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Original Article

A Comparative study on the effects of Yaji (Suya Meat sauce) and its spice constituents on the male reproductive profile of adult male Sprague Dawley rats

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ABSTRACT

Objective: This comparative study was designed to analyze the potential effects of Yaji (suya meat sauce) and its composite spices on male fertility based on testicular histology, serum testosterone level, and semen analysis parameters.

Methods: The study included 70 adult male Sprague Dawley rats with an average weight of 120 g. They were divided into two experimental study groups, respectively analyzed for 28 and 56 days. Each group featured 35 rats, further subdivided into seven treatment groups (A - G; n=5 each). Group A - Control; Group B: 200 mg/kg of Yaji; Group C: 200 mg/kg of red pepper; Group D: 200 mg/kg of black pepper; Group E: 200 mg/kg of clove; Group F: 200 mg/kg of ginger; and Group G: 200 mg/kg of garlic given orally using an oral cannula. At the end of the experiment, the animals were euthanized. Blood samples collected via cardiac puncture and their testes were excised and weighed. The cauda epididymis was excised for semen analysis using a Neubauer Counting Chamber (hemocytometer) and the testes were fixed in Bouin solution, processed, and stained with Hematoxylin and Eosin.

Results: Significant increases ($p<0.05$) were seen in body weight, testicular weight, serum testosterone level, sperm count and motility in the Yaji treated groups, in addition to significant increases in serum testosterone level, sperm counts, and sperm motility, and enhanced spermatogenesis and proliferation of Leydig cells in vivo as compared to the groups given isolated component spices (groups C-G), which also showed significant changes in testosterone and semen analysis when compared with the control groups.

Conclusions: Yaji or its spice components can boost male fertility parameters when consumed in moderated quantities without the known cytotoxic additives or condiments such as monosodium glutamate.

Keywords: Yaji, spermatogenesis, Leydig cells, testosterone, sperm count

INTRODUCTION

Yaji, made from a mixture of different pungent tasting spices and also referred to as local curry powder, is used for spicing various local dishes such as *Suya* (a meat delicacy), gusgus, fatte, and daffa-duja in the Hausa community in Nigeria (Wall, 1998; Erhirhie, 2019). In Nigeria, spices are rarely consumed as a single flavoring agent, but rather combined with several spices with or without additives or condiments (Ugwuja *et al*., 2007; Erhirhie, 2019). Yaji contains the following spices: finely ground roasted peanuts; red pepper; black pepper; garlic; onion powder; groundnut cake; ginger; and cloves with or without additives or condiment such as white Maggi - popularly called Ajinomoto- in Nigeria (Igene & Mohammed, 1983; Okonkwo, 1987; Nwaopara *et al*., 2004; Omojola, 2008); other ingredients include table salt (sodium chloride) and sometimes fine cornmeal (Wright, 2005). Yaji component spices have been documented in the treatment of male sexual dysfunction in Ayurvedic medicine (Vijayakumar *et al.,* 2004). It is important to note that Hausa male folks believe that some traditional or herbal aphrodisiacs such as Yaji, hakinmaye, and stimi help to enhance sexual performance, a belief that has made these spices highly consumed by men, most especially in the Northern part of Nigeria and in Niger Republic, for their supposed effects on sexual appetite stimulation and stamina (Uzeh *et al*., 2006; Nwaopara *et al.,* 2008; Ezejindu *et al*., 2014). Scientific reports documented that the active compounds present in Yaji spices are potentially harmful when consumed in excess (Southgate, 1993; Nwaopara *et al*., 2008; Ukoha *et al*., 2014). The active compounds in Yaji include gingerol found in ginger (Wichtl, 2004), eugenol in cloves, capsaicin in red peppers, piperine in black pepper (Surh & Lee, 1995; Krishnaswamy & Raghuramulu, 1998; McGee, 2004) and allicin in garlic (Macpherson *et al.,* 2005), each with documented adverse effects on testicular and male fertility parameters when consumed in isolation (Nwaopara *et al*., 2004). Some studies have documented liver adverse effects, with mild patchy necrosis of the hepatocytes and signs of hepatitis (Vijayakumar *et al.,* 2004; Nwaopara *et al*., 2007), whereas in the kidneys distinct basophilic bodies have been found in the interstitium of the renal cortex (Nwaopara *et al.,* 2008). Considering the inherent chemical properties/potential of all active constituents present in Yaji, it might be fitting to say that Yaji is indeed a chemically complex spice (Akpamu *et al.,* 2011a). The spice combination found in Yaji makes it a good flavoring ingredient for different foods and dishes, which explains its massive consumption in Northern Nigeria (Uzeh *et al*., 2006).

A wide range of studies have reported deleterious effects of Yaji in major organs such as the pancreas, liver, kidneys, and brain, with effects ranging from cellular distortion, necrosis, to degeneration in the mentioned organs (Nwaopara *et al*., 2004; 2007; 2008; 2009; 2010). In Nigeria, there is a growing concern about the excessive consumption of this spice, which has been apportioned without a standard dosage or serving method (Nwaopara *et al*., 2010) and consumed mostly by men (Alley & Burroughs, 1991). The side effects of dieting on the male reproductive system also include some beneficial effects in the treatment of reproductive system disorders associated with flavonoid and antioxidant compounds (Hosseini, 2018). Oxidative stress can potentiate male infertility, thereby affecting parameters such as sperm motility, sperm count, and spermatogenesis (Eskenazi *et al*., 2005). However, consumption of food, spice, or diets containing vitamin C, vitamin E, zinc, folic acid, copper, selenium, and other phytochemicals with antioxidant properties may improve and protect one's fertility status (Robbins *et al*., 2012). Scientists need to advocate for continuous fertility health awareness and the importance of nutrition in reproductive health and in the prevention of fertility problems

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(Lo, 2000; Safarinejad *et al*., 2010), by further connecting the science of nutrition to preventive medicine. Regulation of male dietary pattern is known to be an important component of reproductive health (Bhargava & Guthrie, 2002; Kopelman & Caterson, 2005).

Hence, this study was designed to evaluate the effects of Yaji and its isolated constituent spices on male fertility. This study presents protocols and results of a wider and deeper evaluation of the effect of Yaji and its isolated constituent spices, including elements such as hormonal assays (for testosterone levels), semen analysis, testicular histology, and assessment of effects on body and organ weights to further the knowledge about the extent to which *Yaji and its spice mixture* may affect male fertility and add to scarce literature produced on the subject. Semen analysis is a significant concept in infertility investigation of herbal compounds, drugs, or supplements taken as a measure to improve male fecundity in clinical andrology and male fertility (Vasan, 2011).

MATERIAL AND METHODS

Experimental animals

Seventy healthy adult male Sprague Dawley rats aged about two months with an average weight of 120 g were included in this study. They were cared for according to the animal care guidelines documented in the "*Guide for the Care and Use of Laboratory Animals"* (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). The animals were procured from Covenant Farm (Nig.) Enterprises, Gbolasire Estate; Iwo road Ibadan, Oyo State, Nigeria. They were authenticated in the Department of Zoology, University of Lagos, Nigeria, and then housed in the Animal House of the Department of Anatomy, University of Lagos, Nigeria, where they were kept in well-ventilated rat metallic cages under standard laboratory condition (12hrs light: 12hrs dark cycle; temperature-: 37.5°C; 40-55% humidity). They were allowed to acclimatize for two weeks before experimentation and fed pelleted rat feed from UAC, Vital Feeds Lagos, Nigeria, and water *ad libitum*.

Research material: Yaji (Suya Sauce)

Yaji spice preparation was performed according to the method described by Nwaopara *et al*. (2004) without additives. The composition of Yaji for this study was as follows: dried fruits of *Zingiber officinale* (rhizomes of ginger), *Syzygium aromaticum* (clove buds), dried *Capsicum annum* (Red pepper), *Allium sativum* (garlic), and *Pipier guineese* (black pepper) as reported by Erhirhie (2019) procured in Lawanson market in Surulere, Lagos, Nigeria. Spice fruits were taken to the Botany Department, University of Lagos, Nigeria, for identification and authentication where a voucher specimen was deposited.

Preparation of the aqueous extract of Yaji (Suya Sauce) and its spice constituents

The quantities of ingredients in commercially produced Yaji sauce are usually not standardized (Ezejindu *et al.,* 2014). In this study, Yaji was made by mixing dried powdered spice constituents, which were later macerated into an aqueous solution. Equal portions of each ground spice [dried fruits of *Zingiber officinale* (rhizomes of ginger), *Eugenia aromaticum* (dried clove buds), *Capsicum annum* (Red pepper), *Allium sativum* (garlic) and *Pipier guineese* (black pepper)] were mixed to form the *Yaji* sauce. Then, 10 g (10,000 mg) of Yaji and each of the six isolated ingredients named above were macerated in 50 ml of distilled water in different labeled containers, to obtain a concentration of 200 mg/ml. No previous studies have looked into the therapeutic effect of this dose of Yaji, although Memudu *et al*. (2015) analyzed the effects of garlic and black pepper at 200 mg/kg.

Experimental Animals Grouping

Group A – Control; Group B: 200 mg/kg of Yaji; Group C: 200 mg/kg of red pepper; Group D: 200 mg/kg of black pepper; Group E: 200 mg/kg of cloves; Group F: 200 mg/kg of ginger; and Group G: 200 mg/kg of garlic. The ingredients wee administered orally using an oral cannula for 28 days and 56 days, respectively

Oral administration of aqueous extract of Yaji sauce and its spices

Each animal was given 1ml of the crude extract of Yaji and its isolated spice components for 28 days according to the method described by Zodape & Gaikwad (2019) and for 56 days based on the method described by Chinta *et al*. (2017) and Ekaputri *et al*. (2018) via oral cannula. The lengths of administration were based on the spermatogenic cycle of rats, which lasts for 55 days on an average (Chinta *et al*., 2017).

Euthanasia of experimental animals: A day after the administration of the last dose, the experimental animals final body weights were taken before they were euthanized via intraperitoneal injection of 50 mg/ml of ketamine (Claris life Sciences Ltd, India).

Blood collection for serum biochemical analysis: Blood was collected via cardiac puncture using a 5 ml capacity syringe inserted into the left ventricle. The collected blood sample were transferred into a labeled plain specimen bottles. They were arranged in a centrifuge machine and spun at 2,500 r.p.m. for 10 minutes to obtain serum. Decanted serum was placed in a plain cuvette and frozen for Serum testosterone Spectrophotometric analysis using Enzyme-linked immunoassay (ELISA) test kits made by BIOTEC Laboratories, U.K. The test kits used were made by BIOTEC Laboratories Ltd, 32 Ansons Road Martlesham Heath, UK.

Testicular tissue collection for histopathology analysis

The animals were dissected, an incision was made in the lower abdominal wall and the testes pushed out from the scrotal sac. The testes were quickly excised and their wet weight measured using an analytical weighing scale. The cauda epididymis was excised for semen analysis. The testes were preserved in Bouin solution. Longitudinal sectioned testicular tissue specimens were processed for histology studies according to the method described by Bancroft & Gamble (2008). The tissue specimens were dehydrated with concentrated ethanol, cleared with xylene, and embedded in paraffin wax. Tissue blocks were serially sectioned using a Leica Rotary microtome set at 5 µm. Testicular tissue sections were stained using Hematoxylin and Eosin stain to assess the general cell architecture of the testicular tissue specimens.

Semen analysis: The cauda epididymis was excised and transferred into normal saline; the spermatozoa were carefully teased out. A drop of normal saline containing spermatozoa was placed on a Neubauer Counting Chamber (hemocytometer) for semen analysis under a light microscope at 10x magnification. Semen analysis and sperm counts were done for each group based on the protocol described in the World Health Organization (WHO) manual (WHO, 2010).

Sperm motility: Sperm motility was assessed using the WHO classification system. Each sample was assessed twice. For consistency, all readings were carried out at 37°C (WHO, 2010).

Tissue Analysis and photography: Photomicrographs of the stained sections were viewed using an Olympus light microscope (Olympus, Germany) at 400x magnification. Micrograph images were taken with a digital camera (MV500 Cameroscope) attached to the Light Microscope using 10x and 40x objective lenses.

Statistical analysis

The data obtained were analyzed using Graphpad Prism version 8.4.3 (686) for Windows. The column statistics were used for one-way analysis of variance (ANOVA) and the Tukey post-hoc test for parametric tests. Statistical significance (*) was set at $p<0.05$, i.e., a *p*-value < 0.05 was considered statistically significant. Data were expressed as Mean±Standard deviation (Mean±SD). Comparative analysis involving two continuous variables was done using an independent sample t-test, while those involving more than two variables were done using one-way analysis of variance (ANOVA).

RESULTS (Tables 1 and 2)

The effects of Yaji and its constituents on body weight

This study focused on weight changes in experimental animals given Yaji and its constituents for 28 days (4 weeks) and 56 days (8 weeks), respectively. There was a significant weight increase ($p<0.05$) between the initial and final weights of each group (Groups A-G) in the 28 days study. At 28 days, the group given Yaji had a significant increase in body weight (Figure 1) when compared to controls and treated in the groups given cloves and garlic ($p<0.05$). However, there was no statistically significant increase in body weight when the group given Yaji (B) was compared against the rats fed Red Pepper (C), Black Pepper (D) or Ginger (F) ($p>0.9996$). In the groups studied for 56 days (Figure 2), a statistically significant increase was seen in the body weights of the rats given Yaji as compared with the groups given black pepper and garlic ($p<0.05$); no significant weight change was seen when controls (A) and the groups given red pepper (C), cloves (E), or ginger (F) were compared to the groups given Yaji (B), according to the Tukey's multiple comparison test ($p<0.05$).

Yaji induced an increase in testicular weight

Testicular weight change was evaluated to vividly describe the effects of Yaji and its spice constituents on testicular tissue mass. In the 28-days study group (Figure 3A), there was a statistically significant increase in testicular. In the 28-day study group (Figure 3A), there was a statistically significant increase in testicular weight in the group given Yaji and red pepper as compared with the controls and treated groups given black pepper, cloves, ginger, and garlic ($p<0.05$); however, there was no statistically significant difference between the groups given Yaji when compared with the groups administered black pepper (D) or red pepper (C) ($p>0.05$). In the 56-days study group, significant increases were seen in the testicular weight of rats given Yaji (B) and red pepper ($p<0.5$); the difference in testicular weight between the rats given Yaji (B) and red pepper (C) was not statistically significant ($p<0.7453$).

Yaji and its constituents mediate steroidogenesis via elevated serum testosterone levels

Serum testosterone levels were assayed to determine the integrity of the interstitial cells of Leydig. In this study, serum testosterone levels were studied to evaluate the interplay between Yaji and its isolated spice components on Leydig cell activity concerning testosterone secretion. A significant increase in serum testosterone secretion in Leydig cells was observed in the rats given Yaji in the 28-days study group (Figure 4A) when compared with controls and rats given red

Table 1. Mean initial and final body weights, testicular weights, serum testosterone levels, and semen analysis of the rats treated for 28 days.

Group (n=5)	Initial Body weight (g)	Final Body weight (g)	Testicular weight (g)	Serum testosterone level (ng/dl)	Sperm count (millions/ml)	Sperm motility (%)
Control (A)	122±2.4	163±5.4	0.93±0.04	1.5±0.05	193±2.2	59±3.5
Yaji (B)	123±2.9	182±3.5	1.1±0.08	2.1±0.07	216±5.4	69±3.7
Red Pepper (C)	121±2.6	181±6.4	1.1±0.13	1.9±0.01	209±7.8	68±2.5
Black Pepper (D)	123±5.5	183±4.7	0.99±0.03	1.9±0.05	206±2.6	68±3.2
Cloves (E)	123±2.6	166±7.0	0.94±0.02	1.9±0.05	198±4.7	63±2.1
Ginger (F)	127±5.6	182±4.9	0.96±0.04	1.9±0.03	196±3.0	68±3.0
Garlic (G)	126±2.5	157±5.6	0.97±0.03	1.9±0.02	206±1.5	67±2.9

Table 2. Mean initial and final body weights, testicular weights, serum testosterone levels, and semen analysis of the rats treated for 56 days.

Group (n=5)	Initial Body weights (g)	Final Body weights (g)	Testicular weights (g)	Serum testosterone level (ng/dl)	Sperm count (millions/ml)	Sperm motility (%)
Control (A)	123±1.1	204±9.0	0.93±0.40	1.6±0.038	223±1.7	70±3.7
Yaji (B)	128±1.9	219±4.1	1.1±0.11	2.1±0.14	245±3.3	76±4.5
Red Pepper (C)	122±2.1	213±4.7	1.1±0.069	2.0±0.045	233±1.0	71±2.7
Black Pepper (D)	122±2.5	203±3.1	0.98±0.03	2.1±0.090	235±3.8	70±2.5
Clove (E)	124±2.3	206±3.0	0.93±0.03	2.0±0.037	228±1.3	70±4.0
Ginger (F)	121±1.6	204±4.8	0.92±0.019	2.0±0.057	216±4.2	66±3.6
Garlic (G)	121±2.6	167±4.4	0.96±0.03	2.0±0.022	235±8.5	66±3.1

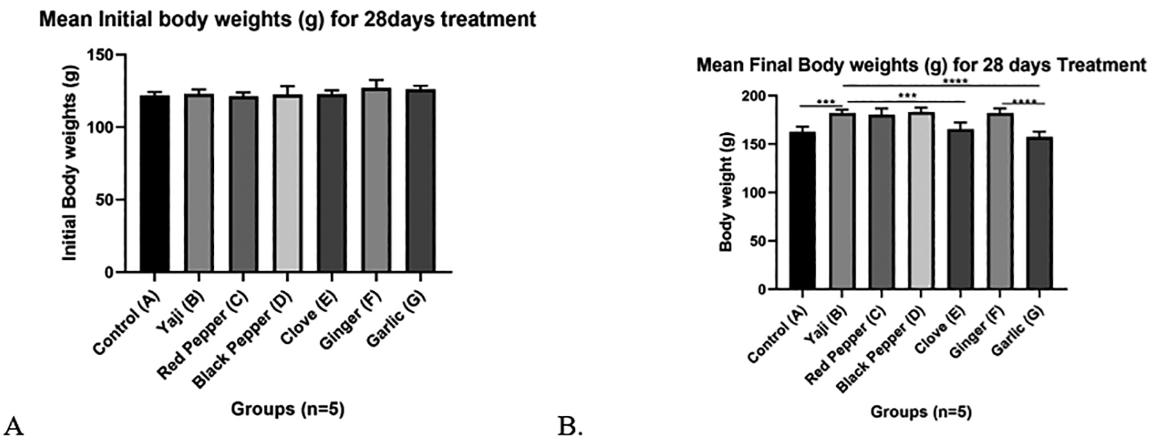


Figure 1. Graphical Representation of Mean Initial and Final Body Weights of Adult Sprague-Dawley rats treated for 28 days. Data analyzed using one-way ANOVA and expressed as Mean±Standard Deviation (Mean±SD). Statistical Significance is taken at $p<0.05$ (*) using the Tukey's Post-hoc test. There is a significant difference in the mean final weights at $p<0.05$ in Control (A) *vs*. Yaji (B) (***$p<0.0001$), Yaji (B) *vs*. Cloves (E) (***$p<0.0010$); Yaji (B) *vs*. Garlic (G) (***$p<0.0001$); no significant difference was found in Yaji (B) *vs*. Red Pepper (C); Yaji (B) *vs*. Black Pepper (D) or Yaji (B) *vs*. Ginger (F) (ns: $p>0.9996$).

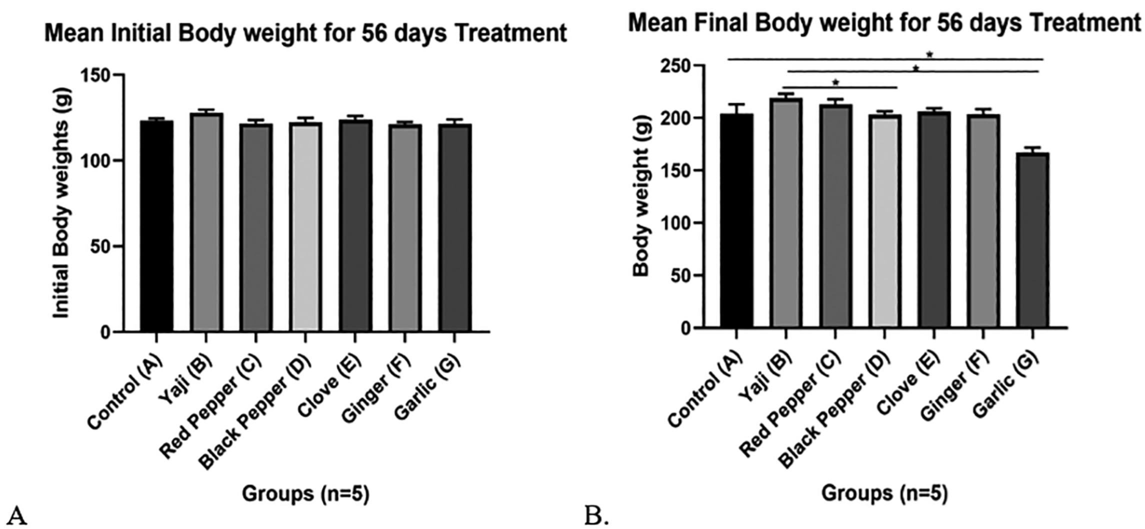


Figure 2. Graphical Representation of Mean Initial and Final Body Weights of Adult Sprague-Dawley rats treated for 56 days. Data analyzed using one-way ANOVA and expressed as Mean±Standard Deviation (Mean±SD). Statistical Significance is taken at $p<0.05$ (*) using the Tukey's Post-hoc test. There is a significant difference in mean final weight at $p<0.05$ for Yaji (B) *vs*. Black Pepper (D) (**$p=0.0091$) and Yaji (B) *vs*. Garlic (G) (****$p<0.0001$); no statistical difference was found in Control (A) *vs*. Yaji (B); Yaji (B) *vs*. Red Pepper (C); Yaji (B) *vs*. Cloves (E); or Yaji (B) *vs*. Ginger (F) using Tukey's multiple comparisons tests.

pepper, garlic, black pepper, clove, or ginger. In the 56-day study group (Figure 4B), Yaji caused a significant increase in serum testosterone levels as compared with controls; no significant difference was seen in the comparison performed with rats given Red Pepper (C), Black Pepper (D), Ginger (F), Garlic (G) or Cloves (E) ($p<0.05$).

Yaji and its constituents improved sperm count

Sperm count (SC) evaluation is used as an index to measure fertility or the fertility potential of a therapy. In the 28-days study group (Figure 5A), there was an increase in the sperm count of rats given Yaji Yaji when compared with the control and rats given black pepper, ginger, clove, red pepper, or garlic. Significant increases were also seen when rats given red pepper, black pepper, garlic, cloves, and ginger were compared with the control group. There was no statistical difference in the group given Yaji (B) when compared with the groups given red pepper (C) or garlic (G). The rats given Yaji for 56 days (Figure 5B) had a significant increase in sperm count. The rats treated with ginger had significant decrease in sperm count ($p<0.05$). There was no statistically significant difference in sperm count of the control (A), rats given cloves (E), red pepper (C), or garlic (G) were compared to the rats given Yaji (B).

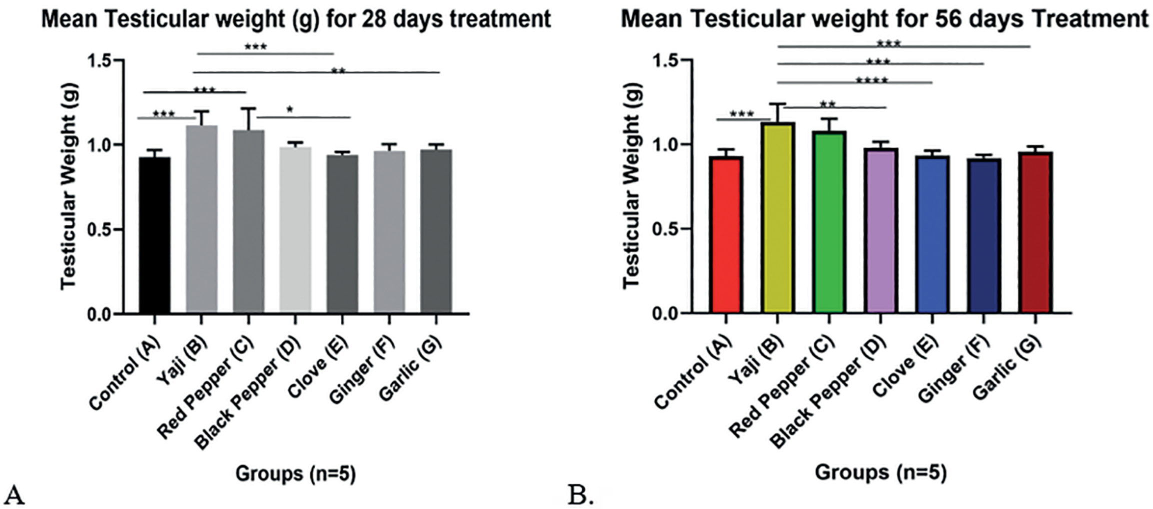


Figure 3. Graphical Representation of Mean Testicular weight of Adult Sprague-Dawley rats treated for 28 and 56 days. Data analyzed using one-way ANOVA and expressed as Mean±Standard Deviation (Mean±SD). In the group treated for 28 days (Figure 3 A), we observed a significant difference at $p<0.05$ for Control (A) *vs*. Yaji (B) (**$p=0.0015$), Yaji (B) vs. Cloves (E) (**$p=0.0037$), Yaji (B) vs. Ginger (F) (*$p=0.0160$), and Yaji (B) *vs*. Garlic (G) (*$p=0.0228$). No significant difference was found in Yaji (B) *vs*. Black Pepper (D) or Yaji (B) *vs*. Red Pepper (C) ($p>0.05$). In the group treated for 56 days (Figure 3B), significant differences were found in Control (A) *vs*. Yaji (B) (****$p<0.0001$), Yaji (B) *vs*. Cloves (E)(****$p<0.0001$), Yaji (B) *vs*. Ginger (F)(**** $p<0.0001$), and Yaji (B) *vs*. Garlic (G)(*** $p=0.0004$); no statistical difference was found in Yaji (B) *vs*. Red Pepper (C) (ns: $p=0.7453$).

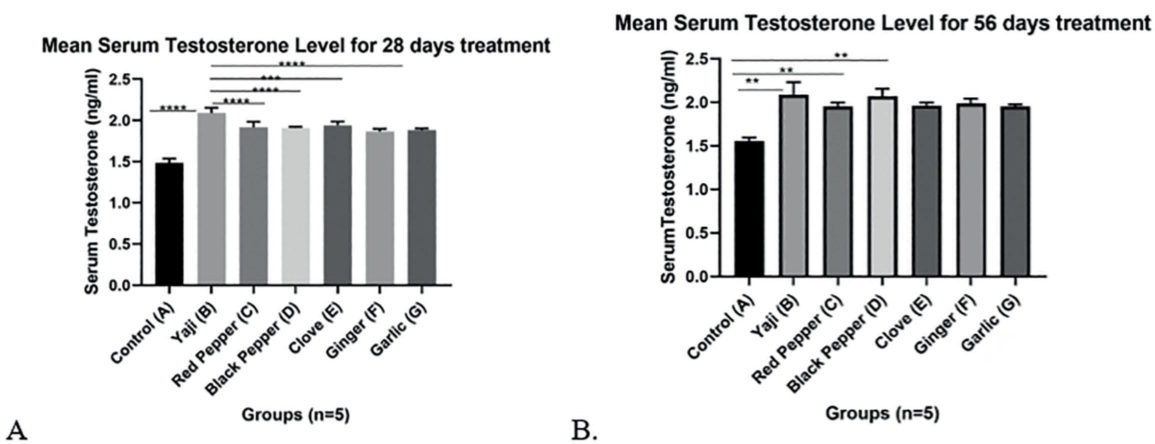


Figure 4. Graphical Representation of Mean Serum testosterone level (mg/ml) of Adult Sprague-Dawley rats treated for 28 and 56 days. Data analyzed using one-way ANOVA and expressed as Mean±Standard Deviation (Mean±SD). In the group treated for 28 days (Figure 4A), we observed a significant difference in Control (A) *vs*. Yaji (B) (****$p<0.0001$), Yaji (B) vs. Red Pepper (C) (****$p<0.0001$), Yaji (B) vs. Black Pepper (D), (****$p<0.0001$), Yaji (B) *vs*. Ginger (F), Yaji (B) *vs*. Garlic (G) (**** $p<0.0001$), and Yaji (B) *vs*. Cloves (E) (***$p=0.0004$); in the group treated for 56 days (Figure 4B), a significant difference was found in Control (A) *vs*. Yaji (B),(**$p=0.0063$); no significant difference was found in Yaji (B) *vs*. Red Pepper (C), Yaji (B) *vs*. Black Pepper (D), Yaji (B) *vs*. Ginger (F), Yaji (B) *vs*. Garlic (G), or Yaji (B) *vs*. Cloves (E).

Yaji and its constituents increased the number (percentage) of motile sperm

Sperm motility is used to evaluate the strength or viability of spermatozoa. A significant increase in sperm motility was seen in the 28-days study group (Figure 6A) given Yaji when compared with control (A), rats given cloves (E), and ginger (F) (*$p<0.00056$); no statistical difference was observed when rats given Yaji (B) were compared to animals administered red pepper (C) or garlic (G) ($p<0.05$). The rats treated with Yaji (B) for 56 days (Figure 6B) had a significant increase in sperm motility when compared to rats given ginger (F) (*$p<0.0211$); no statistical difference was seen when rats given Yaji (B) were compared with control (A), rats given red pepper (C), black pepper (D), cloves (E) or garlic (G) ($p<0.05$).

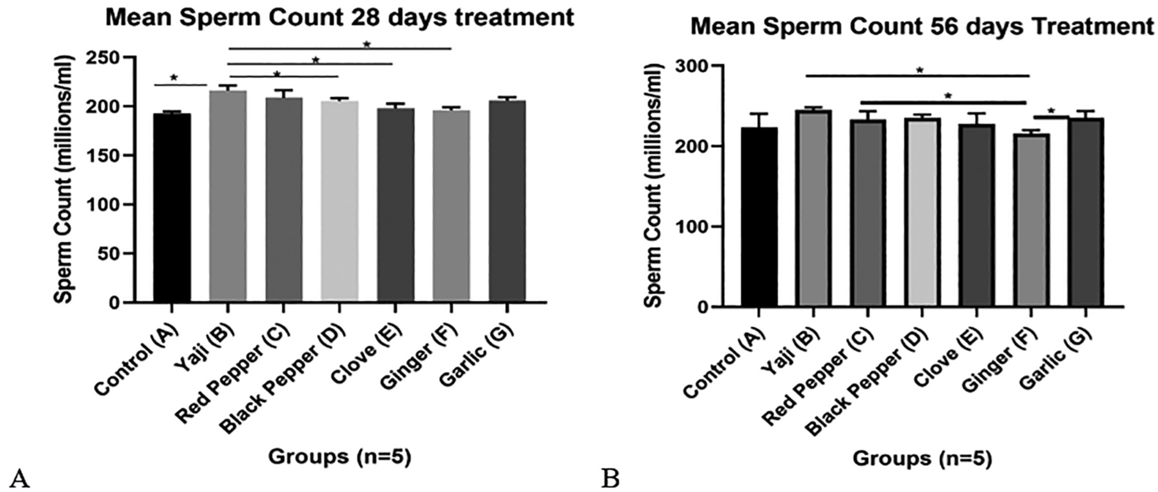


Figure 5. Graphical Representation of Mean Sperm count (millions/ml) of Adult Sprague-Dawley rats treated for 28 and 56 days. Data analyszd using one-way ANOVA and expressed as Mean±Standard Deviation (Mean±SD). In the group treated for 28 days (Figure 5A), we observed a significant difference in Control (A) *vs*. Yaji (B),(** p=0.0013), Yaji (B) *vs*. Black Pepper (D) (*p=0.0100), Yaji (B) *vs*. Ginger (F) (*p=0.0448), and Yaji (B) *vs*. Cloves (E) (*p=0.0396); no significant difference was seen in Yaji (B) *vs*. Red Pepper (C) or Yaji (B) *vs*. Garlic (G). In the group treated for 56 days (Figure 5B), we found a significant difference in Yaji (B) *vs*. Ginger (F) (*p=0.00056); no significant difference was seen in Control (A) *vs*. Yaji (B), Yaji (B) *vs*. Cloves (E), Yaji (B) *vs*. Red Pepper (C) or Yaji (B) *vs*. Garlic (G).

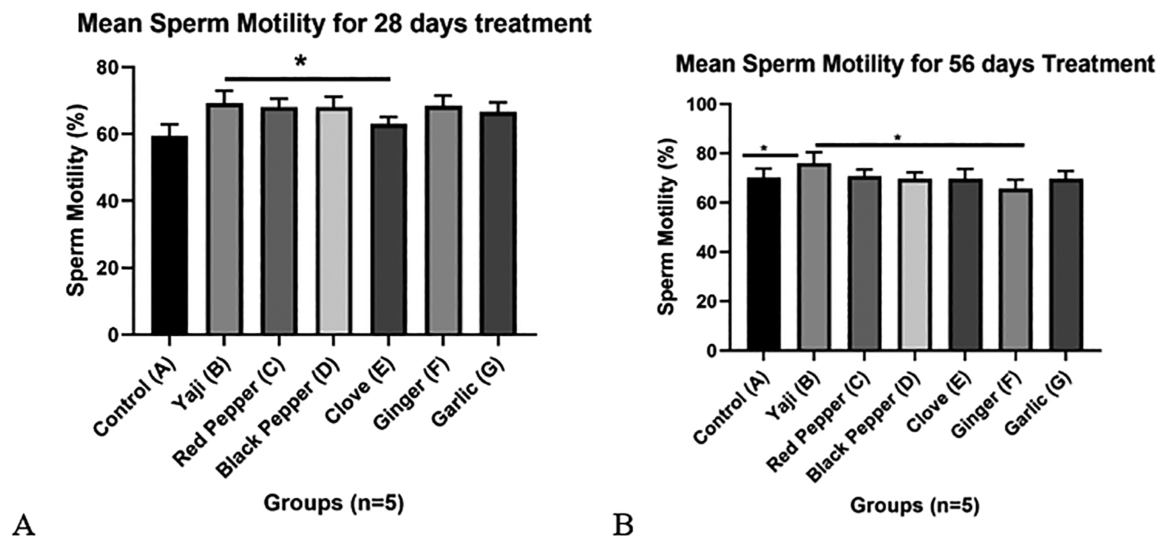
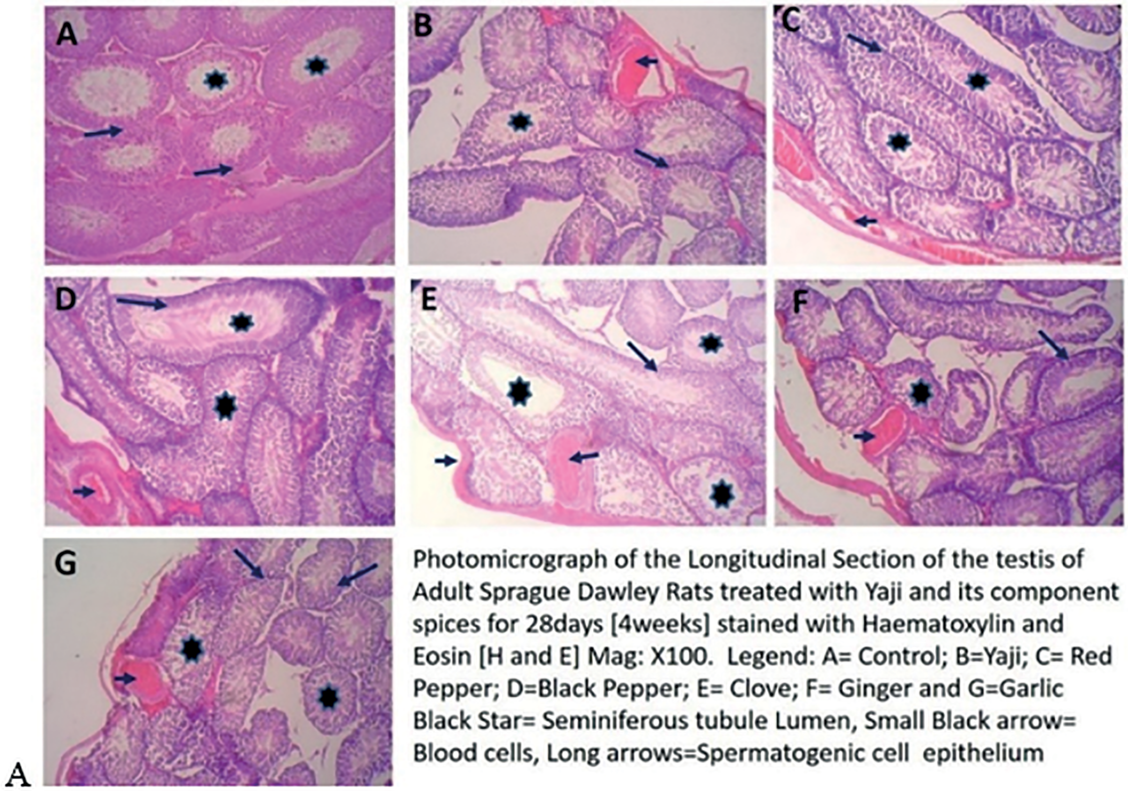


Figure 6. Graphical Representation of Mean Sperm motility (%) of Adult Sprague-Dawley rats treated for 28 and 56 days. Data analyzed using one-way ANOVA and expressed as Mean ± Standard Deviation (Mean±SD). In the group treated for 28 days (Figure 6A), we observed a significant difference in Control (A) vs. Yaji (B), Yaji (B) *vs*. Ginger (F) (*p=0.00056), Yaji (B) *vs*. Red Pepper (C) and Yaji (B) *vs*. Garlic (G) are not significant at p<0.05 but Yaji (B) *vs*. Clove (E) (*p=0.0433) was significant. In the group treated for 56 days (Figure 6B), a significant difference was seen in Yaji (B) *vs*. Ginger (F) (*p=0.0211); no statistical difference was seen in Control (A) vs. Yaji (B), Yaji (B) vs. Red Pepper (C), Yaji (B) vs. Black pepper (D), Yaji (B) vs. Cloves (E) or Yaji (B) vs. Garlic (G).

Yaji and its spice constituents influence spermatogenesis and Leydig Cell proliferation

Hematoxylin and Eosin (H&E) staining was used to demonstrate testicular cells inclusive of spermatogenic cell series and Leydig cells. The micrographs are displayed in 100x (Figures 7A and 8A) and 400x magnification (Figures 7B and 8B). The control group showed the presence of all spermatogenic cell series, interstitial cells of Leydig, and less occluded seminiferous tubule lumens with no disruption of the spermatogenic cell lineage. The groups given Yaji (B), red pepper (C), black pepper (D), clove (E), and ginger (F) for 28 days (Figure 7) showed mild histological disruption of the spermatogenic cell series as compared to control and rats given garlic. However, the interstitial cells were not disrupted. In the group treated for 56 days (Figures 8A and B), control (A) had presence of proliferative



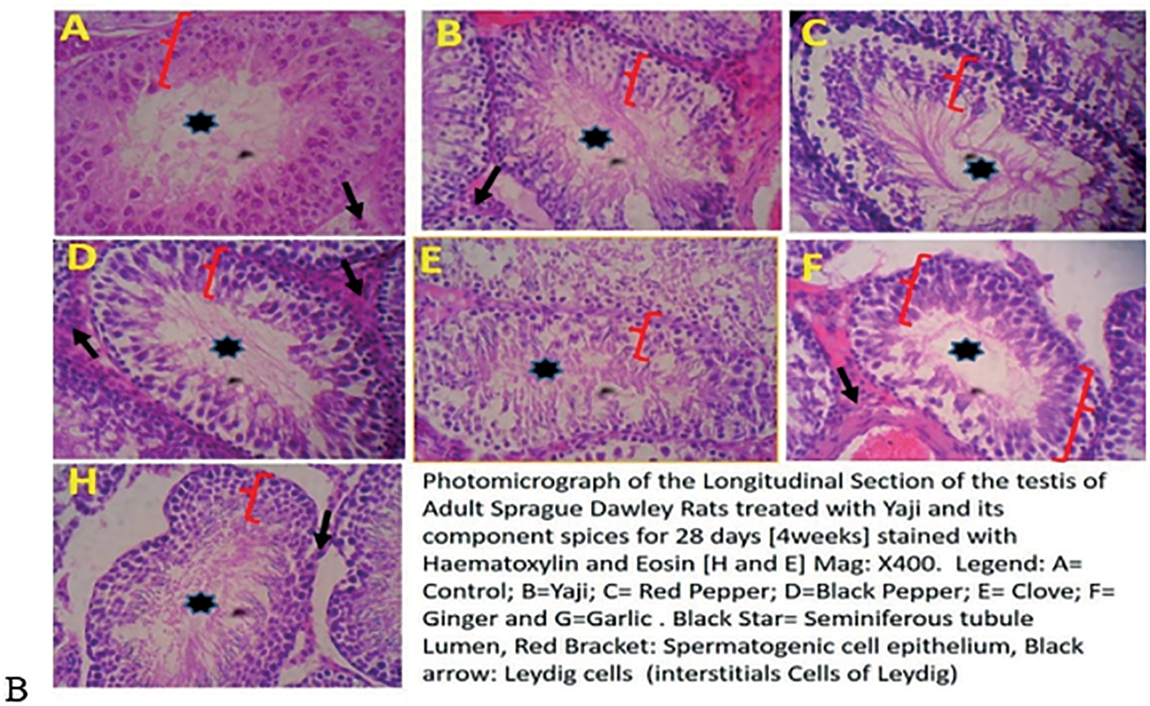
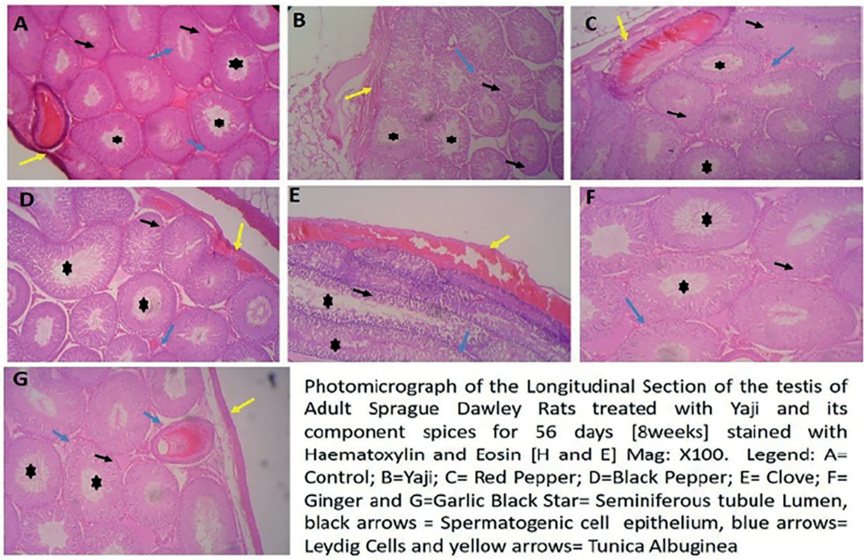
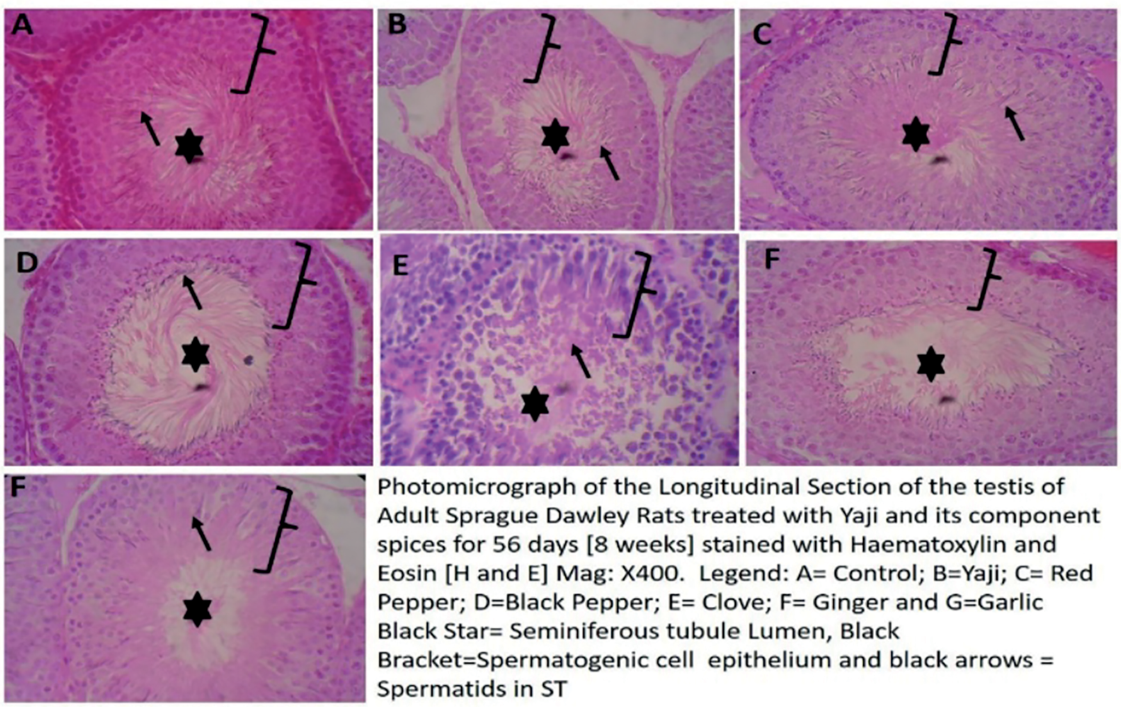


Figure 7. Photomicrograph of a section of the longitudinal section of the testes of Adult Sprague-Dawley rats treated for 28 days. H&E stain captured at 10x (Figure 7A) and 40x (Figure 7B) magnification. Legend: A=Control, B=Yaji, C=Red pepper, D=Black pepper, E=Cloves, F=Ginger, and G=Garlic. Black arrows=Leydig cells (Interstitial cells); black asterisk=seminiferous tubule lumen and red bracket: basal and adluminal membrane of the seminiferous tubule made up of spermatogenic cell lineage. The control group had all spermatogenic cell series, interstitial cells of Leydig and less occluded seminiferous tubule lumen. Rats given Yaji, red pepper, black pepper, cloves, and ginger had mild histological disruptions of the spermatogenic cell series as compared to controls and rats given garlic. However, the interstitial cells were undisrupted.



A



B

Figure 8. Photomicrograph of a section of the longitudinal section of the testes of Adult Sprague-Dawley rats treated for 28 days. H&E stain captured at 10x (Figure 8A) and 40x (Figure 8B) magnification. Legend: A=Control, B=Yaji, C=Red pepper, D=Black pepper, E=Cloves, F=Ginger, and G=Garlic. Yellow arrows=Tunica vaculosa, Blue arrows=Leydig cells (Interstitial cells); black asterisk=seminiferous tubule lumen, Black arrows=spermatogenic cells, and Black bracket: basal and adluminal membrane of the seminiferous tubule made up of the spermatogenic cell lineage. Controls had all spermatogenic cell series, spermatids in the adluminal compartment, proliferative interstitial cells of Leydig, and waves of spermatids in the seminiferous lumen. Similar histological features were observed in rats given Yaji, red pepper, black pepper, and ginger. The group given cloves had disruptions of spermatogenic cells series in the adluminal compartment as compared to the basal compartment of the seminiferous tubule. However, the interstitial cells were undisrupted in all study groups treated for 56 days.

interstitial cells of Leydig, well-aligned spermatogenic cell series in contact with the basement membrane, and brush-like waves of spermatids in the seminiferous tubule ad luminal junction and its lumen, in addition to waves of spermatids in the ST lumen. These histological findings were also seen in the groups given Yaji, red pepper, black pepper, and ginger. However, the rats given cloves showed disruption of spermatogenic cells series in the ad luminal compartment as compared to the basal compartment of the seminiferous tubule. However, the interstitial cells were undisrupted in all study groups treated for 56 days.

DISCUSSION

The diets and food supplements available globally give ample opportunity to procure and provide intervention, considering therapeutics and supplements for fertility problems (Sinclair, 2000; Cheah & Yang, 2011; Robbins *et al*., 2012). These food, herbs, or spices are consumed in mixtures featuring other spices or herbs (Rahmawati & Bachri, 2012). The foods and diets that show promise at improving male fertility possess micronutrients, such as folic acid, selenium, zinc, and vitamins C and E, with the ability to improve sperm quality. Other foods, if consumed in excess and even in minute quantities, may disrupt sperm integrity (Eslamian *et al*., 2012).

Mostly, men consume Yaji hence this study was designed to evaluate whether there is a synergistic effect derived from the consumption of the mixture of spices present in Yaji upon male fertility and describe its mechanism, in a bid to demonstrate its effects on male reproductive and nutritional health awareness.

This study showed that Yaji caused a significant increase in body weight in the rats treated for 28 and 56 days when comparisons were made between rats given Yaji and subjects in the control, clove, garlic, red pepper, ginger, and black pepper groups. This implies Yaji has anabolic potentials. According to Agbo *et al*. (2013), Yaji caused a dose-dependent increase in body weight, i.e., the dose of Yaji was directly proportional to weight gain. This elevated weight of rats given Yaji is contrary to what Akpamu *et al*. (2011b) found; in their study, 9 g of Yaji caused a significant reduction in body weight as compared with rats given cloves, ginger, red pepper, black pepper, and groundnut. Ukoha *et al*. (2014) reported that Yaji caused a dose-dependent body weight loss, contrary to observations in our study. The increase in body weight seen in our study might be linked to the dose of Yaji administered. Its constituent spices – Red Pepper, Black pepper, Cloves, and Ginger – caused an increase in body weight, as reported by Mbongue *et al*. (2005), Hassan *et al*. (2010), and Akpamu *et al*. (2011a). Black pepper as an ingredient that increases body weight and induces increased eating contradicts reports by Vijayakumar *et al*. (2004), in which it was described as a lipid-lowering spice. Garlic caused a significant decrease in body weight gain in our study on account of its potential in altering body fat deposition, adipose tissue weight, serum lipid profiles, and regulating lipid metabolism, coupled with its active compound allicin potential to mediate cascade reactions involved in the reduction of visceral fat gain in rats, thereby increasing its effectiveness at lowering weight gain and triglycerides levels (Memudu *et al*., 2015; Yang *et al*., 2018; Quesada *et al*., 2020) via an increase in fat metabolism. Ginger caused an elevation in body weight, a finding contrary to its described weight reducing potential documented by Micheal *et al*. (2008). Rats given cloves had significant decreases in body weight, as reported by Agbaje *et al*. (2009). Red pepper induced body weight gain in our study, contrary to reports made by Erdost *et al*. (2009), in which a diet containing 1% red hot pepper (10 g/kg diet) caused decreases in body weight. It was deduced from our study that Yaji potentiates increase in body weight due to constituents such as Red Pepper, Black pepper, Cloves, and Ginger. These spices have anabolic and androgenic potentials linked with an increasing eating patterns linked to increase in body weight.

The evaluation of the changes in organ weight is an indicator to ascertain whether a tested substance or drug is toxic or therapeutic (Nirogi *et al*., 2014). A significant decline or increase in the absolute or relative weight of an organ after administration of a drug/supplement indicates the toxicity of that particular drug or substance (Maina *et al*., 2008; Simmons *et al*., 1995). Testicular weight change was evaluated to vividly describe the effects of Yaji and its spice constituents on testicular tissue mass. In regard to testicular weight, Ogwo *et al*. (2016) mentioned that declines in testicular weight might be attributed to falling testosterone levels, as testosterone plays a role in the enhancement of testicular growth; therefore, severe declines in testosterone concentration may cause testicular atrophy. Furthermore, the weight of the reproductive organs always gives a good measure of the degree of spermatogenesis in rats (Raji *et al*., 2005). In the 28- and 56-days study group, the rats treated with Yaji and red pepper had increases in testicular weight as compared with the control and rats given cloves, ginger, and garlic ($p<0.05$); however, no significant difference was seen between the rats given Yaji (B) and the groups treated with Black Pepper (D) and Red Pepper (C). The observed increase in testicular weight in the groups given Yaji, Red pepper, and Black Pepper as compared with other study groups was accompanied by increases in body weight due to fat deposition and proliferative testicular tissue cells as observed in testicular histology tests. The higher testicular weight seen in the rats given these spices supports reports made by Agrawal & Bhide (1987) and Final report on the safety assessment of capsicum annuum extract, capsicum annuum fruit extract, capsicum annuum resin, capsicum annuum fruit powder, capsicum frutescens fruit, capsicum frutescens fruit extract, capsicum frutescens resin, and capsaicin (2007). Hence, there was an appreciable degree of spermatogenesis progress in the testes (Raji *et al*., 2005). Contrary to Ukoha *et al*. (2014), in our study Yaji caused increases in testicular weight. This is probably linked to a lower dose of 200 mg in our study *versus* the 5 to 15 g used by Ukoha *et al*. (2014). Black pepper and its active compound piperine may potentiate a significant reduction in testicular weight (Chinta *et al*., 2017), but this was not evident in our study. Some studies linked a reduction in testicular weight in rats to decreases in their testosterone levels (Nirogi *et al*., 2014), since testosterone plays a role in the enhancement of testicular growth; hence, a marked decline in its concentration might cause testicular atrophy (Ogwo *et al*., 2016).

Serum testosterone levels were assayed to determine the integrity of the interstitial cells of Leydig, since testosterone, a hormone responsible for male sexual libido, is secreted by Leydig cells (Wallen, 2001). In our study, serum testosterone levels were studied to evaluate the interplay between Yaji and its isolated spice components on Leydig cells proliferative and secretory activity. The group given Yaji for 28 days had a significant increase in serum testosterone secretion by Leydig cells when compared with the control group and the rats given red pepper, garlic, black pepper, cloves, and ginger; however, there was no significant difference between the rats given Yaji and Red Pepper (C), Black Pepper (D), Ginger (F), Garlic (G) and Cloves (E) treated for 56 days. This shows that there is a correlation between Yaji and the steroidogenic process involving testosterone production in the Leydig cells (Kochhar, 2008; Memudu *et al.,* 2015). Black pepper, red pepper, and Ginger induced steroidogenesis possibly via their high contents of fatty acids (Meghwal & Goswami, 2012), including auric acid, myristic acid, and palmitic acid, all required in androgen synthesis (Gromadzka-Ostrowska, 2006). It is worth noting that piperine in black pepper may reportedly cause significant decreases in the weight of the testes, an event attributed to a histological regression in the seminiferous tubule cell mass resulting from cell death or disruption of the spermatogenic cell series, subsequently leading to spermatogenesis arrest and impaired testosterone synthesis by Leydig cells (Ukoha *et al*., 2014). Piperine's alleged potential to damage the epididymal environment and sperm function occurs via increases in reactive oxygen species levels, which thus lower the activity of antioxidant enzymes in the epididymis (D'Cruz & Mathur, 2005; Mishra & Singh, 2009). Nevertheless, reports have often described a biological role for piperine as an antioxidant, anticancer, antipyretic, anti-inflammatory (Ahmad *et al*., 2012) agent, shedding light on the conflicting scientific reports on black pepper and its active compound piperine. This study showed a direct proportional increases in testicular weight and serum testosterone levels in rats given cloves, Yaji, red pepper, and black pepper, contrary to Ukoha et al. (2014) but corroborated by reports made by Nirogi *et al*. (2014), Ogwo *et al*. (2016) and Orieke *et al*. (2019), in which testicular weight changes were directly proportional to testosterone levels, a reflection of the role testosterone has in testicular growth. Semen analysis is used as input in investigations about infertility potentially caused by drugs and supplements, since it is a measure of male fertility in clinical and basic andrology (Vasan, 2011). Routine semen analysis provides basic, useful health information concerning sperm production, sperm count, sperm motility, and viability (Jequier, 2010; Vasan, 2011) that may be used in research and studies on fertility potential. Male infertility is linked to a decline in semen quality (Abarikwu, 2013). Infertility may be caused due to low sperm count, impaired sperm motility, decreased testosterone levels, and abnormal sperm morphology (Kumar & Singh, 2015). Our study used sperm count (SC) as an index to measure the fertility potential of Yaji and its constituent spices (Guzick *et al*., 2001). As mentioned earlier, Yaji is mostly consumed by male folk in Nigeria. Reports have shown that diet is a contributing factor to the male infertility index (Jahan *et al*., 2009). In this study, Yaji caused an increase in the sperm counts of rats treated for 28 and 56 days.

The mammalian epididymis plays a significant role in the functional maturation of spermatozoa due to its ability to secrete an optimal level of sialic acid, an essential element in spermatozoa functional integrity (Prasad & Rajalakshmi, 1976; Rajalakshmi, 1992). Hence, Yaji probably induced spermatogenesis and improved the epididymal environment for sperm maturation and function by lowering reactive oxygen species levels as a result of its spices mixture potential to increase the activity of antioxidant enzymes in the epididymis. The antioxidant properties present in its spices protect spermatozoa from oxidative stress during sperm development (Maneesh *et al*., 2005), thus increasing the number of spermatocytes. Our study showed that Yaji's spice components red pepper, black pepper, garlic, cloves, and ginger significantly increased the sperm counts of treated rats as compared with the control group. However, there was no statistically significant change in the sperm counts when rats given Yaji were compared with the control (A) and the rats given cloves (E), red pepper (C), or garlic (G). This study assessed sperm motility as an indicator for spermatozoa strength or viability. In the groups treated for 28 and 56 days, Yaji caused a statistically significant increase in sperm motility as compared with controls and rats given cloves (E) and ginger (F). No significant difference was found when rats given Yaji (B) were compared with their counterparts given red pepper (C), black pepper (D), cloves (E), or garlic (G). This suggests that all the active compounds in Yaji work in synergy to improve semen quality. However, contrary to reports by Hammami *et al*. (2008; 2009), in which garlic was described to mediate decreases in serum testosterone levels and to cause degenerative changes in testicular histology, our findings indicated that garlic induces serum testosterone level increases. Cloves reportedly increase sperm count, motility, and viability (Mishra & Singh, 2009). Garlic (Memudu *et al*., 2015), red pepper (Babaei Garmkhany & Yousofvand, 2015) and black pepper (Sutyarso *et al.,* 2015; Zodape & Gaikwad, 2019) have also been reported to improve sperm count and motility due to their androgenic and antioxidant properties. Hence, their interplay caused an elevation in sperm count and motility in rats given Yaji, contrary to reports made by Ukoha *et al*. (2014) that Yaji might impair testicular function. The observed increases in testosterone levels imply functional, secreting Leydig cells and pro-fertility effects (Okereke & Onuoha, 2015) of Yaji, red pepper, black pepper, ginger, and cloves.

The testis is a male reproductive organ involved in spermatogenesis in the seminiferous tubules and steroidogenesis (testosterone synthesis) in the interstitial cells of Leydig (Payne & O'Shaughnessy, 1996; Ogbuewu *et al*., 2011; Sembulingam & Prema, 2016). In the testes, spermatogenic cells are formed in the seminiferous tubules, and atrophy in this tubule results in disturbed spermatogenesis (Ahmed-Farid *et al*., 2017), decreased spermatids leading to declines in fertility and decreased reproductive performance (Cheah & Yang, 2011; Ahmed-Farid *et al*., 2017). Hematoxylin and Eosin (H&E) stain has been used to demonstrate testicular cells inclusive of spermatogenic cell series and Leydig cells (Chinta *et al*., 2017). Histopathology tests performed in the testicular tissue of the rats treated for 28 days using H&E stain revealed the presence of all spermatogenic cell series, interstitial cells of Leydig, and less occluded seminiferous tubule lumen with no disruption of the spermatogenic lineage in the control. However, the groups given Yaji (B), red pepper (C), black pepper (D), cloves (E), and ginger (F) for 28 days (Figures 7A and 7B) showed mild histological disruption of the spermatogenic cell series as compared with controls and rats given garlic, although interstitial cells of Leydig were not disrupted. The micrographs of the specimens taken from the rats treated for 56 days (Figures 8A & 8B) revealed spermatids in the adluminal compartment of the seminiferous tubules in comparison with the rats treated for 28 days, in addition to proliferative interstitial cells of Leydig, and well-aligned spermatogenic cell series in contact with the basement membrane. The rats given Yaji, red pepper, black pepper, and ginger showed similar histological features, including brush-like waves of spermatids in the seminiferous tubule adluminal compartment, presence of Type A and B spermatogonia in the basal membrane of the seminiferous tubules, primary spermatocytes, and distinguishable spermatids.

This study showed that Yaji improved spermatogenesis, supports seminiferous tubule cells and Leydig cell proliferation, by means of synergistic reactions between spice constituents. This phenomenon is best explained based on Yaji's ability to stimulate the production of testosterone from Leydig cells in the testis as reported in this study to support male reproductive function. Testosterone promotes the development of reproductive organs (Sembulingam & Prema, 2016). The observed increase in testosterone levels may have resulted from either the direct effect of Yaji and its spices on the proliferation and secretory activity of Leydig cells or the indirect effects arising from the increasing activity of luteinizing hormone level (Okereke & Onuoha, 2015). Medicinal plants, their flavonoids and antioxidant components have been used in the treatment of reproductive system disorders (Hosseini, 2018). Plants with high antioxidant potential protect the testicular milieu from reactive oxygen species (ROS). Ginger's antioxidant and pro-spermatogenic activity have been documented by (Ugbogu *et al*., 2018). Antioxidant properties (Viuda-Martos *et al*., 2010) have been described for eugenol – an active constituent of cloves (Jaganathan & Supriyanto, 2012; Kabuto *et al.,* 2007; Scherer *et al.,* 2009). Red pepper extracts showed strong antioxidant activity (Sim & Sil, 2008; Ademoyegun *et al.,* 2011). Red pepper reportedly enhanced the proliferation of Leydig cells that secrete testosterone and are involved in spermatogenesis (Erdost *et al*., 2009). Histology findings of improved testicular function have been linked to red pepper's ability to increase FSH activity and the proliferation of spermatogenic cells and Leydig cells linked to testosterone (Erdost *et al.*, 2006), in addition to presenting with antioxidant properties (Materska & Perucka, 2005; Liu & Nair, 2010). Yaji is a spice with super antioxidant activity, since each of its constituent spices has high antioxidant and pro-spermatogenic properties. Testicular histology of rats given Yaji revealed the presence of all spermatogenic cells and absence of atrophy in the seminiferous tubules that might disturb spermatogenesis (Ahmed-Farid *et al*., 2017).

Rats in the group given Yaji had appreciable wave-like or brush-like border appearance of the spermatids in the adluminal compartment of the ST, similar to those displayed in the testes of rats treated with red pepper and black pepper, leading to an increased number of sperm cells and thus demonstrating that Yaji, red pepper, black pepper, and ginger may improve fertility and enhance reproductive performance (Ahmed-Farid *et al*., 2017). Spermatogenesis is an energy-dependent process that requires a significant intake of antioxidants, minerals, vitamins, and flavonoids (Ogungbemi, 2006). In this present investigation, the increased level of testosterone may have been caused by the zinc and antioxidant properties present in Yaji and its constituent spices, which may have also been responsible for the improvement in the histological architecture of the testes of the rats (Sutyarso *et al*., 2015; Zodape & Gaikwad, 2019). Zinc supplementation can improve the antioxidant status and increase serum levels of sex hormones, including testosterone in rats (Kumar & Singh, 2015).

CONCLUSION

Yaji is imbued with pro-fertility potential that manifests itself via improvements in male fertility function associated with Leydig cell proliferation, increased synthesis of testosterone, enhanced spermatogenesis, and consequently improved sperm counts and motility. This study provided scientific support to the relevance and potential therapeutic applications of Yaji in male fertility enhancement.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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