BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 13, Number 1, April 2024 | Pages: 109-119 | DOI: 10.14421/biomedich.2024.131.109-119 ISSN 2540-9328 (online)

Comparative Effects of Ripe and Unripe Lime (*Citrus aurantifolia*) on Spermatozoa and Gonadosomatic Index in Matured Male Wistar Rats

Obioma Ogbonnaya[1], Azubuike Nwankwo[2], Daniel Chidi Ifenkwe[3], Chikera Samuel Ibe[4], Ekele Ikpegbu[4,5], Osah Martins Onwuka[6,*]

[1]College of Agriculture, Gregory University Uturu, Abia state, Nigeria.
[2]Department of Human Physiology, Faculty of Basic Medical and Health Sciences, Abia State University Uturu, Abia State, Nigeria.
[3]Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.
[4]Department of Veterinary Anatomy, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.
[5]Department of Comparative Pathology, Cummings School of Veterinary Medicine, Tufts University, United State of America.
[6]Department of Human Physiology, Gregory University Uturu, Abia State, Nigeria.

Corresponding author*
osahmartinz@gmail.com

Manuscript received: 17 January, 2024. Revision accepted: 08 May, 2024. Published: 16 May, 2024.

Abstract

To ascertain the comparative effects of ripe and unripe Lime (*Citrus aurantifolia*) on spermatozoa and gonadosomatic index evaluation in matured male Wistar rats; exploring the idea that both ripe and unripe Lime (*Citrus aurantifolia*) might or might not positively affect semen quality, crucial for male fertility. Twenty-eight (28) sexually mature male Wister rats, aged 9–10 weeks and weighing between 211.50g and 217.00g, were divided equally into seven groups (1 to 7); with Group 1 serving as the control and Groups 2 to 7 receiving 25%, 50%, and 75% concentrations of ripe lime juice (RLJ) and unripe lime juice (ULJ) respectively. The findings indicated that ULJ had a higher concentration (0.1mg/ml) compared to ripe lime RLJ at 0.08mg/ml, although both had approximately the same LD50 value of 1581.138mg/kg. RLJ, at different concentrations, adversely impacted the reproductive performance of rats, leading to decreased progressive motility, livability, sperm count, testicular size, and sexual drive. However, ULJ did not exhibit these effects. A 75% concentration of RLJ showed anti-prostatic activity, causing a reduction in prostate size, which was more pronounced than that of the same ULJ concentration. Importantly, both RLJ and ULJ did not have a significant impact on the sizes of the liver, spleen, heart, kidneys, and lungs, with these visceral organs maintaining normal sizes comparable to the control group (statistically, $p>0.05$).The findings suggest that RLJ or ULJ consumption, particularly at the highest concentration, may lead to alterations in reproductive performance, hence such consumption should be discouraged.

Keywords: *Citrus aurantifolia*; Lime, Fertility; Male reproduction; spermatozoa; Testis.

INTRODUCTION

Infertility is a phenomenon associated with offspring production (Rahayu and Hanizar, 2021). Globally, the incidence rate is approximately 30%, with males accounting for about 50% prevalence (Orieke et al., 2019). In Nigeria, male infertility is reported to contribute to 20-50% of all infertility cases across different regions (Eze, 2012). The worldwide increase in male infertility cases has been linked to a decline in semen quality (Abarikwu, 2013).

Infertility can result from factors such as low sperm count, inadequate sperm motility, abnormal morphological structures, or a combination of these issues (Kumar and Singh, 2015). Another significant contributing factor is the decline in or inappropriate secretion of male sex hormones (Aprioku and Obianime, 2014; Majzoub and Agarwal, 2018). A normal sperm should have a concentration of at least 15 million/ml of ejaculate, with 72% motility indicating progressive movement and 94% normal morphology (World Health Organization, 2010).

Abnormalities in sperm, particularly in concentration and motility, have been identified as major causes of male infertility in various studies (Sharma, 2017; Wdowiak, 2019; Babakhanzadeh, 2020). Other factors affecting sperm quality include exposure to radiation (Gorpinchenko, 2014; Kesari et al., 2018), nutrition (Martinez-Soto, 2016; Salas-Huetos et al., 2018), lifestyle choices (Ilacqua, 2018), age (Silea, 2019), psychological factors (Janevic, 2014), and deletions in the Y chromosome (Colaco and Modi, 2018).

Several plants have a historical reputation for influencing male reproductive functions. Some of these plants can impact spermatogenesis at the testicular level or alter the body's hormone profile. The use of fruits like *Citrus aurantifolia* in the general diet is gaining popularity globally due to its nutritional and health

benefits (Nnenne et al., 2020). Commonly known as lime or bitter orange in Nigeria, this fruit belongs to the Rutaceae family. Lime is recognized for its rich content of phytochemicals, antioxidants, vitamins, minerals, and dietary fiber, which are known to reduce the risk of various health issues, as utilized in ethno-medicine (Septembre-Malaterre et al., 2018). It is considered a promising fruit with health-promoting qualities and is widely consumed (Adegoke et al., 2017).

Lime, a sour, round, and bright green citrus fruit, is extensively used in West Africa, particularly in Nigeria, for various purposes such as food, refreshing drinks, desserts, and seasoning (Enejoh et al., 2015). Known for its health benefits, including immune system enhancement (Kim, 2023) and weight loss due to its hypolipidemic property (Enejoh et al., 2015), lime has become popular among young men for its perceived impact on weight management (Saini et al., 2022).

This research aims to investigate the potential effects of lime consumption on the male reproductive system, including semen parameters, libido, hormonal levels, and testicular weight, using a rat model. Given lime's significant presence in herbal preparations in Nigeria (Ayinde et al., 2012; Enejoh et al., 2015), the findings will contribute to understanding how lime extract may influence physiological parameters and fertility functions in male Wister rats. This information is crucial for researchers, ethno-medicinal healers, and end-users, providing insights into the appropriate use of lime, especially among individuals of reproductive age in rural areas.

MATERIAL AND METHODS

Study Location

Fresh lime fruits were collected and identified at the mini-market of Abia State University, Uturu Campus. The extraction of lime juice and the animal studies, including semen evaluation, were conducted in the Department of Physiology and Pharmacology at the College of Veterinary Medicine (CVM), Michael Okpara University of Agriculture, Umudike (MOUAU), where study approval was obtained.

Experimental Animals and Management

A total of 28 sexually matured male Wister rats, aged 9–10 weeks and weighing between 211–217g, were obtained from the Department of Veterinary Physiology and Pharmacology animal house at the College of Veterinary Medicine (CVM), MOUAU. The animals underwent a 14-day acclimatization period in the animal laboratory, during which they were dewormed using Paraquantel (at 10mg/body weight), and their physiological parameters were measured and recorded.

Under standard laboratory conditions with a 12-hour light–dark cycle at a room temperature of 25 ± 2oC, the rats were provided with a standard diet (pelleted Chukun growers feed) and had access to water ad libitum. Regular weighing was conducted using a sensitive electronic scale (digital electronic balance, Citizen Scales [1] PVT Ltd., South Patel Nagar, New Delhi, sensitivity: 0.01 g) before the start of the study and on a weekly basis.

Preparation of lime juice extract

Fresh lime fruits, both ripe and unripe, were washed, weighed, and halved. The juice from each half was expressed into a glass beaker. After filtration using muslin, the filtrate was collected into labeled bottles. The lime juice extract was considered 100%, and dilutions of 25%, 50%, and 75% were prepared by adding distilled water using volumetric flasks. These different concentrations were orally administered to the animals in separate groups, each receiving a dose of 5ml/kg of the respective lime juice concentration (Khan et al., 2010).

The percentage yield of the juice extract was calculated using the formula:

$$\%\ \text{yield} = \frac{\text{Weight of lime juice extracted}}{\text{Weight of lime fruit used}} \times 100$$

(Soffwora, 1993)

Acute toxicity study of the lime extract

The Lorke method (1983) was employed in this study, which involved a total of 35 matured mice conducted in two phases. In the first phase, groups A-C, each comprising 5 mice, received oral doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg of ripe lime juice (RLJ) extract, respectively. In the second phase, groups D-F, also with 5 mice in each group, were orally administered doses of 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg of unripe lime juice (ULJ) extract, respectively. Additionally, a control group (group G) consisting of 5 mice was given 5ml/kg of distilled water. All mice had unrestricted access to food and water ad libitum. The animals were observed for signs of toxicity and mortality over 72 hours post-administration and for an additional 14 days for delayed toxicity. The median lethal dose (LD50) of the lime juice extracts, both ripe and unripe, was calculated using the formula recommended by Khan et al. (2013).

$$\text{LD50} = \sqrt{(\text{least dose with mortality} \times \text{Highest dose without mortality})}$$

Experimental Design

The 28 male Wistar rats were evenly divided into 7 groups, including a control group, as well as groups receiving ripe lime juice (RLJ) and unripe lime juice (ULJ) at concentrations of 25%, 50%, and 75% from the stock solution (Table 1).

Table 1. Experimental design.

Group	
1	Normal male rat (5ml/kg D/W)
2	RLJ at $LD_{25\%}$ stock
3	RLJ at $LD_{50\%}$ stock
4	RLJ at $LD_{75\%}$ stock
5	ULJ at $LD_{25\%}$ stock
6	ULJ at $LD_{50\%}$ stock
7	ULJ at $LD_{75\%}$ stock

NOTE: RLJ: Ripe lime juice; ULJ: Unripe lime juice; LD: Lethal dose; D/W: Distilled water.

The animals were dosed based on their body weight using a gastric tube (gavage) over a period of 28 days, during which continuous monitoring took place. After the dosing period, euthanasia was carried out by placing each rat in a small transparent bucket with a cover, containing cotton wool soaked in mild chloroform. The epididymis was then harvested, and sperm samples were collected from the caudal portion of the epididymis. Smears were prepared on preheated glass slides for subsequent evaluation.

Male Fertility Study

Libido (sex drive) evaluation

Libido in the rats was assessed through physical observation, focusing on their reactions and sexual behavior when introduced to a female rat in estrus. Sexual behavioral reactions, including general grooming, sniffing, mounting, and thrusting on the female rats, were noted and recorded according to a scoring pattern described by Chibundu (2013) (Table 2). Scores were allocated based on these observed reactions.

Table 2. Libido grading for male rats.

Sexual behavior	Score	Grading
Vigorous grooming, Sniffs and attempt to mount	5	Very high libido
Grooms, sniffs but no attempt to mount	4	High libido
Sniffs only	3	Moderate
Grooms only	2	Low
Does not pay attention to the female rat	1	Poor libido

Source: Chibundu (2005).

Sperm colour and consistency

The color and consistency of the sperm samples were assessed through macroscopic observation and recorded. The evaluation followed the consistency scale (1-4) as defined by Chibundu (2013), where scores 1 and 2 represented milky white and creamy white, respectively. The consistency scale utilized included scores 1 (watery), 2 (slightly thick), 3 (thick), and 4 (very thick) for assessing the thickness of the sperm samples.

Sperm motility

The methodology outlined by El-Sherbiny (1987) was followed for evaluating sperm samples collected through epididymal washings in each treatment group. Progressive motile sperm cells were assessed immediately after collection. A smear of one drop of the sperm sample was created on a preheated glass slide and examined under light microscopy at low magnifications (×10 and ×40). The assessment was conducted subjectively and scored as a percentage. Only sperm cells demonstrating straight-forward movement were considered in the motility count, while those moving in circles, backward, or displaying pendulum-like movement were excluded. Individual sperm motility was scored according to the following scale: 1 (vigorously progressive), 2 (progressive), 3 (cycling movement), and 4 (stationary movement).

Sperm viability (live proportion)

The viability of sperm cells was determined by staining a drop of the collected sperm sample with Eosin-Nigrosin stain. The stained glass slide was allowed to air-dry for 30 seconds, followed by fixation with ethanol. The slide was then examined under a light microscope at a magnification of X100 (oil immersion). Using a hand-held mechanical stopwatch counter, the proportion of viable sperm cells was counted. A total of 300 sperm cells were counted, and the number of viable cells was expressed as a percentage of the total count. Viable sperm cells, which remain unstained, were differentiated from dead cells, which picked up the stain, following the method described by El-Sherbiny (1987).

Sperm concentration

The sperm concentration was determined using a haemocytometer following the method described by Ukar et al. (2016). A dilution of 1:200 was created using a red blood cell pipette. In this procedure, a 1% buffered formalin solution was employed as the semen diluting fluid to immobilize the sperm cells. The haemocytometer was loaded with a drop of the sperm solution, allowed to settle for 2 minutes on a wet paper (to facilitate sperm cell settling), and then examined under the microscope at ×40 magnification. The formular; Sperm concentration per ml = No. of cells counted × Dilution Factor × 0.04 × 106 (Egbuka, 1995) was used to determine sperm concentration.

Abnormal sperm proportion

The percentage of abnormal sperm proportion was assessed following the method outlined by El-Sherbiny (1987). A drop of the sperm sample was stained with Eosin-Nigrosin stain, and the resulting mixture was smeared on a glass slide. Under a lower magnification of ×40, the slide was examined to identify primary and secondary abnormal sperm cells. The percentages of differential abnormalities, including head, mid-piece, and

tail abnormalities, were determined according to the criteria specified by El-Sherbiny (1987).

Statistical Analysis

All collected data underwent statistical analysis, and the test values were presented as Mean ± SEM (Standard Error of Mean). Statistical differences among the means of different groups were analyzed using one-way analysis of variance (ANOVA), followed by the LSD test for mean separation. The level of significance was set at $p < 0.05$ compared to the control group. Values at $p < 0.05$ were considered statistically significant.

RESULTS

Comparing juice percentage yield of ripe and unripe lime juice

The RLJ yielded (72.50%), more than the ULJ (61.25%) (Figure 1).

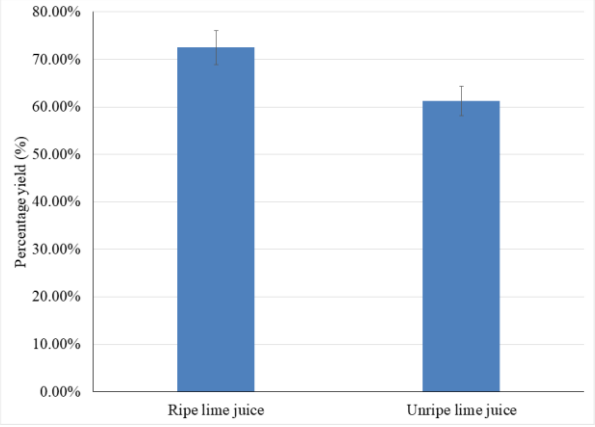

Figure 1. Comparing juice percentage yield of ripe and unripe lime juice.

Comparing juice concentration yield of unripe lime juice

On the contrary, the ULJ was more concentrated (100mg/ml; $p<0.05$) than the RLJ (80mg/ml) (Figure 2).

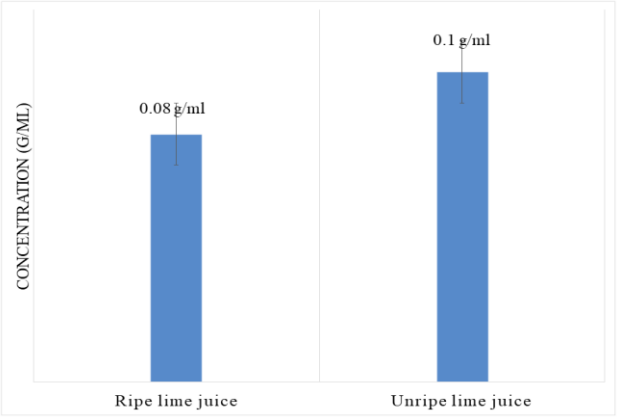

Figure 2. Comparing juice concentration yield of unripe lime juice.

Acute toxicity testing of ripe and unripe lime juice

The lethality results indicated that, after 72 hours post-administration of both ripe lime juice (RLJ) and unripe lime juice (ULJ) to rats at high (5000mg/kg) and low (500mg/kg) doses, no deaths or signs of toxicity were recorded in the low dose (500mg/kg) groups for both RLJ and ULJ. However, in the 5000mg/kg dose groups, 100% mortality was observed in RLJ, and 75% mortality was recorded in ULJ. The calculated LD50 was 1581.138mg/kg, falling below the 2000mg/kg threshold, indicating significant lethality in both RLJ and ULJ. Based on these findings, convenient concentration gradients of 25%, 50%, and 75% reconstitution were prepared and utilized in the study for both RLJ and ULJ.

Effect of graded concentration of ripe and unripe matured lime juice extract on spermatozoa assessment of male wistar rats

Table 3 illustrates the significant effects of both ripe lime juice (RLJ) and unripe lime juice (ULJ) administration on the spermatozoa qualities (color, pH, progressive motility, livability, and count) of Wistar male rats. However, the thickness/consistency of the epididymal reserve was not affected. The color of the epididymal reserve in male Wistar rats administered ULJ at 25% and 50% concentrations shifted from milky white (normal color) to creamy white. In contrast, the color of the epididymal reserve in those administered RLJ at various concentrations remained unchanged and retained a milky white appearance similar to that of normal rats. The pH of the epididymal reserve in male Wistar rats administered graded concentrations of RLJ (25%, 50%, and 75%) and 25% concentration of ULJ fell within the normal pH range (7.00±0.00) of rat semen (pH 6.99±0.00). However, the pH of the epididymal reserve was significantly lower ($p < 0.05$) at 6.98±0.01 in the group of Wistar rats administered the highest concentration (75%) of ULJ compared to the normal pH.

The results of spermatozoa movement revealed that RLJ at various concentrations significantly ($p < 0.05$) negatively affected the progressive motility of harvested sperm cells from the caudal epididymides. This resulted in a concentration-dependent decrease, reducing from 85.27±1.8% (normal rats' progressive motility) to a range of 62.47±4.40–68.30±1.95%. In contrast, rats administered ULJ at 25%, 50%, and 75% concentrations recorded spermatozoa progressive motility of 79.73±2.86%, 73.98±3.49%, and 73.80±4.45%, respectively. These values were comparable ($p > 0.05$) with the normal rats' progressive motility (Table 3).

The results of the spermatozoa live proportion in Wistar rats, as presented in Table 3, exhibited a direct relationship with the percentage of motile spermatozoa. The administration of RLJ had a negative effect ($p < 0.05$) on the livability of spermatozoa harvested from the caudal epididymides, while ULJ did not affect the live proportion.

The spermatozoa concentration results showed gradual ($p < 0.05$) decreases in the number of sperm cells counted in each caudal epididymis of all the Wistar rats administered various concentrations of RLJ and in the rats administered 75% ULJ. However, the number of spermatozoa counted in the Wistar rats administered 25% (104.59±7.95 × 10^6/caudal epididymis) and 50% (84.42±15.90 × 10^6/caudal epididymis) ULJ were statistically comparable ($p > 0.05$) to the number counted in the control rats (111.19±7.37 × 10^6/caudal epididymis).

Table 3. Effect of graded concentration of ripe and unripe matured lime juice extract on spermatozoa assessment of male wistar rats.

	Parameters					
Treatment group	**Colour**	**Consistency**	**pH**	**Progressive motility (%)**	**Spermatozoa livability (%)**	**Spermatozoa concentration (×10⁶/CE)**
5 ml/kg D/W	1.00±0.00^{b}	4.00±0.00	7.00±0.00^{a}	85.27±1.8^{a}	94.72±0.72^{a}	111.19±7.37^{a}
25% RLJ	1.00±0.00^{ab}	3.33±0.33	6.99±0.00^{ab}	68.30±1.95^{bc}	82.47±1.38^{bc}	64.33±3.84^{b}
50% RLJ	1.66±0.33^{ab}	3.33±0.67	6.99±0.00^{ab}	64.67±6.64^{c}	79.17±5.42^{bc}	62.75±3.36^{b}
75% RLJ	1.66±0.33^{ab}	3.33±0.66	6.99±0.00^{ab}	62.47±4.40^{c}	75.51±4.55^{c}	58.60±9.21^{b}
25% ULJ	2.00±0.00^{a}	3.66±0.00	6.99±0.00^{ab}	79.73±2.86^{ab}	87.54±2.96^{ab}	104.69±7.95^{a}
50% ULJ	2.00±0.00^{a}	4.00±0.00	6.98±0.01^{b}	73.96±3.49^{abc}	84.99±3.75^{abc}	84.42±15.90^{ab}
75% ULJ	1.33±0.33^{ab}	3.66±0.33	6.98±0.01^{b}	73.80±4.45^{abc}	84.77±3.21^{abc}	77.54±5.00^{b}

Note: Values are presented as mean± S.E (standard error of mean). Different superscript letters along treatment groups shows significant ($p<0.05$) differences. RLJ: Ripe lime juice; ULJ: Unripe lime juice; D/W: Distilled water.

Effect of graded concentration of ripe and unripe lime juice extract on the spermatozoa morphology of Wister rats

The results of spermatozoa morphology in the experimental male rats (Table 4) revealed that administering 50% and 75% of RLJ caused significant ($p < 0.05$) damage to the tail, head, and mid-piece of the sperm cells. Administration of ULJ at 75% also caused significant ($p < 0.05$) damage to the sperm cell head. However, the percentage of normal sperm cells in 25% RLJ concentration and 25%, 50%, and 75% ULJ were statistically comparable ($p > 0.05$) to the control rats. It is noteworthy to mention that 75% of RLJ caused the most significant ($p < 0.05$) damage to the cells, with the highest number of total abnormalities (4.71±0.50%) and the least ($p < 0.05$) number of normal cells (95.28±0.50%) compared to others. However, it's important to note that the total abnormal sperm cells obtained in 75% RLJ are less than the 25% concentration, which is considered to affect fertility.

Table 4. Effect of graded concentration of ripe and unripe matured lime juice extract on spermatozoa morphology of male Wistar rats.

Treatment group	**Tail abnormality**	**Head abnormality**	**Mid-piece abnormality**	**Cytoplasmic droplets**	**Total abnormal sperm cells**	**Percentage normal sperm cells**
5 ml/kg D/W	0.19±0.03^{de}	0.27±0.02^{c}	0.13±0.02^{c}	1.16±0.05^{b}	1.76±0.05^{c}	98.23±0.05^{a}
25% RLJ	0.27±0.02^{cd}	0.26±0.04^{c}	0.26±0.03^{bc}	1.12±0.01^{b}	1.91±0.05^{c}	98.08±0.05^{a}
50% RLJ	0.36±0.02^{b}	1.25±0.03^{b}	0.55±0.11^{b}	1.12±0.00^{b}	3.29±0.13^{b}	96.71±0.13^{b}
75% RLJ	0.44±0.03^{a}	1.60±0.24^{a}	1.41±0.26^{a}	1.25±0.04^{a}	4.71±0.50^{a}	95.28±0.50^{c}
25% ULJ	0.17±0.29^{e}	0.23±0.01^{c}	0.26±0.01^{bc}	1.16±0.01^{b}	1.83±0.02^{c}	98.17±0.02^{a}
50% ULJ	0.21±0.01^{de}	0.31±0.04^{c}	0.34±0.04^{bc}	1.21±0.02^{ab}	2.07±0.01^{c}	97.92±0.00^{a}
75% ULJ	0.33±0.01^{bc}	0.32±0.02^{c}	0.28±0.01^{bc}	1.19±0.01^{ab}	2.13±0.03^{c}	97.87±0.03^{a}

Note: Values are presented as mean± S.E (standard error of mean). Different superscript letters along treatment groups shows significant ($p<0.05$) differences. RLJ: Ripe lime juice; ULJ: Unripe lime juice; D/W: Distilled water.

Effect of graded concentration of ripe and unripe matured lime juice extract on libido assessment of male Wistar rats

The results regarding the effect of administering both RLJ and ULJ on the libido of male Wistar rats, as presented in (Table 5), indicated a concentration-dependent decrease in sex drive (libido) in both RLJ and ULJ administered groups compared to the high sex drive observed in the normal control Wistar rats. The reaction time outcomes revealed that it took an average of 7 minutes and 27 seconds, 5 minutes and 29 seconds, 6 minutes and 40 seconds, and 6 minutes and 49 seconds for the rats administered 25% RLJ, 25%, 50%, and 75% ULJ, respectively, to start grooming and sniffing the introduced female. In comparison, it took an average of 4 minutes and 26 seconds for those in the control group to complete the sexual behavioral reactions from general grooming to an attempt to mount.

Table 5. Effect of graded concentrations of ripe and unripe lime juice extract on libido of Wister rats.

Treatment group	Libido score	Reaction Time (minutes)
5 ml/kg D/W	4.66±0.33^{a}	4.26±0.45^{c}
25% RLJ	1.66±0.26^{cd}	7.27±0.64^{a}
50% RLJ	1.00±0.00^{d}	23.32±0.22^{b}
75% RLJ	1.00±0.00^{d}	24.47±0.53^{ab}
25% ULJ	3.66±0.28^{b}	5.29±0.86^{bc}
50% ULJ	2.33±0.33^{c}	6.40±0.48^{ab}
75% ULJ	2.00±0.00^{c}	6.49±0.72^{ab}

Note: Values are presented as Mean ± SEM (Standard Error of Mean). Different superscript letters across treatment groups shows significant ($p<0.05$) differences. RLJ: Ripe lime juice; ULJ: Unripe lime juice; D/W: Distilled water

Effect of graded concentration of ripe and unripe lime juice extract on body weight changes of Wister rats

The body weight of the Wistar rats before the commencement of the lime juice administration (week 0) indicated that all the Wistar rats had the same body weight ($p > 0.05$) (Table 6). Furthermore, there was no significant ($p > 0.05$) difference in their body weight at week 1 during the administration of both RLJ and ULJ. However, by week 2, except for the average body weight of the rats administered a low concentration (25%) of RLJ, which weighed the same ($p > 0.05$) as the control rats, there was a significant and steady weight reduction across all the administered groups from week 2 to week 4. This was in contrast to the consistent ($p < 0.05$) body weight increase observed in the control rats during the same period.

Table 6. Effect of graded concentration of ripe and unripe lime juice extract on the weight gain of Wister rats.

	Duration				
Treatment group	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
5 ml/kg D/W	217.00±17.32	228.50±8.74	245.00±9.65^{a}	258.50±5.10^{a}	264.50±20.10^{a}
25% RLJ	216.25±10.76	223.00±6.79	218.25±23.26^{ab}	213.50±4.97^{b}	198.00±11.45^{b}
50% RLJ	212.75±7.70	210.00±3.18	208.00±5.35^{b}	193.25±5.26^{b}	184.75±7.39^{bc}
75% RLJ	214.25±10.47	210.50±3.52	210.00±3.18^{b}	192.75±11.77^{b}	159.75±6.28^{c}
25% ULJ	211.50±9.04	210.75±4.92	192.25±6.77^{b}	187.50±4.99^{b}	173.25±11.85^{bc}
50% ULJ	216.25±1.54	216.00±6.74	207.25±2.92^{b}	192.75±11.77^{b}	189.00±1.63^{bc}
75% ULJ	216.75±3.68	219.00±7.12	210.25±1.70^{b}	200.25±23.28^{b}	196.25±6.01^{b}

Note: Values are presented as mean± S.E (standard error of mean). Different superscript letters along treatment groups shows significant ($p<0.05$) differences. RLJ: Ripe lime juice; ULJ: Unripe lime juice; D/W: Distilled water.

Effect of graded concentration of ripe and unripe lime juice extract on gonadosomatic index evaluation of Wister rats

Table 7 displayed the relative weight of the testes in all the ULJ groups, and that of the 25% RLJ groups, which weighed the same ($p > 0.05$) as the size of the testes in the control group relative to their body weights. There was a significant ($p < 0.05$) reduction in the relative size of the testes in the rats administered 50% and 75% RLJ compared to the normal relative size of rat testes. However, the relative size of the prostate gland in the 75% RLJ administered rats was significantly ($p < 0.05$) reduced, indicating a smaller average prostate size compared to the control rats.

Table 7. Effect of graded concentration of ripe and unripe lime juice extract on the Gonadosomatic index of Wister rats.

	Gonads		
Treatment group	**% Testes**	**% Prostate**	**% Seminal vesicle**
5 ml/kg D/W	1.43±0.19^{a}	0.16±0.03^{a}	0.58±0.11^{a}
25% RLJ	1.03±0.02^{abc}	0.14±0.02^{ab}	0.36±0.02^{b}
50% RLJ	0.92±0.01^{c}	0.07±0.01^{ab}	0.21±0.06^{b}
75% RLJ	0.94±0.02^{bc}	0.05±0.04^{b}	0.21±0.03^{b}
25% ULJ	1.36±0.17^{ab}	0.07±0.00^{ab}	0.28±0.06^{b}
50% ULJ	1.21±0.20^{abc}	0.13±0.02^{ab}	0.30±0.01^{b}
75% ULJ	1.19±0.07^{abc}	0.15±0.02^{a}	0.28±0.07^{b}

Note: Values are presented as mean± S.E (standard error of mean). Different superscript letters along treatment groups shows significant ($p<0.05$) differences. RLJ: Ripe lime juice; ULJ: Unripe lime juice; D/W: Distilled water.

Effect of graded concentration of ripe and unripe lime juice extract on relative organ weight of Wister rats

The results presented in Table 8 indicate that both RLJ and ULJ did not have a significant effect on the size of the liver, spleen, heart, kidneys, and lungs of the rats. The size of these visceral organs in the administered groups was statistically the same ($p > 0.05$) as the normal size of these organs in the control rats. This suggests that both RLJ and ULJ, at the various concentrations, did not cause any gross damage to the evaluated organs.

Table 8. Effect of graded concentration of ripe and unripe lime juice extract on the relative organ weight of Wister rats.

	Visceral organs				
Treatment group	**Liver**	**Spleen**	**Heart**	**Kidneys**	**Lungs**
5 ml/kg D/W	3.38±0.72	0.36±0.09	0.35±0.05	0.62±0.04	0.62±0.05
25% RLJ	3.51±0.46	0.47±0.13	0.34±0.02	0.65±0.12	0.77±0.07
50% RLJ	3.39±0.15	0.51±0.03	0.38±0.03	0.65±0.05	0.70±0.01
75% RLJ	2.93±0.09	0.44±0.02	0.33±0.02	0.48±0.01	0.58±0.04
25% ULJ	3.07±0.32	0.50±0.09	0.38±0.01	0.62±0.03	0.79±0.05
50% ULJ	3.48±0.41	0.45±0.10	0.33±0.03	0.54±0.04	0.68±0.03
75% ULJ	3.18±0.10	0.63±0.16	0.31±0.00	0.54±0.04	0.75±0.14

Note: Values are presented as mean± S.E (standard error of mean). Different superscript letters along treatment groups shows significant ($p<0.05$) differences. RLJ: Ripe lime juice; ULJ: Unripe lime juice; D/W: Distilled water.

DISCUSSION

The yield and concentration of RLJ and ULJ exhibited an inverse relationship. RLJ yielded 72.50% with a concentration of 0.08g/ml, while ULJ had a yield of 61.25% with a concentration of 0.1g/ml. This variation in both percentage yield and concentration gradient might have impacted the observed activity or effects of the two lime juices in this study. The chemical composition of RLJ includes essential components such as vitamins, minerals, phenolic compounds, saponins, cardiac glycosides, reducing sugars, flavonoids, and a minimal amount of steroids, which are recognized as crucial precursors to reproductive hormones (Enejoh et al., 2015; Navabi et al., 2018; Khan et al., 2018; Suntar et al., 2018; Saini et al., 2022). RLJ also contains a high level of tannins. Lime is known to have secondary metabolites like limonoids, phenylethylamine alkaloids, with p-synephrine being the most abundant (Adokoh et al., 2019). As indicated by Kim (2023), a medium-sized lime (67g) contains varying amounts of riboflavin, niacin, folates, phosphorus, magnesium, and trace amounts of iron, calcium, vitamin B6, and thiamine, along with a moderate amount of potassium and a substantial 22% of vitamin C. These phytoconstituents are scientifically believed to contribute to the physiological and pharmacological effects induced by lime juice on the body system (Jayaprakasha et al., 2006; Boshtam et al., 2011; Nallely et al., 2012; Akhtar, 2013; Saini et al., 2022).

The alteration of physiological parameters, including rectal temperature, heart rate, weakness, behavioral pattern, skin fur, and appetite, used for toxicity evaluation, deviated from normal at the highest dose (5000 mg/kg) in both RLJ and ULJ. This deviation occurred shortly before their demise, within less than 1 hour post-administration, indicating an LD50 (lethal dose for 50% of the population) of 1581.138 mg/kg (<2000 mg/kg) for both lime juices. This suggests that both RLJ and ULJ have a narrow margin of safety. The findings of this study on LD50 align with the research conducted by Chijoke-Nwachukwu and Dede (2010), who reported an oral single dose LD50 of 1500 mg/kg for RLJ and 2000 mg/kg for lemon juice. Additionally, Oboma et al. (2020) and Saini et al. (2022) reported LD50 values for lime leaf extract in separate acute and subacute studies (1480 mg/kg, 1655 mg/kg, and 1532.64 mg/kg, with an average of 1555.88 mg/kg). This average did not significantly differ from the LD50 value obtained in the current study. The toxicity results imply that RLJ, with a 100% mortality rate, was more toxic to the mice than ULJ, even though RLJ is sourer than ULJ. This observation underscores the notion that ripeness does not necessarily correlate with safety in this context.

The results obtained from the anti-fertility study revealed that ULJ at low to medium concentrations had a significant ($p<0.05$) effect on the color of the epididymal reserve in male Wistar rats, while RLJ did not exhibit any impact on the color. In Wistar rats, the normal color of semen is typically milky white, and changes to a creamy white color can indicate the influence of diet or treatment on semen color (Peters et al., 2008). There is a direct relationship between semen color and sperm concentration, with creamy white semen indicating a higher sperm count compared to milky white semen (Alkan et al., 2001; Peters et al., 2008). This observation aligns with the findings of the current study, where the rat group with creamy white epididymal reserve recorded a higher sperm count than those with milky white epididymal reserve. Similar correlations between semen color and sperm concentration have been reported in previous studies by Singh and Singh (2016), Ahmed et

al. (2021), and Ezeigwe et al. (2022) in their fertility studies involving lemon fruits. However, Rahaju and Hanizar (2021) reported no direct relationship between semen color and sperm concentration in mice treated with a 500mg/kg dose of lime leaf extract. These findings suggest that ULJ, particularly at low to medium concentrations, may have a noticeable impact on the sperm count and epididymal reserve color in male Wistar rats, highlighting the importance of considering concentration levels in the evaluation of such effects.

The observed interaction between higher concentrations of ULJ and spermatozoa stored in the caudal epididymides may have led to a decrease in pH, ultimately affecting sperm quality. Adokoh et al. (2019) reported a pH value of 6.67 in rat blood when ULJ was administered at a dose of 1000mg/kg for 58 days. This pH value is more acidic compared to the pH values of 6.98 obtained in the ULJ at 5% and 75% concentrations in the current study. The variation in pH values of ULJ in semen, as found in this study, could be attributed to differences in the buffering capacities of various body fluids or variations in dosage levels. The buffering capacity of a fluid refers to its ability to resist changes in pH when an acid or base is added. Differences in dosage levels might also influence the extent of the observed pH changes. It's worth noting that pH is a critical factor influencing the activity and function of sperm. Sperm function optimally within a specific pH range, and deviations from this range can adversely affect sperm quality and fertility. Therefore, the observed decrease in pH associated with higher concentrations of ULJ suggests a potential impact on sperm quality, emphasizing the importance of considering pH levels in the evaluation of reproductive effects.

The findings from this study suggest that the administration of RLJ for 28 consecutive days led to a decrease in the progressive motility of spermatozoa. This result aligns with existing literature, including studies by Imade et al. (2005), Obidi et al. (2008), Sagay et al. (2009), Loizzo et al. (2012), Aprioku and Obianime (2014), Oboma et al. (2020), Ahmed et al. (2021), and Saini et al. (2022). These studies, as mentioned, have reported adverse effects on both female (such as reduction in the length of proestrus and estrus, increased frequency of metestrus, and changes in luteinizing hormone levels) and male (including decreased sperm motility, count, and viability) reproductive performance at higher dosages or concentrations of lime juice and Citrus aurantifolia leaf extracts administered to laboratory animals. While some studies, such as Singh and Singh (2016), have reported adverse effects of lemon and lime extracts on steroidogenic markers in the testes, induction of germ cell apoptosis, drastic decrease in epididymal sperm quality, and no effect on libido, others like Rahaju and Hanizar in 2021 reported improved sperm quality at 25% lemon leaf extract and negative effects at 75% concentration. The current study found that increasing the dose or concentration of lime juice adversely affected body fluids and reproductive performance, including the progressive motility and livability of sperm. The negative impact on the livability of spermatozoa corresponds with the reduction in their progressive motility. This observation is consistent with Bearden et al. (2004), who reported a high correlation between the proportion of live spermatozoa and visual estimates of progressively motile sperm cells. According to Anderson (2001), various external and internal factors may influence the production of semen and spermatozoa concentration, ultimately affecting male fertility. The study revealed that the administration of lime juice, particularly RLJ, for 28 days resulted in a low sperm count in Wistar rats. However, the reason why RLJ affected the spermatozoa indices evaluated more than ULJ remains unclear and warrants further investigation.

From the study results, it is observed that the 75% concentration of RLJ caused significant abnormalities in the tail, head, and mid-piece of sperm, along with a higher percentage of cytoplasmic droplets compared to lower concentrations. The preservation of normal sperm morphology and integrity in the 25% RLJ, 25%, and 50% ULJ administered groups may be attributed to better antioxidant properties in both lime juices at lower concentrations.

Regarding sexual drive, both RLJ and ULJ led to a decrease in sexual drive (libido), suggesting the presence of active ingredients that may have affected the release of interstitial cell-stimulating hormone and testosterone. The decrease in sexual drive was concentration-dependent and followed a similar trend as sperm concentration, indicating a direct relationship between libido and sperm concentration. However, there are variations in findings reported by Singh and Singh (2016), which showed no effect on libido with 1000 mg/kg of lime leaf extract, possibly due to differences in the plant parts used.

Both RLJ and ULJ caused a significant reduction in body weights from week 2 to week 4. This reduction may be attributed to effects on metabolism, increased basal metabolic rate, suppressed appetite, induced lipolysis, or alteration in feed conversion efficiency. The observed reduction in prostate size in the 75% RLJ group is noteworthy, suggesting a potential effect on prostate health. The reduction in testicular size, particularly in the 50% and 75% RLJ concentrations, could be linked to ecobolic compounds in RLJ with estrogenic and spermicidal properties. These findings align with reports by Saini et al. (2022).

While the study did not show significant enlargement, atrophy, or damage to major organs, it is important to note that changes in organ weights can be sensitive indicators of toxicity. The study suggests that RLJ and ULJ may have concentration-dependent effects on reproductive and physiological parameters in male Wistar rats, highlighting the need for further

investigation into the underlying mechanisms and potential health implications.

CONCLUSION

This study, designed to investigate the effect of both RLJ and ULJ administration on the reproductive efficiency of male Wistar rats, revealed that ULJ is more concentrated than RLJ, although both recorded the same LD50 of 1581.138mg/kg, indicating a narrow safety margin. It can be concluded that RLJ at the various concentrations studied affected the reproductive performance of male Wistar rats by slowing down progressive motility, decreasing the percentage of livability, causing low sperm count, reducing testicular size, and diminishing sexual drive (libido) in the male Wistar rats. However, at a 75% concentration, the sizes of the prostate in the male rats were reduced, suggesting anti-prostatic activity, and it was more effective than the 75% concentration of ULJ.

***Conflict of interest*:** None

***Ethics*:** The study was performed in adherence to ethical standards and was in accordance with the principles outlined in the 1975 Helsinki Declaration as reviewed in 2013.

REFERENCES

Abarikwu SO. 2013: Causes and risk factors for male-factor infertility in Nigeria: A review. Afri J Rep Health 1: 150-166.

Adegoke SA, Oyelami OA, Olatunya OS, Adeyemi LA. 2017. Effects of Lime Juice on Malaria Parasite Clearance. Phy Res 25: 1547–50.

Adokoh CK, Asante D, Acheampong DO, Kotsuchibashi Y, Armah FA, Sirikyi IH, Kimura K, Gmakame E, Abdul-rauf S. 2019. Chemical profile and *in vivo* toxicity evaluation of unripe *Citrus aurantifolia* essential oil. Toxicol Rep 6: 692-702.

Ahmed TT, Kunle-alabi OT, Akindele OO, Raji Y. 2021. Effects of *Citrus limon* Linn Juice on Reproductive Functions in Female Wister Rats. Inter J Health Sci and Res 11: 262-271.

Akhtar SS. 2013. Evaluation of Cardiovascular Effects of *Citrus aurantifolia* (Linn.) Fruit. Social Science Research Network 2013: Retrieved from: http://ssrn.com/abstract=2279447. Retrieved June 13, 2013.

Alkan S, Baran A, Ozdas BO, Evecen M. 2001. Morphological Defects in Turkey Semen. Turkish J Vet Ani Sci 26: 1087-1092.

Alli LA, Adesokan AA, Salawu OA, Akanji MA. 2015. Toxicological studies of aqueous extract of Acacia nilotica root. Interdiscip Toxicol 8: 48-54.

Anderson J. 2001. *The Semen of Animals and Its Use for Artificial Insemination.* Green World Publishers, India. pp. 15-22.

Aprioku JS, Obianime AW. 2014. Evaluation of the effects of *Citrus aurantifolia* (Lime) juice in lead-induced haematological and testicular toxicity in rats. Pharmacol 5: 36-41.

Ayinde O, Ogunnowo C, Ogedegbe RA. 2012. Influence of vitamin C and Vitamin E on testicular zinc content and testicular toxicity in lead exposed Albino rats. Pharmacol & Toxicol 13: 13-17.

Babakhanzadeh E, Nazari M, Ghasemifar S, Khodadadian A. 2020. Some of the factors involved in male infertility: a prospective review. Inter J Gen Med 13: 29–41.

Bearden JJ, Fuquay JW, Willard ST. 2004. Applied Animal Reproduction, Mississippi State University. p. 183-196.

Boshtam M, Moshtaghian J, Naderi G, Asgary S, Nayeri H. 2011. Antioxidant effects of *Citrus aurantifolia* (Christm) juice and peel extract on LDL oxidation. J Res and Med Sci 16: 951-955.

Chibundu UC. 2013. Response of pre-pubertal bucks to administration of estradiol. Dissertation: Federal University of Technology Owerri, Nigeria. p 30.

Chijoke-nwauche IN, Dede EB. 2010. Effects of lemon juice on the reproductive hormones of female Wister rats. J Pharm and All Sci 7: 1-8.

Colaco S, Modi D. 2018. Genetics of the human Y chromosome and its association with male infertility. Repro Biol and Endocrinol 16: 1-24.

Colado-Velázquez J, Mailloux-Salinas P, Medina-Contreras JML, Cruz-robles D, Bravo G. 2015. Effect of Serenoa repens on oxidative stress, inflammatory and growth factors in obese Wister rats with benign prostatic hyperplasia. Phyto Res 29: 1525-1531.

Egbuka CG. 1995. New Approach for estimating sperm concentration in farm animals. Niger Press Plc. p 16-24.

El-sherbiny AM. 1987. Seasonal variation in seminal characteristics of rabbits. M.Sc. Dissertation, Faculty of Agriculture, Ain-Shams University.

Enejoh OS, Ogunyemi IO, Bala MS, Oruene IS, Suleiman MM, Ambali SF. 2015. Ethnomedical Importance of *Citrus Aurantifolia* (Christm) Swingle. Pharm Inno J 4: 01-06.

Eze KN. 2012. Anti-fertility effects of ethanolic extract of *Xylopia aethiopica* on male reproductive organ of Wister rats. Amer J Med Sci 2: 12-15.

Ezeigwe OC, Oladejo AA, Iloanya EL. 2022. The Impact of *Citrus aurantium* Fruit Juice on Bodyweight and Haematological Parameters of Wister Rats. Inter J Res and Rep in Haematol 5: 102-113.

Gobello C, Corrada YA. 2002. Non-infectious prostatic diseases in dogs. Compendium on Continuing Education for the Practising Veterinarian- North American. 24: 99-108.

Gorpinchenko I, Nikitin O, Banyra O, Shulyak A. 2014. The influence of direct mobile phone radiation on sperm quality. Cen Euro J Urol 67: 65-71.

Hafez B, Hafez ESE. 2000. Reproduction in Farm Animals. New York. Lippincott Williams and Wilkins, USA. p. 4-9.

Ilacqua A, Izzo G, Emerenziani GP, Baldari C, Aversa A. 2018. Lifestyle and fertility: the influence of stress and quality of life on male fertility. Reprod Biol Endocrinol 16: 1-11.

Imade GE, Sagay AS, Onwuliri VA, Egah DZ, Potts M, Short RV. 2005. Use of lemon or lime juice douches in women in Jos, Nigeria. Sex Health 2: 237-279.

Janevic T, Kahn LG, Landsbergis P, Cirillo PC, Cohn BA, Liu X, Factor-litvak P. 2014. Effects of work and life stress on semen quality. Fertil and Steril 102: 530–538.

Jayaprakasha GK, Mandadi KK, Poulose SM, Jadegoud Y, Patil BS. 2008. Novel triterpenoid from *Citrus aurantium* L.

possesses chemopreventive properties against human colon cancer cells. Bio Med Chem 16: 5939-5951.

Kale J, Osterlund E, Andrews D. 2018. BCL-2 family proteins: changing partners in the dance towards death. Cell Death Differ 25: 65–80.

Kesari KK, Agarwal A, Henkel R. 2018. Radiations and male fertility. Rep Biol and Endocrinol 16: 1-16.

Khan MH, Nabavi SM, Habtemariam S. 2018. Anti-diabetic potential of peptides: future prospects as therapeutic agents. Life Sci 193: 153–158.

Khan IN, Jahan S, Bhuiya MA, Mazumder K, Saha BK. 2013. Anti-diarrheal Potential of Ethanol and Water extracts of *Euphorbia hirta* whole plant on Experimental animals: A Comparative Study Scholars. J Appl Med Sci 1: 199-204.

Khan IA, Abourashed EA. 2010. Leung's Encyclopedia of Common Natural Ingredients. John Wiley and Sons Publication, New Jersey. p. 422-423.

Khan Y, Rafeeq AK, Syeda A. 2010. Evaluation of hypolipidemic effect of *Citrus limon*. J. Basic and Appl Sci 6: 39-43.

Kim C. 2023. Limes: A citrus fruit with powerful benefits. Retrieved from https//www.healthline.com/nutition.limes on 21st July, 2023.

Kumar N, Singh AK. 2015. Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Rep Sci 8: 191–196.

Loizzo MR, Tundis R, Bonesi M, Menichini F, De luca D, Colica C, Menichini F. 2012. Evaluation of *Citrus aurantifolia* peel and leaves extracts for their chemical composition,antioxidant and anti-cholinesterase activities. J Sci Food and Agric 24: 1893-18937.

Lorke D. 1983. A new approach to practical acute toxicity testing. Arc Toxicol 54: 275 -287.

Majzoub A, Agarwal A. 2018. Systematic review of antioxidant types and doses in male infertility: Benefits on semen parameters, advanced sperm function, assisted reproduction and live-birth rate. Arab J Urol 16: 113–124.

Martinez-Soto JC, Domingo JC, Cordobilla B, Nicolas M, Fernandez L, Albero P, Gadea Landeras J. 2016. Dietary supplementation with docosahexaenoic acid (DHA) improves seminal antioxidant status and decreases sperm DNA fragmentation. Sys Biol In Rep Med 62: 387-395.

Mbaka O, Anunobi C, Ogunsina S, Osiagwu D. 2017. Histomorphological changes in induced benign prostatic hyperplasia with exogenous testosterone and estradiol in adult male rats treated with aqueous ethanol extract of Secamone afzelii. Egypt J Bas and Appl Sci 4: 15-21.

Nabavi SF, KHAN H, D'onofrio G. 2018. Apigenin as neuroprotective agent of mice and men. Pharmacol Res 128: 359–365.

Nallely E, Sandoval M, Abraham G, Elizondo-Treviño E, Garza-González E, Alvarez L. 2012. Chemical Composition of Hexane Extract of *Citrus aurantifolia* and Anti-Mycobacterium tuberculosis Activity of Some of Its Constituents. Molecules 17: 11173-11184.

Nnenne SK, Ubaoji KI, Ogbodo UC, Enemor VHA, Oladejo AA. 2020. Comparative Study on the Nutritional and Antioxidant Components of Fruit Parts of *Citrullus lanatus*. Eur J Nutr and Food Saf 12: 39-51.

Obidi JA, Onyeanusi BI, Rekwot PI, Ayo JO, Dzenda T. 2008. Seasonal Variations in Seminal Characteristics of Shikabrown Breeder Cocks. Inter J Poul Sci 7: 1219-1223.

Oboma Y, Beredugo S, Archibong AM, Zipamone E. 2020. *Citrus aurantifolia* (Lime) juice extract reverses poly cystic ovary in Cadmium chloride exposed Sprague Dawley rats. Gen Intern Med and Clin Inno 5: 1-4.

Orieke D, Ohaeri OC, Ijeh II, Ijioma SN. 2019. Semen quality, hormone profile and histological changes in male albino rats treated with *Corchorus olitorius* leaf extract. Avicen J Phytomed 9: 551-562.

Peters SO, Shoyebo OD, Ilori BM, Ozoje MO, Ikeobi CON, Adebambo OA. 2008. Semen Quality Traits of Seven Strain of Chickens Raised in Humid Tropics. Inter J Poult Sci 7: 949-953.

Rahayu VG, Hanizar E. 2021. The Effect of Lemon (*Citrus limon*) Extracts On The Quantity and Quality of Mice (*Mus musculus*) Sperm. J Islam Sci and Tech 7: 300-316.

Sagay AS, Imade GE, Onwuliri V. 2009. Genital tract abnormalities among female sex workers who douche with lemon/lime in Nigeria. Afri J Rep Heal 13: 37-45.

Saini RK, Ranjit A, Sharma K, Prasad P, Shang X, Gowda KGM, Keum YS. 2022. Bioactive Compounds of Citrus Fruits: A Review of Composition and Health Benefits of Carotenoids, Flavonoids, Limonoids, and Terpenes. Antioxi 11: 239.

Salas-Huetos A, Rosique-Esteban N, Becerra-Tomas N, Vizmanos B, Bullo M, Salas-Salvado J. 2018. The effect of nutrients and dietary supplements on sperm quality parameters: a systematic review and metaanalysis of randomized clinical trials. Advan in Nutri 9: 833–848.

Septembre-Malaterre A, Remize F, Poucheret P. 2018. Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation. Food Res Intern 104: 86–99.

Sharma A. 2017. Male infertility; evidences, risk factors, causes, diagnosis and management in human. Annals of Clin and Lab Res 5: 188.

Silea C, Cucu I, Zarnescu I, Stoian AP, Motofei IG, Bratu OG, Pircalabioru GG, Chifiriuc M. 2019. Influence of age on sperm parameters in men with suspected infertility. Roman Biotech Let 24: 82-90.

Sirinarumitr K, Johnston SD, Kustritz MR, Johnston GR, Sarkar D, Memon MA. 2021. Effects of finasteride on size of the prostate gland semen quality in dogs with benign prostatic hypertrophy. J Ameri Vet Med Ass 218: 1275-80.

Sriplakich S, Lojanapiwat B, Chongruksut W, Phuriyaphan S, Kitirattakarn P, Jun-Ou J, Amantakul A. 2018. Prospective performance of the Prostate Health Index in prostate cancer detection in the first prostate biopsy of men with a total prostatic specific antigen of 4-10 ng/mL and negative digital rectal examination. Pro Int 136-139.

Singh N, Singh SK. 2016. *Citrus limon* extract: possible inhibitory mechanisms affecting testicular functions and fertility in male mice. Sys Biol in Rep Med 62: 39-48.

Sofowara A. 1993. Medicinal Plants and Tradition Medicine in Africa. Ibadan, Nigeria. Spectrum Books Ltd. p. 25-30.

Suntar I, Khan H, Patel S, Celano R, Rastrelli L. 2018. An Overview on *Citrus aurantium*: Its Functions as Food Ingredient and Therapeutic Agent. Oxi Med and Cell Long 1-12.

Ukar AI, Herbert U, Umeze EM. 2016. Effect of exogenous gonadotropin releasing hormone on semen characteristics and libido of rabbit bucks. Proceedings of the 42nd Annual Conference of Nigerian Society of Animal Production, Kwara State. p 853-859.

Wdowiak A, Skrzypek M, Stec M, Panasiuk L. 2019. Effect of ionizing radiation on the male reproductive system. Annals of Agric and Environ Med 26: 210–216.

World Health Organization 2010. WHO laboratory manual for the Examination and processing of human semen-5th Ed, WHO Press, Switzerland, 2010.

THIS PAGE INTENTIONALLY LEFT BLANK