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# Aqueous extract of Tetrapleura tetraptera fruit peels influence copulatory behavior and maintain testicular integrity in sexually mature male Sprague-Dawley rats: Pro-fertility evaluation and histomorphometry evidence

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ABSTRACT

Tetrapleura tetraptera (TT) has been used as a spice, dietary supplement and medicine for various ailments. This study evaluate influence of Tetrapleura tetraptera extract on testis and copulatory behavior in sexually mature male rats.

Thirty-two male and sixty-four virgin female rats weighing 150–200 g were used for this study. Male rats randomly divided into four groups of eight (n = 8) rats each. Group A: Control given 2 ml distilled water, group B, C and D received 50, 300 and 700 mg/kg bwt TT for 56 days through oral gavage. The female rats were used for fertility test. Testicular histology, histomorphology, copulatory behavior, sperm parameters, testosterone (TET), luteinizing hormone (LH), follicle stimulating hormone (FSH), glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) and fertility test were investigated.

Tetrapleura tetraptera significantly increase sperm count, motility, normal morphology, daily sperm production, efficiency of sperm production, sperm (average path velocity, straight line velocity and curvilinear velocity), TET, LH, FHS, SOD, GPx, CAT, number of pregnant females, number of fetuses, seminiferous diameter, epithelium thickness and decrease abnormal morphology, seminiferous height, tubule lumen and MDA across the group as compared with control group. Improved testicular histological integrity, sexual behaviour and libido by increased frequencies of mount, intromission, ejaculation and ejaculatory latency. Latencies of mount, intromission and post-ejaculation were significantly reduced. Also, observed increase spermatocytes and spermatids showed no significant difference in spermatogonia cell counts. Tetrapleura tetraptera therefore, enhance steroidogenesis, spermatogenesis, and improved testicular histological integrity and boost sexual competence in male rats.

## 1. Introduction

Tetrapleura tetraptera is a deciduous plant native to West Africa that belongs to the family Fabaceae Mimosoideae (Jimmy and Ekpo, 2016). The plant commonly found more in rainy forest where it can attain a height of about 20–25 m and a girth of about 1.2–3 m (Aladesanmi et al., 2007). The stem bark quite smooth and thin, leaves are sessile and glabrous, fruits are persistent and hangs at branches ends (Odesanmi et al., 2010). Unripe fruit is green and ripe is dark-purple-brown of about 15–25 cm in length and 4–5 cm breadth (Akin-Idowu et al., 2011). Tetrapleura tetraptera is known as Prekese in Twi language, Aridan in Yoruba, Uhiokrihio in Igbo and Dawo in Hausa (Uyoh et al., 2013). The dry fruit is shiny, glabrous, dark purple brown with pleasant aroma, rich in flavonoid, alkaloid and hydrogen cyanide and it pleasant aroma makes it useful as a spice and pepper soup for combating cold weather (Abii and Amarachi, 2007) and also, taken as soup by nursing mothers to prevent postpartum contraction (Adesina et al., 2016). The fruit is used as popular seasoning in Nigeria and has been shown to cause significant reduction in hematological indices in male rabbits and elevate hematological indices in female rats (Onuka et al., 2017). It serves as local remedies for a variety of diseases such as diabetes mellitus, inflammation, rheumatism, jaundice, fever, malaria, asthma and epilepsy

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(Udourioh, and Etokudoh, 2014). Due to its antimicrobial properties it is used in making traditional black soaps (Adebayo et al., 2000). It contain higher quantity of proteins, lipids and minerals compared to popular spices such as onion and ginger (Adesina et al., 2016). The fruit is rich in essential oils which is noticed in its aromatic smell and fatty acids (both saturated and unsaturated), carbohydrates, ash, minerals such as (Na, Mg, Fe, P), phytochemicals and crude fibre (Tsala et al., 2014; Adesina et al., 2016). Tetrapleura tetraptera fruit posse various pharmacological benefits such as antimalarial, anticonvulsant, antibacterial, molluscicidal, antidepressant, analgesic and anti-inflammatory activity (Agomuo et al., 2011). In vitro research extract of several part of TT demonstrated to be free radical scavengers, antioxidant and protective effects of an enzyme involved in oxidative stress (Irondi et al., 2013, Atawodi et al., 2014). The fruits and bark extracts from TT contain a higher concentration of polyphenol mainly eugenol, quercetin, tyrosol, and catechin (Moukette et al., 2015). The testes is involved in two processes; spermatogenesis and steroidogenesis (Sutyarso et al., 2016). Spermatogenesis describes the processes involved in production of gametes (spermatozoa) and Steroidogenesis is all enzymatic reactions involved in production of male steroid hormones (O'Shaughnessy et al., 2010). These two processes take place in two different compartment of the testis, different in morphology and function: the tubular compartment and interstitial compartment respectively. These compartments although anatomically distinguished are connected closely (Abel et al., 2008). The testis is vulnerable to Various factors such as medication, chemotherapy, toxins, polluted air, lack of nutrients and vitamins can adversely affect spermatogenesis and sperm production, leading to testicular toxicity and cause infertility (Mosher and Pratt, 1991). Tetrapleura tetraptera aqueous fruit extract has not been shown to have an adverse effect on copulatory behavior and testis and it benefits to testis has not been proven either. This study therefore, investigate influence of aqueous fruit peels extract of Tetrapleura tetraptera on copulatory behavior and maintain testicular integrity in sexually mature male Sprague-Dawley rats.

## 2. Materials and methods

### 2.1. Chemicals, reagents and assay kits

Estradiol benzoate and progesterone were procured from Sigma Chemical (St. Louis, USA) and Shalina Laboratories (Mumbai, India), respectively. Assay kits for testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were obtained from Monobind Inc. (Lake Forest, USA). All other reagents used were of analytical grades.

### 2.2. Plant material

Tetrapleura tetraptera were purchased from a local market in Iwo, Osun State, Nigeria. Samples of TT were identified and authenticated by Mr. Omomoh Bernard of Herbarium section of the Centre for Research and Development, Federal University of Technology, Akure, Ondo state, Nigeria. Sample of the plant voucher deposited for reference purpose.

### 2.3. Preparation of tetrapleura tetraptera

Tetrapleura tetraptera fruit were thoroughly washed in sterile water and air dried under shade for four weeks in the laboratory. The air-dried roots were weighed using CAMRY (EK5055, Indian) electronic weighing balance and milled with automatic electrical Blender (model FS-323, China) to powdered form, 400 g, the milled plant sample was later soaked in 2.8 L of phosphate-buffered saline (PBS) for 72 h, at room temperature, and filtered through cheese cloth and then through Whatman #1 filter paper, the filtrate was concentrated using a rotary evaporator (Rotavapor® R-220) at 42–47 °C. The filtrate was refrigerated at $25^{O}$C until used.

### 2.4. Phytochemical screening

Qualitative and quantitative phytochemical analysis of aqueous leaf extract of Tetrapleura tetraptera was done in accordance with (Harborne, 1993; Boham and Kocipai, 1994). While modifications on the report by Grindberg and Williams (2010) on high performance liquid chromatography was adopted to quantify the vitamins.

### 2.5. Animals

Sexually mature male and virgin female Sprague-Dawley rats were procured from Research Farm, Federal University of Technology, Akure, Ondo state, Nigeria. The animals were kept in cages and acclimatized for two weeks before the commencement of the experiment. The rats were maintained under standard natural photoperiodic condition of 12 h of darkness and 12 h of lightness (D:L; 12:12 h dark/light cycle) at room temperature (25–32 °C) and humidity of 50–55%. The rats were fed with standard rat chow 100 g/kg daily as advised by International Centre of Diarrheal Disease Research; Bangladesh (ICDDR, B). Drinking water was supplied ad libitum.

### 2.6. Experiment design and animal grouping

Thirty-two (32) healthy male adult (12–14 weeks old) Sprague-Dawley rats weighing 150–200 g and sixty four (64) virgin female rats were used for this study. The rats were randomly divided into four groups (A, B, C and D) of five (n = 8) rats each. Group A, which served as control, were given 2 ml of distilled water, group B, C and D rats were administered 50, 300 and 700 mg/kg bwt TT, each respectively for 56 days. The extract was administered once daily for six days within a week through oral gavage and the experiment lasted for 56 days (Duration of spermatogenesis in rat being 51.6–56 days (Sasso-Cerri et al., 2009). The female rats were used for fertility test.

### 2.7. Determination of gross anatomy parameters

The weight of male rats was documented at procurement, during the period of acclimatization, at commencement of administrations and once a week throughout the period of experiment. Testis, epididymis, preputial gland, prostate gland, seminal vesicle, levator ani muscle and sphincter ani were collected and weighed. Also, foetal, placental, ovarian and empty uterine weights were measured using an electronic analytical and precision balance CAMRY (EK5055, Indian). Organ weights were recorded in g and expressed as g/100 g body weight. Testicular length and diameter were measured using vainer caliper. Testicular volumes was estimated by water displacement method, volumes of the two testes from each rat was determine and average value obtained regarded as one observation, the values expressed as g/100 g body weight. (Acott, 1999).

### 2.8. Copulatory behavior test

Eight male rats from each group were subjected to the sexual behavior test. The procedure was carry out during the dark phase of the light/dark cycle and 24 h after the last administration. A single male rat was put into a rectangular Plexiglas observation chamber (45 × 40 × 30 cm) and acclimated for 6 min. A sexually receptive female rat was then introduced into the chamber. Mount frequency (MF), intromission frequency (IF), ejaculation frequency (EF), mount latency (ML), intromission latency (IL), ejaculation latency (EL) and post-ejaculation latency (PEI) were monitored for 30 min post pairing period and measured. The experiment was perform in a sound-attenuated room. (Mohammed et al., 2015).

### 2.9. Induction of oestrus phase in female rats

The procedures of Tajuddin et al.,(2004) and OECD (2009) were adopted in this study. Female rats were brought to oestrus by consecutive

**Table 1**
Qualitative and quantitative phytochemical analysis of aqueous extract of Tetrapleura tetraptera.

| S/N | Phytochemicals | Status | Phytochemicals | Quantity |
|---|---|---|---|---|
| 1 | Tannin | + | Vitamin C (mg/g) | 3.14 |
| 2 | Flavonoid | + | Vitamin A (mg/g) | 5.25 |
| 3 | Saponin | + | Vitamin E (mg/g) | 6.65 |
| 4 | Alkaloid | + | Total Phenols (%) | 18.23 |
| 5 | Reducing sugar | + | Total Alkaloids (%) | 1.36 |
| 6 | Cardaic glycoside | + | Total Tannins (%) | 19.35 |
| 7 | Terpenoid | + | Total Flavonoids (%) | 16.71 |
| 8 | Phenol | + | Total Saponins (%) | 2.61 |
| 9 | Phlobatanin | + | Quercetin (%) | 15.74 |
| 10 | Steroid | + | Total cardiac glycolysis | 21.02 |
| 11 | Coumerins | - | - | - |
| 12 | Quinones | - | - | - |
| 13 | Resin | - | - | - |

Key: + = present; − = not present.

subcutaneous administration of estradiol benzoate (10 μg/100 g) and progesterone (0.5 mg/100 g) at 48 h and 6 h respectively prior to pairing. Vaginal smears examination was done to confirmation of oestrus phase.

### 2.10. Fertility test

The oestrous cycle of each rat was characterized for four weeks, vaginal smears between 8:00–9:00 a.m. before the commencement of the experiment. Cyclicity was determined by the modification of the method previously reported by Bazzano et al. (2017). Briefly, normal saline was inserted into the vaginal canal 1 mm deep and irrigated with plastic Pasteur pipette. The lavage was then smeared on a microscopic slide and viewed under microscope. The presence of large nucleated cells with a few leucocytes on the slide was marked the pre-estrous day of the cycle. On the Pre-estrous day of each rat's cycle, a marked male was introduced into a marked female cage at a 1:2 ratio. These mating animals were left together overnight. Vaginal lavage was taken on the morning (estrous day of the cycle) following pairing between 8:00–9:00 am The presence of sperm spermatozoa in the lavage was marked as day 1 of pregnancy (Abraham et al., 2018).

### 2.11. Animal sacrificed and sample collection

At the end of the treatment, blood samples were collected through orbital venous sinus of live animals with microhematocrit tubes within the hours of 7:00 a.m. and 8:00 a.m. and immediately centrifuged at 3000×*g* for 10 min for serum separation to estimate reproductive hormone. Thereafter, animals were euthanized and testes were immediately removed, cleaned from the adhering tissue, and weighed. The epididymal content for each rat was instantly collected for semen analysis. Furthermore, the left testis was fixed in Bouin's fluid for histological procedure. Whereas, the right testis was decapsulated, homogenized in 0.05 M potassium phosphate buffer (pH 7.4), and processed for the estimation of oxidative satures and daily sperm production. All samples were stored at −80 °C until analysis.

### 2.12. Sperm analysis

Cauda epididymis was minced and incubated in 4 ml M199 at 37 °C for 5 min, to allow sperm cells to swim out of the epididymal tubules. Total of 10 μl sperm suspension was added to the sperm counting plate. Sperm parameters were collected by computer-assisted sperm analysis and evaluated using toxicology software program version 12.0.2.

### 2.13. Daily sperm production

Testicular homogenate 20 μl was placed into Neubauer chambers and number of late spermatids was counted under microscope at × 400 magnification. Value obtained was used calculate total number of spermatids per testis. The number was used to determine efficacy of sperm production that is, the number of spermatids per each gram (g) of testicular tissue. Daily sperm production (DSP) evaluated by the quantity of spermatids resistant to homogenization (per testis and per g of testis) was divided by 6.3 (DSP = X/6.3) (Jahan et al., 2018).

### 2.14. Hormonal determination

Serum testosterone, follicle stimulating hormone and luteinizing hormone concentration was determine by enzyme immunoassay using commercial kit (Catalog numbers: EIA-6K2E9, EIA-4K1B0 and EIA-37K3B0 respectively – Monobind Inc. Lake Forest USA). According to manufacturer instruction.

### 2.15. Testicular histological evaluation

Testes were removed from treated and control rats and fixed in Bouin's. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin. Sections of 5 μm thickness were cut using rotary microtome and mounted on clean slides. For histological evaluation, sections were stained with haematoxylin and eosin in accordance with standard procedure and were examined under light microscope (Olympus-BX63 Japan). Microphotography was done by Leica LB microscope (Germany) equipped with an automatic micro photographic system (Japan). Spermatocytes and spermatids counts were done at 10 seminiferous tubules of each experimental unit and then averaged. Images were analyzed by using image J software.

### 2.16. Statistical analysis

The results reported as mean ± SEM.Instat-3 computer program (v2.04, GraphPad Software Inc., San Diego, CA, USA) used to analyze the numbers and to evaluate the significant differences; the comparison of means between each two experimental groups was made using one-way ANOVA with Newman-Keuls Multiple Comparison significant difference test. The differences considered significant if $p < 0.05$.

**Table 2**
Effects of aqueous extract of Tetrapleura tetraptera on mating behavioural parameters in male rats.

| Parameters | Treatment groups | | | |
|---|---|---|---|---|
| | A (Control) | B(50 mg/kg of TT) | C(300 mg/kg of TT) | D(700 mg/kg of TT) |
| Mount frequency | 15.34 ± 1.96 | 18.57 ± 1.79 | 24.52 ± 2.79* | 25.83 ± 3.15* |
| Intromission frequency | 10.96 ± 1.57 | 10.74 ± 1.56 | 18.72 ± 1.34* | 21.29 ± 1.51* |
| Ejaculatory frequency | 3.56 ± 0.51 | 4.10 ± 0.68 | 4.74 ± 0.39 | 5.18 ± 0.42 |
| Mount latency (s) | 63.42 ± 10.00 | 93.85 ± 14.72* | 115.10 ± 14.73* | 124.10 ± 15.25* |
| Intromission latency (s) | 113.80 ± 12.89 | 158.20 ± 13.07* | 172.50 ± 10.60* | 180.10 ± 9.87* |
| Ejaculatory latency (s) | 130.00 ± 16.04 | 135.80 ± 16.65 | 139.70 ± 16.31 | 139.20 ± 16.36 |
| Post-ejaculatory latency (s) | 152.40 ± 14.33 | 158.90 ± 14.30 | 165.00 ± 14.88 | 166.10 ± 15.10 |

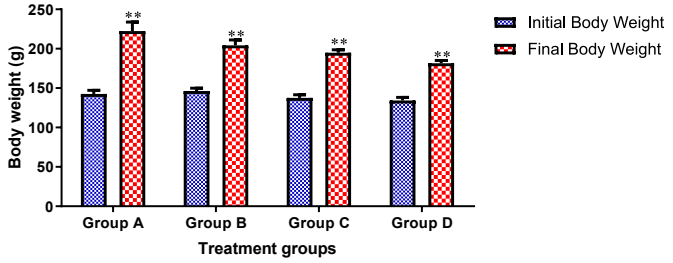


**Fig. 1.** Histogram shows the effects of Tetrapleura tetraptera extract on body weight of Adult Sprague-Dawley rats. Values are expressed as Mean ± S.E.M, n = 8 in each group, *: represent significant different from control, **: represent significant different from initial body weight at $p < 0.05$. A: 2 ml distil water, B: 50 mg/kg bwt, C: 300 mg/kg bwt, D: 700 mg/kg bwt.

**Table 3**
Effects of Tetrapleura tetraptera extract on body and organs weight of Adult Sprague-Dawley rats.

| Parameters | Treatment groups | | | |
|---|---|---|---|---|
| | A (Control) | B(50 mg/kg of TT) | C(300 mg/kg of TT) | D(700 mg/kg of TT) |
| Initial body weight (g) | 142.6±4.58 | 146.4±3.44 | 137.6±3.98 | 134.2±3.94 |
| Final body weight (g) | 222.4±11.44 | 204.2±6.88 | 194.8±3.94 * | 181.6±3.56 * |
| Weight Difference (g) | 79.8±6.86 | 57.8±3.44* | 57.6±0.04* | 47.4±0.38* |
| Testicular weight (g) | 1.22±0.06 | 1.14±0.02 | 1.18±0.04 | 1.10±0.03 |
| Testis/body weight (%) | 0.55±0.02 | 0.56±0.02 | 0.61±0.01 | 0.61±0.01 |
| Testicular volume ($cm^3$) | 1.93±0.24 | 2.00±0.22 | 2.10±0.20 | 2.20±0.20 |
| Testicular length (mm) | 21.36±1.80 | 22.15±1.63 | 22.66±1.61 | 23.53±1.70 |
| Testicular diameter (mm) | 10.84±0.62 | 10.45±0.35 | 11.65±0.39 | 11.94±0.67 |
| Epididymis (g) | 0.14±0.04 | 0.19±0.02* | 0.20±0.01* | 1.00±0.01* |
| Preputial gland (g) | 0.08±0.01 | 0.09±0.01 | 0.010±0.02 | 0.010±0.02 |
| Prostate gland (g) | 0.81±0.30 | 1.01±0.24 | 1.10±0.18 | 1.25±0.12 |
| Seminal vesicle (g) | 1.01±0.30 | 1.19±0.32* | 1.28±0.33* | 1.35±0.34* |
| Levator ani muscle (g) | 0.21±0.06 | 0.23±0.06 | 0.33±0.08 | 0.39±0.08 |
| Sphincter ani (g) | 0.14±0.05 | 0.14±0.04 | 0.17±0.05 | 0.19±0.05 |

Values are expressed as Mean ± S.E.M, n = 8 in each group, *: represent significant different from control at $p < 0.05$, One-Way ANOVA. TT: Tetrapleura tetraptera.

## 3. Results

### 3.1. Phytochemical screening

Qualitative analysis of Tetrapleura tetraptera shows the presence of tannin, flavonoid, saponin, alkaloid, reducing sugar, cardaic glycoside, terpenoid, phenol, phlobatanin and steroid. After the quantitative analysis as depicted in Table 2, total cardiac glycoside (21.02%), total tannins (19.35%), total phenol (18.23%), total flavonoids (16.71%) and Quercetin (15.74%) had higher values compared to alkaloids (1.36%) and total Saponins (2.61%). There were also high values of vitamins A, C and E [Table 1].

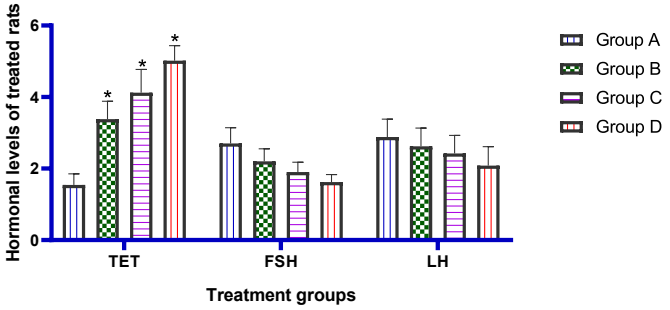


**Fig. 2.** Effects of Tetrapleura tetraptera extract on TET, FSH and LH level of Adult Sprague-Dawley rats. Values are expressed as Mean ± S.E.M, n = 8 in each group, *: represent significant different from control at $p < 0.05$, One-Way ANOVA. TET: Testosterone, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, TT: Tetrapleura tetraptera. A: 2 ml distil water, B: 50 mg/kg bwt, C: 300 mg/kg bwt, D: 700 mg/kg bwt.

### 3.2. Copulatory parameter

Long term exposure to TT significantly increased ($p < 0.05$) mount frequency, intromission frequency (at dose of 300 and 700 mg/kg body weight) and mount latency, intromission latency at dose of 50, 300 and 700 mg/kg body weight when compared with the control group. Increase in mean value of ejaculatory frequency, ejaculatory latency and post-ejaculatory latency across the groups are not significant as compared with the mean value of the control group. Also, administration of 50 mg/kg body of TT shows no significant difference in mean values of mount frequency and intromission frequency in comparison with the control [Table 2].

### 3.3. Changes in body and organs weight

There was significant ($p < 0.05$) decrease in mean value of final body weight at dose of 300 and 700 mg/kg body weight TT when compared to control group, no significant difference between the final body weight at dose of 50 mg/kg body weight and the control group. There was significant ($p < 0.001$) increase in mean value of final body weight across the group and in dose dependent manner when compared with the mean value of initial body weight. [Fig. 1]. There observes increase in testicular weight, volume, length and diameter across the experiment group when compared with the control group but not significant ($p > 0.05$). In addition, mean value of prostate gland, preputial gland, levator ani muscle and sphincter ani weights show no significant difference in treatment and control groups. However, TT significantly increased ($p < 0.05$) the weight of epididymis and seminal vesicle in all the exposed groups as compared with control group. [Table 3].

### 3.4. Serum testosterone, follicle stimulating hormone and luteinizing hormone level

As illustrated in Fig. 2, the mean value of serum testosterone was significantly ($p < 0.05$) increase across the group in dose dependent manner as compared with the control group, however there was observed decrease in mean value of follicle stimulating hormone and luteinizing hormone level across the group when compared to the control group.

### 3.5. Sperm parameters

Tetrapleura tetraptera extract treatment revealed significant ($p < 0.05$) increase in mean value of sperm count, motility, normal morphology, daily sperm production and efficiency of sperm production across the group in dose dependent manner, increased in mean value of normal sperm morphology, average path velocity of sperm, straight line velocity of sperm and curvilinear velocity of sperm at dose of 50 mg/kg

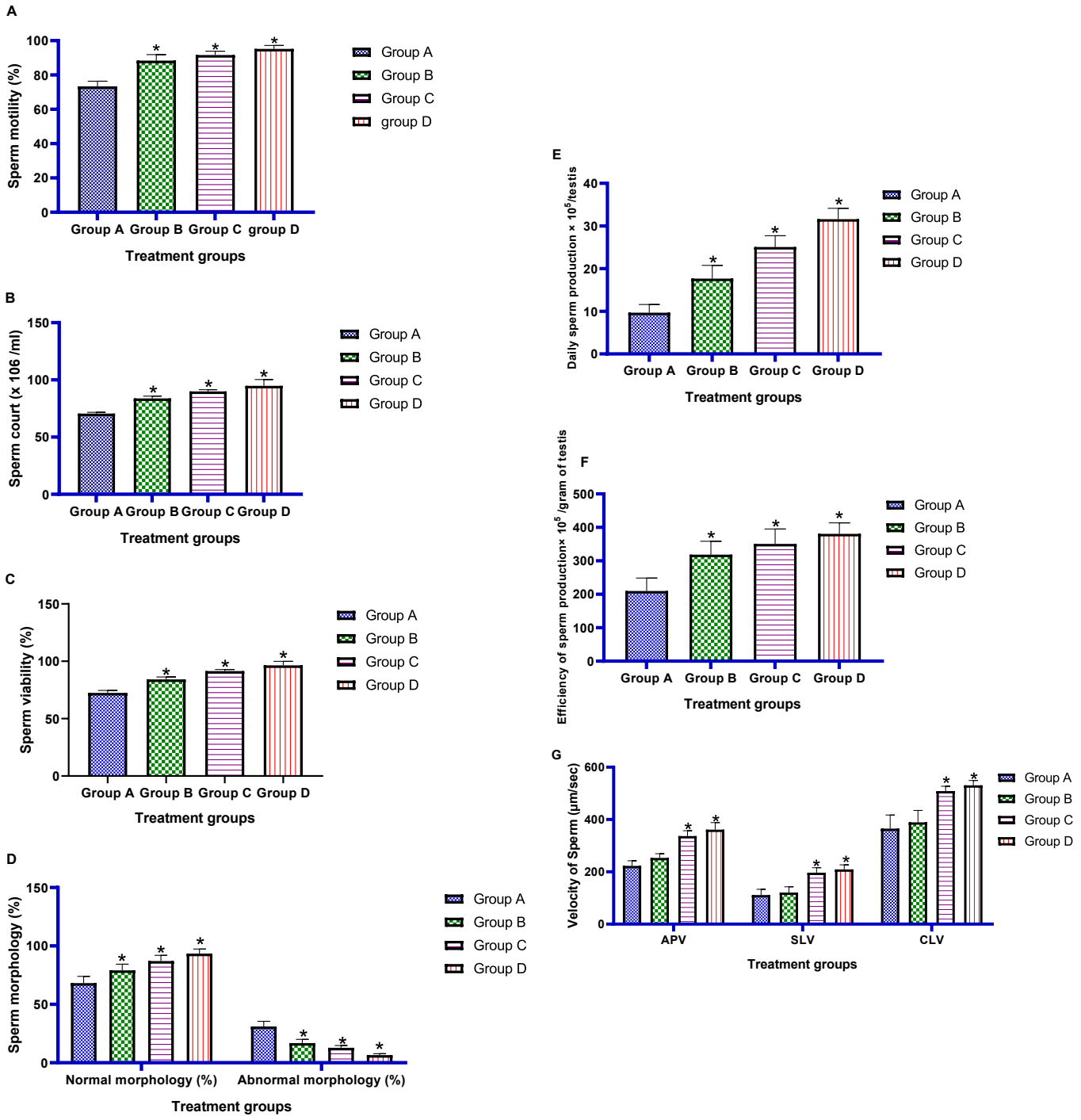


**Fig. 3.** Effects of Tetrapleura tetraptera extract on A: Sperm motility, B: Sperm count, C: Sperm Viability, D: Sperm morphology, E: Daily sperm production, F: Efficiency of sperm production, G: Velocity of sperm of Adult Sprague-Dawley rats. Values are expressed as Mean ± S.E.M, n = 8 in each group, *: represent significant different from control at $p < 0.05$, One-Way ANOVA. TT: Tetrapleura tetraptera, APV: Average path velocity of sperm; SLV: Straight line velocity of sperm; CLV: Curvilinear velocity of sperm; DSP: Daily sperm production; ESP: Efficiency of sperm production.

body weight was not significant but significant at dose of 300 and 700 mg/kg body weight in comparison with the control group. There was statistically significant ($p < 0.05$) decrease in mean value of abnormal sperm morphology in group C and D when compared with the control group, however decrease in abnormal sperm morphology in group B was not significant as compared with the control group [Fig. 3].

### 3.6. Fertility of male and maternal reproductive organs

Long term administration of aqueous extract of TT revealed increased in number of pregnant females and number of foetuses. Number of fetuses and weight of empty uterus significantly increased ($p < 0.05$) in 700 mg/kg bwt TT treated group compared with the control and not

**Table 4**
Effects of aqueous extract of Tetrapleura tetraptera on the fertility of male and maternal reproductive organs.

| Parameters | Treatment groups | | | |
|---|---|---|---|---|
| | A (Control) | B(50 mg/kg of TT) | C(300 mg/kg of TT) | D(700 mg/kg of TT) |
| No. of males | 8 | 8 | 8 | 8 |
| No. of females | 16 | 16 | 16 | 16 |
| No. of pregnant females | 12/ 16(75.00%) | 14/ 16(87.5%) | 15/ 16(93.75%) | 16/ 16(100.00%) |
| No. of foetues | 6.50 ± 0.66 | 6.75 ± 0.45 | 7.42 ± 0.42 | 8.25 ± 0.35* |
| Weight of foetus (g) | 2.41 ± 0.43 | 2.67 ± 0.38 | 3.00 ± 0.30 | 3.22 ± 0.17 |
| Weight of placental (g) | 0.47 ± 0.09 | 0.53 ± 0.08 | 0.61 ± 0.06 | 0.68 ± 0.02 |
| Weight of empty uterus (g) | 0.59 ± 0.09 | 0.60 ± 0.09 | 0.71 ± 0.07 | 0.81 ± 0.07* |
| Weight of ovaries (g) | 0.22 ± 0.04 | 0.31 ± 0.04 | 0.38 ± 0.04 | 0.52 ± 0.02 |
| Crown rump length (cm) | 4.04 ± 0.04 | 4.09 ± 0.10 | 4.11 ± 0.08 | 4.17 ± 0.07 |

Values are expressed as Mean ± S.E.M, n = 8 in each group, *: represent significant different from control at $p < 0.05$, One-Way ANOVA. TT: Tetrapleura tetraptera.

significant at dose 50 and 300 mg/kg bwt of TT. Also, weight of ovaries in 300 and 700 mg/kg bwt TT treated group significantly increase when compared with the control group. No significant difference in mean number of weight of foetus, weight of placental and crown rump length in all the experimental treatment and control groups [Table 4].

### 3.7. Testicular histomorphometry

Administration of aqueous extract of TT at dose of 300 and 700 mg/kg bwt significantly increase the mean seminiferous diameter and epithelium thickness ($p < 0.05$), increased in 50 mg/kg bwt treated group is not significant in comparison to control group. However, there was insignificant decrease in mean seminiferous height and tubule lumen in all the treated as compared with the control group. Tetrapleura tetraptera (300 and 700 mg/kg bwt) treatment significantly increase the mean number of spermatogonia, preleptotene spermatocyte and pachytene spermatocyte. Although, increased in 50 mg/kg bwt treated rats were statistically significant compared to the control rats. In addition, spermatids cell are significantly increased in all the TT treated rats compared to that of control rats ($p < 0.05$). [Table 5].

### 3.8. Testicular biochemical parameters

There was observe significant decreased ($p < 0.05$) in mean value of testicular MDA concentration across the group treated with 50 mg/kg bwt, 300 mg/kg bwt and 700 mg/kg bwt TT respectively in comparison with the mean value of control group treated with normal saline. [Fig. 4A]. There was increase in testicular super oxide dismutase concentration in dose dependent manner when compared with the control and there is significant difference between the control and the group treated with 700 mg/kg bwt of TT however, no significant difference between the control and the groups that received 50 mg/kg bwt and 300 mg/kg bwt TT respectively. [Fig. 4B]. There was significant increased ($p < 0.05$) in testicular $GP_X$ concentration in 300 mg/kg bwt and 700 mg/kg bwt TT treated group when compared to the control group however, increased in testicular $GP_X$ of the group treated with 50 mg/kg bwt TT not significant in comparison with the control group. [Fig. 4C]. There was no significant difference in testicular catalase activity between control group and group B and group C treated with 50 mg/kg bwt and 300 mg/kg bwt of TT respectively however, there was observed significant increased ($p < 0.05$) in testicular catalase activity of group D treated with 700 mg/kg bwt when compared with the control group.[Fig. 4D].

### 3.9. Testicular histology

The microphotograph of testis of animals groups A, B, C and D had a normal cellular composition in their germinal epithelium with sperm cells in the lumen and a normal interstitium. In addition, normal spermatogenesis, better association and higher density of spermatogenic cells, complete maturation of germinal epithelium and lumen contains full mature spermatozoa were evident in both the Control and treated groups, also revealed normal spermatogenic cells, Sertoli and Leydig cells and precise spermatogenesis with abundant spermatids in TT group and control [Fig. 5].

## 4. Discussion

The medicinal property of different bioactive component of plant has been demonstrated to have good health benefits and it is effective in prevention and treatment of chronic ailment. Phytochemicals such as flavonoids, phenols, cardiac glycosides, and terpenoids which were detected in aqueous extract of Tetrapleura tetraptera (TT) have been reported to possess various pharmacological effects such as antioxidants, antidiabetic, antihypertensive and anti-Alzhemic activities (Ochuko et al., 2017).

In this study, TT observed to influence sexual desire and improve sexual performance in male rats. This is occasion by significant surge in serum testosterone (TET) concentration and anterior pituitary hormones level in treated rats compared to control rats thereby, stimulated sexual competence via stimulatory impact on dopamine receptors (Cicero et al., 2002). Observed advances TT-treated male rats toward the females' rat indicate sexual aroused following improved precopulatory behaviours. Mount and intromission frequencies are main indices of libido, vigour, and potency. While MF suggests sexual motivation enhanced, IF is reflective of coherent erection, penile orientation and comfort by which ejaculatory reflexes are stimulated (Agmo, 1997). Therefore, the dose-dependent increases in MF and IF following long term consumption of TT revealed improved libido (Tajuddin et al., 2004). The presence of

**Table 5**
Effects of aqueous extract of Tetrapleura tetraptera on testicular histomorphometry.

| Parameters | Treatment groups | | | |
|---|---|---|---|---|
| | A (Control) | B(50 mg/kg of TT) | C(300 mg/kg of TT) | D(700 mg/kg of TT) |
| Seminiferous diameter (μm) | 234.70 ± 17.28 | 245.80 ± 17.04 | 290.30 ± 12.65* | 296.90 ± 12.64* |
| Seminiferous height (μm) | 101.90 ± 11.70 | 107.90 ± 12.05 | 115.00 ± 11.35 | 124.70 ± 11.63 |
| Epithelium thickness (μm) | 54.17 ± 5.63 | 57.63 ± 4.24 | 75.54 ± 5.08* | 76.69 ± 6.21* |
| Tubule lumen (%) | 3.23 ± 0.46 | 3.21 ± 0.35 | 3.09 ± 0.35 | 3.12 ± 0.29 |
| Spermatogonia | 7.49 ± 0.78 | 8.67 ± 0.72 | 11.01 ± 0.87* | 12.93 ± 1.04* |
| Preleptotene spermatocyte | 15.61 ± 2.59 | 18.53 ± 2.28 | 25.39 ± 2.34* | 28.07 ± 1.39* |
| Pachytene spermatocyte | 20.86 ± 3.02 | 23.56 ± 3.12 | 34.58 ± 2.67* | 35.90 ± 2.44* |
| Spermatid | 80.73 ± 8.46 | 120.5 ± 11.86* | 139.3 ± 11.55* | 155.4 ± 10.04* |

Values are expressed as Mean ± S.E.M, n = 8 in each group, *: represent significant different from control at $p < 0.05$, One-Way ANOVA. TT: Tetrapleura tetraptera.

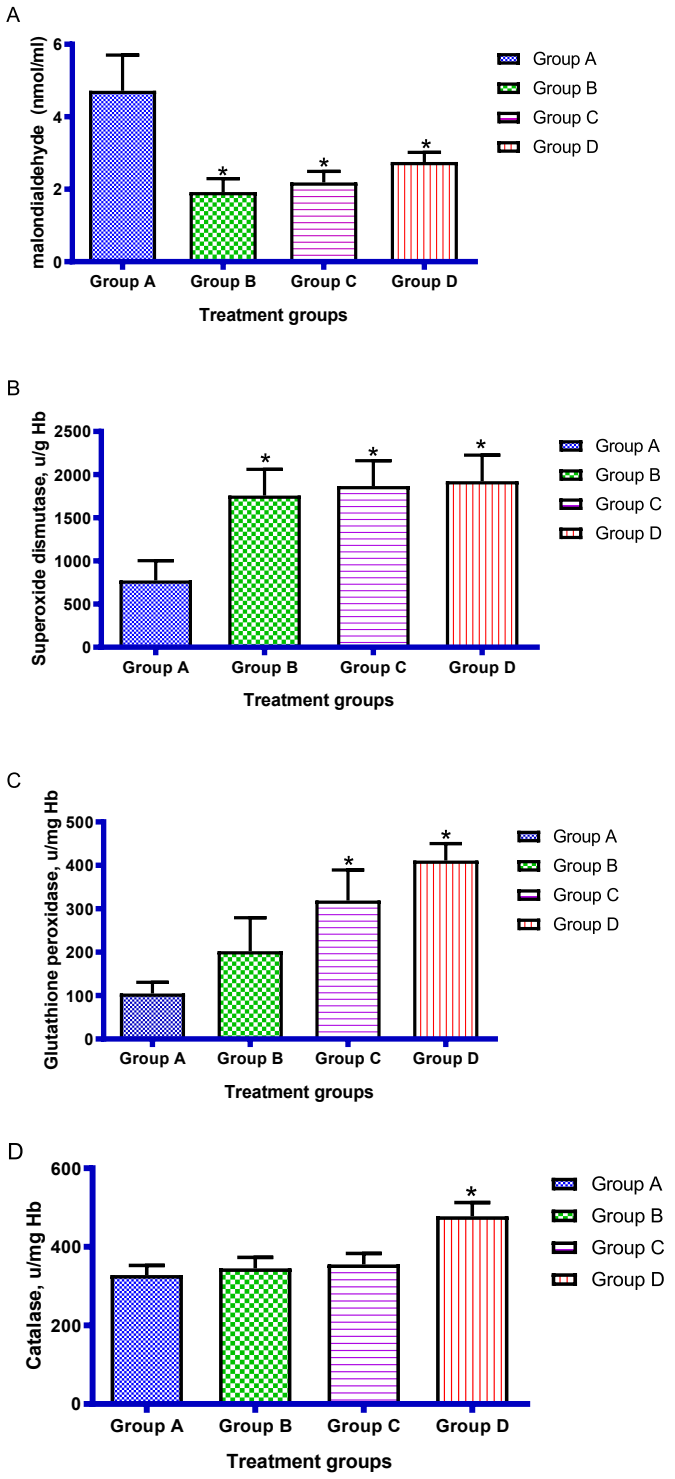


**Fig. 4.** Histogram shows the effects of Tetrapleura tetraptera extract on testicular (A) malondialdehyde concentration (B) Super oxide dismutase concentration (C) Glutathione peroxidase activity (D) Catalase activity. Values are expressed as Mean ± S.E.M, n = 8 in each group, *: represent significant different from control, **: represent significant different from initial body weight at $p < 0.05$. A: 2 ml distil water, B: 50 mg/kg bwt, C: 300 mg/kg bwt, D: 700 mg/kg bwt.

sperm plug in the vagina of the female rats shows that there is an ejaculation, increase in EF by prolong TT administration is an indication of improved aphrodisiac potential of TT. Therefore, Increased MF and IF directly linked to sexual motivation in rats (Cicero et al., 2002). The significant increase ML and IL relative to control after TT treatment could be related to invigorated sexual motivation and enhanced competence. Also, increase in EL following prolong treatment with TT shows that the extract composed bioactive component responsible for prolong coitus and instituted copulatory performance in the rats. Post-ejaculatory latency (PEL) is indices for evaluation of potency, libido and rate of recovery from loss of energy after initial sets of mating (Yakubu et al., 2011). Therefore, increase PEL in TT treated rats shows that the extract enhanced potency and libido.

In present study, there was decrease in mean value of final body weight when compared to control group. Increase in mean value of final body weight across the group and in dose dependent manner when compared with the mean value of initial body weight. There was observes increase in testicular weight, volume, length and diameter across the experiment group when compared with the control group but not significant. In addition, mean value of prostate gland, preputial gland, levator ani muscle and sphincter ani weights show no significant difference in treatment and control groups. However, TT significantly increased the weight of epididymis and seminal vesicle in all the exposed groups as compared with control group. Testis weight depends on the mass of differentiated spermatogenic cells, and its structural and functional integrity requires the adequate biosynthesis of male sex hormones. Therefore, increase in testicular weight in TT treated rats reveals increase spermatogenesis and steroidogenesis function. Also, Increase in testicular and epididymal weights could linked to higher sperm production (Ekaluo et al., 2013, 2015), which could be due to increased androgen biosynthesis as evidenced by a significant increase in TET level (Morakinyo et al., 2008).

Administration of aqueous extract of TT significantly boasted the sperm quality compared with control as evident in increase sperm count, motility, viability, normal morphology, average path velocity of sperm, straight line velocity of sperm and curvilinear velocity and decrease in abnormal sperm morphology these disagree with the findings of Oloyede (2018) that ethanolic extract of TT Pods reduced sperm quality thereby, distorted sperm morphology with some aberrant sperm cells such as sperms without hook, amorphous head, two tails, pin head and clustered tails. Also, significant elevation in daily sperm production (DSP) was noted in all the treated group as compared to control. These results indicate that treatment with TT can shield reduction in DSP within the testis by protecting the testis against adverse effects of any environmental toxin that may present in the body. These effect of TT on sperm may be linked to it active bioactive component. Several studies have reported a protective effect of dietary antioxidants and vitamins A, B, C, and E on sperm DNA against free radicals and improvement of the blood– testis barrier stability. As the administration of TT leads to elevation of TET secretion which could enhance its fertility potent (Khaki et al., 2010; Jedlinska-Krakowska et al., 2006). The spermatogenic activity of TT may be as a result of the carbohydrate content which could have increased sperm motility and viability by increasing glucose metabolism leading to the generation of energy and pyruvate which is the preferred substrate essential for activity and survival of sperm cells. Other components of TT such as arginine, vitamin C and zinc may also, play important roles since studies have shown that nutritional therapies with zinc, vitamin C, vitamin E and arginine proved to be of benefit in treatment of male infertility. The beneficial effect of TT on male fertility may be as a result of it antioxidant components since antioxidants such as vitamins A, C and E have been reported to have good protective effects on the testis. The development of normal and matured sperm is the key to optimum male fertility. The production of sperm cell and TET in testis are mainly regulated by FSH and LH which are released from anterior pituitary (Steinberger, 1971). FSH stimulates spermatogenesis in the steroli cell, while LH stimulates production of TET in the leydig cells of testis (Kerr and De Kretser, 1975). The result of present study suggested that TT treatment increase serum TET concentration across the group in dose dependent manner as compared with control group, however, no significant different in FSH and LH level between the treatment and control group may improve the normal function of the steroli and leydig cells. Sexual cells can occur during the reproductive

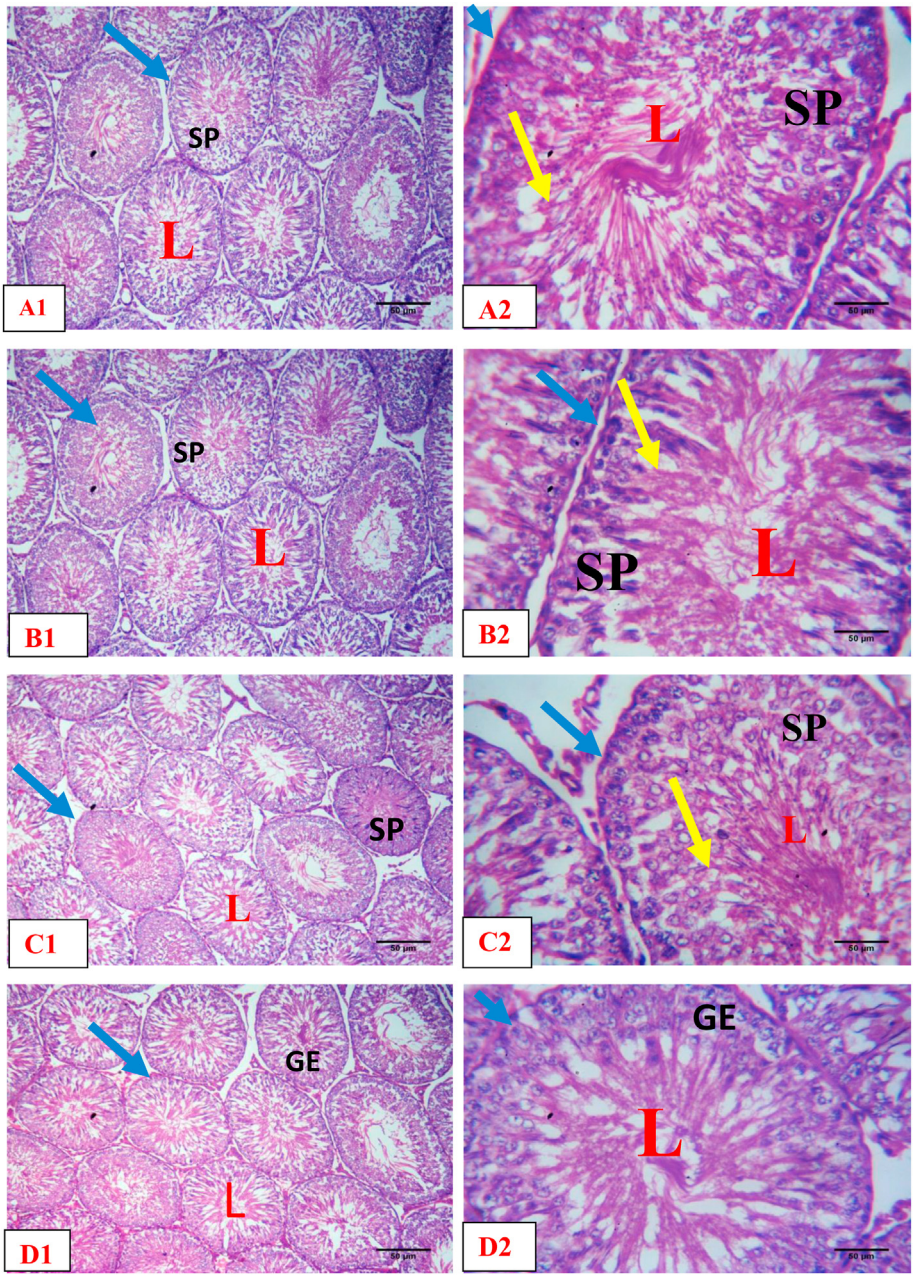


**Fig. 5.** Group A (Control) showing normal histoarchitecture with typically organized layers of spermatogenic cells at different stages of maturation (Yellow arrow), no pathological changes in the lumen (L) of the seminiferous tubules, spermatozoa (SP), basement membrane (blue arrow) and interstitial space (I); Group B revealed a notable restoration of the lumen (L) of the seminiferous tubules with visible spermatozoa (SP) and abundant sperm cell (yellow arrow); Group C showed restored lumen (L) with visible spermatozoa (SP), abundant sperm cell (yellow arrow) and visible and intact basement membrane (blue arrow); Group D showed well preserved cytoarchitecture with normal cellular composition in germinal epithelium (GE), population of immotile sperm cells in the lumen (L) and normal basement membrane (blue arrow).

phase, mitotic division of spermatogonia or during maturation of spermatozoa, thereby, affecting number of quantity of sperm cell production in the testis. In addition, number of pregnant female and viable foetuses were also increased, this reflect and may be due to increase in sperm motility and sperm density. Tetrapleura tetraptera treatment shows no significant impact on crown-rump length, foetal and ovarian weight. This is adduced to nutritional bioactive component of the TT on placental barrier to effectively regulate exogenous maternal change (Blundell et al., 2016; Abraham et al., 2018). We can therefore, deduce that bioactive constituent of TT is effective and safe in management of pregnancy from first trimester to parturition period which is evidence in weight of placental and empty uterus.

Furthermore, the higher membrane lipid content of testes is presumed to make them more vulnerable to oxidative stress (Mahera et al., 2013). In this study TT bioactive component lower the membrane lipid content of the testes occasion by decrease testicular MDA and increase testicular SOD, GSH and CAT in dose dependent manner. Thereby, decreases lipid peroxidation since accumulation of lipid in nonadipose tissues could lead to cell dysfunction and cell apoptosis (Schaffer, 2003). We can therefore, postulate from this study that TT fruit peel can effectively scavenges ROS and inhibit the generation of oxidative injury in testis that may be induce by any toxin. Tetrapleura tetraptera with free radicals scavenging potential can alleviate lipid peroxidation. Our report agree with Ochuko et al., 2017 that the observed dose dependent inhibition of lipid peroxidation by TT extract therefore, insinuates a protective effect of the peels against oxidative damage.

Moreover, spermatogenic cells could be observed and distinguished and spermatogonia in the basal membrane would involve the primary spermatocytes in the mitotic stage. In all the treatment groups, secondary spermatocytes were not observed due to the rapid meiotic stage of the spermatozoa in the seminiferous tubules (Eroschenko, 2010). In addition, the regeneration of Sertoli and Leydig cells was also observed. Sertoli cells play a primary role in spermatogenesis and TET (Kianifard et al., 2011). Spermatogenic, Sertoli, and Leydig cells were correlated to each other in terms of spermatogenesis and TET synthesis. Testicular

dysfunction might interfere with spermatogenesis and TET synthesis due to cell damage. In this study, long term consumption of TT relatively protect and maintain the rats' testicular integrity, protective and maintenance may be directly on spermatogenesis by reducing lipid peroxidation and prevent or decrease the formation of free radicals, by acting as antioxidant. Or indirectly on steriodogenesis through pituitary hypothalamic or sex hormonal effects. Protective nature of TT could be attributed to it bioactive component and antioxidant properties. Our findings therefore, agree that TT fruit peel is a reservoir of medicinal phytochemicals with wide range of potential application (Kuate et al., 2015) and the presences of constituents such as flavonoids, saponins, tannins and alkaloids in the fruit of TT could have contributed to the observed anti-inflammatory and antioxidant roles of the extract in this study. We therefore, deduce that TT fruit peel is capable of protecting testicular pituitary axis against any toxin that may present in the body since exposure to toxic agents could produce pituitary hypothalamic toxicity which in turn could affect spermatogenesis resulting in functional or structural impairment of sperm and testis.

## 5. Conclusion

Aqueous extract of Tetrapleura tetraptera thus, improve steroidogenesis, spermatogenesis and maintain testicular integrity in rats. Also, the extract could improve testicular toxicity with anti-oxidative activity and through free radicals scavenging ability. Antioxidant activity and profertility potential of aqueous extract of this plant could serve as a potent adjuvant in the treatment of male infertility.

## Ethical approval

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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## CRediT authorship contribution statement

**Sunday Aderemi Adelakun:** Conceptualization, Methodology, Validation, Investigation. **Babatunde Ogunlade:** Methodology, Project administration, Supervision, Investigation. **Toluwase Solomon Olawuyi:** Formal analysis, Investigation. **Abdulfatai Olakunle Ojewale:** Investigation.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Values are expressed as Mean ± S.E.M, n = 8 in each group, *: represent significant different from control at $p < 0.05$, One-Way ANOVA. TT: Tetrapleura tetraptera.

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