earlier studies by Ebisintei and Okaba,[7] who reported that flavonoid-rich plant products preserved testicular weight and structure in male albino rats.[7] similarly emphasized that plant-derived antioxidants support tissue integrity by stabilizing cellular membranes and reducing inflammatory damage.

Effects on Sperm Parameters: Sperm count, motility, and morphology are critical determinants of male fertility and are highly sensitive to hormonal imbalance and oxidative stress. The significant reductions in sperm count and motility observed in the alkaloid- and tannin-treated groups indicate compromised spermatogenic efficiency and epididymal sperm maturation. Alkaloids are known to interfere with mitochondrial ATP production in sperm cells, thereby reducing motility and viability.[8] Tannins may further impair spermatogenesis by binding essential trace elements such as zinc, which is crucial for sperm development and membrane stability.[9] The flavonoid fraction, however, produced a marked improvement in sperm count, motility, and normal morphology. This enhancement may be attributed to the potent antioxidant properties of flavonoids, which scavenge reactive oxygen species (ROS) and protect germ cells from lipid peroxidation and DNA damage (15). Similar fertility-enhancing effects of flavonoids have been reported in other medicinal plants, including *Moringa oleifera* and *Uvaria* species [5, 12]

Ebisintei and Okaba[7] further demonstrated that improved antioxidant balance within the testes and epididymis directly correlates with increased sperm output and functional competence. Thus, the observed improvement in sperm parameters in the flavonoid-treated group suggests that this fraction of *Uvaria ovata* promotes both spermatogenesis and sperm maturation.

Effects on Serum Testosterone: Testosterone is a key regulator of spermatogenesis, libido, and the maintenance of male reproductive organs. The significant reduction in serum testosterone levels observed in the alkaloid- and tannin-treated groups suggests suppression of Leydig cell steroidogenic activity. This suppression may result from oxidative damage to Leydig cells or disruption of the hypothalamic–pituitary–gonadal (HPG) axis.[4] Reduced testosterone levels have been consistently associated with impaired spermatogenesis and decreased sperm quality.[8]

In contrast, the flavonoid-treated group exhibited a significant elevation in serum testosterone levels compared to the control. This increase suggests enhanced Leydig cell function and possible stimulation of luteinizing hormone (LH) secretion. Flavonoids have been reported to upregulate key steroidogenic enzymes and protect Leydig cells from oxidative stress.[15] Ebisintei and Okaba similarly observed elevated testosterone levels following administration of plant-derived antioxidants in male rats, reinforcing the role of flavonoids as pro-androgenic agents.[7]

The positive correlation between elevated testosterone levels and improved sperm parameters in this study

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further underscores the central role of androgenic regulation in male fertility.

Histopathological Examination: Histopathological evaluation of the testes provides direct structural confirmation of functional and hormonal findings. Testes from the alkaloid-treated group showed degenerated germinal epithelium, widened seminiferous tubules, and reduced spermatozoa, indicating impaired spermatogenesis. These alterations are characteristic of phytochemical-induced reproductive toxicity and have been reported in alkaloid-rich plant studies.[14]

The tannin-treated group exhibited mild vacuolation and partial seminiferous tubular atrophy, suggesting moderate testicular damage. Tannins are known to exert dose-dependent cytotoxic effects, particularly when administered as isolated fractions rather than as part of whole plant extracts [9]

In contrast, the flavonoid-treated group displayed intact seminiferous tubules, well-organized germinal layers, abundant spermatozoa, and healthy Leydig cells. These histological features indicate enhanced spermatogenic activity and endocrine function. Idam and Ebisintei previously reported that phytochemical-rich extracts with antioxidant properties preserved tissue architecture in experimental models, [7] while Ojewole highlighted the anti-inflammatory role of flavonoids in preventing testicular degeneration.[16]

Implications of the Findings

Overall, the findings demonstrate that *Uvaria ovata* contains phytochemical constituents with contrasting reproductive effects. Alkaloid and tannin fractions exhibit anti-fertility tendencies through suppression of spermatogenesis and testosterone production, whereas the flavonoid fraction exerts fertility-enhancing, antioxidant, and testiculoprotective effects. These results support the conclusions of Ebisintei P. and Idam B. U. that fractionation is critical in determining the safety and therapeutic value of medicinal plants.

CONCLUSION

This study demonstrates that the phytochemical constituents of Uvaria ovata exert differential effects on male reproductive physiology. Alkaloid and tannin fractions impair spermatogenesis, reduce testosterone levels, and induce structural testicular damage, indicating anti-fertility properties. In contrast, the flavonoid fraction enhances sperm quality, increases testosterone levels, and preserves normal testicular architecture, suggesting a protective and fertility-enhancing role. These findings highlight the importance of phytochemical fractionation in determining the safety and therapeutic potential of medicinal plants. Further studies are recommended to characterize the active compounds and elucidate their mechanisms of action.

Declarations

Conflict of interest: The author declares that there are no conflicts of interest regarding the publication of this study.

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