OPEN ACCESS

Scholars International Journal of Anatomy and Physiology
Abbreviated Key Title: Sch Int J Anat Physiol
ISSN 2616-8618 (Print) | ISSN 2617-345X (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Oxidative Stress Markers Evaluation after Prolonged Administration of Aqueous Extract of Raphia Hookeri Fruit Pulp in Male Wistar Rats

Egbono, F. F[1*], Udeh, W. C[2], Ogbonna, U. N[3]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, P.M.B 532, Port Harcourt, Nigeria
[2]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, P.M.B 532, Port Harcourt, Nigeria
[3]Lecturer, Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Nigeria

DOI: 10.36348/sijap.2024.v07i02.001 | **Received:** 18.02.2024 | **Accepted:** 25.03.2024 | **Published:** 03.04.2024

***Corresponding author:** Egbono, F. F
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, P.M.B 532, Port Harcourt, Nigeria

Abstract

The study investigated effect of prolonged administration of Raphia Hookeri fruit pulp on oxidative stress markers in male wistar rats. A total of 24 male wistar rats of weight ranging from 200g to 350g were used. The rats divided in 4 groups, 3 groups administered orally with doses of extract at 500mg/kg, 1000mg/kg and 2000mg/kg body weight while control group fed with feed and water for 28 days. The animals sacrificed, samples taken, laboratory test done for stress markers. Statistical analysis done at $p<0.05$, values expressed as SEM. Results showed Malondialdehyde (MDA) values for group 2(0.77 ± 0.32nm/ml), group 3(1.79 ± 0.73nm/ml) and group 4(3.35 ± 0.20nm/ml) while for group 1(Control group) MDA value is 4.10 ± 0.3nm/ml. Mean values for Superoxide Dismutase (SOD) in treated groups (2-4) were 18.00 ± 1.0 units/ml, 29.50 ± 8.50units/ml, and 33.50 ± 37.25units/ml respectively and mean value for control group was 68.00 ± 4.0units/ml. Mean values for Glutathione Peroxidase (GPx) in test groups (2-4) were 53.50 ± 10.50ng/ml, 63.50 ± 5.50ng/ml, and 82.50 ± 6.50ng/ml respectively. GPx value for control group was 94.00 ± 3.00ng/ml. There was a graded increase from low dose to high dose. All treated groups indicated marginally ($P>0.05$) reduced values of Percentage change in body weight when compared to the control group (Group 1). Elevated levels of MDA indicates an increased oxidative stress and lipid. Higher levels of SOD and Gpx. This research demonstrates the ability of Raphia Hookeri fruit pulp to decrease oxidative stress hence regular consumption is recommended.

Keywords: Raphia Hookeri, Oxidative, Stress markers, Aqueous Extract, Fruit pulp prolong consumption, Wistar Rats.

INTRODUCTION

Plant species belonging to the Arecaceae family (palms) have been extensively used as source of livelihood in different cultures of the world (Gruca *et al.*, 2015). They are a viable source of medicine and raw materials for the construction and beverage industries (Gruca *et al.*, 2015) Raphia Hookeri commonly known as Oghol by the people of abua', is a species native to West Africa and one of the underutilized indigenous palms in West Africa. Raphia hookeri is usually about 10 m in height and has pinnate, feather or fan-like compound leaves up to 12 m long. The plant is also monocarpic in nature, in that, it produces inflorescences that flower, set seeds and die off once the fruits are matured. The fruits of R. hookeri are usually large, cone-shaped and covered with an outer layer of scales, while the yellow seeds are characterized by tough mesocarp (Adeniyi & Akpabio, 2011).

Naturally, R. hookeri is distributed in swampy areas in tropical forests of West Africa, South-East Asia, and Latin America (Umerie, 2000). In West Africa, most especially Nigeria, virtually all the parts of the plant have found relevance in functional uses, socio-cultural activities, and as a remedy for several diseases including diabetes (Ajao *et al.*, 2021).The fruit whose pulp is considered edible in some parts of Nigeria like Rivers state, Abua/Odual LGA, Emoh Community and not edible in other parts which made its consumption rate

low or none in such parts (Egbono *et al*., 2023).The boiled fruit pulp is commonly called Ogbusi by the Abua people and mostly eaten with tapioca (processed cassava) commonly known as Ataka by the Abua people of Rivers state in Nigeria.

Raphia Hookeri fruit pulp is a good source of phytochemicals and some micronutrients and is locally consumed as a snack. In fact, the people of Emoh village in Abua/Odual LGA theorized that the ogbusi boost immunity, inhibit plasma glucose, reduce blood pressure, ameliorate fat and boost hematopoiesis (Egbono *et al*., 2023).

Oxidative stress is produced by the peroxidation and oxidation of many cell lipids, proteins, carbohydrates, and nucleic acids. Oxidative stress in any tissue results from an imbalance between the production of reactive oxygen species (ROS) and their efficient removal by available antioxidant systems (Terry T. Turner & Lysiak., 2008). Testicular oxidative stress is a significant factor contributing to male infertility and reproductive dysfunction. Increased production of ROS is caused by inflammatory injury to the male genital tract. Furthermore, pathogenic bacterial strains that inhabit the reproductive tract may induce ROS or free radical overproduction linked with inflammatory responses (Turvey & Broide, 2009). Free radicals are a class of extremely reactive molecules that have one or more unpaired electrons and can oxidatively alter biomolecules they encounter. When they react almost instantly with any chemical in their surroundings, they set off a chain reaction that causes cellular damage (Jeannette *et al*., 2000). The main ROS found in seminal plasma are superoxide, hydroxyl, and hydrogen hydroxide radicals

There is a need to create a balance between produced free radicals and its metabolism for appropriate function of testicular cells, because if the testicular biological system fails to detoxify or repair the adverse effects of free radicals, the cells and tissue are damaged seriously. In this regard, antioxidants can minimize this damage by counteracting free radicals or preventing their formation in the testicular cells. It is noteworthy that a part of the body's antioxidant defense system, called preventive antioxidant system, is related to antioxidant enzymes such as Superoxide Dismutase (SOD), catalase, and Glutathione Peroxidase (GPX) (Asadi *et al*., 2017). These antioxidants delete Reactive Oxygen Species (ROS) produced in the body's different tissues to prevent peroxidation of plasma membrane lipids (Rafieian *et al*., 2013). Vitamins such as E and C are some examples of these antioxidants. These antioxidants neutralize free radicals and prevent them from damaging the cell and tissues. Given that the antioxidants produced by the body are not able to neutralize all free radicals, then use of antioxidant supplements can play an important role in increasing the body's capacity to fight free radicals. In this research, the evaluation of oxidative stress markers status will be done in other to understand the potential effects of natural compounds such as Raphia Hookeri fruit pulp extract on physiology of male wistar rats.

METHODOLOGY

Materials

These include Syringes, Hand Gloves, Cages, Dissecting Blade, Dissecting Board, Permanent Marker, Animal Feeds, Water, Chloroform, EDTA Bottles, Cannula, 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 (24) health 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 Ayeezi 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.

Oxidative Stress Markers Laboratory Analysis

Glutathione Peroxidase (GSH-Px)

Principle

Glutathione Peroxidase (GSH-Px) can promote the reaction of hydrogen peroxidase (H_2O_2) and reduced glutathione to produce H_20 and oxidized glutathione (GSSG). The activity of the glutathione peroxidase can be expressed by the rate of enzymatic reaction. The activity of glutathione can be calculated by measuring the consumption of reduced glutathione. Hydrogen peroxide and reduced glutathione can react without catalysis of GSH-Px, so the portion of GSH reduction by non-enzymatic should be subtracted. GSH can react with Dinitrobenzoic acid to produce 5-thio-dintrobenzoic acid anion, which showed a stable yellow color.

Materials

Spectrophotometer (412nm), Micropipettor, Incubator, Vortex mixer, Centrifuge, Double distilled water, Normal saline, Phosphate, Dinitrobenzoic acid solution (DTNB), GSH standard.

Procedure:

Enzymatic Reaction

Non-enzyme tube; take 0.2ml of 1mmol/L GSH standard solution into 5ml EP tube. Enzyme tube; take 0.2ml of 1mmol/L GSH standard solution, A*ml of sample into 5ml EP tube and mix fully. (Please note; for serum or plasma, A*ml, while for tissue or cell culture supernatant, A*ml is 0.2ml)

Pre-heat the tubes at 37℃ water bath for five minutes. Pre-heat stock solution at 37℃ for five minutes at the same time.

Add 0.1ml of stock solution to the tubes and mix fully. React at 37℃ for 5minutes accurately

In Non-enzyme tube, add 2ml of acid reagent and A*ml of samples. In Enzyme tube, add 2ml of acid reagent to the tube.

Mix fully with a vortex mixer and centrifuge at 3100g for 10minutes, and take 1ml of the supernatant for chromogenic reaction. If the supernatant contains some sediments, transfer to a new EP tube and centrifuge again

Chromogenic Reaction

Non-enzyme tube: take 1ml of supernatant of non-enzyme tubes to 5ml of EP tube.

Enzyme tube: take 1ml of supernatant of enzyme tubes to 5ml of EP tube.

Blank tube: take 1ml of GSH standard application solution to 5ml of EP tube.

Standard tube: take 1ml of 20 micro mole per litre GSH standard solution to 5ml EP tube.

Add 1ml of phosphate solution, 0.25ml of DTNB solution to each tube.

Mix fully and stand for 15 minutes at room temperature. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 412nm with 1cm optical path curvature

Superoxide Dismutase (SOD)

Principle

The activity of SOD was measured by WST-1 method using the kit used. The principle of the WST-1 is as follows. Xanthine oxidase (XO) can catalyze WST-1 which reacts with O_2 to generate a water soluble formazan dye. SOD can catalyze the deproportion of superoxide anions, so the reaction can be inhibited by SOD, and the activity of SOD is negatively correlated with the amount of formazan dye. Therefore, the activity of SOD can be determined by the colorimetric analysis of WST-1 Products. (Note, WST-1 means water-soluble tetrazolium salt).

Materials

Microplate reader (440-460nm), micropipettor, multichannel pipettor, vortex mixer, incubator, reagents such as; double distilled water, normal saline or phosphate-buffered saline (PBS).

Procedure

Determine wells for diluted standard, blank and sample. Add 50 micro liter each dilution of standard, blank and sample into the appropriate wells (it is recommended that all samples and standards be assayed in duplicate, it is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations). Immediately add 50 micro liter of Biotinylated Detection Ab working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 minutes at 37℃. Note: solutions should be added to the bottom of the

micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

Decant the solution from each well, add 350 micro liter of wash buffer to each well, soak for 1 minute and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Do not allow wells to be dry.

Add 100 micro liter of HRP (Horseradish Peroxidase) working solution to each well. Cover the plate with a new sealer. Incubate for 30 minutes at 37℃.

Decant from each well, repeat the wash process for 5 times as conducted in step 2

Add 90 micro liter of substrate reagent to each well. Cover the plate with a new sealer. Incubate for about 15 minutes at 37℃. Protect the plate from light.

Add 50 micro liter of stop solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.

Determine the optical density (OD value) of each well at once with a micro-plate reader set at 450nm

Malondialdehyde (MDA)

Principle

MDA is the catabolite of lipid peroxide and can react with thiobarbituric acid (TBA) and produce red compound, which has a maximum absorption peak at 532nm.

Materials

Test tube, micropipettor, vortex mixer, centrifuge, spectrophotometer (420nm), reagents such as; clarificant, acid reagent, chromogenic agent, and 10nmol/ml standard.

Procedures:

Blank tube: Add 1ml of clarificant to the 5ml of EP tube

Standard tube: 1ml of 20 micro mile per liter of GSH standard solution to the 5ml of EP tube.

Sample tube: Add 1ml of supernatant to the 5ml of EP tube

Add 1.25ml of acid reagent application solution. 0.25ml of chromogenic agent, 0.05ml of 10nmol/ml standard to each tube

Mix fully and stand for 15minutes at room temperature. Set spectrophotometer to zero with distilled water and measure the OD values of each tube at 420 nm wavelength with 1cm optical path curvature.

Statistical Analysis

The data obtained from the present study were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 21. The values were expressed as mean ± standard error of mean.

RESULTS

The results of the present study are presented in tables and charts and were interpreted accordingly.

Table 1: Effect of administration of aqueous fruit pulp extract of Raphia hookeri (AERHF) on oxidative stress markers in male Wistar rats

Group and Treatment	Malondialdehyde (MDA) (nm/ml)	Superoxide (SOD) (units/ml)	Glutathione Peroxidase (GPx) (ng/ml)
Group 1: Control Group	4.10 ± 0.3	68.00 ± 4.0	94.00 ± 3.00
Group 2: Low Dose treated (500mg/kg b.w AERHF)	0.77 ± 0.32 a	18.00 ± 1.00 a	53.50 ± 10.50 a
Group 3: Medium Dose treated (1000mg/kg b.w AERHF)	1.79 ± 0.73 a	29.50 ± 8.50 a	63.50 ± 5.50 a
Group 4: High Dose treated (2000mg/kg b.w AERHF)	3.35 ± 0.20 b	33.50 ± 37.25 a	82.50 ± 6.50 b

Values represent mean ± SEM, n=3; a Significant at $p<0.05$ compared to Group 1; b Significant at $p<0.05$ when compared to group 2; c Significant at $p<0.05$ when compared to group 3.

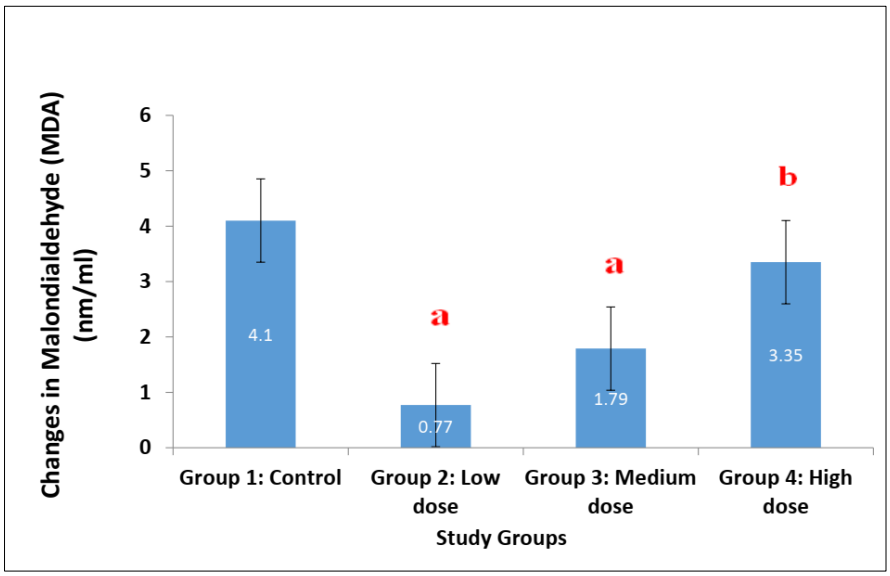

Figure 1: Effect of administration of aqueous extract of Raphia hookeri fruit pulp (AERHF) on Malondialdehyde (MDA) (nm/ml) level in male Wistar rats

Values represent mean ± SEM, n=3; a Significant at p<0.05 compared to Group 1; b Significant at p<0.05 when compared to group 2; c Significant at p<0.05 when compared to group 3.

Key:
Group 1: Control Group
Group 2: Low Dose treated (500mg/kg b.w AERHF)
Group 3: Medium Dose treated (1000mg/kg b.w AERHF)
Group 4: High Dose treated (2000mg/kg b.w AERHF)

The data shown on Figure 4.1represent the result of the effect of administration of aqueous extract of raphia hookeri fruit pulp (AERHF) on malondialdehyde (MDA) level in male Wistar rats.

The low and medium doses treated groups were found to have significantly (p<0.05) reduced when compared to that of the control group. The MDA level in the high dose treated group was seen to be significantly (P<0.05) raised when compared to that of the low dose treated group. Notably too, it was observed that the MDA levels in the treated groups had dose-dependent increase, even if these increases were not uniformly significant.

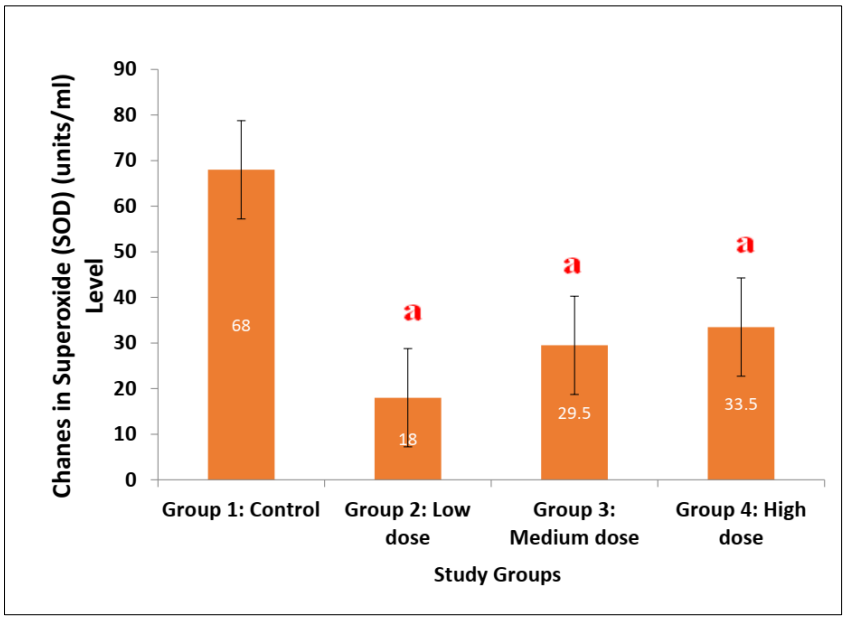

Figure 2: Effect of administration of aqueous extract of Raphia hookeri fruit pulp (AERHF) on Superoxide (SOD) (units/ml) level in male Wistar rats

Values represent mean ± SEM, n=3; a Significant at p<0.05 compared to Group 1; b Significant at p<0.05 when compared to group 2; c Significant at p<0.05 when compared to group 3.

Figure 2 shows the outcome of the effect of administration of extract of raphia hookeri fruit pulp (AERHF) on superoxide (SOD) level in male wistar rats.

All treated groups indicated significant (P<0.05) decreases in their SOD levels when compared to the control/untreated group. There were graded increases in the levels of SOD from low to high doses treated groups; although, these were not statistically significant (P>0.05).

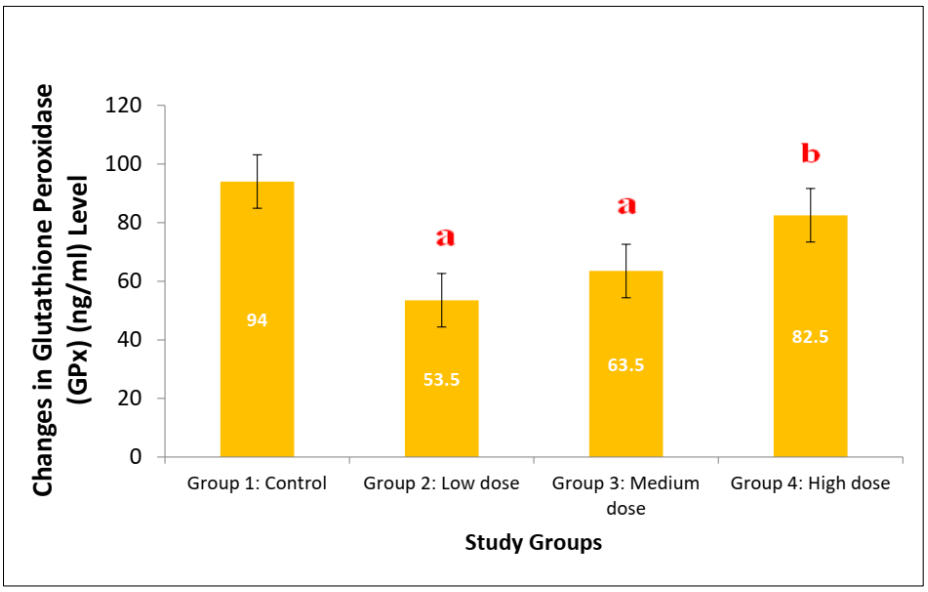

Figure 3: Effect of administration of aqueous fruit pulp extract of Raphia hookeri (AERHF) on Glutathione Peroxidase (GPx) (ng/ml) level in male Wistar rats

Values represent mean ± SEM, n=3; a Significant at p<0.05 compared to Group 1; b Significant at p<0.05 when compared to group 2; c Significant at p<0.05 when compared to group 3.

The result on Figure.1 is the effect of administration of aqueous extract of raphia hookeri fruit pulp (AERHF) on glutathione peroxidase (GPx) level in male Wistar rats.

The low and medium doses treated groups were seen to be significantly (p<0.05) decreased when compared to that of the control group. The GPx level in the high dose treated group was seen to be significantly (P<0.05) raised when compared to that of the low dose treated group. Also too, it was noted that the GPx levels in the treated groups had dose-dependent increase, although these increases were not regularly significant.

Statistical Analysis of Weights of Rats

This section presents the result of the present study with regards to the weight of rats in tables and charts and with the appropriate interpretations. Version 21.0 of the IBM Statistical Product and Service Solutions (SPSS) software was used to analyze the quantitative data obtained from the present study. One-way analysis of variance (ANOVA) followed by LSD Post Hoc tool were used to establish statistical significance, and P value less than 0.05 (P<0.05) indicated the threshold for statistical significance. Mean and standard error of the mean (SEM) were used to represent the values.

Determination of Percentage Change in Body Weight

Determination of Percentage Change in Body Weight

% Change in Body weight = Final Body weight – Initial Body Weight. X 100

Initial Body Weight

Table 2: Effect of administration of aqueous extract of Raphia hookeri fruit pulp (ARERH) 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 ARERH)	0.59 ± 1.91
Group 3: Medium Dose treated (1000mg/kg b.wAERHF)	5.44 ± 2.32
Group 4: High Dose treated (2000mg/kg b.w AERHF)	5.33 ± 1.16

Values represent mean ± SEM, n=8-females; 3-males; a Significant at p<0.05 compared to Group 1; b Significant at p<0.05 when compared to group 2; cSignificant at p<0.05 when compared to group 3

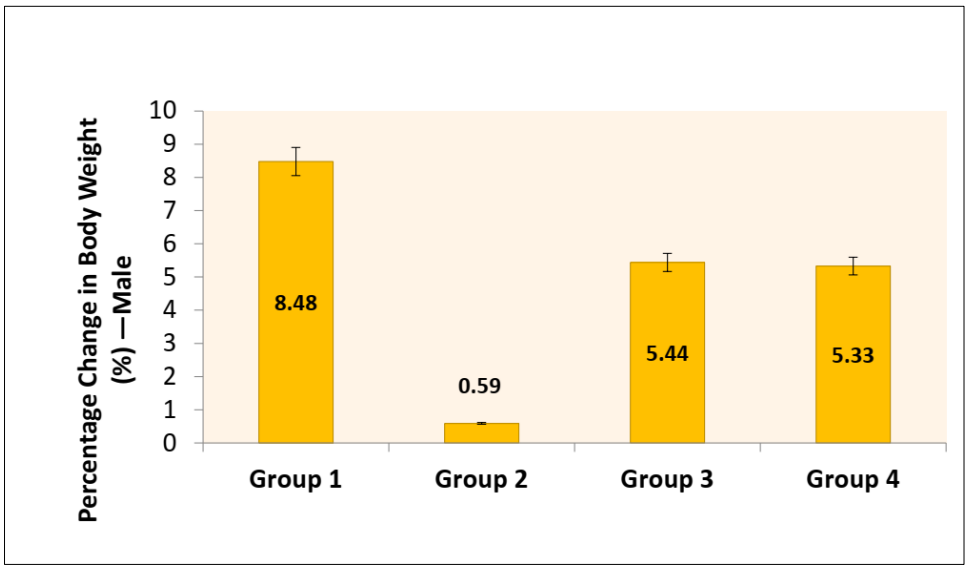

Figure 4: Effect of administration of aqueous extract of Raphia hookeri fruit pulp (AERHF) on Percentage Change in Body Weight of Male Wistar Rats

Values represent mean ± SEM, n=3; a Significant at p<0.05 compared to Group 1; b Significant at p<0.05 when compared to group 2; cSignificant at p<0.05 when compared to group 3.

The data on Figure 4 represent the effects of administration of aqueous extract of Raphia hookeri fruit pulp (AERHF) on the percentage change in body weight male and wistar rats.

Amongst the male Wistar rats, all treated groups indicated marginally (P>0.05) reduced values of Percentage change in body weight when compared to that of the control group (Group 1). Within the treated groups, the variation in percentage change in body weight was not statistically significant (P>0.05) when each were compared to the others and the variation did not follow any significant pattern.

DISCUSSION

The results of the laboratory analysis carried out to investigate the effect of administration of fruit extract of raphia hookeri (AERHF) on some oxidative stress markers in male wistar rats revealed that the levels of MDA, SOD, and GPx were reduced in groups 2, 3, and 4 as compared to the control group. The outcome of the extract treatments on MDA levels indicated that groups 2 and 3 had a significant (p<0.05) decrease when compared to group 1. The MDA levels in group 4 was seen to be significantly (P<0.05) raised when compared to that of group 2. Malondialdehyde (MDA) is a highly reactive compound that is derived from the breakdown of polyunsaturated fatty acids. It is often used as a biomarker to assess oxidative stress. High level of MDA indicates an imbalance between production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. High level of MDA resulting from increased oxidative stress and lipid peroxidation have been associated with impaired sperm quality, infertility, erectile dysfunction as well as other conditions such as; alcoholic liver disease, hypertension, atherosclerosis, kidney diseases and cancer. So decreased levels of MDA as shown from the results of the research could be as a result of vitamins C and E which are present within the raphia hookeri fruit pulp that serve as an antioxidant in reducing reactive oxygen species and thus in reducing oxidative stress. Ascorbic acid, commonly known as vitamin C, is a potent antioxidant that can help reduce oxidative stress. Studies have shown that vitamin C supplementation can improve sperm parameters, including sperm count, motility, and morphology. It also can potentially reduce DNA damage in sperm cells, contributing to better reproductive outcomes (Hajjar *et al*., 2020).

Vitamin E is a fat-soluble vitamin existing in eight different forms (Schmölz, 2016).α-tocopherol is the most effective antioxidant of the tocopherols and is also the plentiful in humans. The main function of Vitamin E is to protect against lipid peroxidation, and there is also evidence to suggest that α-tocopherol and ascorbic acid function together in a cyclic-type of process. During the antioxidant reaction, α-tocopherol is converted to an α-tocopherol radical by the donation of a variable hydrogen to a lipid or lipid peroxyl radical, and the α-tocopherol radical may hence be reduced to the original α-tocopherol form by ascorbic acid (Kurutas, 2016).

Various studies have showcased the effects of both vitamins C and E in reducing oxidative stress. For instance, the results from a research by (Chen *et al*., 2001) on adult stroke prone-spontaneously hypertensive rats (SHRSP) described how both vitamins C and E could improve vascular function and structure and prevent the progression of hypertension by modulating activity of NADPH oxidase and superoxide dismutase (SOD). Another study by (Greco *et al*., 2005) stated that two-month treatment with 1gr vitamin E and C improved intra-cytoplasmic sperm injection (ICSI) success rate in patients with sperm DNA damage and reduced the level of DNA damage in these individuals. Therefore, it can be said that both vitamins C and E inhibit the production of reactive oxygen species and thus reduce oxidative stress in infertile men.

However, while increased MDA levels are generally associated with detrimental effects, excessively low levels of MDA may also have implications for health such as impaired oxidative stress response or disease implications like Alzhemer's disease.

Considering the outcome of the extract treatment on both SOD and GPx levels in the study animals, there was significant ($P<0.05$) decreases in their SOD and GPx levels when compared to the control/untreated group. There were graded increases in the levels of SOD from low to high doses treated groups; although, these were not statistically significant ($P>0.05$). While for GPx, the GPx level in the high dose treated group was seen to be significantly ($P<0.05$) raised when compared to that of the low dose treated group. SOD stands for Superoxide Dismutase, which is an enzyme that plays a crucial role in protecting cells from damage caused by ROS. SOD works by catalyzing the conversion of superoxide radicals, a type of ROS into hydrogen peroxide and molecular oxygen which are less harmful. There are three main forms of SOD found in humans: copper-zinc SOD (Cu/ZnSOD), manganese SOD (MnSOD). And extracellular SOD (Asakura & Kitara, 2013). Each form is localized in different compartments and tissues. A decrease in SOD levels suggest a decreased ability to neutralize harmful reactive oxygen species and protect against oxidative stress. Note SOD is primarily an intracellular enzyme and is typically not found in high concentrations in the blood stream. However, certain conditions can lead to increased release of SOD in the extracellular space. One such condition is acute lung injury or acute respiratory distress syndrome (ARDS).

GPx stands for glutathione peroxidase, which is an enzyme that plays a crucial role in the antioxidant defense system of cells. It functions by catalyzing the reduction of hydrogen peroxide using glutathione as a cofactor. This process helps protects cells from oxidative damage caused by ROS. GPx helps to maintain balance between oxidative stress and antioxidant defense system. A decrease in Gpx may indicate reduced antioxidant defense against oxidative stress.

There was a reduction in body weight of rats in the treated groups (2-4) when compared to the control group. However, this was not statistically significant ($P>0.05$).

From this research, levels of all 3 oxidative stress markers are being reduced, this might suggest that the body is experiencing a shift in its oxidative stress response. It is possible that other factors such as different antioxidants or enzymatic systems are compensating for the decreased levels in SOD and Gpx.

The finding of this work has revealed a possible remedy in reducing oxidative stress that arises due to increase in reactive oxygen species. Hence, consumption is recommended regularly but in moderate consumption.

CONCLUSION

The outcome of this investigation has been able to reveal that extract of raphia hookeri fruit pulp (mesocarp) when consumed in low or moderate intervals regularly can help reduce oxidative stress which can help improve cellular health, reduce inflammation, improve male reproductive system and general body performance.

REFERENCES

- Adeneyi, A. A., & Akpabio, U. D. (2011). Nutritional potential of hard seed of Raphia Hookeri. *Obbeche J*, *29*(2), 366-369.
- Afolayan, A. O., Borokini, T. I., & Afolayan, G. O. (2014). Sublethal effects of methanolic extract of Raphia hookeri on the reproductive capacity of Clarias gariepinus. *Advances in Zoology*, *2014*.
- Agarwal, A., Parekh, N., Selvam, M. K. P., Henkel, R., Shah, R., Homa, S. T., ... & Harlev, A. (2019). Male oxidative stress infertility (MOSI): proposed terminology and clinical practice guidelines for management of idiopathic male infertility. *The world journal of men's health*, *37*(3), 296.
- Aitken, R. J., & Clarkson, J. S. (1987). Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. *Reproduction*, *81*(2), 459-469.
- Aitken, R. J., & Drevet, J. R. (2020). The importance of oxidative stress in determining the functionality of mammalian spermatozoa: a two-edged sword. *Antioxidants*, *9*(2), 111.
- Ajao, A. A. N., Moteetee, A. N., & Sabiu, S. (2021). From traditional wine to medicine: phytochemistry, pharmacological properties and biotechnological applications of Raphia hookeri G. Mann & H. Wendl (Arecaceae). *South African journal of botany*, *138*, 184-192.

- Anderson, J. B., & Williamson, R. C. N. (1990). Fertility after torsion of the spermatic cord. *British journal of urology*, *65*(3), 225-230.
- Asadi, N., Bahmani, M., Kheradmand, A., & Rafieian-Kopaei, M. (2017). The impact of oxidative stress on testicular function and the role of antioxidants in improving it: a review. *Journal of clinical and diagnostic research: JCDR*, *11*(5), IE01.
- Asadi, N., Bahmani, M., Kheradmand, A., & Rafieian-Kopaei, M. (2017). The impact of oxidative stress on testicular function and the role of antioxidants in improving it: a review. *Journal of clinical and diagnostic research: JCDR*, *11*(5), IE01.
- Asakura, H., & Kitahora, T. (2013). Antioxidants in inflammatory Bowel Disease, Ulcerative Colitis, and Crohn Disease. In *Bioactive Food as Dietary Interventions for Liver and Gastrointestinal Disease* (pp. 37-53). Academic Press.
- Asmat, U., Abad, K., & Ismail, K. (2016). Diabetes mellitus and oxidative stress—A concise review. *Saudi pharmaceutical journal*, *24*(5), 547-553. https://doi.org/10.1016/j.jsps.2015.03.013.
- Chen, X., Touyz, R. M., Park, J. B., & Schiffrin, E. L. (2001). Antioxidant Effects of Vitamins C and E Are Associated With Altered Activation of Vascular NADPH Oxidase and Superoxide Dismutase in Stroke-Prone SHR. *Hypertension*, *38*(3), 606–611. https://doi.org/10.1161/hy09t1.094005
- Colacurci, N., Gaia, M. M., Izzo, G., Izzo, P., Trotta, C., Mele, D., & De Franciscis, P. (2012). Recombinant Human FSH Reduces Sperm DNA Fragmentation in Men With Idiopathic Oligoasthenoteratozoospermia. *Journal of Andrology*, 588-593.
- Dada, F. A., Oyeleye, S., Ogunsuyi, O. B., Olasehinde, T. A., Adefegha, S. A., Oboh, G., & Boligon, A. A. (2017). Phenolic constituents and modulatory effects of Raffia palm leaf (Raphia hookeri) extract on carbohydrate hydrolyzing enzymes linked to type-2 diabetes. *Journal of Traditional and Complementary Medicine*, 494-500.
- Darbandi, M., Darbandi, S., Agarwal, A., Sengupta, P., Durairajanayagam, D., Henkel, R., & Sadeghi, M. R. (2018). Reactive oxygen species and male reproductive hormones. *Reproductive Biology and Endocrinology*, *16*, 1-14.
- Dobrzyńska, M. M., Baumgartner, A., & Anderson, D. (2004). *Antioxidants modulate thyroid hormone-and noradrenaline-induced DNA damage in human sperm*. Mutagenesis, 325-330.
- Dos Santos, A. A., Ferrer, B., Gonçalves, F. M., Tsatsakis, A. M., Farina, M., Renieri, E. A., & Rocha, J. (2018). *Oxidative Stress in Methylmercury-Induced Cell Toxicity*. Toxics, 10.3390/toxics6030047.
- Egbono, F. F. (2023). Liver Enzymes Functions Effect of Raphia Hookeri Fruit Pulp (Mesocarp) Extract in Male Wistar Rat‟s Model. *Scholars International Journal of Biochemistry*, 99-105.
- Egbono, F. F., Mene, A. E., & Nwiko, K. M. (2023). Liver Enzymes Functions Effect of Raphia Hookeri Fruit Pulp (Mesocarp) Extract in Male Wistar Rat‟s Model. *Scholars International Journal of Biochemistry*, 99-105.
- Fiorillo, C., Argento, F. R., Becatti, M., Mannucci, A., Fini, E., Coccia, M. E., & Taddei, N. (2021). The Impact of Oxidative Stress in Male Infertility. *Frontier of Molecular Biosciences*.
- García-Díaz, E. C., Enrique, G. Q. L., Arenas, R. E., Aragón-Martínez, A., Ibarra-Arias, J. A., Retana-Márquez, & Socorro, M. d. (2015). Oxidative status in testis and epididymal sperm parameters after acute and chronic stress by cold-water immersion in the adult rat. *Systems Biology in Reproductive Medicine*, 150-160.
- Ghezzi, P., Jaquet, V., Marcucci, F., & Schmidt, H. W. (2017). The oxidative stress theory of disease: levels of evidence and epistemological aspects. *British Journal of Pharmacology*, 1784-1796.
- Gireeja, W., Gaurkar, S. S., Prasad, R., Lohakare, T., & Wanjari, M. (2023). The Impact of Oxidative Stress on Male Reproductive Function: *Exploring the Role of Antioxidant Supplementation*. Cureus, e42583.
- Greco, E., Romano, S., Iacobelli, M., Ferrero, S., Baroni, E., Minasi, M. G., Ubaldi, F., Rienzi, L., &Tesarik, J. (2005). ICSI in cases of sperm DNA damage: beneficial effect of oral antioxidant treatment. *Human Reproduction*, *20*(9), 2590–2594. https://doi.org/10.1093/humrep/dei091
- Hajjar, T., Soleymani, F., &Vatanchian, M. (2020). Protective Effect of Vitamin C and Zinc as an Antioxidant against Chemotherapy-Induced Male Reproductive Toxicity. *Journal of Medicine and Life*, *13*(2), 138–143. https://doi.org/10.25122/jml-2019-0107
- Hanukoglu, I. (2006). Antioxidant Protective Mechanisms against Reactive Oxygen Species (ROS) Generated by Mitochondrial P450 Systems in Steroidogenic Cells. *Drug Metabolism Reviews*, 171-196.
- Ikeda, M., Kodama, H., Fukuda, J., Shimizu, Y., Murata, M., Kumagai, J., & Tanaka, T. (1999). Role of radical oxygen species in rat testicular germ cell apoptosis induced by heat stress. *Biology of reproduction*, *61*(2), 393-399.
- Jan, A. T., Mudsser, A., Arif, A., Choi, I., Kehkashan, S., & Qazi , R. M. (2015). Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. *International Journal of molecular Sciences*, 29592–29630.
- Jarow, J. P., Sharlip, I. D., Belker, A. M., Lipshultz, L. I., Sigman, M., Thomas, A. J., Schlegel, P. N., Howards, S. S., Nehra, A., Damewood, M. D., Overstreet, J. W., &Sadovsky, R. (2002). Best

Practice Policies for Male Infertility. *Journal of Urology, 167*(5), 2138–2144. https://doi.org/10.1016/s0022-5347 (05)65109-9

- Jewell, T. (2018, may 29). Healthline. Retrieved from Health: https://www.healthline.com/human-body-maps/testis
- Keshari, A. K., Akhilesh , V. K., Tarun , K., & Srivastava, R. (2015). Oxidative Stress: A Review. *The International Journal of Science & Technoledge*, 155-161.
- Kozarski, M., Klaus, A., Jakovljevic, D., Todorovic, N., Vunduk, J., Niksic, M., & Vrvic, M. (2015). Antioxidants of Edible Mushrooms. *Oprn Access Journal/ molecules*, doi:10.3390/molecules201019489.
- Kurutas, E. B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. *Kurutas Nutrition Journal*.
- Lipinski, B. (2001). Pathophysiology of oxidative stress in diabetes mellitus. *Journal of Diabetes and Its Complications, 15*(4), 203–210. https://doi.org/10.1016/s1056-8727(01)00143-x
- Luo, L., Chen, H., M. A., Show, M. D., Anway, M. D., & Zirkin, B. R. (2006). Aging and the Brown Norway rat Leydig Cell Antioxidant Defense System. *Journal of Andrology*, 240-247.
- Mallikarjuna M. Reddy, S. V., Subhashini, J., Madhava C. Reddy, K. R., & Pallu Reddanna. (2006). Bacterial lipopolysaccharide-induced oxidative stress in the impairment of steroidogenesis and spermatogenesis in rats. *Reproductive Toxicology*, 493-500.
- Mancini, A., Roberto, F., Silvestrini, A., Nicolotti, N., Di Donna, V., la Torre, G., & Meucci, E. (2009). Hormonal Regulation of Total Antioxidant Capacity in Seminal Plasma. *Jounal of Andrology*, 534-530.
- Mannucci, A., Argento, F. R., Fini, E., Coccia, M. E., Taddei, N., Becatti, M., &Fiorillo, C. (2022). The Impact of Oxidative Stress in Male Infertility. *Frontiers in Molecular Biosciences, 8*. https://doi.org/10.3389/fmolb.2021.799294
- Maritim, A. C., Sanders, R. A., & Watkins, J. B. (2003). Diabetes, oxidative stress, and antioxidants: A review. *Journal of Biochemical and Molecular Toxicology, 17*(1), 24–38. https://doi.org/10.1002/jbt.10058
- Marta, G., Blach-Overgaard, A., & Balslev, H. (2015). African palm ethno-medicine. *Journal of Ethnopharmacology*, 227-237.
- Mbaka, G. O., Ogbonnia, S. O., Olarewaju, O. T., & Duru, F. T. (2013). The effects of ethanol seed extract of Raphia hookeri (Palmaceae) on exogenous testosterone and estradiol induced benign. *Journal of Morphological Sciences*, 235-243.
- Mbaka, G. O., Ogbonnia, S. O., Oyeniran, K. J., & Awopetu, P. I. (2012). Effect of Raphia hookeri Seed Extract on Blood Glucose, Glycosylated Haemoglobin and Lipid Profile of Alloxan Induced Diabetic Rats. *British Journal of Medicine and Medical Research*, 621-635.
- Meucci, E., Milardi, D., Mordente, A., Giacchi, E., Marinis, L. D., & Mancini, A. (2003). Total antioxidant capacity in patients with varicoceles. *Reproductive Biology*, 1577-1583.
- Najla , A., El-Masry, T. A., Alotaibi, B., Tousson, E., & Asmaa, S. (2020). Therapeutic effects of rocket seeds (Eruca sativa L.) against testicular toxicity and oxidative stress caused by silver nanoparticles injection in rats. *Environmental Toxicology*.
- Nasri, H., & Rafieian-Kopaei, M. (2014). Protective effects of herbal antioxidants on diabetic kidney disease. *Journal of Research in Medical Science*, 82-23.
- Ngoula, F., Lontio, F. A., Tchoffo, H., Manfo Tsague, F. P., Djeunang, R. M., Vemo, B. N., ... & Djuissi Motchewo, N. (2020). Heat induces oxidative stress: reproductive organ weights and serum metabolite profile, testes structure, and function impairment in male cavy (Cavia porcellus). *Frontiers in Veterinary Science*, *7*, 37.
- Nowicka-Bauer, K., & Nixon, B. (2020). Molecular changes induced by oxidative stress that impair human sperm motility. *Antioxidants*, *9*(2), 134.
- Owoeye, O., Awoyemi, F. O., & Ajiboye, E. O. (2018). Ameliorative effects of Raffia hookeri pulp extract on cisplatin-induced brain damage and consequent neurobehavioural changes in Wistar rats. *Nigerian Journal of Physiological Sciences*, *33*(1), 75-82.
- Palomba, S., Falbo, A., Espinola, S., Rocca, M., Capasso, S., Cappiello, F., & Zullo, F. (2011). Effects of highly purified follicle-stimulating hormone on sperm DNA damage in men with male idiopathic subfertility: a pilot study. *Journal of endocrinological investigation*, *34*, 747-752.
- Potts, J. M., Sharma, R., Pasqualotto, F., Nelson, D., Hall, G., & Agarwal, A. (2000). Association of Ureaplasma Urealyticum with Abnormal Reactive Oxygen Species Levels and Absence of Leukocytospermia. *Journal of the American Urological Association*, 1775-1778.
- Rafieian-Kopaei, M., Baradaran, A., & Rafieian, M. (2013). Plants antioxidants: From laboratory to clinic. *Journal of nephropathology*, 152-153.
- Rivier, C., & Rivest, S. (1991). Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. *Biology of reproduction*, 523-532.
- Sarica, K., Küpeli, B., Budak, M., Koşar, A., Kavukçu, M., Durak, İ., & Göğ; üs, O. (1997). Influence of experimental spermatic cord torsion on the contralateral testis in rats: Evaluation of tissue free oxygen radical scavenger enzyme levels. *Urologia internationalis*, *58*(4), 208-212.

- Schmölz, L. (2016). Complexity of vitamin E metabolism. *World Journal of Biological Chemistry*, *7*(1), 14. https://doi.org/10.4331/wjbc.v7.i1.14
- Shankar, K., & Mehendale, H. M. (2014). Oxidative stress. *In Encyclopedia of Toxicology* (pp. 735-737). Academic Press. Retrieved from Science Direct: https://www.sciencedirect.com/science/article/abs/pii/B9780123864543003456
- Sharifi-Rad, M., Anil Kumar, N. V., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., ... & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Frontiers in physiology*, *11*, 552535.
- Syntin, P., Chen, H., Zirkin, B. R., & Robaire, B. (2001). Gene expression in Brown Norway rat Leydig cells: effects of age and of age-related germ cell loss. *Endocrinology*, *142*(12), 5277-5285.
- Tesarik, J., Martinez, F., Rienzi, L., Iacobelli, M., Ubaldi, F., Mendoza, C., & Greco, E. (2002). In-vitro effects of FSH and testosterone withdrawal on caspase activation and DNA fragmentation in different cell types of human seminiferous epithelium. *Human Reproduction*, *17*(7), 1811-1819.
- Turner, T. T., & Lysiak, J. J. (2008). Oxidative stress: a common factor in testicular dysfunction. *Journal of andrology*, *29*(5), 488-498.
- Turvey, S. E., & Broide, D. H. (2009). Innate Immunity. *Journal of Allergy and Clinical Immunology*, 24-32.
- Umerie, S. C. (2000). Caramel production from saps of African oil palm (Elaeis guineensis) and wine palm (Raphia hookeri) trees. *Bioresource technology*, *75*(2), 167-169.
- Utiger, R. D. (2923, August 14). Britannica. Retrieved from Britannica: https://www.britannica.com/science/testis
- WikiLectures. (2022, april 6). Retrieved from wikilectures: https://www.wikilectures.eu/w/Oxidative_stress_theory
- Zheng, F., Wang, Y. Z., Wu, Y. X., Zhang, M. Y., Li, F. T., He, Y., ... & Yue, H. (2021). Effect of stabilization malvids anthocyanins on the gut microbiota in mice with oxidative stress. *Journal of Food Biochemistry*, *45*(12), 4892-4902.
- Zini, A., & Schlegel, P. N. (2003). Effect of hormonal manipulation on mRNA expression of antioxidant enzymes in the rat testis. *The Journal of urology*, *169*(2), 767-771.