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TITLE

Effect of Prolonged Consumption of Aqueous Extract of Raphia Hookeri Fruit Pulp /Mesocarp on Sperm Parameters in Male Wistar Rats.

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ABSTRACT

This research was aimed at evaluating the effects of the consumption of aqueous extract of *Raphia Hookeri* mesocarp on sperm parameters in male Wistar rats. 24 male Wistar rats weighing 200g to 355g were grouped into 4 groups, group 1 control which was fed with feed and water, group 2 given 500mg/kg, group 3 given 1,000mg/kg and group 4 given 2,000mg/kg body weight of the extract for 28days. The animals were sacrificed and sperm samples analyzed via selected analysis techniques, $p<0.05$ values. Result showed; sperm count values for group 2 (51.00 ± 3.00), group

3 (22.00 ± 1.00) and group 4 (51.00 ± 3.00), control group (44.00 ± 1.0). Sperm motility in groups 2 to 4 were 52.5 ± 7.50, 67.50 ± 2.50 and 96.5 ± 1.50 respectively, control group was 91.00 ± 1.00. Sperm morphology in groups 2 to 4 were 1.50 ± 0.50, 0.50 ± 0.50 and 2.50 ± 0.50 respectively, control group 4.00 ± 1.00. Sperm viability in groups 2 to 4 were 94.00 ± 3.00, 92.00 ± 7.00 and 88.50 ± 6.50 respectively, control group was 90.00 ± 2.00. Percentage change in weight of rats in groups 2 to 4 were 0.59 ± 1.91, 5.44 ± 2.32, 5.33 ± 1.16 respectively, control group was 8.48 ± 5.71. Increased total sperm count and percentage sperm motility in group 4 and significant when compared to control. Sperm motility, sperm count levels decreased across group 1, 2, no significant change in sperm viability, sperm morphology when compared to control. The results indicated no significant change in weight compared to control. Revealed that extract of *Raphia Hookeri* fruit pulp/mesocarp when consumed frequently (2,000mg/kg) is capable of boosting sperm quality, fertility through it's positive effects on sperm motility and sperm count, this is as a result of its antioxidant activity.

KEY WORDS: Prolonged consumption, Aqueous Extract, Raphia Hookeri, Fruit Pulp/Mesocarp, Sperm Parameters, Male Wistar Rats

INTRODUCTION

The sperm, also known as spermatozoa, is the male reproductive cell or gamete. It is produced in the testes through a process called spermatogenesis. Sperm cells are essential for sexual reproduction as they are responsible for fertilizing the female egg during sexual intercourse (Kumar and Singh, 2015), the structure of a sperm cell consists of three main parts: the head, the midpiece, and the tail. The head contains the genetic material of the sperm, which includes the Deoxyribonucleic acid (DNA) and chromosomes necessary for fertilization. It is covered by a cap-like structure called the acrosome, which contains enzymes that aid in penetrating the outer layer of the egg during fertilization. (Omu, 2013). The midpiece of the sperm contains mitochondria, which provide energy for the movement of the sperm. This region is packed with numerous mitochondria to ensure that the sperm has enough energy to swim towards the egg.(Kumar and Singh, 2015) The tail, also known as the flagellum, is a long and whip-like structure that propels the sperm forward through a rapid and coordinated movement called flagellar motility. Sperm cells are extremely small, measuring about 50 micrometers in length. They are specialized for swimming through fluid environments such as semen and cervical mucus to reach and penetrate the egg. The ability of sperm to move is crucial for successful fertilization.(Kumar and Singh, 2015). Sperm production begins at puberty and continues throughout a man's life. The process of spermatogenesis involves multiple stages of cell division and maturation within the seminiferous tubules of the testes. Spermatogonia, which are undifferentiated cells, undergo mitosis to produce primary spermatocytes. These primary spermatocytes then undergo meiosis I to form secondary spermatocytes, which further divide through meiosis II to produce haploid spermatids. Finally, spermatids undergo a process called spermiogenesis, during which they differentiate into mature sperm cells, once matured, sperm cells are stored in the epididymis, a coiled tube located on the back of each testicle. During ejaculation, sperm are propelled from the epididymis through the vas deferens and mixed with seminal fluid from the seminal vesicles and prostate gland to form semen. The semen is then ejaculated through the urethra during sexual intercourse.(Kumar and Singh, 2015) It is important to note that not all sperm cells are capable of fertilization. Only a small percentage of the millions of sperm ejaculated during sexual intercourse will reach the egg. Factors such as sperm count, motility, and morphology can affect fertility.(Omu, 2013). Sperm cells play a vital role in sexual reproduction by delivering the male genetic material to the female egg for

fertilization. Their unique structure and swimming ability enable them to navigate through the female reproductive tract and reach the egg. Understanding the anatomy and physiology of sperm is essential for comprehending human reproduction.

Sperm parameters refer to the various characteristics and measurements used to assess the quality and fertility potential of sperm. These parameters are crucial in determining male fertility and can provide valuable insights into a couple's chances of conceiving naturally or through assisted reproductive techniques. There are several key parameters that are commonly evaluated when assessing sperm quality. These include sperm count, motility, morphology, and vitality. Sperm count, also known as sperm concentration, refers to the number of sperm present in a given volume of semen. It is typically measured in millions per milliliter (million/mL). A normal sperm count is generally considered to be above 15 million/mL. Low sperm count, known as oligospermia, can significantly reduce the chances of conception. A few commonly used terms regarding sperm density: Oligospermia: any sperm density less than what is considered normal, Severe oligospermia: typically considered to be less than 5 million sperm/cc, Azoospermia: no sperm at all seen in the ejaculate, Virtual azoospermia: only a very small number of sperm (sometimes defined as less than 100,000 sperm/cc) (Russell, 2015) Sperm motility refers to the ability of sperm to move and swim effectively. It is divided into two categories: progressive motility and total motility. Progressive motility refers to the percentage of sperm that exhibit forward movement, while total motility includes both forward and non-forward movement. High motility is essential for sperm to navigate through the female reproductive tract and reach the egg for fertilization. Motility, or the number of sperm that are actually swimming, is important for success with natural intercourse and intrauterine insemination, because the sperm need to be able to swim up the fallopian tubes, where fertilization takes place. For IVF, sperm motility is not quite so important since sperm can be injected directly into an egg in the lab with the use of intracytoplasmic sperm injection, or ICSI. However, the sperm that are injected need to be alive, and motility is an accurate marker of this (i.e. if sperm are moving then they are live, although sperm that are not swimming may be alive as well).

Sperm morphology refers to the appearance or form of the sperm i.e size and shape. Sperm Morphology is said to be a very strong indicator of a man’s testicular health .(Menkveld *et al*; 2011) Multiple aspects are taken into consideration while evaluating sperm’s morphology, including its head, midpiece, tail, and the presence of cytoplasmic droplets. (A small portion of cytoplasm found in the sperm (Arulraj, 2022). As per WHO standards, a sample should have about 4-39% of normally shaped sperms.(W.H.O 2021). A sperm normally possess the following characteristics: Head: A smooth and oval shaped structure with a size less than a needle point (5-6 micrometers in length; 2.5-3.5 micrometers in width). Acrosome: A well-defined cap-like structure covering 40-70% of the head. It is a membrane that contains enzymes which are able to penetrate an egg’s membrane.(.Harris, Fronczak, Roth, and Meacham 2011), Midpiece: A thin structure between the head and the tail , Tail: Also known as the neck, it is said to be a defect-free structure thinner than the midpiece. It is said to be around 50 micrometers in length.(Harris, Fronczak, Roth, and Meacham 2011).A normal sperm will have no visible abnormalities in any of the above structures. Also, there are no fluid droplets (cytoplasmic droplets) in the head of the sperm which are larger than one-half the size of the sperm head. (Menkveld *et al*. 2011) Importance of Morphology: Assessment of sperm morphology has been described by some authors as a good indicator of male fertility. It is understood that there is no hindrance in sperm production and development when no defects arise in a sperm’s overall shape and size. Occurrence of a defect in its morphology indicates disturbance in the process, which in turn may interfere with conception thereby affecting fertility. Having abnormally shaped sperm in higher amounts can be associated with infertility as per some studies. It can also be linked with other irregularities of the semen such

as low sperm count or motility. On the other hand, Men with abnormally shaped sperms may also have no trouble achieving a pregnancy. Sperm vitality refers to the percentage of live sperm in a semen sample. It is an indicator of the overall health and viability of sperm cells. While some dead or non-viable sperm may be present in every sample, a high percentage of live sperm is desirable for successful fertilization (Ohta *et al*; 2010).

The world has been faced with countless health challenges which affects the lives of humans, this has motivated medical professionals and even natives to search for remedies via natural plant consumption to which the *Raphia Hookeri* plant is one of such plants. The *Raphia Hookeri* Plant is of the Arecaceae family (palms) (Gruca, *et al*; 2015) to which it is commonly known as Raffia Palm, and to the indegenous people of Abua local government area known as Oghol. *Raphia Hookeri* plant is a monocotyledonous plant, It has a trunk covered with attractive unusual coils, usually reproduces through seeds and grows up to 12 m tall and 60 cm in trunk diameter (Hutchinson *et al*; 1993). From scientific reports and investigations, it has been shown that the origin of Raphia palms is traceable to West Africa, particularly along swampy and semi swampy area of tropical and equatorial rain forest or derived savannas (Ndon, 2003). Endemic to Africa, its distribution covered many countries of the tropical area like Cameroon, Burkina Faso, Nigeria, Madagascar, Gambia, Ghana, Guinea, Ivory Coast and Kenya.

Raphia Hookeri produces fruits that are oblong-ellipsoid in a scaly cone comprised of rhombus triangular reddish-brown scales (Keay and Hepper, 1953). The fruits contain an important part called pulp or mesocarp which is considered edible when boiled, In addition the pulp is used as a bitter flavouring in meals. Due to its hypothesized medicinal qualities, the pulp is used as a form of native medicine in some certain parts of Africa (Liu, 2004 and Altiok, 2010). Every part of *Raphia Hookeri* tree is useful economically, both in the food industry sector and the art sector. In the food industry sector, the mesocarp of the ripe Raphia Hookei fruit pulp which is rich in many nutrients such as lipid (40-52%), protein (6.1%), carbohydrate (61.4%), vitamins such as niacin (0.27 mg), vitamin A (0.15 mg) and minerals (3%) (Liu, 2004 and Altiok, 2010), cannot only be used as food supplement. It can also be a main source of lipid since it yields edible oil, which can be use and exploit as a cheap and local product which lead to a decrease of resource wasting and environmental pollution (Ndon, 2003).

The *Raphia Hookeri* Plant has a fruit mesocarp commonly called Ogbusi which is boiled and processed for consumption by the people Abua/Odual LGA, Rivers state, Niger Delta Region, southern Nigeria, This fruit pulp is hypothesized to have a wide range of Health impacting effects which include hyperlipidaemia, boost immunity, inhibit plasma glucose, reduce blood pressure, boost haematopoiesis and boost fertility e.t.c (Egbono *et al*; 2023). The boiled fruit pulp ('Ogbusi') is taken as a snack and mostly eaten with tapioca (processed cassava) commonly known as 'Ataka' by the Abua people of Rivers state in Nigeria (Egbono *et al*; 2023). The *Raphia Hookeri* fruit pulp contains very special constituents that affects sperm parameters, it is shown to contain vitamins C and E, carotenes, niacin, alkaloid, saponins, flavonoids and phenols (Egbono *et al*,.2023). All of these constituents are active antioxidants, More specifically the antioxidants vitamin C and E which play a huge role in boosting sperm parameters by decreasing oxidative stress which is the imbalance between the production of reactive oxygen species (ROS) by the spermatozoa, white blood cells and the antioxidant capacity of the seminal plasma (Karande, 2019). Uncontrolled and excessive production of ROS overwhelms the limited antioxidant defenses in semen resulting in seminal oxidative stress (Karande, 2019).

Vitamin E enhances sperm performance. It protects spermatozoa from oxidative damage and loss of motility. Vitamin C (calcium ascorbate) is a water-soluble ROS scavenger with high potency. It is a strong antioxidant destroying free radicals in the body and protects human spermatozoa

against endogenous oxidative damage by neutralizing hydroxyl, superoxide and hydrogen peroxide radicals. Vitamin C prevents sperm agglutination. Vitamin C and E act together to protect against peroxidative attack on spermatozoa (Karande, 2019). As such, taking such an antioxidant rich fruit could hypothetically help boost sperm parameters. Sperm parameters are specific characteristics or qualities of sperm cells that are measured and analyzed to assess their quality, health, and potential for fertilization (Milachich and Dyulgerova-Nikolova, 2020). These parameters provide valuable information about the reproductive health and fertility of individuals. The sperm parameters can offer insights into various aspects of reproductive biology, health, and potential impacts of different factors. Sperm parameters are used as a fertility test in men or to confirm that a vasectomy was successful (Milachich and Dyulgerova-Nikolova, 2020). Many studies have been done on Raphia Hookeri, some of which include: the effects of the *Raphia Hookeri* fruit pulp on Lipid profile, (Egbono *et al*; 2023) the study of vitamin, phytochemicals and toxic elements in the pulp and seed of *Raphia Hookeri* (Ogbuagu, 2008); the qualitative and quantitative evaluation of the phytochemicals of *Raphia Hookeri* and Raphia farinifera fruits (Oluwaniyi *et al*; 2014); some morphological and chemical characteristics of developing fruit of *Raphia Hookeri* (Bassey, 1985);

Although, studies have been done on *Raphia Hookeri* fruit pulp, very few have focused on its effects on sperm health more specifically sperm parameters. This study can be a source of rich knowledge and information for the male reproductive health sector and also the nutrition sector. Moreover, the exploitation of *Raphia* palm tree is increasing over years in Nigeria, due to the high demand of its derived products such as bamboo and *Raphia* fiber, (Ndon, 2003) but awareness on the effects of the fruit on sperm parameters is still lacking.

The world at large and mainly Nigeria, has access to natural remedies like the *Raphia Hookeri* fruit pulp and also countless fruits and herbs which can be used to ameliorate symptoms for a vast variety of illnesses but there is lack of knowledge on the impact of these ubiquitous natural medicines and less consumption of these fruits. This leads the populace both in the higher and lower class of the Nigerian society to spend a lot of money on inorganic and organometallic drugs when there are natural medicines that do affect sperm parameters and other parts of the bodies health,that are not as expensive or hard to find.

METHODOLOGY

Materials

The materials involved in this study includes; Syringe, Hand Gloves, Cages, Dissecting Blade, Dissecting Board, Permanent Marker, Animal Feeds, Water, Chloroform, EDTA Bottles, Cannula, matured Male Wistar Rats, Lab coats, Disinfectants, Dry saw dust etc

Animal Preparations

A total of Twenty four (24) healthy male Wistar rats of weight ranging from 200g to 350g were used for this study. These rats where housed in the animal house, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The animals were maintained in a well-ventilated animal house under optimum condition of humidity, temperature and natural light-dark cycle were allowed free access to food and water.

Acclimatization of Animals

After identification, the animals were weighed using a weighing balance and housed in a clean plastic cage for two weeks so as to acclimatize to the environmental condition of the animal house, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria.

Experimental Extract and Preparation

The Extract of *Raphia Hookeri* fruit pulp was used for the experiment. Maceration method was used for the preparation, the fruit pulp were air- dried in other not to kill the active ingredients, then it was finally crushed and soaked in a maceration jar about 1000gram of the extract was dissolved in 2000ml of water and allowed to stand for 72 hours with a continuous agitation to enable a good yield after which it was filtered and the filtrate was mounted on a water bath to evaporate the liquid content at temperature of 65 degrees Celsius, after evaporation the weight of the extract was taken and it was stored for use.

Study Design

A total of Twenty four healthy male Wistar rats were used for this study. The rats were divided into a control group of 6 animals and three (3) other groups with 6 animals each. The other groups (2,3,4) were administered the extract for 28days after they had been acclimatized for 14 days in the animal house, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria.

Sample Collection

Raphia hookeri fruit pulp used for this study was purchased from the local market called Ayeeziin Abua/Odual LGA,Rivers State, Nigeria,

Mode of Administration of Extract

Aqueous extract of *Raphia Hookeri* was administered orally in low dose, medium dose and high dose daily for 28 days. In the course of oral administration of the extract to the animals the following doses were administered for each group except the control group for twenty-eight (28) days. The Lethal dose (LD 50) of the aqueous extract of *Raphia Hookeri* fruit was calculated using Lorke‟s method, 5000mg/kg body weight of Wistar rats was attained, therefore the male Wistar rats were not given extract beyond 5000mg/kg body weight:

Group 1(Control Group): Were given animal feed and water
Group 2 (Low dose): Were given 500mg/kg body weight of the extract
Group 3 (Medium dose): Were given 1000mg/kg body weight of the extract
Group 4 (High dose): Were given 2000mg/kg body weight of the extract.

Analysis of sample sperm parameters

Estimation of the percentage of motile spermatozoa

Materials: Microscope and Slide

Procedures:

Place one drop of well mixed liquefied semen on a slide and cover with a cover glass.

Using the 40x objective with condensers iris closed sufficiently to give good contrast, examine several fields of the preparation for motile spermatozoa.

Report the approximate percentages that are actively motile.

Morphology

Mateials: Microscope, Slide, 95% Ethanol, Sodium Bicarbonate, Carbon Fuschin, methlere blue

Procedures

Make a thin smear of the liquefied well-mixed semen on a slide. While still wet, fix the smear with 95% ethanol for 5-10minutes and allow to air dry.

Wash the smear with sodium bicarbonate formal solution to remove any mucus present

Rinse the smear with several change of water.

Cover the smear with dilute (1 in 20) carbon fuschin and allow to stain for 3 minutes. Wash off the stain with water.

Counter stain, by covering the smear with dilute (1-20) methylere blue for 2mins. Wash off the stain with water and allow the smear to air dry.

Report staining results in categories of head defects, midpieces and tail

Sperm count and viability

Mateials: Microscope, Slide, Neubauer Chamber and eosin.

Procedures

Using a pasteur pippete, fill an improved Naubauer chamber with the diluted semen. Wait for 3-5 minutes for the spermatozoa to settle.

Using the 10x objective lens with the condenser iris closed suffeciently to give good contrast, count the number of spermatozoa in an area of f 2sqmm, i.e 2 large squares.

Calculate the number of spermatoza in 1ml of fluid by multiplying the number counted by 100.000

Add a drop of eosin to the preparation

Report percentage stained red as non viable and those not stained as viable

In normal specimens, over 70% of the spermatozoa are motile most specimen;s containing over 8% motive forms. The spermatozoa remain motile for several hours

Statistical Analysis

The data obtained from the present study were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) version 21.0. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Post-Hoc multiple comparison test and P value less than 0.05 ($P<0.05$) indicated the threshold for statistical significance. The values were expressed as mean ± standard error of mean (SEM).

RESULTS

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Table 1: Effect of administration of AERHF(Aqueous Extract Of Raphia Hookeri Fruit Pulp) on sperm count in male Wistar rats

Group and Treatment	Sperm Count ($X10^6$/ml)
Group 1: Control Group	44.00 ± 1.00

Group 2: Low Dose treated (500mg/kg b.w AERHF)	17.00 ± 1.00 [a]
Group 3: Medium Dose treated (1000mg/kg b.w AERHF)	22.00 ± 1.00 [a]
Group 4: High Dose treated (2000mg/kg b.w AERHF)	51.00 ± 3.00 [a, b, c]

The low and medium doses (500 and 1000mg/kg AERHF) treated rats indicated significantly (P<0.05) levels of sperm count when their respective values were compared to those of control and high dose (2000mg/kg AERHF) treated rats. Again, it was seen that, the high dose treated rats had significantly (P<0.05) raised mean sperm count when compared to that of the control group.

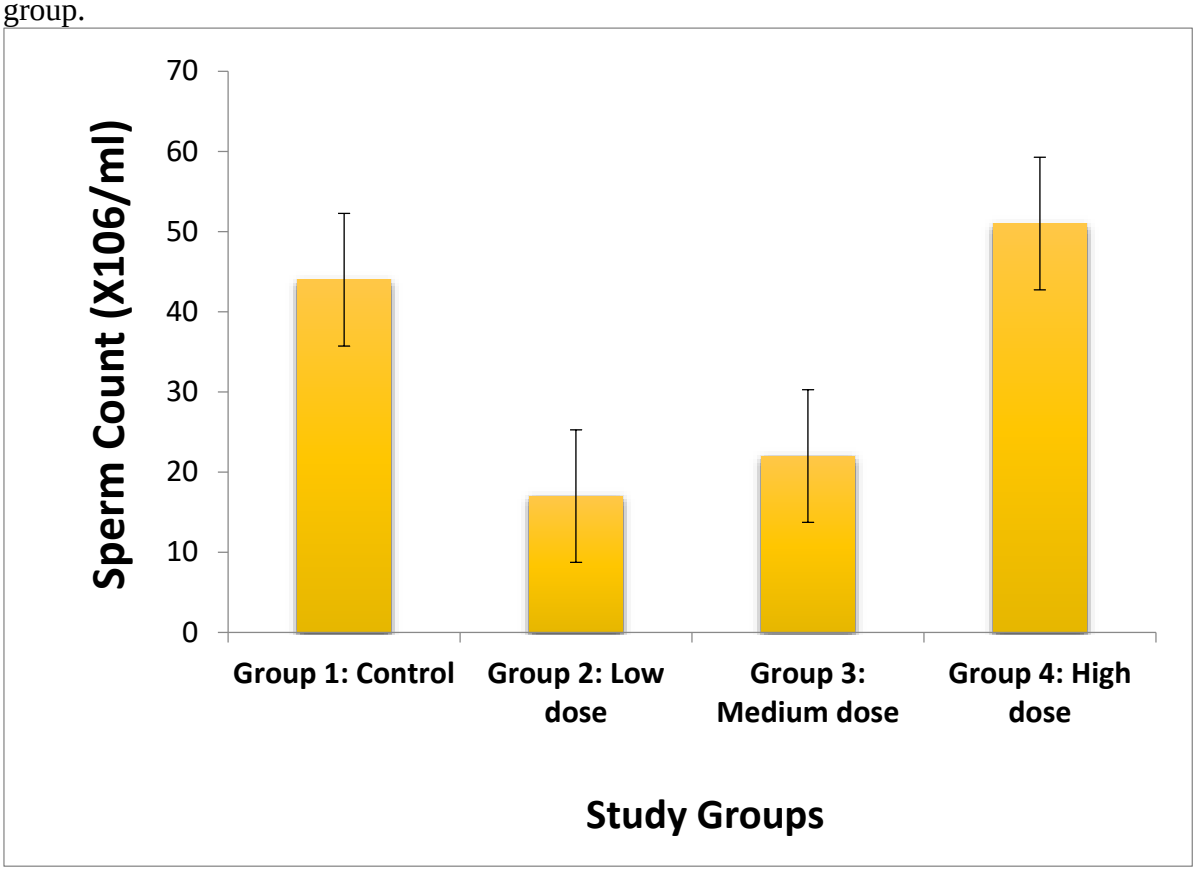

Figure 4.1: Effect of administration of aqueous root extract of *Raphia Hookeri* (AERHF) on Sperm Count (X10^6/ml) level in male Wistar rats.

Table 2: Effects of administration of aqueous extract of *Raphia Hookeri* Fruit pulp (AERHF) on sperm motility in male Wistar rats.

Groups	Motile (%)	Active (%)	Sluggish (%)
Group 1: Control	91.00 ± 1.00	90.00 ± 1.00	7.00 ± 1.10
Group 2: Low dose	52.5 ± 7.50 [a]	40.00 ± 10.00 [a]	12.50 ± 2.50

Group 3: Medium dose	67.50 ± 2.50 [a]	50.00 ± 5.00 [a]	17.5 ± 2.50 [a]
Group 4: High dose	96.5 ± 1.50 [b, c]	87.5 ± 2.50 [b, c]	9.00 ± 100 [c]

The percentage motile and active sperm cells in the low and medium doses treated animals (i.e. Groups 2 and 3) had graded significant ($p<0.05$) decreases when compared to those of the control rats and high dose treated groups respectively (i.e. groups 1 and 4). Similarly, the percentage of sluggish cells was highest in group 3 and when this amount was compared to that of group 4, it was significant ($P<0.05$).

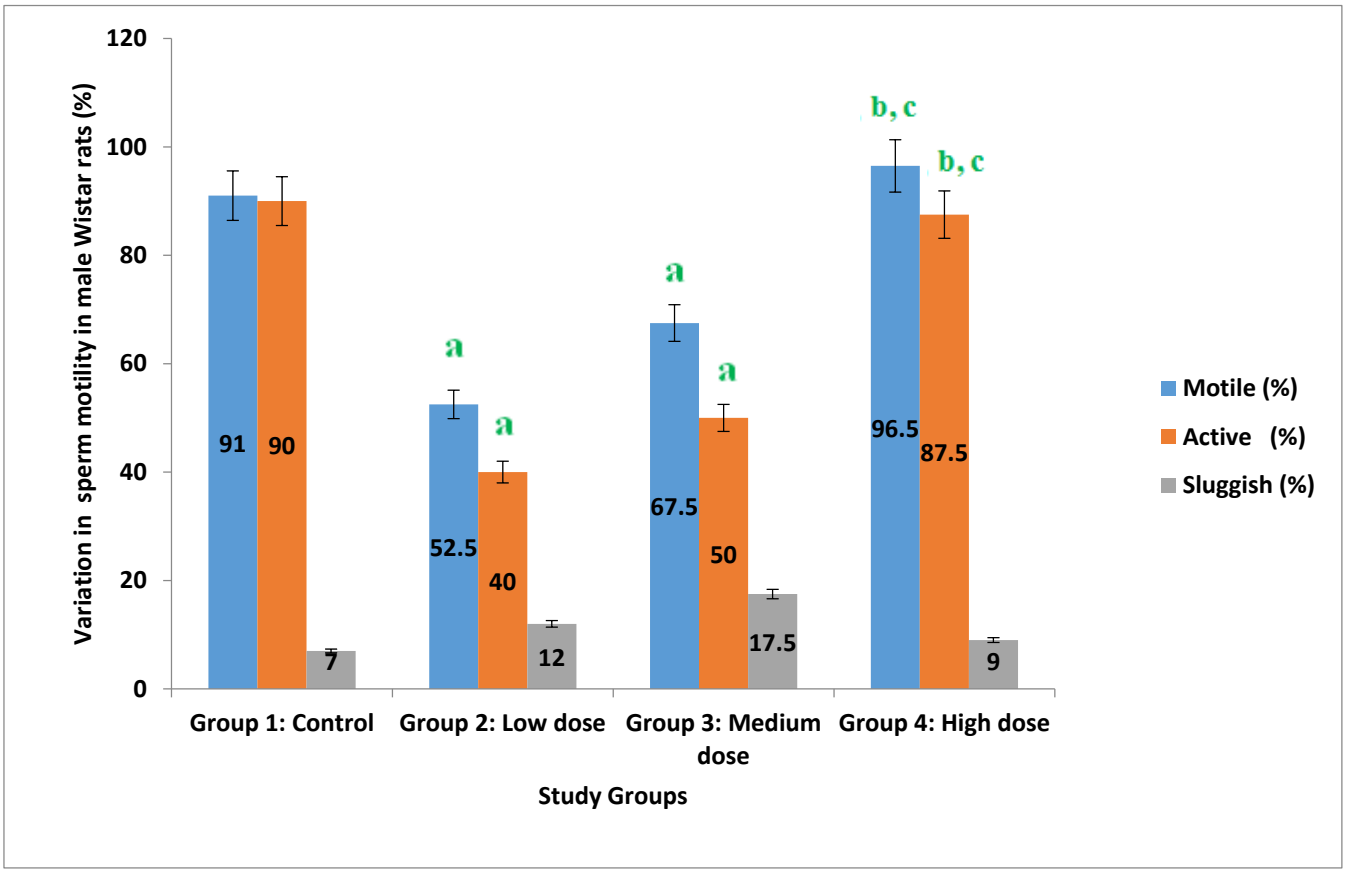

Figure 2: Effects of administration of AERHF on sperm motility in male Wistar rats.

Table.3: Effects of administration of AERHF on sperm morphology in male Wistar rats.

Groups	Dead cells (%)	Head Defects (%)	Tail (%)	Mid-piece (%)
Group 1: Control	3.00 ± 1.00	4.00 ± 1.00	6.00 ± 2.00	0.00 ± 0.00
Group 2: Low dose	47.50 ± 7.50 [a]	1.50 ± 0.50	3.50 ± 2.50	1.00 ± 0.00
Group 3: Medium dose	32.50 ± 2.50 [a]	0.50 ± 0.50 [a]	7.00 ± 6.00	0.50 ± 0.50
Group 4: High dose	3.50 ± 1.50 [b, c]	2.50 ± 0.50	8.50 ± 5.50	0.50 ± 0.50

Table.3 displays the effects of administration of AERHF on sperm morphology in male Wistar rats.

The amount of dead sperm cells was seen to be highest in Group 2 (low dose treated) and this was followed by that of Group 3(medium dose treated); the levels of the dead cells in these groups were found to be significantly ($P<0.05$) higher when compared to those of Groups 1 and four. Considering the results on sperm head defects, Group 3 had the lowest presentation of the defects compared to even Group 1 (control Group).

There were no significant ($P>0.05$) variations in the percentage presentations of the tails and midpiece portions of the sperm cells.

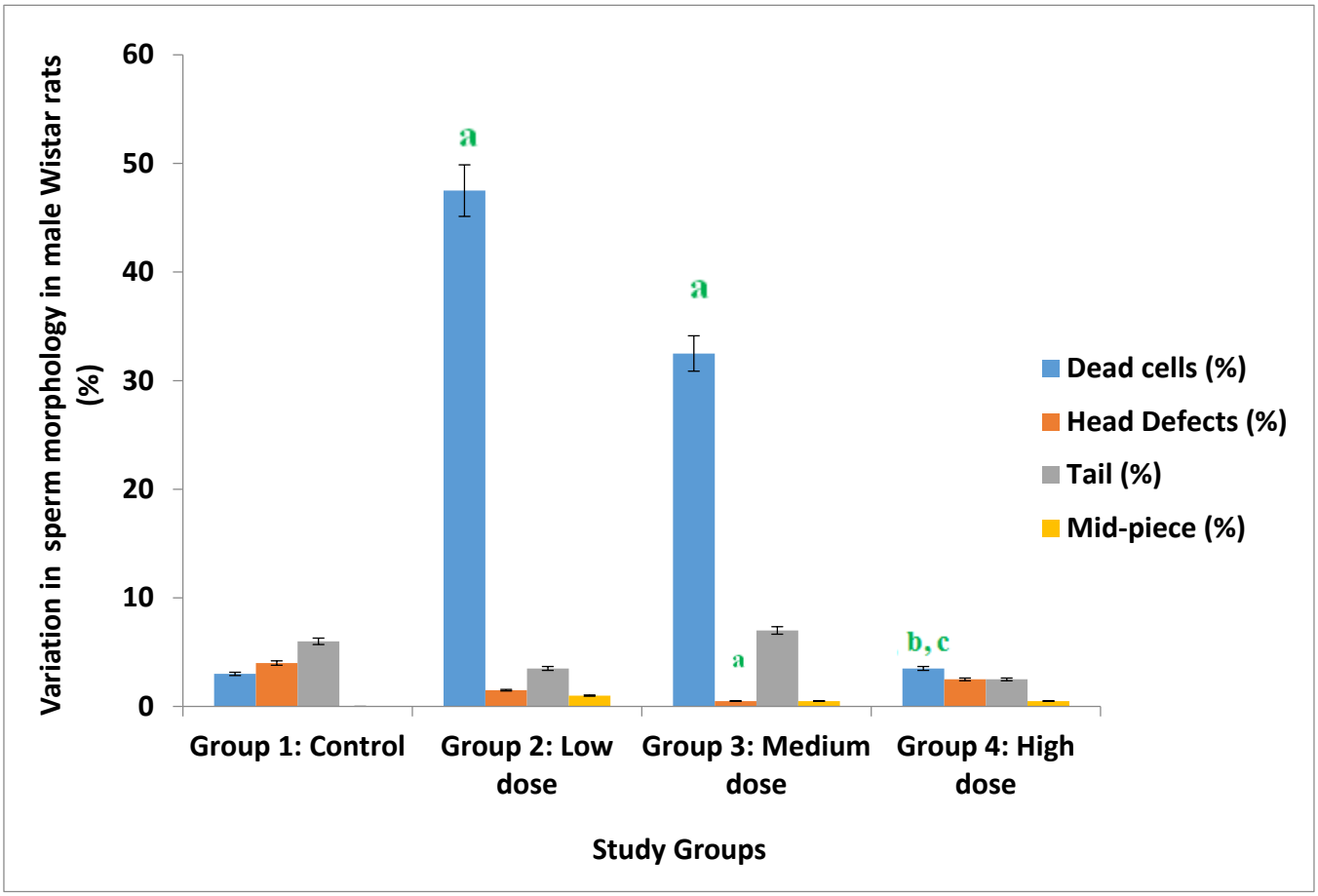

Figure 4.: Effects of administration of AERHF on sperm morphology in male Wistar rats

Table 4: Effects of administration of AERHF on sperm viability in male Wistar rats.

Groups	Viable (%)	Non-Viable (%)
Group 1: Control	90.00 ± 2.00	10.00 ± 3.00
Group 2: Low dose	94.00 ± 3.00	6.00 ± 3.00
Group 2: Medium dose	92.00 ± 7.00	8.00 ± 4.00
Group 2: High dose	88.50 ± 6.50	11.50 ± 6.50

The result on Table 4 shows the effects of administration of AERHF on on sperm viability in male Wistar rats.

The percentage values of viable and non-viable sperm cells did not vary significantly ($p>0.05$). Although notably, the viable cells of the low dose treated animals had the highest value which was followed by that of the medium dose treated group when respectively compared to the control or untreated group and high dose treated group.

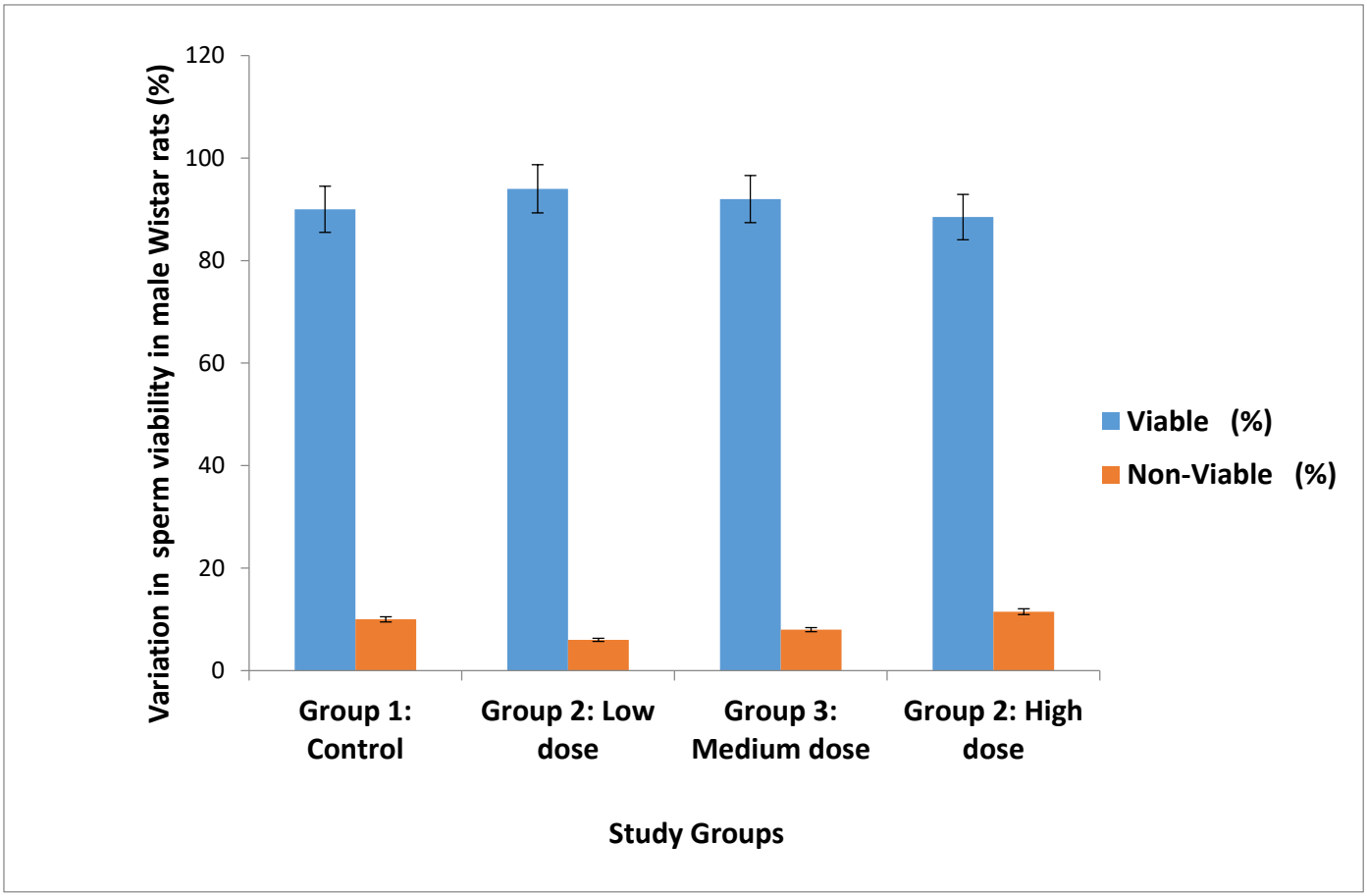

Figure 4: Effects of administration of AERHF on sperm viability in male Wistar rats.

Table 5: Effect of administration of aqueous Fruit extract of *Raphia hookeri* (AERHF) on Percentage Change in Body Weight Male Wistar Rats

Groups and Treatment	Percentage Change in Body Weight (%)
	Male
Group 1: Control Group	8.48 ± 5.71
Group 2: Low Dose treated (500mg/kg b.w AERHF)	0.59 ± 1.91
Group 3: Medium Dose treated (1000mg/kg b.w AERHF)	5.44 ± 2.32

Group 4: High Dose treated (2000mg/kg b.w AERHF)	5.33 ± 1.16

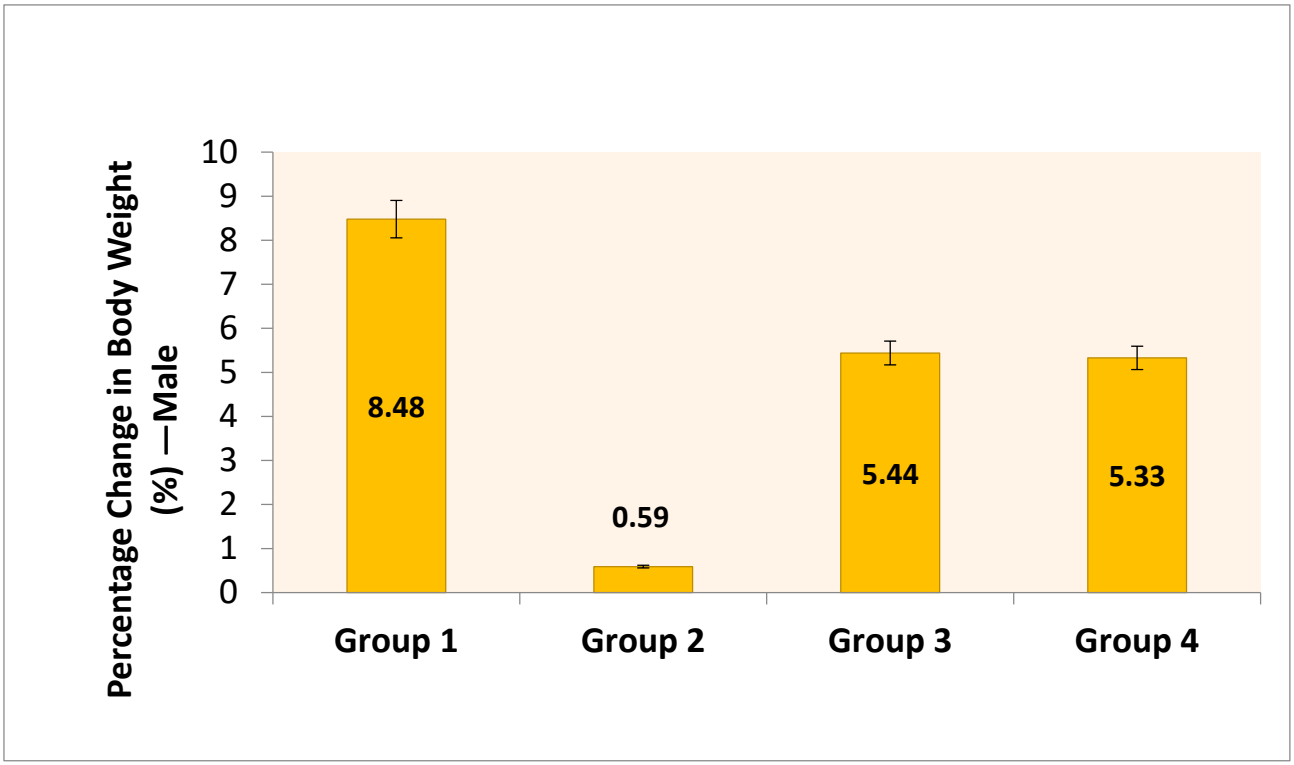

Figure 5: Effect of administration of aqueous Fruit extract of *Raphia hookeri* (AERHF) on Percentage Change in Body Weight of Male Wistar Rats

Discussion

Infertility is a complex medical condition that affects both men and women it is said to be defined as not being able to get pregnant (conceive) after one year (or longer) of unprotected sex. When it comes to male factor of Infertility, one of the key factors that can contribute to difficulties in conceiving is abnormal sperm parameters. Sperm parameters refer to various characteristics of sperm, including sperm count, motility, morphology, and other factors that are assessed during a semen analysis. Sperm count, also known as sperm concentration, refers to the number of sperm cells present in a given volume of semen. A low sperm count, known as oligospermia, can reduce the chances of fertilization as there are fewer sperm available to reach and penetrate the egg. The

World Health Organization (WHO) defines a normal sperm count as having at least 15 million sperm per milliliter of semen (Omu, 2013). Sperm motility refers to the ability of sperm to move and swim effectively. It is crucial for sperm to have good motility in order to reach the egg for fertilization. Poor sperm motility, known as asthenospermia, can hinder the ability of sperm to reach the egg and decrease the chances of successful fertilization. The WHO defines normal sperm motility as having at least 40% of sperm with progressive forward movement. Sperm morphology refers to the size and shape of sperm cells. Abnormalities in sperm morphology, known as teratospermia, can affect their ability to penetrate the egg and result in reduced fertility (Kumar and Singh, 2015). The WHO defines normal sperm morphology as having at least 4% of sperm with normal shape and structure. In addition to these primary parameters, there are other factors that can affect male fertility. These include semen volume, pH level, liquefaction time (the time it takes for semen to change from a gel-like state to a liquid state), and presence of white blood cells or other substances that may indicate infection or inflammation.There are several potential causes for abnormal sperm parameters. These can include genetic factors, hormonal imbalances, testicular disorders, varicocele (enlarged veins in the scrotum), infections, exposure to toxins or chemicals, certain medications, excessive heat exposure (such as from saunas or hot tubs), and lifestyle factors such as smoking, excessive alcohol consumption, drug use, obesity, and stress. It is important to note that abnormal sperm parameters do not necessarily mean that a man is infertile. Many men with suboptimal sperm parameters are still able to conceive naturally. However, abnormal sperm parameters can reduce the chances of successful conception and may require medical intervention or assisted reproductive techniques.

The results of the analysis carried out to examine the effects of *Raphia Hookeri* fruit pulp on Sperm parameters indicated varying changes in all the four selected sperm parameters for this study. The results revealed that for the low and medium doses (500 and 1000mg/kg AERHF) treated rats indicated significantly ($P<0.05$) levels of sperm count when their respective values were compared to those of control and high dose (2000mg/kg AERHF) treated rats. Again, it was seen that, the high dose treated rats had significantly ($P<0.05$) raised mean sperm count when compared to that of the control group. This indicating that taking *Raphia Hookeri* fruit pulp is capable of improving sperm count when taken in high frequency when compared to the control but conversely decreasing sperm count when taken in low to medium frequency when compared to the control.
Normal Sperm count for an adult male is said to be 39 million sperm cells per ml (Pino *et al*., 2020) with the control group having a mean sperm count of 44million well above normal sperm count, though with the low and medium doses there was a significant drop in the sperm count when compared to that of the control (17, 22 million) both not indicating oligospermia (Low sperm count)(Pino *et al*., 2020). This acute drop in sperm count could be hugely attributed to the frequency of intake due to the high lipid content in the *Raphia Hookeri* fruit pulp which is bout 48% of it's nutritional content (Doungue *et al*., 2021). Lipids are capable of causing such due to what is called Dyslipidemia (Pappan and Rehman, 2021). This is caused when the LDL (low density lipoproteins) and triglycerides levels are very high and the HD (high density lipoproteins) level is too low(Pappan and Rehman, 2021). Dyslipidemia leads to oxidative stress, which is known to affect sperm Deoxyribonucleic acid (DNA) quality and in such sperm parameters and sperm count this was backed up by a study done in the 1990s, by Diaz-Fontdevila and Bustos-Obregon, 1993 which indicated a significant decrease in the ability of sperm to undergo acrosomal reaction when rabbits fed a cholesterol-enriched diet. These reports have shown that an overload of dietary cholesterol causes alteration of the acrosomal lipid domains when sperm pass through epididymal maturation, this in turn can drop sperm quality and sperm count (Saez and Drevet, 2019). This was only seen in the low to medium dose but in the high dose the active nutritional antioxidants are at a higher concentration this positively impacts sperm parameters.

These antioxidants present include Vitamin E and vitamin C present in the *Raphia Hookeri* fruit pulp which are both important antioxidants that play a role in protecting cells from oxidative damage (Egbono *et al*., 2023). There are evidences that indicates that these vitamins may have a positive impact on sperm motility and overall male fertility Vitamin E is a fat-soluble antioxidant capable of neutralizing free radicals, thus protecting cell membranes from free radicals. It also prevents the lipid peroxidation cascade (Greco, 2005) (oxidative degradation of lipids leading to cell damage) Furthermore, Vitamin E has been shown to inhibit the production of ROS (reactive oxygen species) in infertile men. Greco, 2005 reported on an intervention study of infertile men. The intervention group in this study received one gram of vitamin E and one gram of vitamin C. The extent of DNA damage was significantly reduced in the intervention group after two months. Therefore, the author stated that two months of treatment with one gram of vitamins C and E improved the intracytoplamic sperm injection success rate in patients with impaired sperm DNA damage and reduced the rate of DNA damage in these individuals (Greco, 2005). Like vitamin E, vitamin C is an antioxidant that helps counteract oxidative stress in the body, including in the reproductive system. Vitamin C has been shown to help protect sperm from oxidative damage, which can lead to improved sperm motility and viability (Greco, 2005).

The following breakdown could also be the reason for the results observed with percentage motile and active sperm cells with the low and medium doses of AERHF treated animals (i.e. Groups 2 and 3) had graded significant ($p<0.05$) decreases when compared to those of the control rats and high dose treated groups respectively). Similarly, the percentage of sluggish cells was highest in group 3 and when this amount was compared to that of group 4, it was significant ($P<0.05$). This indicates that a high intake of *Raphia Hookeri* fruit pulp could have huge effect on boosting percentage of motile sperm when compared to those of lower intake. Backing up these observations were the results of The amount of dead sperm cells which was seen to be highest in Group 2 (low dose treated) and this was followed by that of Group 3(medium dose treated); the levels of the dead cells in these groups were found to be significantly ($P<0.05$) higher when compared to those of Groups 1 and four. Though the results do not indicate any pathological condition like asthenozoospermia (low sperm motility that is above 32%) it does indicate similar outcomes with the results of sperm motility, with low dose and medium dose having a drastic decrease in sperm motility and a hike In the high dose group showing that a high frequency intake of *Raphia Hookeri* fruit can improve sperm motility. This is a can be also as explained as a result of the antioxidants Vitamin E and vitamin C present in the *Raphia Hookeri* fruit pulp. Considering the results on sperm head defects, Group 3 had the lowest presentation of the defects compared to even Group 1 (control Group).

There were no significant ($P>0.05$) variations in the percentage presentations of the tails and midpiece portions of the sperm cells. This did not indicate any significant change between the three groups and the percentage of defects is more than suitable for Fertility. Lastly, the results also indicated.The percentage values of viable and non-viable sperm cells did not vary significantly ($p>0.05$) and with all showing viability percentage fit for fertility (>58%). Although notably, the viable cells of the low dose treated animals had the highest value which was followed by that of the medium dose treated group when respectively compared to the control or untreated group and high dose treated group. This is due the number of motile sperm cell is more in the high dose and control and as such less percentage of viable sperm cells while for the medium and low dose there is less number of motile sperm cells and a such more viable sperm cells. That is the reason for little to no significant change.

Lastly there was no significant percentage change in weight ($P>0.05$) when compared to the control group indicating that *Raphia Hookeri* fruit has no peculiar effect on weight.

The results of this investigation revealed that *Raphia Hookeri* fruit can significantly have a positive effect on overall sperm quality though it needs to be taken frequently so as to not have adverse

effects on the sperm quality. It can play a huge role in positively impacting mainly sperm count and sperm motility.

Conclusion

The outcome of this investigation has been able to reveal that extract of *Raphia Hookeri* fruit pulp (mesocarp) when consumed in High rates can help boost sperm motility and sperm count significantly when compared to taking it at a low to moderate rate of consumption. That said its impact and effect is not as significant on other sperm parameters i.e sperm viability and sperm morphology. The impact of *Raphia Hookeri* Fruit Pulp on sperm motiiility and sperm count shows the effect of traditional medicine in the fight against infertility because sperm motility and sperm count is a huge factor in the fertility of the male reproductive system.

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