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Histomorphological and Hormonal Changes in the Testes of Albino Rats Treated with Herbal Aphrodisiac Formular

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ABSTRACT

Herbal supplements, commonly referred to as botanicals, are plant-derived products utilized for their therapeutic properties. This study aims to examine the histomorphological and hormonal changes in the testes of albino rats treated with a herbal aphrodisiac formula. A total of eight male and two female rats were used in the study, with the males divided into control and test groups, while the females were used for mating with the test groups. Group A (Control) received only normal feed (growers' mash) and distilled water daily for four weeks. Group B was administered 0.2 ml of Men's Formula supplement and sacrificed on day 7, whereas Group C was administered 0.2 ml of Men's Formula supplement and sacrificed on day 14. The body weights of the animals were measured before and after acclimatization, and similar weight assessments were performed at the end of each phase, with the average weights recorded accordingly. At the end of the procedure, the testes of each rat were excised under chloroform anesthesia and fixed in 10% formal saline for subsequent histological examination. Blood samples were collected via cardiac puncture into plain bottles, and sera were later separated for hormonal analysis. The results indicated that the herbal aphrodisiac formula enhanced testosterone levels and increased testis weight. However, no significant change ($P > 0.05$) was observed in body weight, and testicular histology remained unaffected. Further studies with prolonged usage of the herbal aphrodisiac formula are recommended to determine potential long-term effects

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INTRODUCTION

Herbal supplements, also known as botanicals, are plant-derived products recognized for their therapeutic properties. These supplements have been utilized for centuries across various cultures, playing a vital role in traditional medical systems such as Traditional Chinese Medicine (TCM) and indigenous healing practices [1]. They are available in multiple forms, including teas, capsules, tablets, powders, and extracts, and are widely promoted for their potential health benefits. The growing interest in natural and alternative health approaches has contributed to a surge in the popularity of herbal supplements in recent years [2].

LITERATURE REVIEW

Male dysfunction, which encompasses a range of issues related to reproductive and sexual health, remains a significant concern affecting the quality of life for many men worldwide [3]. This category includes conditions such as erectile dysfunction (ED), premature ejaculation, low libido, and other sexual performance-related disorders [4]. These issues often stem from a complex interaction of physical, psychological, and lifestyle factors, which can lead to stress, relationship difficulties, and diminished self-esteem [5]. Erectile dysfunction, one of the most common male dysfunctions, is characterized by a persistent inability to achieve or sustain an erection sufficient for satisfactory sexual activity. It affects millions of men, particularly those over the age of 40, with prevalence increasing with age [6].

Several underlying causes contribute to erectile dysfunction, including cardiovascular disease, diabetes, hormonal imbalances, neurological disorders, and the use of certain medications. Psychological factors such as stress, anxiety, and depression also play a crucial role in its onset and persistence [7]. Another prevalent issue, premature ejaculation, is marked by an inability to control ejaculation, which can lead to dissatisfaction for both partners and emotional distress. This condition arises from both psychological factors, such as performance anxiety, and biological influences, including abnormal neurotransmitter levels [8]. Similarly, low libido, or reduced sexual desire, can be caused by hormonal imbalances, chronic illnesses, mental health disorders, and certain medications [9]. Additionally, lifestyle factors such as poor diet, lack of physical activity, excessive alcohol consumption, and smoking contribute to decreased sexual desire [10]. Addressing low libido often requires a comprehensive approach that combines medical intervention, lifestyle changes, and psychological support [11].

Herbal supplements have gained popularity among men seeking natural remedies for male dysfunction, including erectile dysfunction, premature ejaculation, and low libido. These alternatives to conventional pharmaceuticals appeal to individuals who prefer holistic and integrative health approaches [12]. The use of herbal supplements for male sexual health is deeply rooted in traditional medical systems such as Ayurveda and Traditional Chinese Medicine (TCM), which have long relied on plants and herbs to promote vitality and reproductive function [13].

Among the most well-known herbs for male dysfunction is Panax ginseng, commonly referred to as Korean red ginseng. This herb is recognized for its potential to enhance erectile function, likely by increasing nitric oxide production, which improves blood circulation to the penile tissues [14]. Clinical research has demonstrated that ginseng may significantly enhance erectile function and overall sexual satisfaction. Another widely used herb, Yohimbe, is derived from the bark of the African Yohimbe tree. Its active component, yohimbine, is believed to enhance erectile performance by promoting blood flow and nerve stimulation in the genital area [15].

Maca root, a plant native to the high-altitude regions of Peru, has traditionally been used to improve libido and sexual performance. Studies indicate that maca may help regulate hormone levels and boost energy, which in turn enhances sexual desire and function [16]. Another well-known herb, Tribulus terrestris, has been traditionally used to support sexual health by stimulating the production of hormones such as testosterone, which plays a crucial role in libido and sexual function [17].

Despite their potential benefits, the use of herbal supplements for male dysfunction presents certain challenges and considerations. Variability in the quality and potency of herbal supplements can affect their effectiveness and safety [18]. Unlike prescription medications, herbal supplements are not always subject to strict regulatory oversight, which can lead to inconsistencies in product quality. Additionally, some herbal compounds may interact with conventional medications or cause adverse effects, highlighting the importance of consulting healthcare professionals before initiating any herbal treatment [19].

METHODOLOGY

Animal Model and Handling

Ten adult albino rats (~120g) were obtained and acclimatized for two weeks in a controlled laboratory environment. They were housed in wire mesh cages to prevent contamination and fed Growers' mash with water. Care and usage followed standard laboratory animal guidelines.

Grouping of Animal Model

Eight male and two female albino rats were used, divided into control and test groups.

- Group A: 2 males, not serviced by females.
- Group B: 3 males, serviced by females for 14 days.
- Group C: 3 males, serviced by females for 21 days.

Substance of Study

The aphrodisiac herbal formula was purchased from a government approved pharmacy in Benin, Edo state and diluted to appropriate concentrations.

Substance Administration

The administration of Men's Formula Supplement was done orally as follows:

- Group A (Control): Received only normal feed (growers' mash) and distilled water for four weeks.

- Group B: Given 0.2ml of Men's Formula Supplement, sacrificed on day 7.
- Group C: Given 0.2ml of Men's Formula Supplement, sacrificed on day 14.

Sample Collection and Analysis

The animals' weights were recorded before and after acclimatization, as well as at the end of each phase, with the average weight documented. At the conclusion of the study, the testes were excised under chloroform anesthesia and preserved in 10% formal saline for histological examination. Blood samples were collected via cardiac puncture into plain bottles, and sera were separated for hormonal analysis.

Histological Processing

Tissues were processed using an automatic tissue processor following the histopathology laboratory protocol at Benson Idahosa University. The fixed tissues in 10% formalin underwent sequential processing in formalin, graded alcohols, xylene, and molten paraffin wax. After embedding in paraffin, the blocks were solidified at 5°C for 15 minutes, trimmed at 30 microns, and cut into 5-micron sections using a rotary microtome. The sections were floated in a water bath at 45°C, mounted on frosted-end slides, placed on a hot plate for 40 minutes, then de-waxed, hydrated, air-dried, and stored for staining.

Staining Procedure (Tietz , 1995)

Tissue sections were stained using the Hematoxylin and Eosin technique. They were dewaxed in xylene, hydrated through descending alcohol grades, and stained with hematoxylin for 7 minutes. After rinsing, differentiation in 1% acid alcohol was halted in distilled water, followed by bluing in tap water for 10 minutes. Sections were then counterstained with eosin, rinsed, dehydrated in ascending alcohol grades, cleared in xylene, air-dried, and mounted with dibutyl phthalate propylene xylene (DPX).

Hormonal Analysis

Testosterone levels were measured using the ELISA method (Tietz, 1995). This immunoenzymometric assay utilized high-affinity antibodies with distinct epitope recognition. Immobilization occurred within the microplate well through streptavidin-coated wells interacting with biotinylated monoclonal anti-testosterone antibodies.

Statistical Analysis

Data obtained were analyzed using SPSS version 20 statistical software package. Results generated were expressed as mean ± SD and a P-value of <0.05 was considered significant

RESULT

Table 1 shows the Mean ± Standard Deviation (S.D) of body weight values for Control, Test Group at Day 14 and Test Group at Day 21 for male albino rats.

The Mean ± S.D values for Control, Test Group at Day 14 and Test Group at Day 21 were 234.53±44.49, 203.60±3.37 and 212.85±4.02 respectively. However, there was no statistically ($P>0.05$) significance difference across the groups

Table 1. The Mean ± Standard Deviation (S.D) of Body Weight Values for Control, Test Group at Day 14 and Test Group at Day 21 for Male Albino Rats

	Mean ± S.D	**F – Value**	**P-Value**
CONTROL	234.53±44.49	1.51	0.27
TEST GROUP AT DAY 14	203.60±3.37		
TEST GROUP AT DAY 21	212.85±4.02		

Numbers represent Mean±S.D, *mean significant P≤0.05

Table 2 shows the Mean ± Standard Deviation (S.D) of organ weight (Testes) values for Control, Test Group at Day 14 and Test Group at Day 21 for male albino rats.

The Mean ± S.D values for Control, Test Group at Day 14 and Test Group at Day 21 were 2.50±0.08, 1.83±0.33 and 2.43±0.42 respectively. For the Organ weight, there was a significant (P<0.05) variation across the groups

Table 2. The Mean ± Standard Deviation (S.D) of Organ Weight (Testes) Values for Control, Test Group at Day 14 and Test Group at Day 21 for Male Albino Rats

	Mean ± S.D	**F - Value**	**P-Value**
CONTROL	2.50±0.08	5.63	0.03*
TEST GROUP AT DAY 14	1.83±0.33		
TEST GROUP AT DAY 21	2.43±0.42		

Numbers represent Mean±S.D, *mean significant P≤0.05

Table 3 shows the Mean ± Standard Deviation (S.D) of Testosterone values for Control, Test Group at Day 14 and Test Group at Day 21 for male albino rats.

The Mean ± S.D values for Control, Test Group at Day 14 and Test Group at Day 21 were 0.34±0.02, 0.23±0.22 and 0.44±0.01 respectively.

Table 3 The Mean ± Standard Deviation (S.D) of Testosterone Values for Control, Test Group at Day 14 and Test Group at Day 21 for Male Albino Rats

	Mean ± S.D	**F – Value**	**P-Value**
CONTROL	$0.34\pm0.02_{bc}$	111.56	0.00*
TEST GROUP AT DAY 14	$0.23\pm0.02_{ac}$		
TEST GROUP AT DAY 21	$0.44\pm0.01_{ab}$		

Numbers represent Mean±S.D, *mean significant P≤0.05

Similar subscript alphabets represents a significant statistical variation occurred when compared with each other groups.

Micrographs

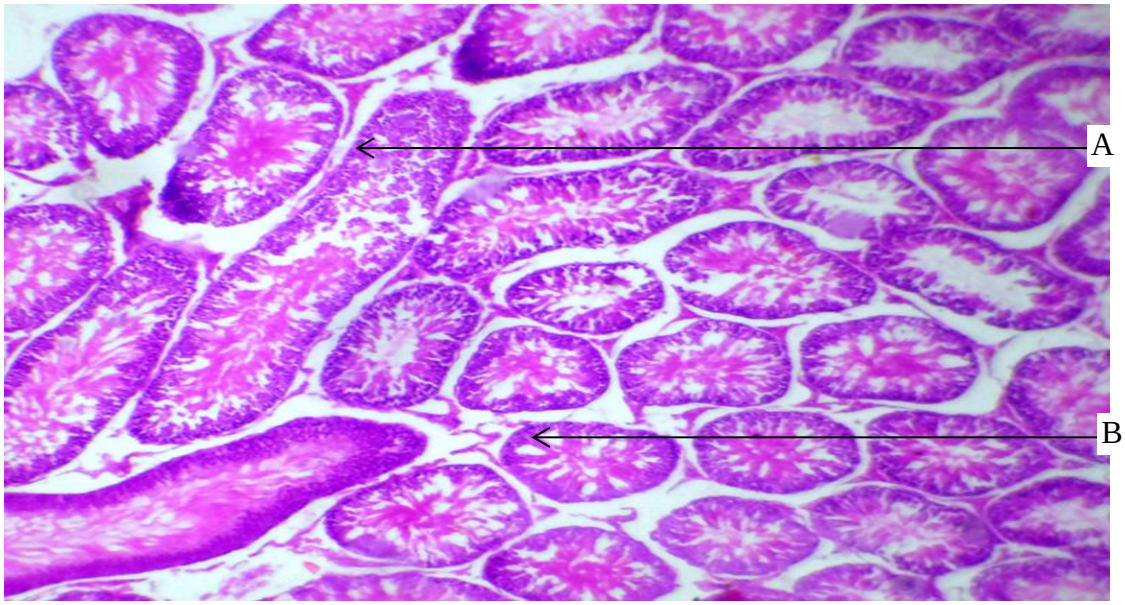

Plate 1a Shows the Normal Histological Feature of the Testis of the Untreated (Control) Albino Rat with Presence of Seminiferous Tubule (A) and Tunica Vasculosa (B) of the Testis. Handex40

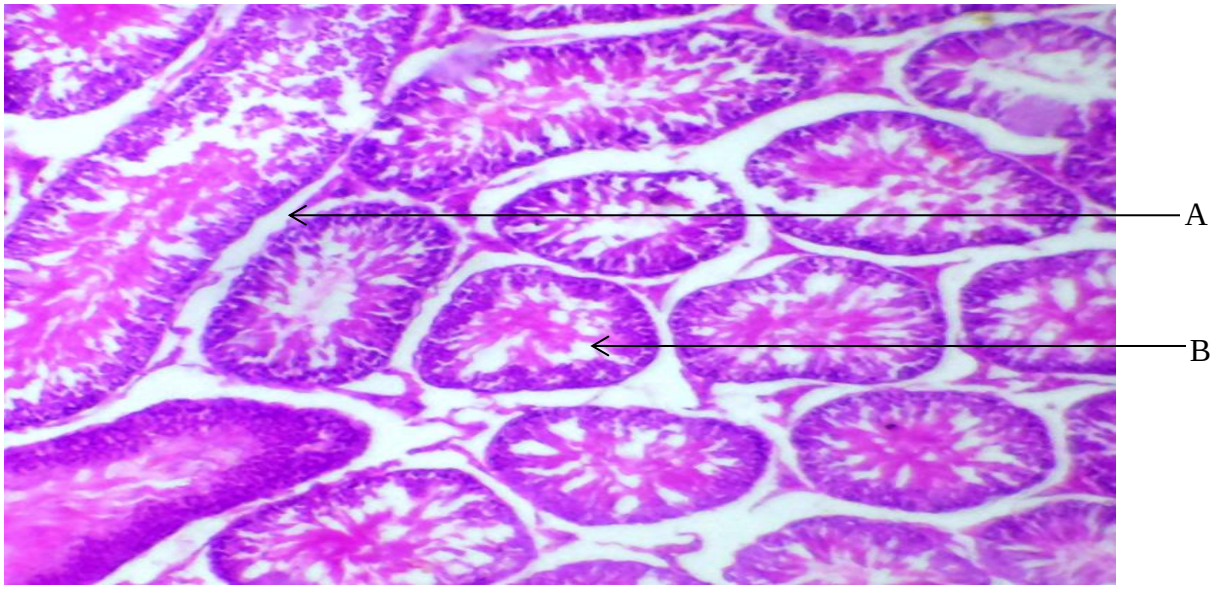

Plate 1b Shows the Normal Histological Feature of the Testis of the Untreated (Control) Wistar Rat with Presence of Seminiferous Tubule (B) and Tunica Vasculosa (A) of the Testis. Handex100

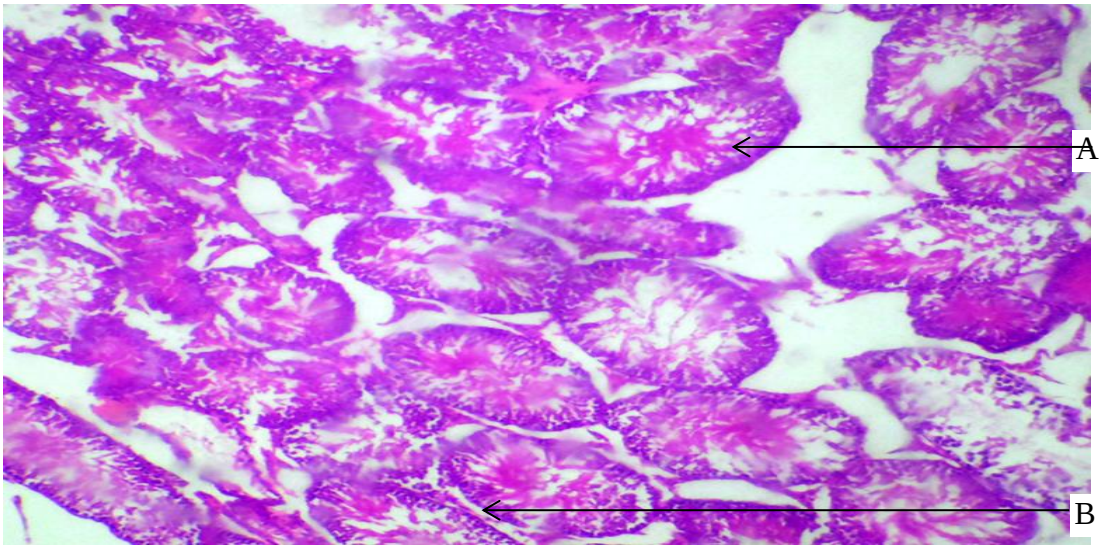

Plate 2a Shows the Normal Histological Feature of the Testis of the Treated (Test Group at Day 14) Wistar Rat with Presence of Seminiferous Tubule (A) and Leydig Cells (B) of the Testis. Handex40

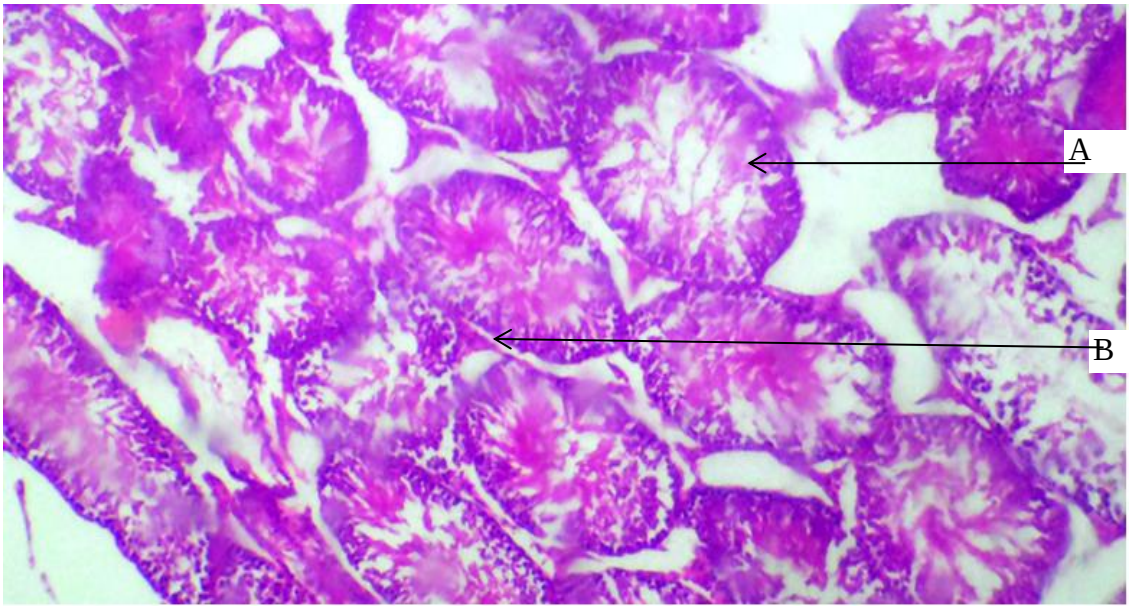

Plate 2b Shows the Normal Histological Feature of the Testis of the Treated (Test Group at Day 14) Wistar Rat with Presence of Seminiferous Tubule (A) and Leydig Cells (B) of the Testis. Handex100

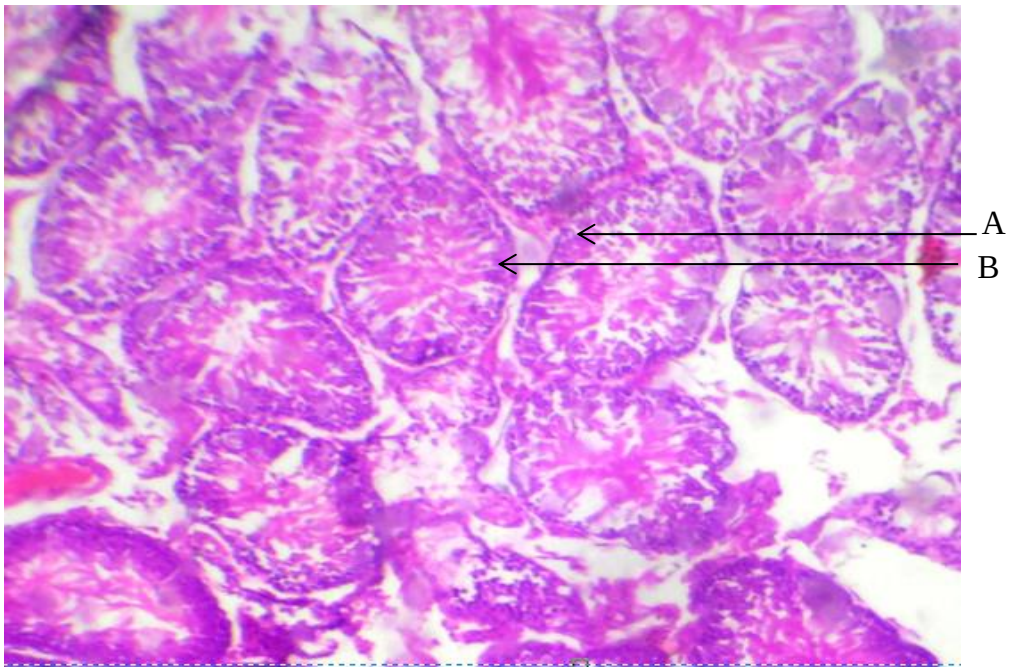

Plate 3a Shows the Normal Histological Feature of the Testis of the Treated (Test Group at Day 21) Wistar Rat with Presence of Connective Tissue Septa (A) and Seminiferous Tubule (B) of the Testis. Handex40

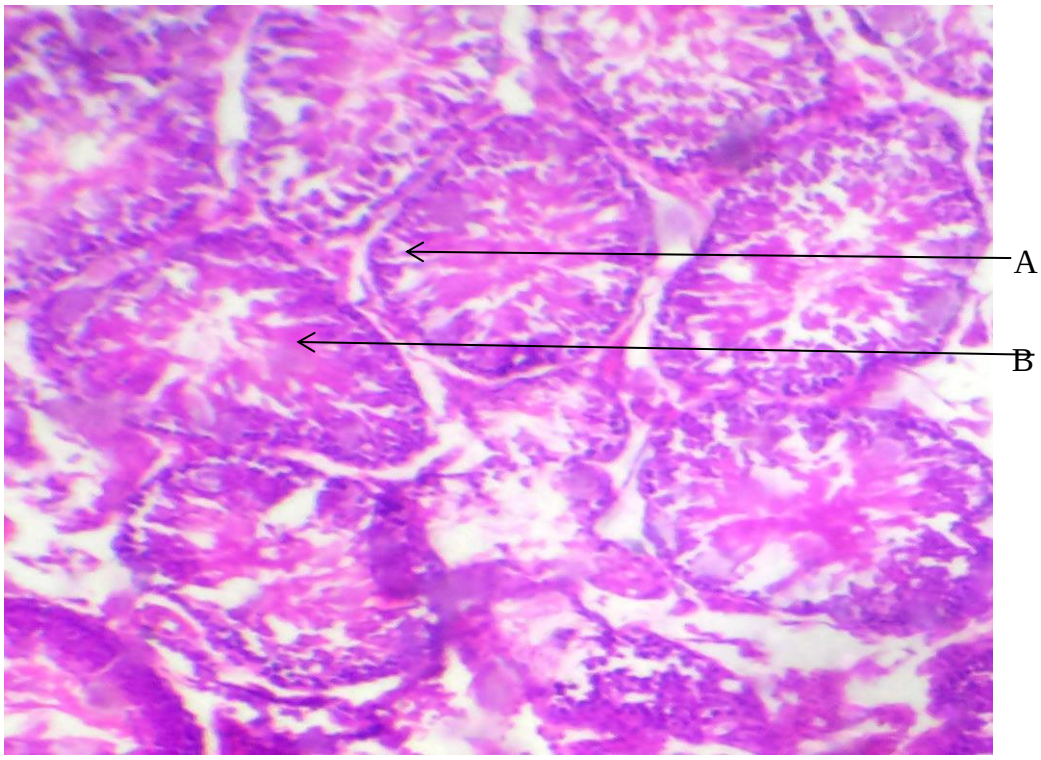

Plate 1b Shows the Normal Histological Feature of the Testis of the Treated (Test Groupat Day 21) Wistar Rat with Presence of Connective Tissue Septa (A) and Seminiferous Tubule (B) of the Testis. Handex100

DISCUSSION

Table 1 presents the mean and standard deviation values for the Control group, Test Group at Day 14, and Test Group at Day 21 of male albino rats administered with the male formula (herbal supplement), showing that P≤0.05 is statistically non-significant across all groups. This indicates that the male formula does not significantly affect body weight, possibly because the concentration and dosage of its active ingredients are optimized for sexual health rather than weight management. Additionally, the formula's ingredients may not influence metabolic processes or energy balance related to body weight [20]. This result aligns with the findings of Boye et al. [21], who assessed Polyscias fruticosa (L.) Harm (Araliaceae) leaf extract on male fertility in rats and found no significant effect on body weight. However, it contrasts with Jang et al. [22], who studied the herbal formula KH-204 and reported a significant change in body weight in a rat model of erectile dysfunction. Similarly, Buhner [23] found a significant impact on body weight in a related study.

Table 2 displays the mean and standard deviation values for the Control group, Test Group at Day 14, and Test Group at Day 21 of male albino rats administered with the male formula, revealing that P≤0.05 is statistically significant across all groups. This suggests that the male formula significantly impacts testes weight, likely due to its ingredients influencing hormone levels, particularly testosterone, which plays a key role in testicular function and size. Additionally, components that support spermatogenesis may contribute to the observed increase in testes weight [24]. These findings are consistent with those of Raji et al. [25], who reported a significant increase in testes weight in male rats treated with Alstonia boonei stem bark extract. Buhner [23] also observed a significant increase in testes weight, as did Dutta and Sengupta [26] in their research on medicinal herbs for male infertility. Similarly, Defo et al. [27] found that Guibourtia tessmannii extract significantly increased testes weight in obese sexually sluggish rats.

Table 3 outlines the mean and standard deviation values for the Control group, Test Group at Day 14, and Test Group at Day 21 of male albino rats administered with the male formula, demonstrating that P≤0.05 is statistically significant across all groups. This indicates that the male formula significantly affects testosterone levels, possibly due to ingredients that influence testosterone production or regulation. The formula may also improve blood flow to the genital area, enhancing sexual function and indirectly impacting hormone levels and overall sexual health [28]. These findings align with those of Buhner [23], who reported a significant increase in testosterone levels. Similarly, Chye [29] found a significant impact on testosterone levels in a study on traditional Asian folklore medicines for sexual health.

The micrographs reveal that both the control and test groups exhibit normal testis morphology. This may be due to the herbal aphrodisiac formula's antioxidant properties, which reduce oxidative stress, hormonal modulation that enhances testosterone levels, and anti-inflammatory effects that mitigate testicular inflammation [30]. These herbs provide essential nutrients like zinc and vitamin E, improve testicular blood flow, and promote cell protection and

regeneration. Adaptogenic herbs help the body manage stress, while certain phytoestrogens and phytosterols offer protective effects on testicular cells, collectively supporting testicular tissue structure and function [31]. These results are consistent with the findings of Rajendar et al. [32], who reported the protective effects of Tribulus terrestris Linn against cadmium-induced testicular damage. Similar observations were made by Msiska et al. [28] and Mansour et al. [33], who documented improvements in sperm parameters, serum hormonal levels, and testicular histology in Wistar rats.

CONCLUSIONS

Herbal aphrodisiac formulas was reported to enhance testosterone levels and testis weight , though no significant change was observed on the body weight.

RECOMMENDATIONS

More studies can be carried out on the Herbal aphrodisiac formulas also for a longer duration to ascertain if long usage have effects. Other organs can also be examined.

FUTURE STUDY

This research still has limitations so further research is needed related to the topic of Histomorphological and Hormonal Changes in the Testes of Albino Rats Treated with Herbal Aphrodisiac Formular to perfect this research and increase insight for readers.

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