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Acetaminophen-Induced Infertility in Male Wistar Rats: Impact of Avocado Leaves on Hormonal Profile and Semen Analysis

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ABSTRACT

Background and Objective: Male infertility is intended as a condition for men who are unable to impregnate their partners for at least one year after intercourse without using a protector. This research work statistically investigates the effects of paracetamol usage on hormonal profile and sperm count by modelling the impact of paracetamol and standard drug, same dosage of paracetamol combinations and the interactions on hormonal profile and sperm count and to determine the effect of consuming avocado on the parameters. **Materials and Methods:** Forty-two male Wistar rats weighing 100-105 g were used to determine the effect of acetaminophen on hormones and sperm count. The avocado leaves were air-dried and blended into powder, mixed with water (100 mL), stirred and mixed intermittently for 72 hrs. The water extract was filtered into a conical flask and stored until when used. The animals were humanely sacrificed after which a caudoventral mid-abdominal incision was made to access the internal organs. The testes were detached from the epididymis and harvested. The concentration was determined by the use of the improved Neubauer hemocytometer. **Results:** The LH and FSH hormones in the groups apart from the control increased significantly due to acetaminophen administration but the administration of standard dose, helped in reviving the damage to a significant level. However, long-term administration of acetaminophen doesn't reduce body weight, avocado extract reduces it to a significant level. However, an excessive increase in FSH concentration as observed in the 3000 mg/kg of acetaminophen treated rats may negatively affect reproduction due to its effect on spermatogenesis. As observed, testosterone levels in the lower doses increased but decreased in the high-dose group. **Conclusion:** The result showed that avocado had a negatively significant influence on semen, sperm motility and viability were reduced and the consumption of high amount of avocado leaf extract has a negative effect on the reproductive system in the male.

KEYWORDS

Male infertility, avocado, hormonal profile, semen, acetaminophen, infertility

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INTRODUCTION

Male infertility is defined as the inability of a man to have a child after sexual activity for a minimum of one year without the use of a protector[1]. Although both the male and female partners can contribute to infertility, an estimated 50% of cases are caused entirely or largely by male factors[2-6]. An individual becomes infertile when sperm are unable to fertilize the ovum due to concentration abnormalities and sperm motility[1].

Acetaminophen, a commonly used analgesic and antipyretic medication, has been reported to have adverse effects on male reproductive health. Prolonged or high-dose exposure to acetaminophen has been associated with altered hormonal balance and reduced semen quality, leading to infertility[3,7]. There is a need to explore potential therapeutic interventions that can mitigate the negative effects of acetaminophen on male fertility. Avocado leaves have been traditionally used in herbal medicine and have shown promising bioactive properties that may offer protection against reproductive dysfunction[8]. However, the effects of avocado leaves on the hormonal profile and semen analysis of acetaminophen-induced infertility in male Wistar rats remain largely unexplored[3,9,10]. It has been suggested that acetaminophen may induce oxidative stress in the testes and other tissues.

Male infertility is a significant health issue affecting couples worldwide, with various factors contributing to its prevalence[11]. In recent years, a decline in semen quality across Africa, Europe, North America and Asia has been reported[3,12]. This seems to suggest that male infertility is a growing global problem. Providing a balanced nutritional intake, such as that found in medicinal plants, is one way to try to address infertility[8,13]. Fruits are one kind of food that supports healthy reproduction.

The avocado fruit (*Persea americana*) includes protein, vitamins and minerals that can enhance the quality of sperm, making it a food with the potential to improve reproductive health. The pure avocado fruit is packed with healthy ingredients like dietary fiber, potassium, folate, thiamine, riboflavin and extremely high levels of vitamin A, B, C, E and K[14]. The quantity and quality of sperm can be decreased by the body's lack of vitamin A, B, C and E[15]. Men who are infertile can also become fertile again by taking 100 mg/kg of vitamin E daily. This vitamin affects testicular weight, sperm count, motility and estrogen production in addition to boosting sperm survival and development[16]. In order to produce spermatozoa with appropriate morphology during spermatogenesis in the testes, proteins serve to protect the sperm plasma membrane[17]. Fatty acids, particularly unsaturated fatty acid groups, are abundant in avocados[18]. According to Prager *et al*.[19] and Orabueze *et al*.[20], rats fed an avocado oil-based diet for 42 days saw a 4-fold increase in the blood concentration of estradiol and a decrease in testosterone levels, which may boost male fertility.

In Nigeria, male infertility is a significant yet underappreciated reproductive health concern. In certain regions of the nation, male factors account for 20-50% of the causes of infertility. Few researches examined the national reasons for infertility[21]. Acetaminophen, a widely used medication, has been associated with adverse effects on male reproductive health[22]. The study evaluated the level of testosterone, Follicle Stimulating Hormone (FSH), Luteinizing (LH) and conduct a seminal fluid assay of a male Wistar rat with acetaminophen-induced infertility and treated with avocado leaves. To evaluate the level of testosterone, FSH and LH, conducted a seminal fluid assay of a male Wistar rat with acetaminophen-induced infertility and not treated with avocado leaves. Ultimately, this study has the potential to improve reproductive health outcomes and offer new avenues for the management of male infertility.

MATERIALS AND METHODS

Collection and authentication of plant material: Ten avocado fruits were procured from botanical garden, at the University of Ibadan, Nigeria. The plant specimen was authenticated by the Department of Zoology. This study was carried out between August, 2023 to November, 2023.

Preparation of plant extract: The leaves were air-dried and 30 g of dried aerial parts of avocado leaves were blended into powder, it was mixed with water (100 mL) and poured inside a bottle, it was stirred and mixed intermittently for 72 hrs. The water extract was filtered using a filter paper into a conical flask and the residue was frozen in a freezer at -84°C. Finally, the filtrate was transferred to a flask and stored until when use (-20°C)[23].

Animals acquisition: Thirty adult male Wistar rats weighing 100-130 g were obtained from the animal house, Lead City, University. The rats were housed in plastic mesh cages and maintained in a well-ventilated room at 25±2°C, on a 12 hrs light/12 hrs dark cycle. Rats had unrestricted access to standard rat chow and tap water. The Wistar rats were acclimatized for 2 weeks[24].

Ethical consideration: The investigation was conducted in accordance with the National Institutes of Health Guide for the care and use of Laboratory Animals and ethical approval was obtained from the Institutional Review Ethical Committee of Lead City University and the University of Ibadan and every effort was made to minimize both the number of animals used and their suffering.

Experimental grouping and treatment: The rats were divided into 7 groups (6 in each group), the rats were acclimatized for 14 days, then the administration was performed for 14 days. Group 1 was used as a negative control, while group 2 was used as a positive control. Groups of rats were given different treatment regimens.

Group 1: Rats were given food and water only; it is a negative control
Group 2: Containing acetaminophen (3000 mg/kg) as a positive control
Group 3: Acetaminophen (3000 mg/kg) and standard drugs (silymarin 140 mg)
Group 4: Acetaminophen (3000 mg/kg) and 300 mg/kg *Persea americana* extract
Group 5: Contains acetaminophen (3000 mg) and 200 mg/kg aqueous extract of *Persea americana*
Group 6: Acetaminophen (3000 mg/kg) and 100 mg/kg aqueous extract of *Persea americana*
Group 7: Acetaminophen (3000 mg/kg), standard drugs (silymarin 140 mg) and 100 mg/kg aqueous extracted from the *Persea americana*

Sample size: The sample size was determined using Fisher's formula for cross-sectional study:

$$n = \frac{Z^2\ pq}{d^2}$$

Where:
n = Desired sample size (for population greater than 10,000)
Z = Standard normal deviation, which is 1.96 (at 95% confidence interval)
p = Prevalence of the problem

Data indicates the global prevalence of male infertility as 2.5 to 12% with 2.5-4.8% infertile men in Sub-Saharan Africa[25]:

p = 2.8%
q = 1-p
q = 1-0.028
d = 0.05 (the precision)

$$n = (1.96)^2 \times 0.028 \times 0.972/(0.05)^2 = 41.82$$

Sample preparation: At the end of treatment (2 months), the rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.). Blood samples were collected from the apex of the heart of the 42 rats into the heparinized bottle and centrifuged at 3000 rpm for 15 min using a bench centrifuge (ERBA) and the plasma will be stored frozen until it is needed for biochemical assay.

Laboratory observation note: The 42 rats were bought from Mr. Gbenga animal house, in which 7 groups were bought and cleaned. Sawdust was placed on the floor of the cage, rat fed was bought and the rats were made comfortable. They were acclimatized for two weeks, to make them adapt to the new environment, however, they were weighed and divided based on their body weight into six groups each in which they were six rats in each cage and were given just food and water. By the 3rd week, the experiment began.

The cages were cleaned and their sawdust was changed every morning. The first group is the control that were given just food and water only for the whole experiment. The 2nd group took 0.5 mL of acetaminophen then with food and water, the 3rd group took 0.5 mL of acetaminophen and 0.5 mL of standard (sylimarin), the 4th group took 0.5 mL of acetaminophen and 0.4 mL of avocado extract, the 5th group took 0.5 mL of acetaminophen and 0.8 mL of avocado extract, the 6th group took 0.5 mL of acetaminophen and the 7th group took 1.5 mL of avocado extract and 0.5 mL of acetaminophen and 0.4 mL of avocado extract and 0.5 mL of standard. They were fed with the aid of gavage.

On observation, group 6 and 7 were observed to be acting reserved, they hardly played nor jumped, then group 4 and 5 were moderately active, they also played with each other and looked like they were fighting.

Group 1, 2 and 3 were super active and also played with each other, but the most active were group 1 and 2. One rat in group 4 died. Whenever they perceive and take the avocado leave extract, they tend to poop more often, both groups 6 and 7 do this the most. However, they prefer to sleep in their food tray and sleep on each other.

Laboratory analysis

Analysis of hormonal profile: Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone (TT) were measured using enzyme-linked immunosorbent assay kits[26] (Elabscience Houston, Texas, 77079, USA).

Principle: The principle of ELISA is based on the specificity of the antigen-antibody interaction. In this technique, a known amount of antigen is immobilized on a solid surface, such as a microtiter plate (10.4" capacitive touch screen, high sensitivity) by Diatek Wuxi City, Jiangsu Province, China. The serum sample containing the target antibody is added to the well and allowed to incubate. If the antigen is present in the serum, it will bind to the immobilized antigen, forming an antigen-antibody complex. After washing, a secondary antibody conjugated with an enzyme is added to the well. This secondary antibody recognizes the primary antibody and binds to it, forming a sandwich-like structure. The excess unbound conjugate is washed off and a substrate solution is added to the well. The enzyme in the conjugate catalyzes a color change in the substrate, which can be measured using a spectrophotometer (ERBA).

Procedures: The FSH, LH and TT-coated microtiter plates were purchased from a commercial source (St Kenny Ventures) and the required reagents were prepared according to the manufacturer's instructions. The wells of the microtiter plate were coated with the FSH, LH and TT antigen by incubating the plate with a known concentration of FSH, LH and TT solution for 1-2 hrs at room temperature or overnight at 4°C. A known volume of patient serum was added to the well and incubated for 1-2 hrs at

room temperature or overnight at 4°C. An enzyme-labeled anti-human IgG antibody was added to the well and incubated for 1 hr at room temperature. A substrate solution (Tetramethylbenzidine (TMB)) was added to the well and incubated for 10-30 min at room temperature. The enzyme in the conjugate catalyzes a color change in the substrate, which was measured using a spectrophotometer. The absorbance values were measured at 450 nm using a spectrophotometer and the results were compared to a standard curve generated using known concentrations of FSH, LH and TT[27,28].

Semen analysis

Semen collection: The animals were humanely sacrifices after which a caudoventral mid-abdominal incision was made using a sterilized scissor in order to access the internal organs. The testes were located once pushed upward from the scrotum. Then, the testes were detached from the epididymis and harvested using the method described by Abdul Ganiyu *et al.*[29].

Sperm motility: A drop of semen was placed on a warm microscopic slide mixed with a drop of sodium citrate and covered with a cover slip. The sample was observed under a microscope (Widaco) at X10 magnification and the percentages were recorded; only sperm cells moving in a unidirectional motion were included in the count, while cells moving in circles, backward direction, or pendulous movements were excluded[29].

Sperm morphology: Determination of sperm morphology comprises the following:

Preparing a smear of semen on a slide:

- Both surfaces of the frosted slides were cleaned by rubbing vigorously with lint-free tissue paper
- Frosted portion of the slide was labelled with the identifying information (e.g., identification number, date) using a pencil with medium-hard lead
- A 5-10 µL aliquot of semen was applied, depending on sperm concentration, to the end of the slide, Use a second slide to pull the drop of semen along the surface of the slide
- Slides were allowed to air dry and then stained[5]

Staining of slide: This was done by using papanicolaou staining technique:

- Fixed the air-dried semen smear by immersing slides in 95% (v/v) ethanol for at least 15 min[5]
- Sequentially immerse the slides in:

  - Ethanol 80% (v/v) for 30 sec
  - Ethanol 50% (v/v) for 30 sec
  - Distilled water for 30 sec
  - Harris's haematoxylin for 4 min
  - Distilled water for 30 sec
  - Acidic ethanol for 4-8 dips*
  - Running cold tap water for 5 min
  - Ethanol 50% (v/v) for 30 sec
  - Ethanol 80% (v/v) for 30 sec
  - Ethanol 95% (v/v) for at least 15 min
  - G-6 orange stain for 1 min
  - Ethanol 95% (v/v) for 30 sec
  - EA-50 green stain for 1 min
  - 2 changes of ethanol 95% (v/v) for 30 sec
  - 2 changes of ethanol 100% for 15 sec

- Mounted the stained semen smears:

    - Two or three small drops of mounting medium were added to the slide
    - A coverslip (24×50 mm or 24×60 mm are most convenient) was placed directly on the smear
    - Coverslip was positioned so that contact with the mounting medium begins from one long side, to prevent air bubbles from being trapped
    - Excess xylene (if used) was wiped off from underneath the slide
    - Mounted smear was allowed to dry horizontally in a slide drying rack or on absorbent paper for 24 hrs in a fume cupboard[5]

- Viewing:

    - Examined the slide with bright field optics at X1000 magnification with oil
    - Immerse and assessed approximately 200 spermatozoa per replicate for the percentage of normal forms or of normal and abnormal forms[5]

Sperm count: The concentration was determined by the use of the improved Neubauer hemocytometer by Kyrios-Soter Scientific (China). Semen was pipetted to the 0.5 mark using the blood cell pipette and this was made up to the 1.0 mark with normal saline. The normal saline serves both to dilute the semen and fix the spermatozoa present. The pipette was introduced into a pipette shaker and allowed to mix. About 2 or 3 drops of diluted sperm were discarded from the pipette before being introduced under the cover slip on the counting chamber from each side of the hemocytometer. The hemocytometer was carefully placed in a closed pre-wetted chamber for 5 min before being viewed under a light microscope by Omax (Germany) at X40 objective. Sperm heads that have more than half the sperm head within the large five squares that formed the diagonal segment of squares of the hemocytometer chambers were counted. The sperm concentration was determined and calculated as:

$$\text{Concentration (mL)} = \text{(Dilution factor) (Count in 5 squares) } (0.05\times10^{6})^{29}$$

Method of data analysis: The primary data was derived from animal studies and presented using simple frequency and percentage tables with explicit narration underneath each table, representing the result derived from the experiment. The statistical software package that was considered for this study is SPSS. The Statistical Package for the Social Sciences (SPSS-IBM, 2020) was used. The mean and standard error of mean (SEM) were calculated for all values. Comparisons between the control and the treated groups were done using the student's t-test. The differences were considered statistically significant at $p<0.05$.

RESULTS

The result of the experimental study was presented in tables and charts. The animals were in 7 groups, group 1 was fed with feed and water, group 2 as the positive control was induced with acetaminophen (300 mg/b.wt.), group 3 was fed with acetaminophen (300 mg/b.wt.) and also given silymarin, group 4 was induced with acetaminophen and half dose of extract, group 5 was given acetaminophen and half of the avocado extract in a standard dose, group 6 was given acetaminophen and double dose avocado's extract while group 7 was given acetaminophen, standard dose and avocado's extract.

Figure 1 showed the result of the animal body weight. There was no significant difference in the body weight of all the animals across all groups ($p = >0.05$; $F = 6, 30$). All the animals were first fed the same way for the 2 week acclimation period. Figure 2 showed the result of the animal's final body weight. There was significant difference in the body weight of all the animals across all groups in comparison to the initial weight ($p = 0.001$; $F = 8.42$) there was an increase in all the groups compared to the initial weight before the experiment. Comparing the weight among the group, there is no significant difference among the group of rats weighed after the experiment $p>0.05$ ($p = 0.493$, $F = 0.920$).

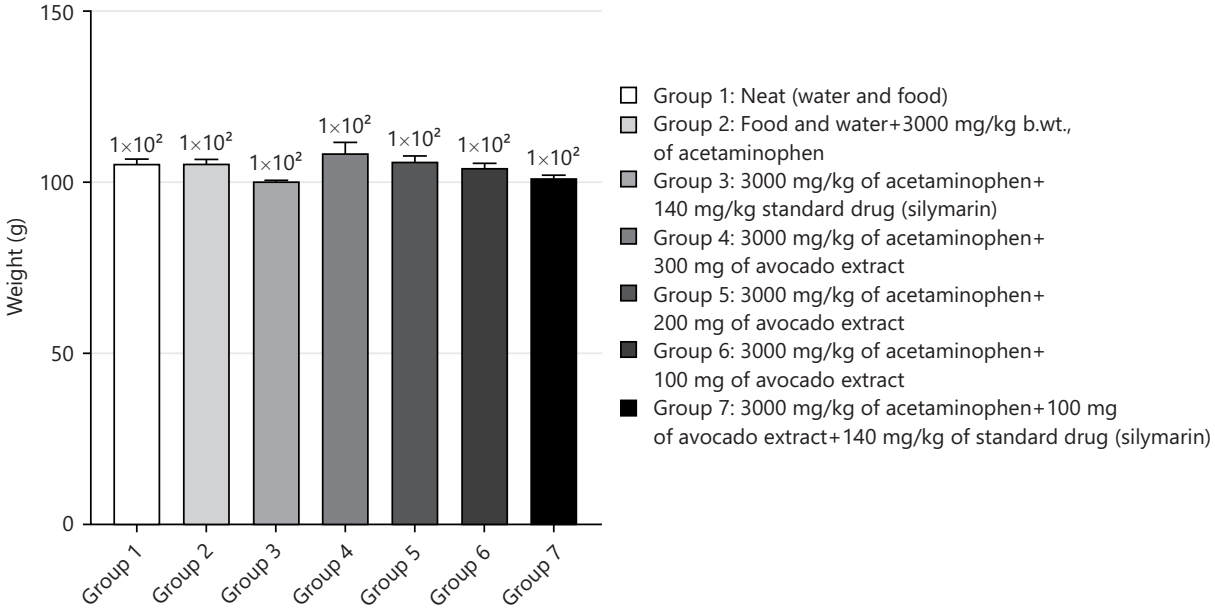

Fig. 1: Initial weight of the experimental rat before treatment
SEM = 1.33

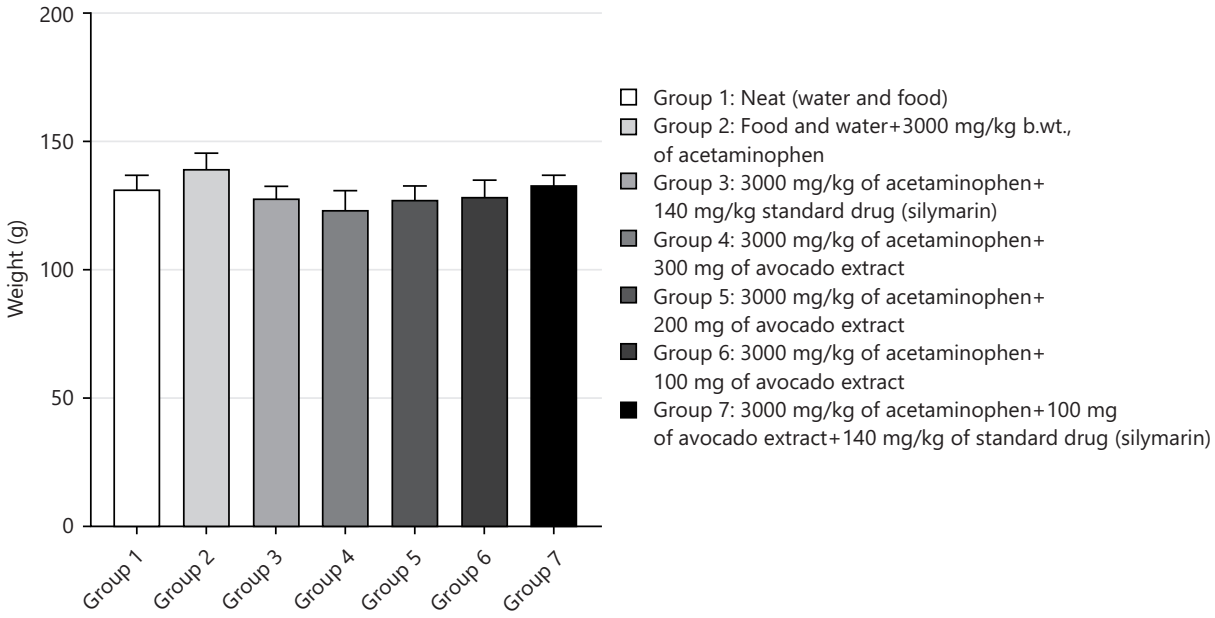

Fig. 2: Bar chart showing the final animal body weight across all groups
Values are mean SEM of data obtained and $p>0.05$

However, comparing the initial weight with the final weight, there is a significant difference ($F = 8.42$, $p<0.001$). It is found that the weight of the rat increased after the experiment (Fig. 3).

Table 1 showed the descriptive details of hormones (testosterone, prolactin, Follicle Stimulating Hormone, (FSH) and Luteinizing Hormones (LH)). The descriptive details were given by Mean±Standard Deviation. Analysis of variance was performed on the data, there are significant differences across the groups of the experimental rats ($p<0.001$). Group 2 has the highest mean value in all the hormonal parameters (i.e., group 2 parameters were elevated above all other groups). Table 2 showed the comparison of the

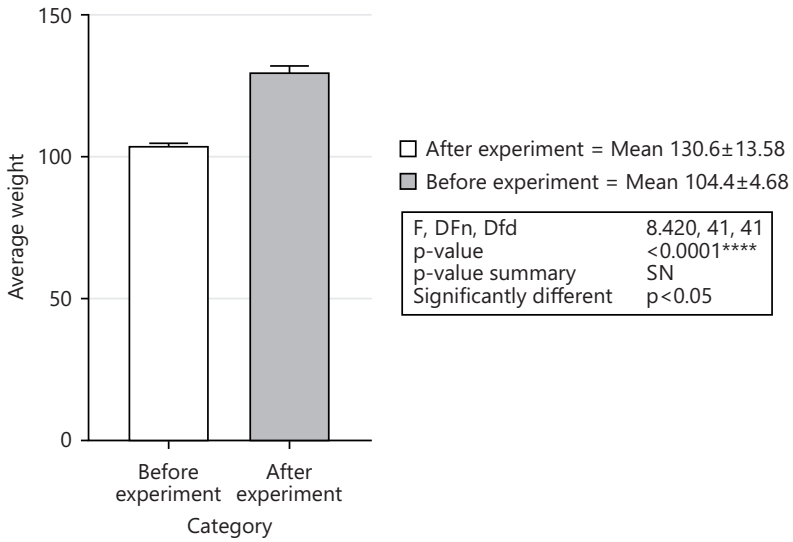

Fig. 3: Bar chart showing the comparison of initial weight with the final weight of the experimental rat

Table 1: Descriptive statistics and analysis of variance for the hormonal parameters across the groups of experimental rats

Hormones	Group 1 (n = 6)	Group 2 (n = 6)	Group 3 (n = 6)	Group 4 (n = 6)	Group 5 (n = 6)	Group 6 (n = 6)	Group 7 (n = 6)	f-value	p-value
Testosterone	16.3±3.1	45.2±1.5	17.5±1.9	21.7±1.6	32.5±1.9	37.5±1.9	20.7±2.2	173.98	0.000*
Prolactin	10.0±3.2	32.5±1.9	12.3±3.8	15.2±3.3	22.0±2.6	27.5±1.9	11.0±4.0	49.93	0.000*
FSH	7.3±2.7	19.5±1.9	5.5±2.9	8.5±2.9	12.1±1.6	16.5±1.5	6.6±2.6	31.44	0.000*
LH	4.8±1.8	13.0±2.0	5.0±2.4	4.4±1.4	7.4±1.5	11.2±1.5	3.8±1.9	24.56	0.000*

±: Mean-Standard Deviation, *Significant difference (p<0.05), FSH: Follicle Stimulating Hormone and LH: Luteinizing Hormone

Table 2: Unpaired t-test of the hormonal parameters of group 1 against the other groups

Hormones	Group	Mean difference	t- value	Df	p-value
Testosterone	2	-28.8333	-30.487	5	0.000*
	3	-1.1667	-0.670	5	0.532
	4	-5.3333	-3.730	5	0.014*
	5	-16.1667	-13.857	5	0.000*
	6	-21.1667	-13.057	5	0.000*
	7	-4.3333	-2.335	5	0.067
Prolactin	2	-22.5000	-14.375	5	0.000*
	3	-2.3333	-1.282	5	0.256
	4	-5.1667	-2.876	5	0.035*
	5	-12.0000	-12.000	5	0.000*
	6	-17.5000	-12.426	5	0.000*
	7	-1.0000	-0.466	5	0.661
FSH	2	-12.2333	-8.315	5	0.000*
	3	1.8167	0.974	5	0.375
	4	-1.2000	-0.804	5	0.458
	5	-4.8500	-4.637	5	0.006*
	6	-9.2667	-8.134	5	0.000*
	7	0.6500	0.458	5	0.666
LH	2	-8.1333	-6.273	5	0.002*
	3	-0.2167	-0.150	5	0.887
	4	0.4000	0.482	5	0.650
	5	-2.5833	-2.550	5	0.051
	6	-6.4167	-9.562	5	0.000*
	7	1.0333	1.494	5	0.195

*Significant difference (p<0.05), FSH: Follicle Stimulating Hormone and LH: Luteinizing Hormone

hormonal parameters value of the experimental group with the negative control (group 1). An unpaired t-test between group 1 and all other groups was performed. This was done to know the effect of the treatment as compared with the no-treatment group. For testosterone, there are significant differences

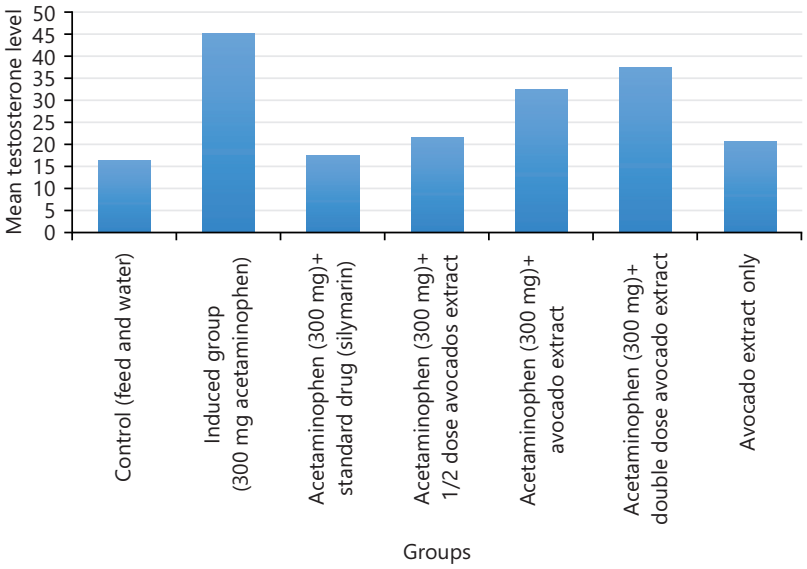

Fig. 4: Effect of avocado extracts on the testosterone of acetaminophen-induced Wistar rat
SEM, n = 6 and p<0.001

Table 3: Unpaired t-test of the hormonal parameters of group 2 (Induced group) against the other groups

Hormones	Group	Mean difference	t-value	Df	p-value
Testosterone	1	28.8333	30.487	5	0.000
	3	27.6667	26.247	5	0.000
	4	23.5000	22.239	5	0.000
	5	12.6667	12.017	5	0.000
	6	7.6667	7.064	5	0.001
	7	24.5000	16.840	5	0.000
Prolactin	1	22.5000	14.375	5	0.000
	3	20.1667	10.049	5	0.000
	4	17.3333	17.529	5	0.000
	5	10.5000	9.151	5	0.000
	6	5.0000	4.038	5	0.010
	7	21.5000	10.597	5	0.000
FSH	1	12.2333	8.315	5	0.000
	3	14.0500	7.872	5	0.001
	4	11.0333	13.343	5	0.000
	5	7.3833	8.554	5	0.000
	6	2.9667	3.027	5	0.029
	7	12.8833	17.484	5	0.000
LH	1	8.1333	6.273	5	0.002
	3	7.9167	5.656	5	0.002
	4	8.5333	13.680	5	0.000
	5	5.5500	4.701	5	0.005
	6	1.7167	1.726	5	0.145
	7	9.1667	10.063	5	0.000

between group 1 and group 2 ($p<0.001$), group 4 ($p<0.014$), group 5 ($p<0.0001$) and group 6 ($p<0.0001$). For prolactin; there is a significant difference between group 1 and group 2 ($p<0.0001$), group 4 ($p<0.035$), group 5 ($p<0.0001$) and group 6 ($p<0.0001$). For FSH; there is a significant difference between group 1 and group 2 ($p<0.0001$), group 5 ($p<0.006$) and group 6 ($p<0.0001$). The LH there is a significant difference between group 1 and group 2 ($p<0.002$) and group 6 ($p<0.0001$).

Table 3 showed the comparison of the hormonal parameters value of the experimental group with the positive control (group 2). An unpaired t-test between group 2 and all other groups was performed. This was done to know the effect of overdosed acetaminophen as compared with the treatment method specified in other groups. There is a significant difference between group 2 and other groups in all the parameters ($p<0.05$).

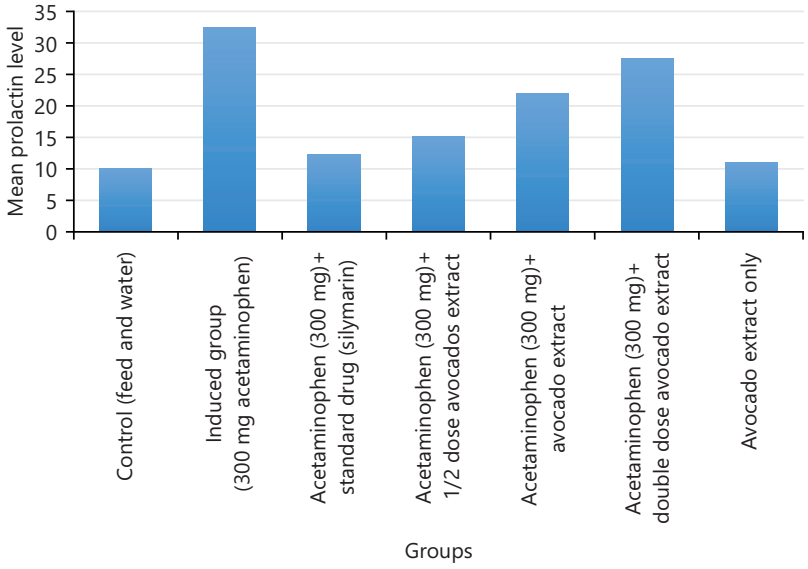

Fig. 5: Effect of avocado extracts on prolactin of acetaminophen-induced Wistar rat
SEM, n = 6 and p<0.001

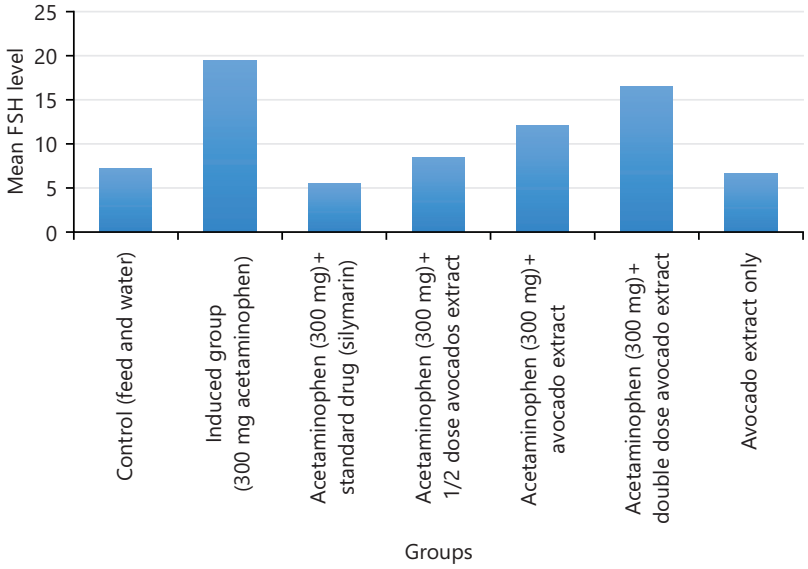

Fig. 6: Effect of avocado extracts on follicle stimulating hormone of acetaminophen-induced Wistar rat
SEM, n = 6 and p<0.001

Figure 4 to 7 shows the graphical representation of the mean value of each parameter across the 7 groups.

Semen analysis: Calculating the SEM analysis, there is a significant variation in group 2 compared to the other group. Meanwhile, other groups that involves in the treatment group exhibit variation too but not to a significant level (Table 4).

DISCUSSION

Avocado leaf effects observed highly significant increase in values of testosterone (ng/mL) in all concentrations of avocado leaf extract (group 4, 5 and 6) when compared with negative control groups (group 1). When compared with control groups, highly significant increase ($p<0.001$) in testosterone level was illustrated in experimental treated groups with concentrations of 3000 mg/kg of acetaminophen (group 2). However, male infertility is intended as a condition for men who are unable to impregnate their partners for at least one year after intercourse without using a protector[1]. Low sperm

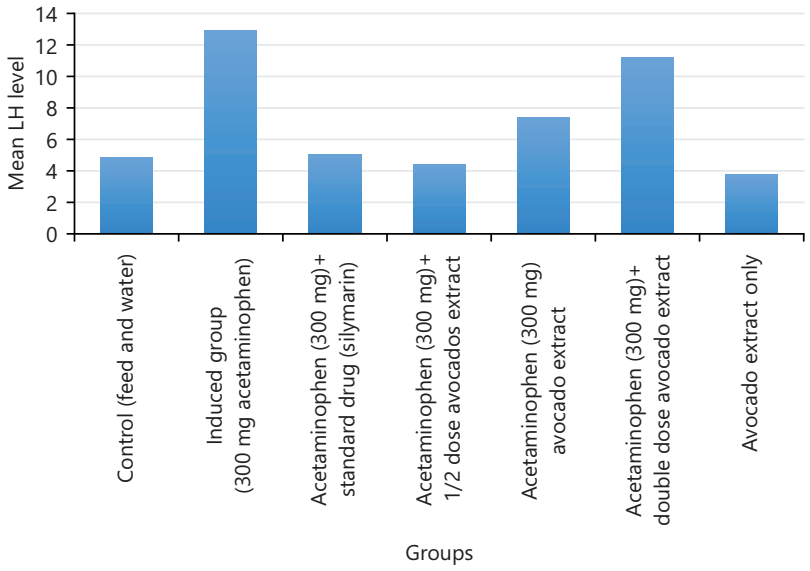

Fig. 7: Effect of avocado extracts on luteinizing of acetaminophen-induced Wistar rat
SEM, n = 6 and p<0.001

Table 4: Description of the semen obtained from the experimental rats

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
pH	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline
Consistency	Moderately visious	Highly visious	Moderately visious	Moderately visious	Moderately visious	Moderately visious	Moderately visious
Colour	Grayish	Grayish	Grayish	Grayish	Grayish	Grayish	Grayish
Total count[6]	55×10^6 sperm/cell	10×10^6 sperm/cell	95×10^6 sperm/cell	-	60×10^6 sperm/cell	12×10^6 sperm/cell	96×10^6 sperm/cell
Motility							
Actively motile	80	12	82	77	60	43	90
Sluggishly motile	10	58	12	10	25	28	5
Dead/immotile	10	30	6	13	15	29	5
Morphology							
Normal shape	80	10	76	80	64	40	88
Abnormal shape	15	70	10	12	26	44	10
Partial shape	5	20	14	8	10	16	2

count or an imbalance in hormones could be the cause of this. The gonads are the primary site of production for sex hormones, especially testosterone in males, where FSH and LH have an influence. It is well recognized that elevated levels of sex hormones have a positive feedback effect at the pituitary gland level, where they control gonadotropin output.

The study discovered that giving avocado (*Persea americana*) leaf extract to male rats raised their blood levels of testosterone, FSH, LH and prolactin. In the current investigation, however, there was a marginally significant difference in the serum levels of LH, FSH and prolactin between the rats in the silymarin and *Persea americana* leaf extract-treated groups and the rats in the control group. Based on the study's findings, it appears that silymarin and *P. americana* leaf extract primarily benefit the gonads, promoting the release of these gonadal hormones into the bloodstream in men. Rats given acetaminophen (group 2) were found to have a greater overall hormone secretion stimulating effect than rats given *P. americana* extract (group 4, 5, 6 and 7).

The results of this present study revealed that some of the chemical agent(s) which are contained in *P. americana* leaf extracts possess stimulatory activity on the reproductive functions in rats model. Serum concentration of FSH significantly increased in a dose-dependent manner following treatment with

P. americana in all test groups when compared with control ($p<0.05$) but fell when given acetaminophen+standard drugs (silymarin) and aqueous extract from the *Persea americana* (group 7). Testosterone concentrations in all test groups were considerably greater when compared with those of the control group.

The extract may have enhanced the male rats' reproductive activity, as evidenced by the rise in Follicle Stimulating Hormone (FSH) concentration after treatment. In men, free sperm production is stimulated and maintained to a normal degree, which in turn controls sexual development, growth, pubertal maturation and reproductive capabilities[30]. However, because acetaminophen induces spermatogenesis, an excessive rise in FSH levels, as seen in rats given 3000 mg/kg of the drug, may have a deleterious effect on reproduction. It has been determined that high FSH concentration, which typically denotes a main testicular malfunction and spermatogenesis impairment, is the most prevalent endocrine problem linked to male infertility or subfertility[31].

The administration of high-dosage extract may be harmful to the male reproductive system, as evidenced by the fact that the concentrations of prolactin and testosterone hormones rose in the rats treated with lower doses but decreased in the group given a higher dose. Prolactin hormone may be the cause of the rise in testosterone concentration in the low and moderate dose treated groups. Prolactin hormone stimulates testosterone production from Leydig cells in the testis, which increases male reproductive functions[31]. In males, testosterone, a primary sex hormone, stimulates the growth of the reproductive organs and secondary sexual traits[31]. The observed reduction in testosterone concentration after high-dose treatment could have been caused by the extract's direct impact on the Leydig cells or indirectly by the group's lower prolactin hormone levels. A significant indicator of infertility is said to be a decline in testosterone concentration[32].

The color of the sperm samples did not substantially change between the test groups and the control, indicating that the extract treatment had no effect on their expectedly creamy hue, which is associated with improved reproductive performance. Sperm sample pH is a well-known indicator of the survival rate of sperm cells and a crucial parameter in the assessment of sperm sample quality. Acidic environments are known to raise sperm mortality, impair sperm function and conception and contribute to infertility[33]. This can occur in the female vagina or in the sperm sample itself. Because alkaline media neutralizes the acidity of the female vagina and creates an environment that maximizes sperm motility and improves the processes leading to ovum fertilization, it is therefore the most optimal medium for sperm performance[31]. Therefore, although the low pH seen in all groups may correlate with an inhibitory effect on both sperm survival and reproduction, the alkaline semen environment identified in all groups implies that the dose favors reproduction in rats. Notably, individual sperm motility, progressiveness and viability in this therapy group were all identical to those in the control group. Progression in sperm motility has been associated with a high fertility index, according to Love[34]. The fructose content of sperm samples has been shown to increase sperm motility; however, at the greatest dose, the extract may have decreased the fructose content of the sperm sample, resulting in a decrease in sperm motility and viability. These results seem to corroborate a research which showed that large dosages of *Persea americana* extract had detrimental effects on male rats' reproductive systems[35].

CONCLUSION

The study revealed positive changes in hormonal profiles and semen analysis parameters, suggesting a potential protective role of avocado leaves against the detrimental impacts of acetaminophen on reproductive health. In view of the pending observation of the effectiveness of the leave *Persea americana* and the ability to reverse the damage caused by acetaminophen, it is recommended that *Persea americana* leave should be incorporated into spectrum of herbal drugs for the management of hormonal imbalance.

SIGNIFICANCE STATEMENT

This study aims to investigate and evaluate the effects of avocado leaves on hormones and semen of acetaminophen-induced infertility in male Wistar rats and to conduct a seminal fluid assay on the rats treated with avocado leaves. The avocado extract reduces body weight by a significant level. Excessive increase in FSH concentration as observed in the 3000 mg/kg of acetaminophen-treated rats may negatively affect reproduction due to its effect on spermatogenesis. As observed, testosterone in the lower doses increased but decreased in the high dose group. It is recommended that *Persea americana* leaves should be incorporated into a spectrum of herbal drugs for the management of hormonal imbalance, however, high doses should not be taken into consideration to prevent oligospermia and azoospermia.

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