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Physiologic Investigation of frequent Administration of Aqueous Extract of Raphia Hookeri Fruit Pulp on Selected Reproductive Hormones in Female Wistar Rats

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Abstract: *This research is aimed at evaluating the effect of frequent consumption of aqueous extract of raphia hookeri fruit pulp on selected reproductive hormones in female Wistar rats. A total of 32 apparently healthy rats weighing 130g to 180g grouped into 4, group1 as control fed with distilled water and feed, group 2 given 500mg/kg, group3 given 1000mg/kg, group 4 given 2000mg/kg body weight of the extract for 28days. Animals sacrificed and blood samples taken for laboratory analysis,results analyzed using statistical tool SPSS version 21.0, one-way ANOVA,P<0.05 considered significant, values expressed as mean, standard error of mean (SEM). Outcome displayed significant (P>0.05) reduction in Estrogenlevels in groups 2 (17.65±1.25) and 3 (23.00±0.00), but group 4 (43.15±3.85) showed a significant increase when compared to control (37.00±4.00). Follicle Stimulating Hormonelevels in all treated groups did not indicate significant (P>0.05) changes when compared to the control group (2.10±0.30), however, group 4 (3.45±1.15) showed a significant increase when compared to groups 2 (0.65±0.25) and 3 (1.00±0.10). No significant (P>0.05) variations in luteinizing hormone levels when treated groups compared to the control group (1.40±0.30), only group 4 (2.25±0.15) indicated significant (P<0.05) increase when compared to groups 2 (0.55±0.45) and 3 (1.35±0.15). Hence, it can be inferred that the frequent consumption of aqueous extract from Raphia Hookeri fruit pulp has the potential to alleviate female reproductive disorders associated with hormonal imbalances or dysfunctions*

Keywords: Raphia Hookeri

I. INTRODUCTION

Raphia Hookeri is a palm species native to Western and Central Africa, where it grows in lowland coastal freshwater swamps and river banks. This plant is classified under the palm tree and belongs to the family of Palmae or Palmacea which originated from tropical West Africa where it extended (Imogie et al., 2008). Raphia Hookeri grows to a height of 9m and is adapted to live with its roots in water-logged soil, by possessing breathing roots (Okwu and Nnamdi, 2008). It is a monocarpic plant that produces inflorescence only once and then dies. It is grown mainly for its production of palm wine. Raphia Hookeri is considered to be one of the most economically valuable plants in Africa. The plant is highly valued by locals, who use every part of it for a variety of purposes. It also bears fruits (Raphia

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Hookeri) whose pulp is considered edible in some parts of the country and not edible in other parts(Ogbuagu, 2008).The fruits of this plant can be consumed after boiling, they are toxic if eaten raw. Raphia fruit pulp is a good source of phytochemicals and some micronutrients and is locally consumed as a snack (Tata et al., 2023). Its fruit is large, cone-shaped with a single hard nut having an outer layer of overlapping reddish brown scales and in-between the outer layer of scales and the hard seed is a yellow, mealy, oil-bearing mesocarp or pulp (Mbaka et al., 2013). Similarly, Ndon (2003), described Raphia Hookeri fruit as the large, cone-shaped with a hard nut having an outer layer of rhomboid-triangular and overlapping reddish-brown scales.Between the outer layer and the seed, is a yellow, oil-bearing mesocarp or pulp (Ndon B. A., 2003). The fruit is poisonous when raw and is crushed for use as a fish poison. However, the oily mesocarp of the fruit can be boiled and eaten as a vegetable or used as a laxative and liniment for pains (Facciola, 1998).

According to Egbono et al. (2023), the ripe boiled Raphia Hookeri fruits pulp locally called 'Ogbusi' by Abua people in the Abua/Odual local government area of Rivers State, Niger Delta region, Southern Nigeria, is usually soaked in water or stored in the refrigerator to maintain nutrients and commonly consumed with tapioca. The inhabitants of Emoh village in Abua and the people of Abua/Odual LGA hypothesized that the Ogbusi boost immunity, inhibit plasma glucose, reduce blood pressure, ameliorate fat, and boost hematopoiesis, etc (Egbono, et al., 2023).The pulp extract of Raphia Hookeri was shown to contain vitamins C and E, carotenes, niacin, alkaloids, saponins, flavonoids, and phenols which explains its antioxidant activity (Edem et al., 1984; Akpan et al., 2004; Dada et al., 2017). The health benefits of the plant may be attributed to the various biological constituents and chemical compounds it contains.However, the effect of Raphia Hookeri fruit pulp on female reproductive hormones is not well known. Bioactivecompoundscontainedin Raphia Hookeri fruit pulp mayalsohavesome beneficial effectson female reproductivehormones. This leads to the evaluation of the nutritional value of Raphia Hookeri fruit on selected female reproductive hormones by using female Wistar rats. However, there is limited scientific evidence on the possible effects of the Raphia Hookerifruit pulp on the reproductive hormones of females. Due to the lack of awareness and appreciation of its benefits, the fruits are often discarded or left to rot. This not only leads to wastage of valuable resources but also threatens the survival of the species, which is already endangered by habitat loss and overexploitation. Therefore, there is an urgent need to raise awareness and promote the utilization of Raphia Hookeri fruit pulp as it could have potential benefits on selected female reproductive hormones, hence this investigation.

II. MATERIALS

The following materials were used for this research study; Animal cages, feeding and drinking plates, brooms and parker, disinfectants, animal feed, laboratory coats, hand sanitizers,nose mask, masking tape, weighing balance, baskets, hand gloves, water, hand towel, syringe, dry sawdust, and cannula.

ANIMAL PREPARATION

A total of thirty-two (32) apparently healthy female Wistar rats of weights ranging between 130g and 180g were used for this study. These rats were all housed in the preclinical animal house in the Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The animals were maintained in a well-ventilated animal house under optimum conditionsof humidity, temperature and natural light-dark cycle and were allowed free access to food and water. The experimental protocols and procedures used conform to the international guidelines for the care and use of animals in research and teaching. (American Physiological Society, 2002).

ACCLIMATIZATION OF THE ANIMAL

The animals were placed on a scale for weight measurement. They were then accommodated in a cage that maintained a 12-hour cycle of light and darkness, to adapt to the environmental conditions of the University of Port Harcourt's animal house, the rats underwent a period of acclimatization lasting fourteen (14) days, the study was generally conducted in accordance with recommendation from the 1983 declaration of Helsinki on guiding principles in the care and use of animals.

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COLLECTION AND PREPARATION OF PLANT MATERIALS

Collection of Extracts

The aqueousextract which was gotten Raphia Hookeri Fruit was purchased from Emoh community, Okpeden (ward 8) in Abua/ Odual Local Government Area, Rivers State, Niger Delta region, the southernpart of Nigeria.

Preparation of Raphia Hookeri Fruit Extract

The maceration technique was employed, the mesocarp (fruit pulp), was air-dried to preserve the active ingredients, then it was finally crushed and immersed in a maceration jar. Approximately 1000 grams of the extract was dissolved in 2000 milliliters of water and left to stand for 72 hours as continuous agitation was applied during this period to ensure a high yield. The mixture was then filtered, and the filtrate was placed on a water bath to evaporate the liquid content at a temperature of 65 degrees Celsius. After evaporation, the weight of the extract was recorded and it was stored for future use.

ADMINISTRATION OF THE EXTRACT

The Raphia Hookeri fruit aqueous extract was orally administered via a syringe and cannula to the rats for twenty-eight (28) days.

STUDY DESIGN

A total of thirty-two (32) healthy female Wistar rats weighing 130g to 180g were used for this study. The animals were divided into two major groups; Control group and Test group

The Test groups were further divided into 3 groups, each of the 3 groups contained eight animals in each cage compartment.

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 female Wistar rats were not given extract beyond 2000mg/kg body weight:

Mode of Administration

GROUP	DOSE OF EXTRACT
Group 1 (control)	Distilled water and feed only
Group 2	Low dose 500mg/kg body weight of extract
Group 3	Moderate dose 1000mg/kg body weight of extract
Group 4	High dose 2000mg/kg body weight of extract

SAMPLING METHOD

The rats were euthanized under the influence of diethyl ether anaesthesia. Bloodwas then drawn using syringes and needles through a venous puncture. The collected samples weresubsequently transferredappropriately into well labelled Ethylenediaminetetraacetic acid (EDTA) bottles for further laboratory analysis.

III. LABORATORY TESTS AND ANALYSIS

The following laboratory tests were carried out:

Estimation of Estrogen (Estradiol or E2)

Principle of the Test

The Estradiol (E2) enzyme immunoassay (EIA) is based on the principle of competitive binding between E2 in the test specimen and E2-HRP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti-rabbit IgG-coated wells are incubated with E2 standards, controls, patient samples, Estradiol-HRP Conjugate Reagent and rabbit anti-Estradiol reagent at room temperature for 90 minutes. During the incubation, a fixed amount of HRP-labeled E2

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competes with the endogenous E2 in the standard, sample, or quality control serum for a fixed number of binding sites of the specific E2 antibody. E2 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Unbound E2 peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance.

Components

Materials for the Estimation of Estrogen (Estradiol or E2)

Materials	**96 Tests**
Microwell coated with Goat Anti-Rabbit IgG	12×8×1
Estradiol Reference Standards: 6 vials (ready to use)	0.5ml
Rabbit Anti-Estradiol Reagent (pink color)	7ml
Estradiol-HRP Conjugate Reagent (blue color)	12ml
Estradiol Control 1, Liquid (ready to use)	0.5ml
Estradiol Control 2, Liquid (ready to use)	0.5ml
TMB Reagent	11ml
Stop Solution	11ml
Wash Concentrate 20×: 1 Bottle	25ml

Assay Procedure

1. Select numbers of coated wells for blank, calibrators, control and samples.
2. Bring to room temperature.
3. Dispense 25µl of calibrators, controls and sample into appropriate wells.
4. Dispense 100µl of Estradiol-HRP Conjugate Reagent into each well.
5. Dispense 50µl of rabbit anti-Estradiol reagent to each well.
6. Mix thoroughly for 30 seconds and incubate at room temperature for 90 minutes.
7. Wash of reagents from the wells 5 times with distilled/ deionized water.
8. Dispense 100µl of TMB reagent into each well.
9. Incubate at room temperature for 20 minutes.
10. Read at 450nm with a microtiter-well reader within 15 minutes.

Estimation of Follicle-Stimulating Hormone (FSH)

Principle of the Test

The FSH is a solid phase direct sandwich ELISA method. The samples and diluted anti-FSH-HRP conjugate are added to the wells coated with Mab to FSH beta subunit. FSH in the patient's serum binds to anti-FSH Mab on the well and the anti-FSH-HRP second antibody then binds to the FSH. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of FSH in the samples. A standard curve is prepared relating color intensity to the concentration of the FSH.

Materials Provided

1. Microwells coated with FSH, Mab (12×8×1 wells). 96 wells.
2. FSH Standard: 6 vials 0.0, 5,10, 25, 50, 100 **mIU/ml** (0.7ml each). Ready to use.
3. Enzyme Conjugate: 1 bottle (12ml). Ready to use.
4. TMB Substrate: 1 bottle (12ml). Ready to use.
5. Stop Solution: 1 bottle (11ml). Ready to use.
6. 10× Wash Concentrate: 1 bottle (50ml).

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Assay Procedure

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Pipet 50µl of FSH standards, control and patient's sera.
3. Add 100µl of enzyme conjugate to all wells.
4. Cover the plate and incubate for 30 minutes at room temperature (18-26˚C).
5. Remove liquid from all wells. Fill wells with working wash buffer. Wash three times. Blot on absorbent paper towels.
6. Add 100µl of TMB substrate to all wells.
7. Incubate for 10 minutes at room temperature.
8. Add 50µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA reader at 450nm within 20 minutes after adding the stopping solution.

Estimation of Luteinizing Hormone (LH)

Principle of the Test

The LH Quantitative Test is based on a solid phase enzyme-linked Immunosorbent assay (ELISA). The assay system utilizes a mouse monoclonal anti-α-LH antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-β-LH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies resulting in LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45 minutes incubation at room temperature, the wells are washed with water to remove unbound-labelled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, and the color is changed to yellow and measured spectrophotometrically at 450nm. The concentration of LH is directly proportional to the color intensity of the test sample.

Components

Materials provided with the kit:

1. Mouse monoclonal anti-α-LH antibody coated microtiter plate with 96 wells.
2. Enzyme Conjugate Reagent, 13ml.
3. LH reference standards, containing 0, 5, 15, 50, 100 and 200 mIU/ml (WHO, 1st IRP, 68/40), Lyophilized.
4. TMB Reagent (One-Step), 11ml.
5. Stop Solution (1N HCl), 11ml.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 50µl of standard, specimens and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Gently mix for 30 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-25˚C) for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into sink.
7. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water).
8. Strike the wells sharply onto absorbent paper to remove all residual water droplets,
9. Dispense 100µl TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature in the dark for 20 minutes.
11. Stop the reaction by adding 100µl of Stop Solution to the well.
12. Gently mix for 30 seconds. It is important to make sure all the blue color changes to yellow color completely.
13. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

IV. STATISTICAL ANALYSIS

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Version 21.0 of the IBM Statistical Product and Service Solutions (SPSS) software was used to analyse 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.

V. RESULTS OF FINDINGS

Table.1: Effect of Administration of Aqueous Extract of Raphia HookeriFruit (AERHF)onReproductive Hormonal Levels in Female Wistar Rats

Group and Treatment	Estrogen (ng/ml)	FSH (mlU/ml)	LH (mlU/ml)
Group 1: Control Group	37.00 ± 4.00	2.10 ± 0.30	1.40 ± 0.30
Group 2: Low Dose treated (500mg/kg b.w AERHF)	17.65 ± 1.25 [a]	0.65 ± 0.25	0.55 ± 0.45
Group 3: Medium Dose treated (1000mg/kg b.w AERHF)	23.00 ± 0.00 [a]	1.00 ± 0.10	1.35 ± 0.15
Group 4: High Dose treated (2000mg/kg b.w AERHF)	43.15 ± 3.85 [b, c]	3.45 ± 1.15 [b, c]	2.25 ± 0.15 [b]

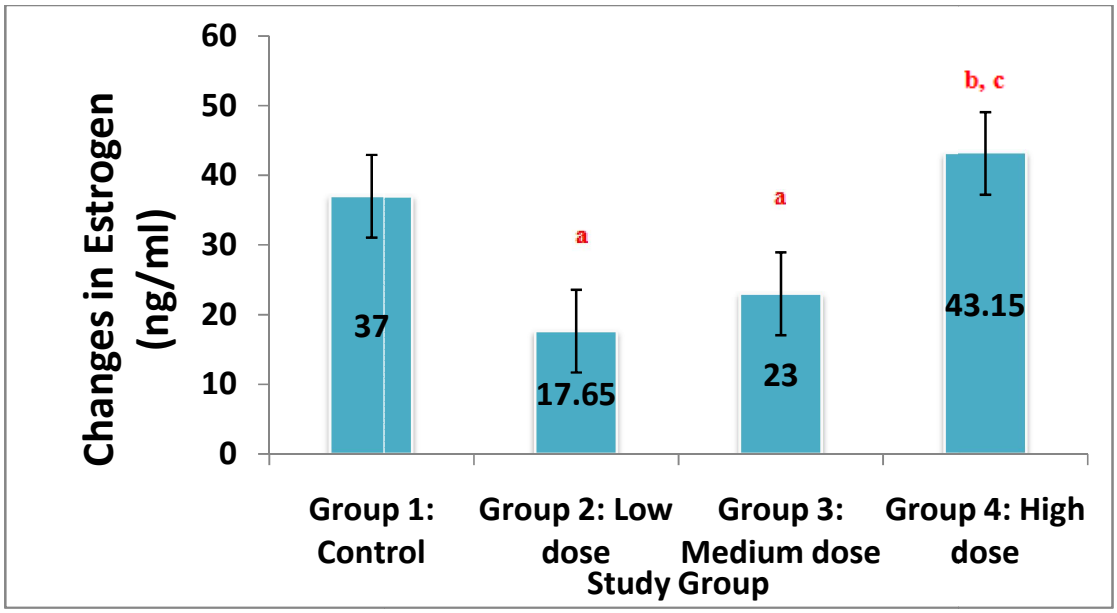

Figure 4.1: Effect of Administration of Aqueous Extract of Raphia Hookeri Fruit(AERHF)on Estrogen (ng/ml) Level in Female Wistar Rats

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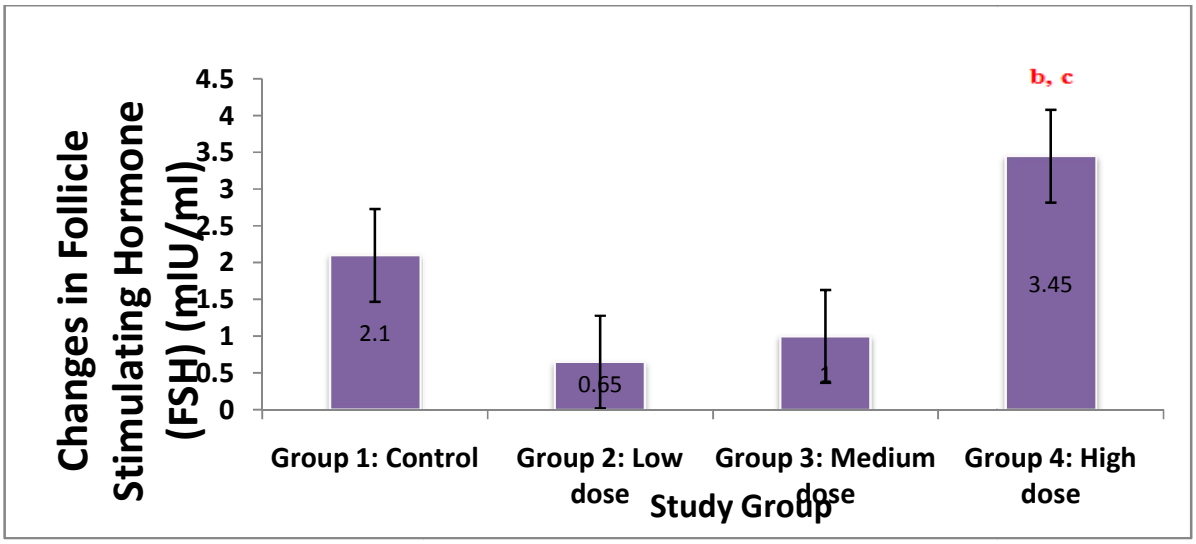

Fig 2: Effect of Administration of Aqueous Extract of Raphia Hookeri Fruit(AERHF)on Follicle Stimulating Hormone (FSH) Level in Female Wistar Rats

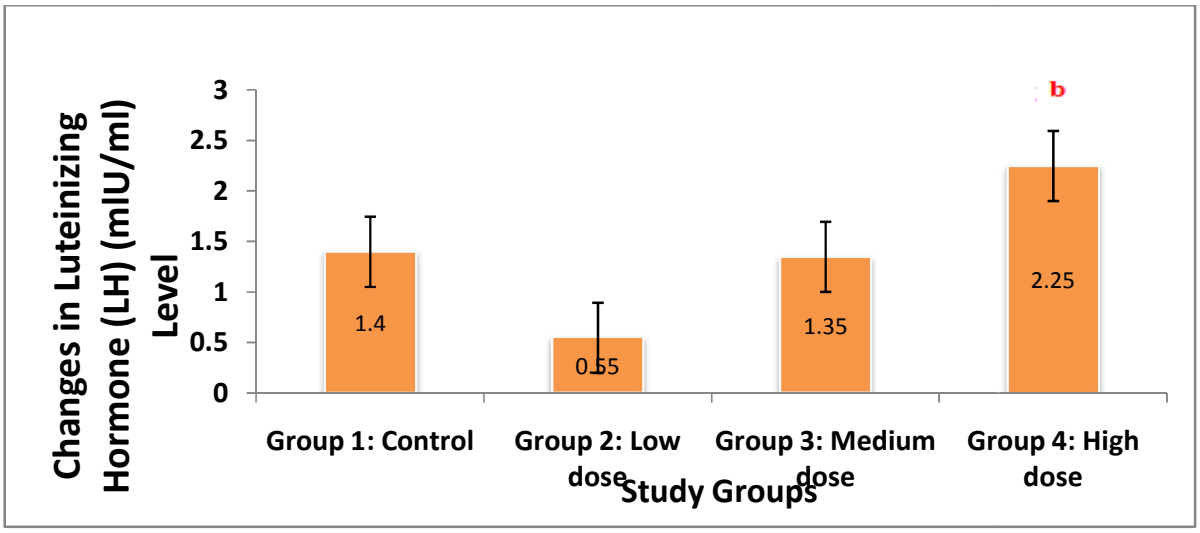

Figure 3: Effect of Administration of Aqueous Extract of Raphia HookeriFruit (AERHF) on Luteinizing Hormone (LH) Level in Female Wistar Rats

VI. DISCUSSION OF FINDINGS

The result of the analysis of estrogen revealed that low(17.65±1.25) and medium (23.00±0.00) doses treated groups showed significant (P>0.05) reductions when they were respectively compared to those of the control group (37.00±4.00) and high-dose treated group (43.15±3.85). Notably, increasing the dose of the extract elicited graded increasing levels ofestrogen.The findings suggest that at low and medium doses, the extract reduces estrogen levels, which could be beneficial in conditions where estrogen reduction is desired, such as decreased sex drive, light or heavy menstrual bleeding, worsening of premenstrual syndrome (PMS), estrogen receptor-positive breast cancer, ovarian cancer, endometrial (uterine) cancer and other estrogen-dependent diseases.In people assigned male at birth, low and medium doses of the extract could be beneficial in conditions where estrogen reduction is desired, such as infertility, or erectile dysfunction. According to Ogbuagu (2008), the extract of Raphia Hookeri fruit contains phytochemicals. One

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group of phytochemicals, called phytoestrogens, are plant compounds that can mimic or modulate the action of estrogen in the body(Healthline, 2020) and the extract of Raphia Hookeri fruit may contain phytoestrogen.This result may also indicate that the extract has an anti-estrogenic effect at lower doses, possibly by competing with endogenous estrogen for binding to estrogen receptors or by inhibiting estrogen synthesis or metabolism. The anti-estrogenic effect may have beneficial implications for preventing or treating estrogen-dependent diseases, such as breast cancer, endometriosis, or uterine fibroids.However, the result also shows that the high dose of the extract increased the estrogen levels significantly compared to the other groups. This indicates that the extract has a pro-estrogenic effect at higher doses, possibly by stimulating estrogen production or enhancing estrogen receptor activity. The pro-estrogenic effect may have adverse implications for increasing the risk of estrogen-related disorders, such as ovarian cancer, thromboembolism, or cardiovascular disease.Conversely, the high dose's increase in estrogen levels indicates potential use in conditions like menopause, where estrogen supplementation might be necessary.Sometimes, low estrogen is a sign of a condition that slows sexual development, which can make it harder to become pregnant, the frequent consumption of aqueous extract of Raphia Hookeri fruit pulp in high doses could be beneficial in conditions like this. Therefore, the aqueous extract of Raphia Hookeri fruit pulp has a biphasic effect on estrogen levels, depending on the dose administered. The result suggests thatthe extract can modulate estrogen levels in the body, either by acting as an estrogen antagonist or an estrogen agonist, depending on the dose.

The result shows that the aqueous extract of raphia hookeri fruit pulp has a dose-dependent effect on follicle-stimulating hormone (FSH) levels in female Wistar rats. The result revealed that all treated groups did not indicate significant ($P>0.05$) changes when they were respectively compared to that of the control group (2.10±0.30). However,the high dose treated group (3.45±1.15) showed a significant ($P<0.05$) raised level of FSH when compared to thosetreated with low (0.65±0.25)and medium (1.00±0.10) doses of the extract. Remarkably also, increasing doses of the extract elicited graded increasing levels of FSH. This indicates that the extract stimulates FSH production or secretion at this dose. The stimulatory effect of Raphia Hookeri fruit pulp extract on FSH levels could have potential applications in the treatment of various female reproductive health conditions, such as infertility, polycystic ovary syndrome (PCOS), premature ovarian failure, and menopause. High doses of the extract could be used to enhance ovulation and fertility in women with low FSH levels or anovulatory cycles.

On theproduction of luteinizing hormone (LH), it was observed that there were no significant ($P>0.05$) variations when the respective treated groups were compared to the control group (1.40±0.30). Only the high-dose treated group (2.25±0.15) indicated a significant ($P<0.05$) increase when compared to that of the low-dose treated group (0.55±0.45) medium dose treated group (1.35±0.15). Similar to the outcomes on estrogen and FSH, increasing doses of the extract produced graded increases in the level of LH in the treated rats.The result shows that increasing doses of the extract are associated with increasing levels of LH, suggesting a dose-response relationship.The ability of Raphia Hookeri fruit pulp extract to stimulate LH levels suggests it could also be potentially useful in managing various conditions related to female reproductive health, including infertility, polycystic ovary syndrome (PCOS), premature ovarian failure, and menopause. For instance, administering high doses of the extract will promote ovulation and fertility in women who have low LH levels or do not ovulate regularly. The fruit pulp of Raphia Hookeri could also be potentially useful in managing various conditions such as dyslipidemia. This is in line with a work done by Kengne et al. (2020) on the "Investigation of the antihyperlipidemic property of Raphia Hookeri mesocarp oil in high-lard diet (HLD) fed rats.

VII. CONCLUSION

The aqueous extract of Raphia Hookeri fruit pulp has a dose-dependent effect on estrogen, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels in the body. The extract of can affect estrogen levels in different ways, depending on the dose. At low and medium doses, it lowers estrogen levels, which could be useful for treating conditions that require estrogen reduction, such as some cancers, menstrual disorders, or sexual dysfunctions. At high doses, it increases estrogen levels, acting as an estrogen booster, the high dose can be useful for alleviating estrogen deficiency symptoms or enhancing fertility. At high doses, it could also be harmful for people with estrogen-sensitive diseases or who want to avoid estrogen stimulation. The extract can act as either an estrogen blocker or booster, depending on the dose. This shows that the extract has a complex and versatile effect on estrogen regulation in the body. The study also demonstrated that the high-dose treated group had significantly higher FSH levels than the low

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and medium-dose treated groups. The extract could have potential applications in the treatment of female reproductive health conditions that are related to low FSH levels or impaired ovulation. This study demonstrated that the extract has a stimulatory effect on LH secretion in female rats and that this effect is dose-dependent. This finding suggests that the extract has therapeutic potential for various female reproductive disorders that are associated with low LH levels, such as infertility, polycystic ovary syndrome (PCOS), premature ovarian failure, and menopause. The aqueous extract of Raphia Hookeri fruit pulp has a dose-dependent and multifaceted effect on the hormonal regulation of female reproduction in rats. The findings of this study further confirm the effects that the frequent consumption of aqueous extract of Raphia Hookeri fruit pulp has in the treatment of various female reproductive disorders that are related to hormonal imbalances or dysfunctions.

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