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Research Article

Effect of Co-administration of Aqueous Extract of Cyperus Esculentus (Tiger Nuts) and Vitamins (Vitamin C And Vitamin E) on Male Reproductive Organ Histology and Fertility Indices in Albino Rats

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Abstract

Infertility poses multifaceted challenges, encompassing emotional, psychological, and socio-economic dimensions to humans. This study investigated the effect of the co-administration of aqueous extract of Cyperus esculentus (tiger nuts) and vitamins (vitamin C and vitamin E) on male reproductive organ histology and fertility indices in albino rats. The experimental design involved administering an oral median lethal dose (LD50) of tiger nuts to 91 male albino rats. They were divided into thirteen groups, each comprising seven animals. Groups included a Control Group for baseline comparison, Aqueous Extract Groups (2-4) to study dose-dependent effects, Aqueous Extract + Vitamin C Groups (5-7) to explore combined effects, Aqueous Extract + Vitamin E Groups (8-10) to examine antioxidant influence, and Aqueous Extract + Vitamins C and E Groups (11-13) for a comprehensive understanding of potential interactions. The study spanned 21 days, evaluating the impact on testicular conditions. The serial sections were stained using hematoxylin and Eosin staining techniques for the testes tissue. The study found a significant difference in testicular weight among different groups of albino rats. Group 11 had the highest testicular weight, while the control group had the least. Testicular weights in groups fed with Vitamin E and a combination of vitamin C and E were significantly higher than those fed with tiger nut only and Vitamin C. Additionally, testosterone levels varied significantly among groups, with higher levels in groups receiving Vitamin E and a combination of vitamin C and E. Vitamin C levels did not show a significant difference among groups, but vitamin E levels were significantly higher in specific groups (12 and 13) compared to others. Histological examinations revealed enhanced spermatogenic maturation and preserved testicular architecture. Overall, the study observed vacuolations and Leydig cell changes, suggesting physiological effects on testicular weight, hormonal levels, and vitamin concentrations in albino rats. The study finds that tiger nut extract, combined with vitamin E or vitamins C and E, significantly boosts testicular weight and serum testosterone in albino rats, supporting spermatogenesis. Particularly, vitamin E enhances spermatogenic maturation, interstitial cells, and blood vessels. Histological examination shows preserved testicular morphology, indicating a healing effect. In conclusion, administering tiger nut extract with vitamins, notably vitamin E, improves fertility indices in male albino rats.

Keywords: Aqueous Extract, Cyperus Esculentus, Vitamin C, Vitamin E, Male Reproductive Organ Histology, Fertility, Albino Rats.

INTRODUCTION

Tiger nuts, scientifically known as Cyperus esculentus, are a perennial plant from the sedge family commonly found in the Mediterranean region [1]. These plants have served as a food source for humans for centuries, with evidence of their cultivation by ancient Egyptians dating back to 5000 BC [2]. Tiger nuts are predominantly grown in the middle belt and northern regions of Nigeria and are known by different names such as "aya" in Hausa, "aki awusa" in Igbo, "ofio" in Yoruba, and "isipisong" in Efik. These tubers, also referred to as chufa and earth almond, are packed with fat,

carbohydrates, minerals, as well as vitamins E and C, making them a promising food source for humans and animals alike [3]. Furthermore, tiger nuts have versatile applications - they can be pounded and baked, used in the production of a traditional drink called "kunu," and incorporated into confections like candy, chocolate, biscuits, and cookies [4]

Consuming milk can provide the body with sufficient amounts of Vitamin E, which is vital in promoting fertility for both men and women [5]. Additionally, Vitamin E can contribute to slowing down the aging process of cells, enhancing skin elasticity, and alleviating skin issues such as wrinkles, acne, and other blemishes. Moreover, research indicates that pork burgers containing liquid co-products from tiger nut tubers exhibit improved cooking properties compared to those without co-products [7]. Tiger nut milk is utilized in China for its various medicinal properties, including its role as a liver tonic, heart stimulant, treatment for stomach pain, promotion of menstruation, and healing of mouth and gum ulcers [8]. Traditional medicine also recognizes tiger nut tuber as a remedy for colon evacuation [9] and potential treatment for ailments such as boils, the common cold, and poliomyelitis [10] Additionally, it has been reported to have both sedative and stimulant effects, aiding digestion, treating diarrhea, and alleviating intestinal inflammation [11]. Tiger nut extract is believed to play a significant role in preventing heart disease, thrombosis, and improving blood circulation. It is also known to help prevent and treat urinary tract infections and other bacterial infections. In the Middle East, tiger nuts are commonly referred to as "Hab Al-zulom" (Arabic), which translates to "the seeds of men," due to their reputation for enhancing male sexual activity. They are often given to grooms during their honeymoons as a sexual invigorator [12]. suggesting a potential effect on the testes.

The testes, or male reproductive glands, are located within the scrotum, an extension of the anterior abdominal wall [13]. These organs are crucial to the male reproductive system as they produce androgens, including testosterone, through steroidogenesis. Additionally, they are responsible for spermatogenesis, the process of producing sperm cells [13]. The function of the testes is regulated by the adenohypophysis, or anterior pituitary gland, where luteinizing hormone (LH) stimulates testosterone production and follicle-stimulating hormone (FSH) promotes sperm production [14]

Sperm cells fuse with egg cells during fertilization, leading to the formation of a zygote. Testosterone, on the other hand, is responsible for the development of male sexual characteristics, maintenance of muscle mass, red blood cell production, bone growth, overall well-being, and sexual function [15] Deficiencies or abnormalities in sperm cell production or testosterone levels can result in male infertility [16].

Scientific research has indicated that any factors affecting the testes can have a significant impact on sexual characteristics and fertility [17]. Infertility is a reproductive system disorder defined as the inability to achieve pregnancy after 12 or more months of regular unprotected sexual intercourse [17]. It can be classified as primary infertility, where the man has never impregnated a woman, or secondary infertility, which refers to cases where the man has previously impregnated a woman but is currently unable to do so. Male infertility can be complete or partial, also known as subfertility, and can result from various factors such as reduced sperm count (oligozoospermia), decreased sperm motility (asthenozoospermia), diminished sperm vitality (necrozoospermia), abnormal sperm morphology (teratozoospermia), or a combination of these factors. Intrinsic testicular disorders are the primary cause of subfertility in most cases.

In recent years, the significance of male factors in infertility has been increasingly recognized, thanks to advancements in diagnostic tools and a comprehensive assessment of male reproductive function. A previous study suggested that treatment with C. esculentus (tiger nut) methanolic extract improved sperm count and motility in male rats, accompanied by increased levels of gonadotropins and testosterone in the bloodstream [18]. Building upon these findings, the present study aims to investigate the impact of C. esculentus (tiger nut) on the testes of albino rats using histological and biochemical techniques.

Infertility is a significant public health issue in Nigeria and other developing countries due to its high prevalence and the profound social implications it has on affected couples and families. It is worth noting that these conditions often stem from underlying diseases, which pose additional health risks to both couples and place an extra burden on the healthcare system [19] Moreover, infertility contributes to depopulation in certain regions and hampers social and economic development in affected areas.

When infertile couples are unable to conceive despite their efforts, they commonly experience feelings of helplessness, frustration, and despair, making it a major life crisis. The emotional and psychological distress they face is immense, particularly as they witness their friends and peers starting families. It is now widely recognized that male factor infertility is equally significant as female factor infertility. Although various diagnostic tests are currently in use, many of them have not been proven effective in predicting the likelihood of pregnancy [20]

For many individuals, the realization of their reproductive potential and achieving their desired number of children is considered a major accomplishment in life and an essential aspect of a fulfilling and meaningful existence [21].

Therefore, it is not surprising that infertility has a profound psychological impact on couples, with up to 20% of them requiring support to cope with their inability to conceive [22]

Most couples seek medical intervention, and even those in low-resource settings are willing to endure significant financial hardship to afford assisted reproduction [23]. Infertility care has made significant advancements in recent decades and has gained increased attention from healthcare providers. Various treatments, including costly options like Assisted Reproductive Techniques (ART), are now routinely available in several public healthcare systems. However, the adoption of these treatments has largely occurred without thorough economic assessments. Additionally, the issue of technology management, i.e., the long-term cost-effectiveness of a treatment when implemented across a broader range of conditions, has received insufficient attention [24]

Once an intervention has been accepted for clinical use in a specific population for a particular indication, it is often utilized in other patient groups without formal evaluation of cost-effectiveness. Healthcare expenditures are increasing worldwide, necessitating the need to control costs while simultaneously achieving efficiency and supporting healthcare systems [25]. Therefore, robust economic evaluations are essential to justify treatments that require ongoing investment. This is particularly critical in modern medicine, given the economic crises impacting several Western countries. In the field of infertility care, economic considerations are even more vital as it deals with improving quality of life rather than solely focusing on survival, thus making it vulnerable to financial cutbacks in healthcare spending [26].

Tiger nut contains essential vitamins, minerals, trace elements, and a high level of amino acids contributing to overall health and well-being. Among its properties, tiger nut is believed to improve male fertility due to its protective role, particularly vitamin C, against oxidative stress [27]. Additionally, the high quantity of vitamin E in tiger nuts enhances semen quality and promotes the viability of sperm cells. In view of limited histology-based research on male fertility indices of tiger nuts, the present study focuses on examining the impact of tiger nut on the testes of albino rats using histological and biochemical parameters.

Tiger nuts have received much attention due to their potential health benefits, availability, and affordability. It is believed to improve sperm count and sperm quality (semen pH, sperm motility, sperm viability, and sperm head abnormality), which could be attributed to its ability to increase testosterone levels. Testosterone stimulates spermatogenesis, sperm maturation, and secretory activity of the accessory sex gland [28]. Since Tiger nuts can improve semen quality, the study aims to investigate their effect on the testes using histological and biochemical techniques.

MATERIAL AND METHODS

Plant procurement and identification

Tiger nuts were bought from the local dealer at ama Hausa area in Owerri, Imo, and the Imo State University, Owerri, Botany Department identified the plant.

Tiger nut extract preparation

The tiger nut was washed to remove sand and debris. They were air-dried at room temperature for 2 days and then blended into powdered form using an electric blender. A measured amount of 6 kg of blended nuts was extracted using 3 liters of warm distilled water. The extract was first double-filtered with cheesecloth, then with filtered paper (Whatman 4 filtered paper). The extract was concentrated at 45°C in a rotary evaporator to 10% volume and then to complete dryness using a vacuum water bath, yielding 600 g of crude extract. The crude extract obtained was stored in a refrigerator until required. The tiger nut crude extract was soaked in distilled water, filtered, and administered in 2 ml daily using the gavage method.

Experimental animals

Ninety-one (91) male albino rats weighing 150g were purchased from local dealers and kept in well-standardized ventilated cages made of aluminum pan and aluminum wire gauge with wood shavings as bedding for the rats. The animals were allowed to acclimatize for 2 weeks, during which they were provided with food, water, and bedding. The experiment lasted for 21 days, and during this time, the animals were fed regularly and provided with food and water. The bedding was changed daily, and any change in weight, feeding habits, behavior, vital signs, and irritability were observed and noted during the process of the experiment.

Experimental design and administration

After administering an oral median lethal dose (LD50) of tiger nuts. Ninety-one (91) male albino rats were used, and they were grouped into thirteen (13) of seven (7) animals per group as follows.

Control Group (Group 1):

It serves as a baseline to compare with the treated groups, providing a reference for normal testicular conditions under these experimental conditions.

Aqueous Extract Groups (Groups 2-4):
Investigate the impact of the aqueous extract of Cyperus esculentus at different concentrations, allowing assessment of dose-dependent effects.
Group 2: Aqueous extract of C. esculentus (500 mg/kg /b.wt) daily for 21 days
Group 3: Aqueous extract of C. esculentus (1000 mg/kg /b.wt) daily for 21 days
Group 4: Aqueous extract of C. esculentus (2000 mg/kg/b.wt) daily for 21 days

Aqueous Extract + Vitamin C Groups (Groups 5-7):
Explore the combined effects of the extract and Vitamin C, providing insights into potential interactions and synergistic effects.
Group 5: Aqueous extract of C. esculentus (500 mg/kg/b.wt) with vitamin C daily for 21 days.
Group 6: Aqueous extract of C. esculentus (1000 mg/kg/b.wt) with vitamin C daily for 21 days.
Group 7: Aqueous extract of C. esculentus (2000 mg/kg/b.wt) with vitamin C daily for 21 days.

Aqueous Extract + Vitamin E Groups (Groups 8-10):
Investigate the combined effects of the extract and Vitamin E, examining the influence of another antioxidant on the observed outcomes.
Group 8: Aqueous extract of C. esculentus (500 mg/kg/b.wt) with vitamin E daily for 21 days.
Group 9: Aqueous extract of C. esculentus (1000 mg/kg/b.wt) with vitamin E daily for 21 days.
Group 10: Aqueous extract of C. esculentus (2000 mg/kg/b.wt) with vitamin E daily for 21 days.

Aqueous Extract + Vitamins C and E Groups (Groups 11-13):
Assess the combined effects of the extract with both Vitamin C and Vitamin E, offering a more comprehensive understanding of the potential interactions among these substances.
Group 11: Aqueous extract of C. esculentus (500 mg/kg/b.wt) with vitamin C and E daily for 21 days.
Group 12: Aqueous extract of C. esculentus (1000 mg/kg/b.wt) with vitamin C and E daily for 21 days.
Group 13: Aqueous extract of C. esculentus (2000 mg/kg/b.wt) with vitamins C and E daily for 21 days.

Sample collection
The rats were sacrificed at the end of the experiment using anesthesia. The blood was collected through cardiac puncture, and the testes were excised. The gross anatomy of the excised testes organs was performed, which involved taking the weight of the organs and examining, describing, and reporting any structural anatomical features observed. The blood was carefully separated and used for testosterone, vitamin C, and E analyses.

Tissue processing
Before processing, the tissue (testes) specimen was fixed in 10% neutral buffered formalin. Surgical grossing of the tissue sample was performed, and the weight and dimensions of the organ (testes) were recorded. A macroscopic examination was conducted, and representative bits were cut from the tissue. These tissue bits will be placed into cassettes and appropriately labeled. Tissue processing was carried out by passing through different ascending grades of alcohols (70% alcohol, 90% alcohol, Absolute alcohol I, Absolute alcohol II, Absolute alcohol III, and Absolute alcohol IV) for 2 hours each. The tissues were cleared with three changes of xylene (xylene I, xylene II, and xylene III) for 2 hours each. The tissue was infiltrated with two changes of molten paraffin wax for 2 hours each. Then, the tissues were embedded using hard paraffin wax and sectioned at 3 microns using the LEICA RM2125RT rotary microtome (Heidelberger- Germany). The sections were floated out on a water bath, picked using albumenized slides, and fixed on a hot plate for 15 minutes.

Clinical Chemistry Preparation
The determination of serum testosterone levels using immunosorbent assay (ELISA) and vitamin E and C levels using High-Performance Liquid Chromatography (HPLC) technique.

Staining method
The serial sections were stained using hematoxylin and Eosin staining technique for the testes tissue.

Statistical analysis
The data obtained was analyzed using a student ANOVA.

RESULTS

Testicular weight
The result of the testicular weight measurement is shown in Table 1. Group 1 to Group 13 shows a mean weight as measured. The statistical analysis revealed a significant difference in testicular weight among different groups. Group 11 had the highest testicular weight (3.06±0.07), while the control group 1 had the least (1.27±0.26). The analysis of variance (ANOVA) indicated a statistically significant difference ($p<0.05$). Specifically, the mean testicular weight of

groups fed with Vitamin E, and a combination of vitamin C and E (8, 9, 10, 11, 12, and 13) was significantly higher than those fed with tiger nut only (2, 3, and 4) and Vitamin C (5 and 6).

Clinical chemistry measurement

The result of testosterone measurement is shown in Table 2. The statistical analysis indicates a significant difference in average testosterone levels among groups. Group 13 had the highest testosterone level (10.00±0.10), while control group 1 had the least (5.90±0.30). The ANOVA demonstrated a statistically significant difference ($p<0.05$). Specifically, groups (8, 9, 10, 11, 12, and 13) fed with Vitamin E, and a combination of vitamin C and E had significantly higher testosterone levels than the control group, those fed with tiger nut only (2, 3, and 4), and vitamin C (5, 6, and 7). Testosterone levels of groups fed with Vitamin C and tiger-nut were significantly higher than the control groups but not significantly different from those feds with tiger-nut only.

The result of Vitamin C measurement is shown in Table 3. The control group (group 1) has the lowest Vitamin C level (0.9±0.3) while group 13 has the highest (1.83±0.15). The measure of the mean difference between the groups was statistically not significant ($p=0.71$) with a p-value greater than 0.05.

The result for the measurement of vitamin E is shown in Table 4. The analysis indicated varying vitamin E levels among groups. Group 12 had the highest vitamin E level (16.27±1.19), followed by group 13 (16.20±0.70), while the control group (group 1) had the lowest (8.50±3.18). The mean difference between groups was statistically significant ($p<0.001$). Pairwise comparisons revealed that vitamin E levels in groups 12 and 13 were significantly higher than in groups 1, 2, and 5, with no significant differences observed between other groups in post-hoc analysis.

Overall, the findings showed vacuolations and Leydig cell changes and their physiological effects on testicular weight and hormonal and vitamin levels in albino rats.

Table 1 shows the average testicular weight of the animals across the groups. The highest testicular weight was reported in group 11 (3.06±0.07) and the least was in control group 1 (1.27±0.26). The measure of mean difference by analysis of variance (ANOVA) showed that there was statistically significant difference ($p<0.05$). The mean testicular weight of the groups fed with Vit E (8, 9,10,11, 12 and 13) was significantly higher than those fed with tiger-nut only (2,3, and 4) and and Vit. C, group (5 and 6).

Table 1: Average Testicular Weight Across the Groups

variables	Groups	N	Mean±Sd	F-value	p-value
Testes Weight	1	3	1.27 ± 0.26^{a}	13.15	<0.001
	2	3	1.37 ± 0.30^{a}		
	3	3	1.47 ± 0.40^{a}		
	4	3	1.50 ± 0.39^{a}		
	5	3	1.59 ± 0.44^{a}		
	6	3	1.66 ± 0.50^{a}		
	7	3	$1.95\pm0.77^{a,b}$		
	8	3	$2.92\pm0.25^{b,c}$		
	9	3	$2.91\pm0.07^{b,c}$		
	10	3	$2.92\pm0.15^{b,c}$		
	11	3	3.06 ± 0.07^{c}		
	12	3	$2.96\pm0.16^{b,c}$		
	13	3	$2.95\pm0.20^{b,c}$		

* Values with same superscript are not statistically significantly difference

Table 2: Shows the average testosterone level across the groups. The mean testosterone level was highest in group 13 (10.00±0.10), while the least testosterone level (5.90±0.30) was in control group 1. The measure of mean difference between groups by ANOVA showed statistically significant difference ($p<0.05$). The mean testosterone level of groups (8, 9, 10, 11, 12 and 13) fed with Vit E were significantly higher than the control group, those fed with tiger nut only (2, 3 and 4), and vitamin C (5, 6 and 7). The testosterone level of the groups fed with Vit C and tiger-nut was statistically significantly higher than the control groups but not significantly different from group fed with tiger-nut only.

Table 2: Comparison of Mean Testosterone Level Between Groups

Variables	Groups	N	Mean±Sd	F-value	p-value
Testosterone	1	3	5.90±0.30[a]	15.29	<0.001
	2	3	7.53±0.55[a,b]		
	3	3	7.67±0.50[b]		
	4	3	7.70±0.79[b]		
	5	3	7.77±0.90[b]		
	6	3	7.60±0.52[b]		
	7	3	7.77±0.25[b]		
	8	3	9.47±0.49[c]		
	9	3	9.43±0.76[c]		
	10	3	9.70±0.46[c]		
	11	3	9.67±0.72[c]		
	12	3	9.69±0.30[c]		
	13	3	10±0.10[c]		

*Value with same superscript is not statistically significantly difference

Table 3: Showed the average Vitamin C level across the groups. The control group (group 1) has the lowest Vitamin C level (0.9±0.3) while group 13 has the highest (1.83±0.15). The measure of mean difference between the groups was statistically not significant (p=0.71) with p-value greater than 0.05.

Table 3: Average Vitamin C Across the Groups

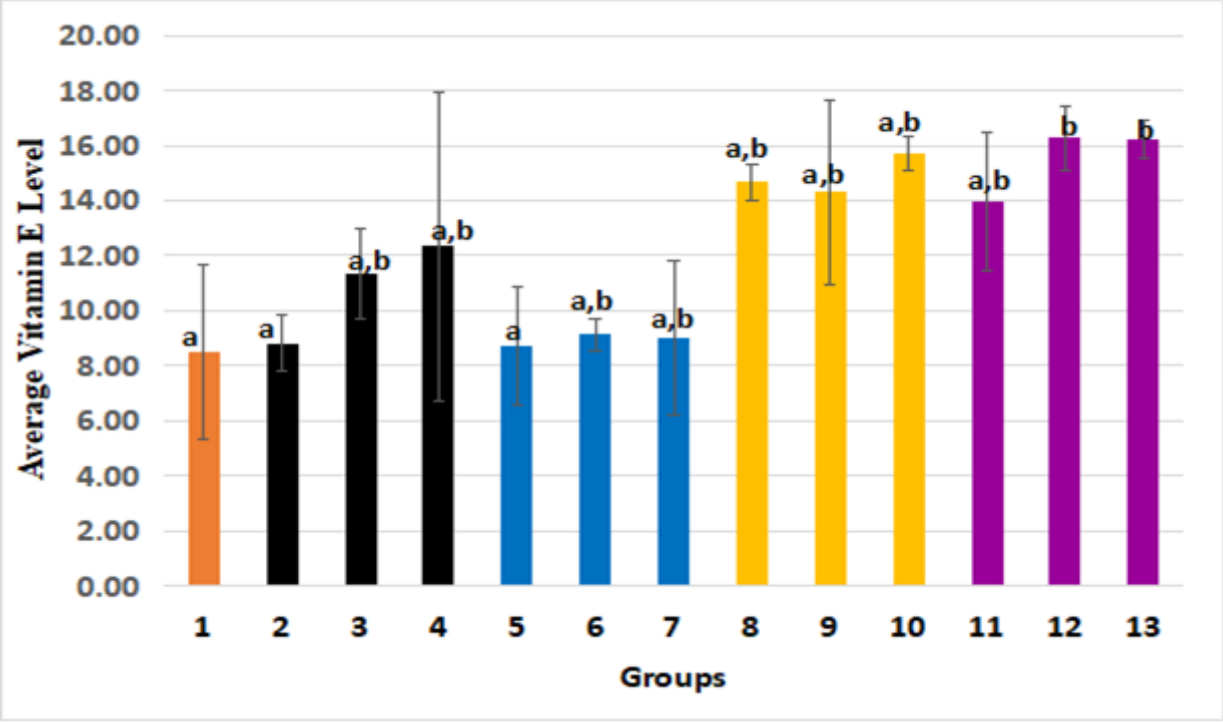
variables	Groups	N	Mean±Sd	F-value	p-value
Vitamin C	1	3	0.90±0.30	0.72	0.71
	2	3	1.4±0.56		
	3	3	1.43±0.55		
	4	3	1.6±0.36		
	5	3	1.53±0.42		
	6	3	1.57±0.57		
	7	3	1.60±0.26		
	8	3	1.37±0.40		
	9	3	1.13±0.15		
	10	3	1.47±0.5		
	11	3	1.53±0.32		
	12	3	1.63±1.01		
	13	3	1.83±0.15		

Table 4 and Chart: Depict the mean Vitamin E level across the groups. It showed different vit E level across various groups. Group 12 showed the highest Vit E level (16.27±1.19), followed by group 13 (16.20±0.70). The lowest vit E was reported in the control group (group 1) (8.50±3.18). The measure of mean difference between group was statistically significant (p<0.001). The pairwise comparison showed that the vit E level of group 12 and 13 were statistically significantly higher than group 1, 2 and 5. The difference between other groups was statistically not significant at post-hoc analysis.

Table 4: Comparison of Mean Vitamin E Level Between Groups

variables	Groups	N	Mean±Sd	F-value	p-value
Viamin E	1	4	8.50±3.18[a]	4.77	<0.001
	2	4	8.83±1.01[a]		
	3	4	11.37±1.65[a,b]		
	4	4	12.33±5.64[a,b]		
	5	4	8.70±2.16[a]		
	6	4	9.13±0.60[a,b]		
	7	4	9.00±2.80[a,b]		
	8	4	14.67±0.68[a,b]		
	9	4	14.30±3.38[a,b]		
	10	4	15.70±0.6[a,b]		
	11	4	13.97±2.51[a,b]		
	12	4	16.27±1.19[b]		
	13	4	16.20±0.70[b]		

* Value with same superscript is not statistically significantly difference



4.3 Histological observations

The harvested testes of the control group, group 2 to group 13, were processed and stained with haematoxylin and eosin technique to look out for any tissue changes. The findings were arranged in plate numbering.

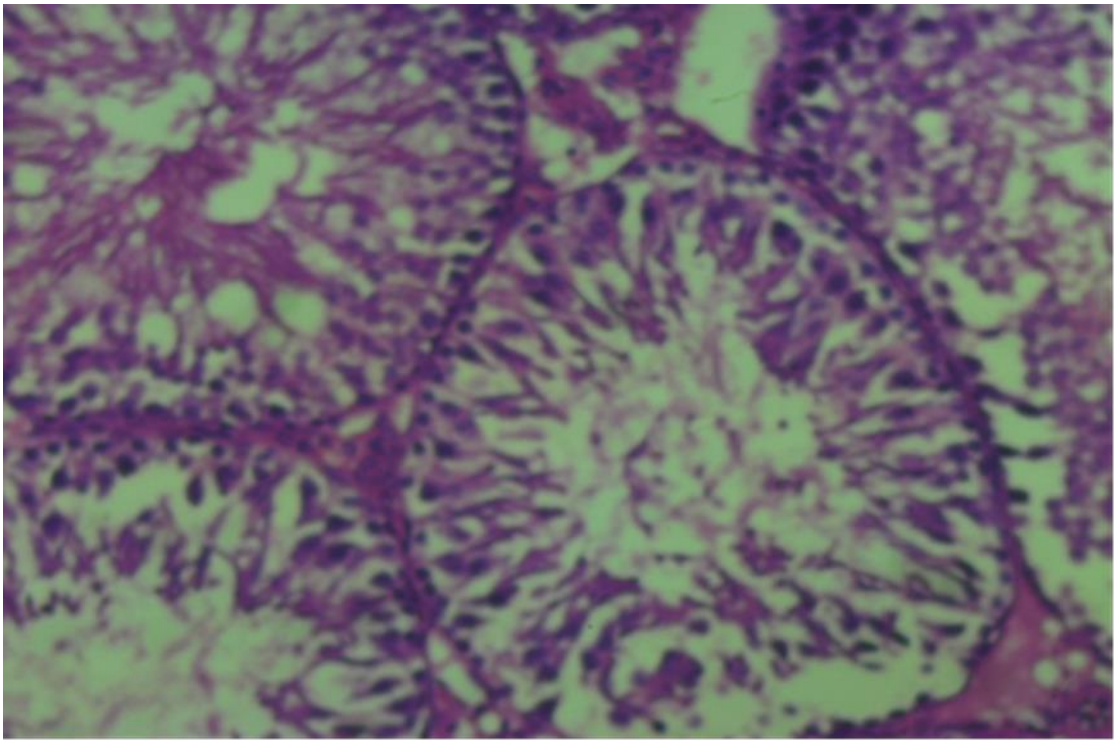


PLATE 1: Photomicrograph of the testis of the control group showing capsule of tunica albunigea and vasculosa containing blood vessels surrounding the testicular tissue. The seminiferous follicles are arranged and separated by interstitial tissue. Features are consistent with that of a normal testis (H & E x 100).

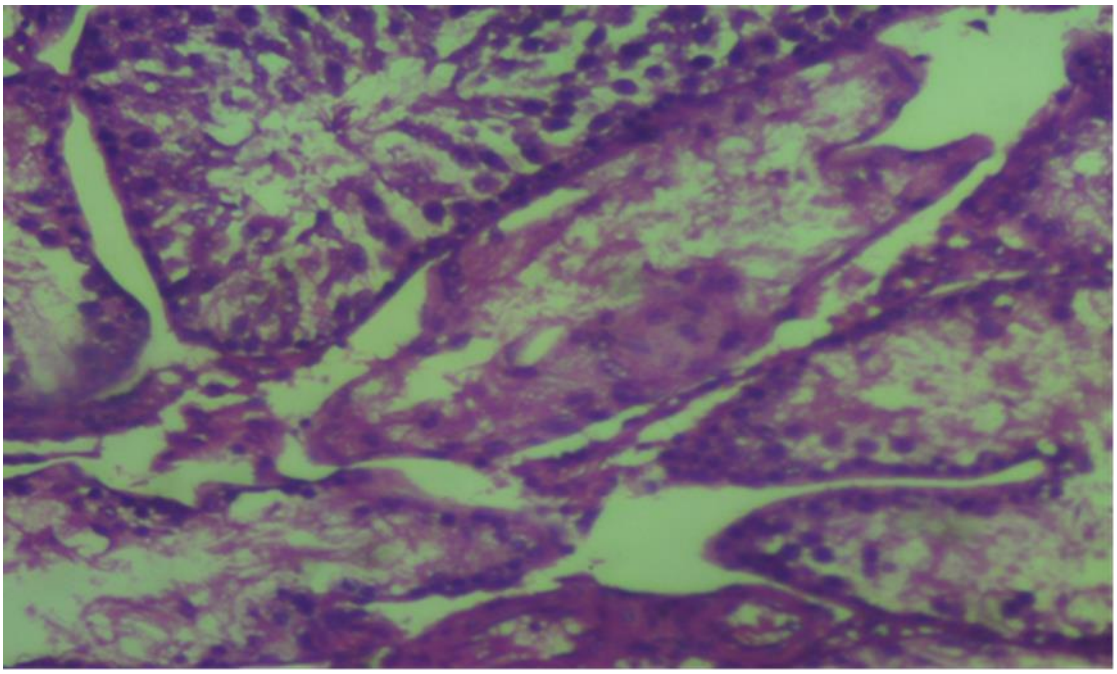


PLATE 2: Photomicrograph of the testis of group 2 showing mild interstitial vacoulations and degenerative changes in seminiferous tubules and testicular architecture (H & E x 100).

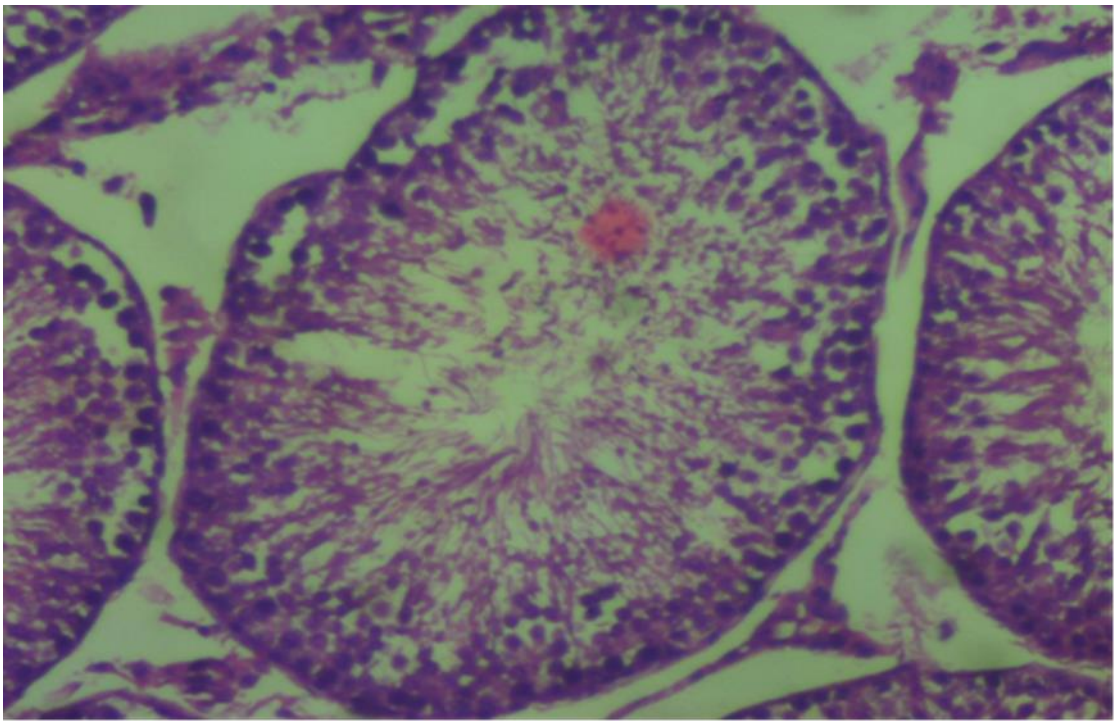


PLATE 3: Photomicrograph of the testis of group 3 showing moderate interstitial vacoulations, marked hemorrhagic necrosis and moderate degenerative changes in seminiferous tubules and testicular architecture (H & E x 100).

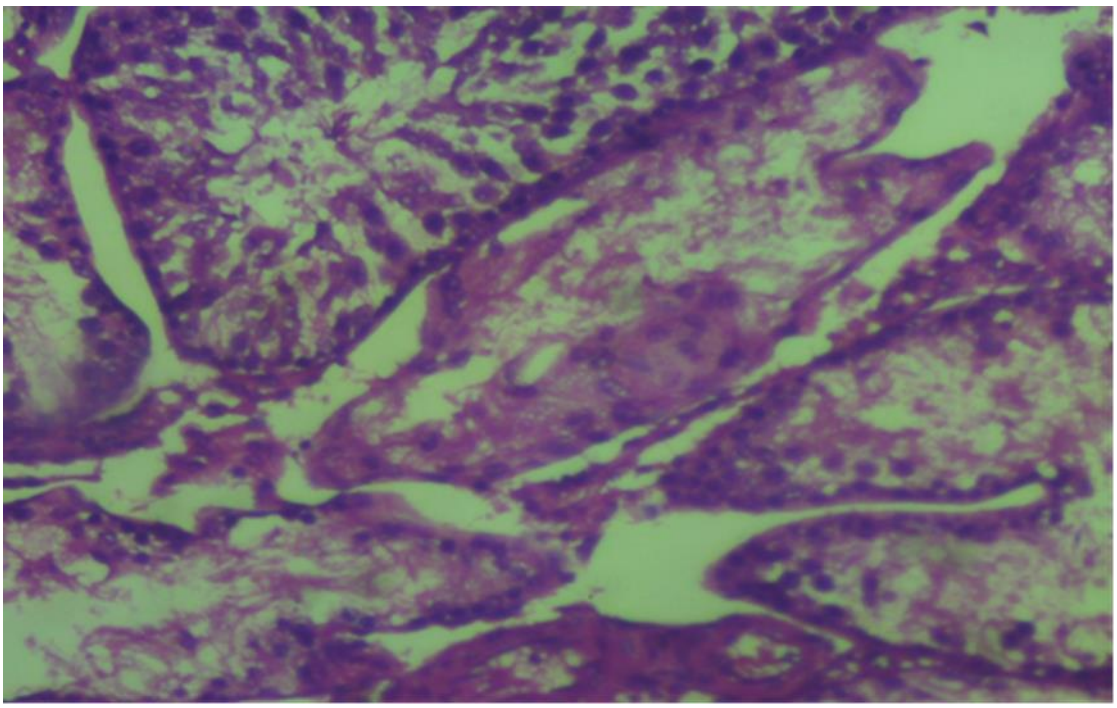


PLATE 4: Photomicrograph of the testis of group 4 showing fibrosis and unremarkable changes in seminiferous tubules and testicular architecture (H & E x 100).

DISCUSSION

The challenges posed by infertility are enormous; this involves emotional stress, psychological distress, socio-economical, etc. The consumption of tiger nut to improve fertility has been known for ages. Thus, effect of coadministration of aqueous extract of cyperus esculentus (tiger nuts) and vitamins (vitamin C and vitamin E) on male

reproductive organ histology and fertility indices in albino rats were investigated in this study. This study showed a significant increase in the testicular weight following the administration of tiger nuts. Therefore, an increase in testicular weight in the treated groups reveals increased spermatogenesis and steroidogenesis. Increase in testicular and epididymal weight have been link to higher sperm production [29]

Increased weight of the testis and epididymis have been reported to mediate in the production of high amounts of spermatozoa in some studies, which was attributed to androgen biosynthesis elevation resulting from spontaneous rise in testosterone levels in rats. Consequently, tiger nut extract as it affects testicular weight and epididymis in rats is in agreement with [16], which was linked to vitamin C rich content and defensive function against oxidative stress [11] and morphological changes of the testes in particular [17] Also vitamin E enhances the functions of testes in the form of increase in weight of testes and width of seminiferous tubules as well as more number of Sertoli and Leydig cells [22]

It was observed in the present study that tiger nuts aqueous extract caused a significant increase in serum testosterone levels and was dose dependent. This finding also similar with that of [18] who reported an increase in serum testosterone level in rats treated with tiger nuts extract. The exact mechanism by which tiger nuts enhances testosterone levels may be attributed to some findings. It was stated that tiger nut may act directly on testicular cells, and not through the hypothalamus-pituitary axis, since no variations in follicle stimulating hormone and luteinizing hormone levels were observed due to tiger nut treatment. The phytochemical analyses revealed the presence of several components (quercetin, vitamins E and C, and the mineral zinc) in tiger nut that could positively contribute to testosterone production and improve the erectile function. Quercetin is a dietary flavonoid that exhibits strong antioxidant activity. A previous study revealed that oral administration of quercetin was associated with a significant increase in serum testosterone level in male rats [23]. It was suggested that the enhancement of testosterone by tiger nut is enabled by the element of zinc and vitamins it contains which work to facilitate the production of testosterone. The trace element zinc is speculated to play a critical role in sexual development. Zinc supplementation improved the sexual behavior of adult male rats in a dose-dependent manner by enhancing testosterone secretion [30]

Also, an increase in vitamin C and E levels were observed in the groups treated with tiger nuts. Vitamin C is known to protect spermatogenesis and it plays a major role in semen integrity and fertility both in humans and animals. It increases testosterone levels and prevent sperm agglutination. It is an important chain breaking antioxidant, contributing up to 65 % of the total antioxidant capacity of seminal plasma found intracellularly and extracellularly [26]. Vitamin C is a strong antioxidant that facilitates the formation of testosterone. It has been reported that vitamin C increases testosterone content in rat testis *in vitro* [23]. This vitamin acts on the hypothalamus-pituitary testicular axis to cause an elevation of testosterone levels, which promotes spermatogenesis [30]

On the other hand, vitamin E is an essential nutrient speculated to enhance testosterone synthesis and was found to increase serum testosterone in an experimental aged mice model [7]. It was reported that daily oral administration of 40 mg/kg vitamin E for 3 weeks restored serum testosterone concentration to normal value in an atherosclerotic rat model. Also, daily supplements of vitamin E in a dose of 75 mg/kg for 50 days ameliorated serum testosterone levels in rats exposed to noise stress [12] Furthermore, antioxidant therapy with vitamin E ameliorates the age-associated erectile dysfunction in male rats. In a recent study, that vitamin E therapy can improve the poor semen quality and increase plasma testosterone levels in dogs [28]

Vitamin E on the other hand is known to improve the motility and fertilizing ability of sperm in the penetration of hamster [23] This vitamin acts on precursors of thrombxanes, prostaglandins and immunoglobulins to promote spermatogenesis [10] Hence lack of vitamin E may damage the reproductive organs like damages in the spermatogenesis, testicular dysfunction and shrinkage of seminiferous tubules. This vitamin also enhances the functions of testes in the form of increase in weight of testes and epididymis. The supplementation of vitamin E at 80 IU / day in Boer goats caused the improvement through the number of epithelium, width of semineferous tubules as well as more number of Sertoli and Leydig cells [19]. The sertoli cells also play an important role in the maintenance as well as the transport of androgens in the testes [26]. This explains the increase in width of seminiferous tubules, sertoli and leydig cells observed in this study.

The histological examination of the testis of control group revealed normal testicular morphology showing normal spermatogonia cells, interstitial space, intact walls of seminiferous tubules and as well as numerous spermatozoa in the lumen. It also shows intact fibromuscular tissue with normal collagen deposits in the interstitial space, capasule and around the blood vessels.

In the present study, histological examination of the testicular tissue revealed an increase in the maturation of spermatogenic cells to spermatozoa and preserved interstitial cells of the treated groups as numerous spermatozoa were seen in the lumen of the treated group and were dose-dependent when compared with the control group as seen in the

photomicrograph of group 11, 12, and 13. This confirms the ability of tiger nuts to improve the maturation of sperm cells to spermatozoa, thereby improving spermatogenesis. However, at higher doses 1000 and 2000 mg/kg of tiger nuts, it was observed that the walls of the seminiferous tubules were replaced with interstitial spaces. There was marked proliferation of the interstitial spaces (more Leydig cells), maybe as a result of inducement from tiger nuts with vitamin E to increase testosterone production. These changes were consistent with testicular spermiogenesis.

Enlargement of the blood vessels was also observed, which enhanced the circulation of testosterone. This may be caused by vitamin E, which has been reported to stimulate the production of vascular nitric oxide, which is critical for the regulation of blood flow and vasodilation and consequently improves erectile function [26] The hypertrophy of the seminiferous tubules was also observed, as shown by the histometric measurements, which may be caused by vitamin E, which has been reported to increase the width of seminiferous tubules [30] Also, the histometric measurements of the length of the seminiferous tubules show a significant reduction in length of the seminiferous tubules when compared with the control group, which could be the result of the interstitial tissue seen replacing the wall of the seminiferous tubule leading to a reduction or cushion of the seminiferous tubules. The collagen fibres in the testicular interstitium of the treated groups, when compared with Control, are intact, showing well-preserved histo architecture. This implies that treatment with an aqueous extract of tiger nut caused no distortions in testicular tissue architecture, even at increased doses. The deeply stained collagen fibers found in the testicular interstitium and around the blood vessels suggest that tiger nut had preservative and protective effects on the collagen fibers and, in turn, the testicular interstitium. This may be dose-dependent as the group treated with the highest dose showed more collagen fibers as compared with groups treated with lower doses as well as a control group.

Conclusion

In conclusion, the present study has shown that the administration of an aqueous extract of tiger nut with vitamins, especially vitamin E, and combined administration of vitamins C and E with tiger nuts to albino rats revealed enhanced fertility indices due to an observed increase in serum levels of testosterone, vitamin C, and E as well as increased in testicular weight, proliferation and maturation of spermatogenic cells.

References

1. Ezeh, O., Gordon, M. H. & Niranjan, K. (2014). Tiger nut oil (*Cyperus esculentus* L.): a review of its composition and physic-chemical properties. *European Journal of Lipid Science Technology*, 116: 783-794.
2. Ashraf, A. E. (2014). Histological and morphometric study on the possible protective effect of vitamin e on the cadmium intoxication of adult male albino rat testis. *Medicine Journal of Cairo University*, 82(2): 321-330.
3. Fernandes, G. S. A., Fernandez, C. D. B., Campos, K. E., Damasceno, D. C., Anselmo-Franci, J. A. & Kempinas, W. D. G. (2011). Vitamin C partially attenuates male reproductive deficits in hyperglycemic rats. *Reproduction and Biological Endocrinology*, 9: 100.
4. Gambo, A. & Dáu, A. (2014). Tigernut (*Cyperus esculentus*): Composition, products, uses and health benefits - A review. *Bayero Journal of Pure Applied Science*, 7(1): 56-61.
5. Agbaje, R. B., Oyetayo, V. O. & Ojokoh, A. O. (2015). Effect of fermentation methods on the mineral, amino and fatty acids composition of *Cyperus esculentus*. *African Journal of Biochemical Research*, 9(7): 89-94.
6. Ahmad, H., Vahid, R. & Rohullah, A. (2016). Effects of intramuscular injections of vitamin e-selenium and a gonadotropin releasing hormone analogue (Gnrha) on reproductive performance and blood metabolites of postmolt male broiler breeders. *Asian Pacific Journal of Reproduction*, 5(2): 156-160.
7. Belewu, M. A., Belewu, K. Y. & Bamidele, R. A. (2010). Cyper-coconut yoghurt: Preparation, compositional and orgaanoleptic qualities. *African Journal for Food Science and Technology*, 1(1): 010-012.
8. Krausz, C. & Riera-Escamilla, A. (2018). Genetics of male infertility. *Nature and Revelation Urology*, 15(6): 369-384.
9. Lin, Y. F., Chang, S. J., Yang, J. R., Lee, Y. P. & Hsu, A. L. (2005). Effects of supplemental vitamin E during the mature period on the reproduction performance of Taiwan native chicken cockerels. *British Poultry Science*, 46: 366-373
10. Boivin, J., Bunting, L. & Collins, J. A. (2007). International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Human Reproduction*, 22(6): 1506-1512.
11. Canyurt, M. A. & Akhan, S. (2008). Effect of dietary vitamin E on the sperm quality of rainbow trout (*Onchorynchus mykiss*). *Aquaculture Research*, 39: 1014-1018.
12. Hashem, N. M., El-Hady, A. A. & Hassan, O. (2013). Effect of vitamin E or propolis supplementation on semen quality, oxidative status and hematobiochemical changes of rabbit bucks during hot season. *Livestock Science*, 157: 520-526.
13. Houguo, X., Huang, L., Liang, M., Zheng, K. & Wang, X. (2015). Effect of dietary vitamin E on the sperm quality of turbot (*Scophthalmus maximus*). *Journal of Oceanic and Coastal Sea Research*, 14(4): 695-702.
14. Chukwuma, E. R., Obioma, N. & Christopher, O. I. (2010). The phytochemical composition and some biochemical effects of Nigerian tiger nut (*Cyperus esculentus* L.) tuber. *Pakistan Journal of Nutrition*, 9: 709-715.

15. Delzenne, N. M. (2003). Oligosaccharides: State of the art. *Proceedings of Nutrition Society*, 62: 177-182.
16. Ebojele, F. O. & Ezenwanne, E. B. (2014). The effect of aqueous extract of *Cyperus esculetus* on some liver functional indices in rabbit. *International Journal for Basic Applied Innovative Research*, 3(1): 8-13.
17. Jerysz, A. & Lukaszewicz, E. (2013). Effect of dietary selenium and vitamin E on Ganders' response to semen collection and ejaculate characteristics. *Biological Trace and Elementary Research*, 153, 196-204.
18. Adejuyitan, J. A. (2011). Tiger nut processing: Its food uses and health benefits. *American Journal for Food Technology*, 6(3): 197-201.
19. Wilson, M. J., Kaye, D., Edward, S. W., Quach, H. T., Sinha, A. A. & Vatassery, G. T. (2013). Effect of vitamin E deficiency on the growth and secretory function of the rat prostatic complex. *Experimental Molecular Pathology*, 74: 267-275.
20. Muhammad, N. O., Bamishaiye, E. I., Bamishaiye, O. M., Usman, L. A., Salawu, M. O., Nafiu, M. O. & Oloyede, O. B. (2011). Physicochemical properties and fatty acid composition of *Cyperus esculentus* (Tiger nut) tuber oil. *Bioresear*. *Bulletin*, 5: 51-54.
21. Wolf, H. G. (1995). A review of female *Fertility;* Important etiological factors and management. J Microbial Biotech 63: 1143-1157
22. Adgidzi, E. A., Ingbian, E. K. & Abu, J. O. (2011). Effects of storage on the quality of tiger nut (*Cyperus esculentus*) products. *Product of Agriculture and Technology*, 7(1): 131-147.
23. Khan, R. U., Rahman, Z. U., Javed, I. & Muhammad, F. (2012). Effect of vitamins, probiotics and protein on semen traits in post-molt male broiler breeders. *Animals Reproductive Science*, 135: 85-90.
24. Achoribo, E. S. & Ong, M. T. (2017). Tiger nut (*Cyperus esculentus*): Source of natural anticancer drug? Brief review of existing literature. *European Medication and Biomedical Journal*, 12: 91-94.
25. Maduka, N. & Ire, F. S. (2018). Studies on tiger nut-ogi incorporated into basal feed as a potential animal growth enhancer using wistar albino rats as experimental animal. *Journal of Advanced Microbiology*, 8(2):1-15.
26. Wang, S., Wang, G., Barton, B. E., Murphy, T. F. & Huang, H. F. (2007). Beneficial effects of vitamin E in sperm functions in the rat after spinal cord injury. *Journal of Andrology*, 28: 334-341.
27. Matough, F. A., Budin, S. B., Hamid, Z. A., Louis, S. R., Alwahaibi, N. & Mohamed, J. (2012). Palm vitamin E reduces oxidative stress, and physical and morphological alterations of erythrocyte membranes in streptozotocin-induced diabetic rats. *Oxidants and Antioxidants Medicine Science*, 1(1): 59-68.
28. Nwaoguikpe, R. N. (2010). The phytochemical, proximate and amino acid compositions of the extracts of two varieties of tiger nut (*Cyperus esculentus*) and their effects on sickle cell hemoglobin polymerization. *Journal of Medicine Science*, 1(11): 543-549.
29. Okoye, J. I. & Ene, G. I. (2018). Effects of processing on the nutrient and anti-nutrient contents of tiger nut (*Cyperus esculentus* Lativum). *Journal of Food Technology and Food Chemistry*, 1(104): 1-7.
30. Qi, X., Wang, K., Zhou, G., Xu, Z., Yu, J. & Zhang, W. (2016). The role of testicular artery in laparoscopic varicocelectomy: A systematic review and meta-analysis. *International Urology and Nephrology*, 48(6): 955-965.

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