PRO-FERTILITY INVESTIGATION OF PINEAPPLE JUICE (*Ananas Comosus*) AND COCONUT (*Cocos nucifera*) MILK ON ADULT MALE WISTAR RATS

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LSC2007286

(PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES)

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN,

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NOVEMBER, 2025

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCE, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.SC) IN SCIENCE LABORATORY TECHNOLOGY (PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES)

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project work Titled "**PRO-FERTILITY INVESTIGATION OF PINEAPPLE JUICE (*Ananas Comosus*) AND COCONUT (*Cocos nucifera*) MILK ON ADULT MALE WISTAR RATS"** was carried out and submitted by **Destiny Osemudiamen EHIOMHEN** with matriculation number, **LSC2007286** in the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

This work is dedicated to Almighty God for His mercy and wisdom during the course of this project work and throughout this journey.

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ABSTRACT

Infertility remains a significant global health challenge, with male factors contributing to nearly half of all reported cases. The search for natural, safe and cost-effective alternatives to conventional fertility drugs has led to increasing interest in medicinal plants. This study investigated the Pro-fertility potential of extracts of *Ananas Comosus* (Pineapple juice) and *Cocos Nucifera* (Coconut milk) on adult male Wistar rats. The extracts were administered orally at doses of 50 ml/kg and 250 ml/kg for 28 days with distilled water and Proviron (25 mg/kg) serving as negative and positive controls, respectively. Sperm parameters, reproductive organ indices and hormonal profiles were assessed following standard procedures. The extract-treated groups, especially at 250 ml/kg, showed significant increases in sperm count, motility, morphology and testosterone levels compared to the control ($P < 0.05$). These improvements suggest enhanced spermatogenesis and androgenic activity. The Observed effects are attributed to Phytochemical such as bromelain, flavonoids, phenolic acids, and medium-chain fatty acids, which possess antioxidant and anti-inflammatory properties. Overall, the Combination of *Ananas Comosus* and *Cocos Nucifera* demonstrated strong pro-fertility potential, indicating its promise as a natural alternative to conventional fertility treatments.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

1.1.1 Medicinal plants and their benefits

Medicinal plants are plants that possess bioactive compounds capable of preventing, Alleviating, or curing diseases in humans and animals. They are widely recognized as a Primary source of therapeutic agents, with approximately 25-50% of modern drugs derived from plant-based molecules or their synthetic analogues. Globally, especially in developing Countries, medicinal plants play a crucial role in healthcare due to their accessibility, Affordability and cultural acceptability (Newman and Cragg, 2020).

1.1.2 Phytochemical Composition of Medicinal plants

Medicinal plants owe their therapeutic properties to secondary metabolites, including:

Flavonoids: plant pigments with antioxidant, anti-inflammatory and cardiovascular-protective properties (karak, 2019).

Saponins:glycosides with cholesterol-lowering, antifungal and fertility modulating properties (Moghimipour and Handali, 2015).

Phenolic acids: antioxidants that protect against oxidative stress and chronic diseases (Ghasemzadeh and Ghasemzadeh, 2011).

Sterols: This include beta-sitosterol and stigmasterol which have antioxidant and anti-inflammatory properties (Genser *et al.,* 2012).

1. 2 BIOLOGY OF PINEAPPLE

1.2.1. DESCRIPTION OF PINEAPPLE

Pineapple (*Ananas comosus*) is a tropical plant native to South America, Specifically the area between southern Brazil and Paraguay. It is a multiple fruit, formed by the fusion of multiple flowers, and is characterized by its tough, waxy leaves and sweet, juicy flesh (Hossain, 2016). Pineapple is widely cultivated in tropical and subtropical regions around the world, with Costa Rica and the Philippines being among the top producers. It is a popular fruit, enjoyed fresh, or as a juice and is also used in a variety of products, including jam, preserves and Cosmetics (Hossain *et al.,* 2015).

1.2.2 TAXONOMIC CLASSIFICATION OF THE PLANT

Kingdom: plantae

Division: Angiospermophyta

Class: Liliopsida

Order: Poales

Family: Bromeliaceae

Genus: Ananas

Species: *Ananas comosus*

1.3 MORPHOLOGY OF THE PLANT

Botanical description: *Ananas comosus* is a perennial monocotyledonous plant that grows up to 1 meter high and 0.5 meters wide, although some varieties like 'Smooth Cayenne' can grow up to 1.5 meters high and 1 meter wide.

Leaves: The leaves are long, pointed and usually needle- tipped, measuring 50-180cm in length, with sharp, up-curved spines on the margins (except for some spineless varieties).

Inflorescence: A compact, terminal cluster of 50-200 individual hermaphrodite flowers with tubular Corolla.

Fruits: The shape of a pineapple is cylindrical or oval. The sizes varies typically from 20-30cm long. The rind is tough, waxy, and segmented. The flesh is often Juicy, sweet and tangy. The crown consists of rosette of leaves at the top.

Name: The botanical name of the pineapple plant is "*Ananas comosus*". The name is used universally to identify the pineapple plant in Scientific and horticultural contexts.

Biology: The pineapple plant's morphology is characterized by its fibrous roots, monocotyledonous structure, short stem, Rosette leaf arrangement, waxy leaves. The plant's biology also includes: CAM Photosynthesis and self- sterility.

Ecology: The pineapple plant's morphology is adapted to its ecological environment in several ways such as its waxy leaves reduce water loss through transpiration, it's spiny leaves provide protection from herbivores and other predators. Its fibrous roots allows for efficient water and nutrient uptake in well- draining soils.

Biophysical limits: The pineapple plant's growth and productivity are influenced by biophysical limits, Including: Temperature, water availability, light and soil. These biophysical limits affect the plant's growth rate, fruit production and disease susceptibility.

Soil type: Pineapple plants prefer well- draining soil with the following characteristics: pH that is slightly acidic, sandy loam or loamy soil, Good drainage to prevent water logging, Adequate Organic matter for nutrient supply.

1.4 NUTRITIONAL COMPOSITION AND THEIR HEALTH BENEFITS OF PINEAPPLE JUICE

Pineapple Juice has some amazing nutritional benefits and some of them include: High in Vitamin C. One cup of pineapple juice provides about 131% of the recommended daily intake of vitamin C, which plays a vital role in immune function, collagen production and iron absorption (Hossain *et al*., 2015). Pineapple Juice are rich in manganese. Pineapple juice is an excellent source of manganese, a mineral that supports bone health, wound healing and metabolism. One cup of pineapple juice provides more than 50% of the recommended daily intake of manganese (Hossain, 2016). Pineapple juice are good sources of fiber. Pineapple juice contains both soluble and insoluble fiber, which can help regulate bowel movements, promote satiety and support healthy blood sugar levels (Chaudhary *et al*., 2019). Pineapple juice may aid digestion. The bromelain in pineapple juice can help break down protein and aid digestion, although more research is needed to confirm its effectiveness (Hossain, 2016). Pineapple juice Support Eye Health. The Vitamin C and beta- Carotene in pineapple juice may help reduce the risk of age-related macular degeneration and cataracts (Chaudhary *et al*., 2019).

1.5 PHYTOCHEMICAL CONTENTS OF PINEAPPLE JUICE

Phytochemicals are natural compounds found in plants that have therapeutic effects when consumed as medicine or as part of our daily diet (Leitzmann, 2016). We can find bioactive compounds like ascorbic acid, bromelain, flavonoids, phenolic acid and lignans in different amounts in pineapple juice (Karak, 2019). The proportion and concentration of these components can vary greatly depending on factors such as post- harvest handling, storage, soil conditions and where the fruit come from.

1.5.1 Ascorbic acid

A water-soluble Vitamin that has antioxidant properties and plays a role in immune function and collagen production. It is present in pineapple juice (Uckiah *et al*., 2009).

1.5.2 Bromelain

A mixture of proteolytic enzymes, including cysteine proteases, aspartic proteases and serine proteases, which are present in pineapple juice and they have anti-inflammatory and anti-cancer properties (Kwatra, 2019).

1.5.3 Flavonoids

A class of plant compounds that include kaempferol, quercetin and isorhapontigenin which are present in pineapple juice and they have antioxidant, anti-inflammatory and anti-cancer properties (Karak, 2019).

1.5.4 Lignans

A class of plant compounds that include lariciresinol and other secoisolariciresinol which are present in pineapple juice and they have antioxidant and anti-cancer properties (Vidinamo, *et al*., *2022*).

1.5.5 Phenolic acids

A class of plant compounds that include ferulic acid, sinapic acid and caffeic acid, which are present in pineapple juice and they have antioxidant and anti-inflammatory properties (Ghasemzadeh and Ghasemzadeh, 2011).

1.6 PHARMACOLOGICAL ACTIVITIES OF PINEAPPLE JUICE

Pineapple juice in general are good source of chemical compounds such as; phenolic acids, ascorbic acid, flavonoids, lignans and bromelain. These compounds which are present in different proportions aid their nutraceutical capabilities which includes; Antioxidant activity, Anti-inflammatory effects, aids Digestion, Antimicrobial properties (Leitzmann, 2016).

1.7 BIOLOGY OF COCONUT

1.7.1 DESCRIPTION OF COCONUT MILK

Coconut milk is a nutritious and versatile liquid extracted from the meat of mature coconuts (*Cocos nucifera*). It has been a staple ingredient in tropical cuisines, particularly in Southeast Asian and pacific Island cultures, for centuries. Coconut milk is rich in medium- chain triglycerides (MCTs), protein, fiber and various essential minerals, including potassium, magnesium and iron.Coconut milk is available in various forms, including full-fat, low-fat and skim milk, as well as powdered and canned versions. It is a popular ingredient in cooking and baking, and is often used in soups, curries, desserts and beverages (Patil and Benjakul, 2018).

1.7.2 TAXONOMY OF COCONUT

Kingdom: Plantae

Division: Angiospermophyta

Class: Liliopsida

Order: Arecales

Family: Arecaceae

Genus: Cocos

Species: *Cocos nucifera*

1.8 NUTRITIONAL BENEFITS OF COCONUT MILK

Some of the nutritional benefits of coconut milk are; Good Source of fiber. Coconut milk contains a small amount of dietary fiber, approximately 2-3% (patil and Benjakul, 2018). Coconut milk is also rich in Minerals. Coconut milk is a good source of minerals, including potassium, magnesium and iron. Coconut milk are high in Saturated fat. Coconut milk is high in saturated fat, particularly Lauric acid, which has been shown to increase high-density lipoprotein (HDL) cholesterol and potentially reduce the risk of cardiovascular disease Coconut milk may also aid in weight loss. The MCTs in coconut milk may aid in weight loss by increasing energy expenditure and enhancing fat oxidation. Coconut milk also contain antimicrobial properties. The Lauric acid in coconut milk has been shown to have antimicrobial properties, which may help support immune function and reduce the risk of illness (Raghavendra and Raghavarao, 2010).

1.9 PHYTOCHEMICALS CONTENTS OF COCONUT MILK

Phytochemicals are natural compounds found in plants that have therapeutic effects when consumed as medicine or as part of our daily diet. We can find bioactive compounds such as;

Lignans, Saponins, sterols, phenolic acids, flavonoids etc. (Ojobor *et al.,* 2018). The proportion and concentration of these components can vary greatly depending on some factors such as when the fruit was plucked, post-harvest handling, soil conditions etc.

1.9.1 Phenolic acids

A class of plant compounds that include ferulic acid, sinapic acid and caffeic acid, which are present in coconut milk and they have antioxidant and anti-inflammatory properties (Karunasiri *et al.,* 2020).

1.9.2 Lignans

A class of plant compounds that include lariciresinol and secoisolariciresinol which are present in coconut milk and they have antioxidant and anti-cancer properties (Sun *et al.,* 2024).

1.9.3 Flavonoids

A class of plant compounds that include kaempferol, quercetin and isorhapontigenin which are present in coconut milk and they have antioxidant, anti-inflammatory and anti-cancer properties (Kim *et al.,* 2023).

1.9.4 Saponins

Coconut milk contains saponins, which have antioxidant and anti-inflammatory properties (Odenigbo and Otisi 2011).

1.9.5 Sterols

Coconut milk contains sterols, including beta-sitosterol and stigmasterol, which have antioxidant and anti-inflammatory properties (Hammouda *et al.,* 2018).

1.10 PHARMACOLOGICAL ACTIVITIES OF COCONUT MILK

Coconut milk in general are good source of chemical compounds such as; phenolic acids, Lignans, flavonoids, Saponins and sterols. These compounds which are present in different proportions aid their nutraceutical capabilities which include; antimicrobal activities, enhanced anti- inflammatory effects, antioxidant properties, Improved digestive health, improved bone health and weight management (Hammouda *et al.,* 2018).

1.10.1 Antimicrobial activities

Coconut milk contains lauric acid, which has antimicrobial properties and can help fight against bacteria, viruses and fungi (Ebisintel and Salim, 2024).

1.10.2 Enhanced anti-inflammatory effects

Coconut milk may offer enhanced anti-inflammatory effects due to the presence of MCTs present in coconut milk which may reduce inflammation and oxidative stress (Ajeigbe *et al.,* 2022).

1.10.3 Antioxidant properties

coconut milk contains a range of antioxidants including flavonoids, phenolic acids, which can help protect cells from oxidative damage and reduce the risk of chronic diseases such as heart disease and cancer (Alyaqoubi *et al.,* 2015).

1.10.4 Improved digestive health

Coconut milk contains fiber and an enzyme which can help aid digestion and reduce symptoms of irritable bowel syndrome (Chen *et al*., 2024).

1.10.5 Improved bone health

Coconut milk contains manganese and copper which are essential for bone health and density. They also support the connective tissue (Chen *et al*., 2024).

1.10.6 Aids in weight management

Coconut milk contains medium- chain triglycerides (MCTs) that can help increase energy expenditure and aid in weight loss (Karunasiri *et al.,* 2020).

1.11 AIM OF STUDY

The aim of this study is to evaluate the profertility activity of pineapple juice (*Ananas comosus)* and Coconut (*cocos nucifera)* milk on adult male wistar rat.

1.12 OBJECTIVES OF STUDY

To achieve the aim of this study the following objectives were carried out.

1. Extraction
2. Phytochemical analysis.
3. Purchase of laboratory animal/acclimatization of mice for 7 days from the pharmacology/toxicology departmental animal house.
4. Administration of juice and standard drug.
5. Induction of ailment.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MALE FERTILITY

2.1.1 DEFINITION OF MALE FERTILITY

Male fertility is the capacity of a sexually mature man to successfully contribute to conception by producing, maintaining and delivering healthy sperm capable of fertilizing a mature female egg, ultimately leading to the establishment of a viable pregnancy. The ability depends on several interconnected biological and functional factors which include; Spermatogenesis, semen quality, hormonal balance, reproductive tract integrity and functional competence. It is usually considered normal when sperm parameters meet or exceed reference thresholds and when conception can be achieved with a fertile female partner within one year of regular, unprotected sexual intercourse. When these conditions are not meant due to sperm production, quality, delivery or functional problem, the male is classified as infertile (Mazzilli *et al*., 2022).

2.1.2 DEFINITION OF INFERTILITY IN MALE

Male infertility is a medical condition in which a sexually mature man is unable to cause pregnancy in a fertile female partner despite 12 months or more of regular, unprotected Sexual intercourse. This condition reflects a defect in one or more stages of the male reproductive process which includes the production, maturation, transport and function of sperm cells. At the biological level, male infertility can result from quantitative defects such as low sperm count (oligozoospermia) or complete absence of sperm (azoospermia) or qualitative defects such as poor motility and abnormal morphology. It can also occur when there are obstructions in the reproductive tract preventing sperm delivery, hormonal imbalances that impair sperm production, genetic

abnormalities that disrupt spermatogenesis or lifestyle and environmental factors that damage sperm quality (borght and Wyns, 2018).

2.2 FACTORS RESPONSIBLE FOR MALE FERTILITY

Several biological, lifestyle and environmental factors can affect a man's fertility and they include;

- **OBESITY AND METABOLIC EFFECTS**

Being overweight or obese can negatively affect male fertility in several ways and they include: Hormonal imbalance, sexual dysfunction, Heat stress and Epigenetic effects (Davidson *et al.,* 2015).

- **OXIDATIVE STRESS**

Oxidative stress happens when the body produces too many reactive oxygen species (ROS), which can damage cells, including sperm. In sperm, oxidative stress can damage the DNA, reduce motility (movement), and impair the ability to fertilize an egg. It is caused by infections, poor diet, smoking, alcohol, heat and environmental pollutants. Antioxidants from food or supplements can help protect sperm from oxidative damage (Walczak-Jedrzejowska *et al.,* 2013).

- **DIET AND LIFESTYLE**

A healthy diet rich in vitamins (C, E, folate) and minerals (Zinc, Selenium) supports sperm production and function. Diets high in processed foods, sugar and unhealthy fats can lead to obesity, diabetes and hormonal problems that impair fertility. Smoking, alcohol and drug use can damage sperm DNA and reduce sperm count. Exercise in moderation improves fertility, but excessive strenuous training can lower testosterone (Ilacqua *et al.,* 2018).

- **ENVIRONMENTAL AND OCCUPATIONAL EXPOSURES**

Chemicals like pesticides, heavy metals (lead, cadmium) and industrial toxins can harm sperm production. Long exposure to heat (e.g hot baths, saunas, and laptops on lap) can lower sperm

count and motility. Radiation and certain medications can also impair sperm quality (Maric *et al.,* 2021).

2. 3 STANDARD DRUG USED FOR FERTILITY

A standard drug used for fertility refers to a clinically recognized medication that has been proven, through research and medical practice, to enhance reproductive function by improving the quality, quantity or function of gametes (sperm in males, Ova in females), regulating hormonal balance or correcting specific physiological abnormalities that impair conception. One widely studied example is Proviron (Semet *et al*., 2017).

2.3.1 PROVIRON AS A STANDARD DRUG USED FOR FERTILITY

Proviron, known chemically as mesterolone, is a synthetic androgen derived from dihydrotestosterone (DHT). It is sometimes prescribed to address male infertility associated with low sperm count or reduced testicular function. Proviron works by stimulating androgen receptors, potentially enhancing the structural and functional integrity of sperm or supporting accessory reproductive organs. However, clinical outcomes have been mixed, and it's use while supported by some studies remains somewhat controversial in fertility treatment. In the context of male fertility, Proviron has historically been used especially in cases of idiopathic male infertility (infertility without a known cause) with the aim of improving spermatogenesis (the production of sperm) and enhancing semen quality. Because it does not suppress the hypothalamic–pituitary gonadal (HPG) axis to the same extent as some other androgens, it can maintain or even stimulate follicle-stimulating hormone (FSH) and luteinizing hormone (LH) activity in certain individuals. This allows for the potential improvement of sperm concentration, motility, and morphology without markedly reducing endogenous testosterone production (Adeniji *et al.,* 2010).

2.3.2 PHARMACOLOGY ACTION OF PROVIRON

2.3.2.1 PHARMACOKINETICS OF PROVIRON

Pharmacokinetics explores how a drug is absorbed, distributed, Metabolized and eliminated by the body. When taken orally, mesterolone Is rapidly and almost completely absorbed, with peak serum levels which reach around 1.6 + or -0.2 hours after ingestion. However it's absolute oral bioavailability is quite low, about 3% of the administered dose actually enters systemic circulation. Mesterolone is highly bound to serum proteins approximately 98%. This high binding affinity means it may help displace testosterone from SHBG, potentially increasing free (active) testosterone Levels. The body rapidly inactivates mesterolone via liver metabolism. There is no conversion into estrogens or corticosteroids. Most of the drug is excreted in metabolized form: approximately 85% in urine and 14% in feces. Within seven days, around 93% of the administered dose is eliminated, half of that within the first 24 hours. The terminal half-life is roughly 12-13 hours, so the drug level declines steadily over that timeframe. Clinically, this suggests multiple daily doses (e.g two to three times) are needed to maintain stable levels (Adeniji *et al*., 2010).

2.3.2.2 PHARMACODYNAMICS OF PROVIRON

Pharmacodynamics explains how a drug interacts with the body and how the body responds. Mesterolone's pharmacodynamics reflect its unique properties as an androgen, which can be particularly relevant in fertility contexts. Proviron functions as an androgen receptor (AR) agonist, meaning that it binds directly to ARs much like naturally occurring androgens. This mode of action stimulates androgen-sensitive tissues and supports male reproductive functions. Proviron has a weak anabolic effect meaning it's not particularly effective for muscle growth. This is due to rapid

inactivation by 3α-hydroxysteroid dehydrogenase (3α-HSD) in skeletal muscle. However, it retains relative androgenic potency, making it useful for supporting reproductive tissues like the epididymis. Because it cannot be aromatized, Proviron does not lead to estrogen-related side effects such as gynecomastia or fluid retention. It also has no progestogenic activity unlike some other androgens. Mesterolone is already in a 5α reduced form, so it isn't further activated by 5α-reductase in tissues like skin or prostate differentiating it from testosterone. It is not 17α-alkylated, which means it carries a lower risk of liver toxicity than many oral androgens. This androgen has a very high binding affinity for SHBG up to 440% that of DHT in some studies. By strongly attaching to SHBG, mesterolone can displace natural testosterone, thereby increasing the free (active) estosterone available in circulation which may enhance androgenic effects (Adeniji *et al*., 2010).

2.3.*2.3*. ADVERSE EFFECTS OF PROVIRON

Adverse effects of proviron include; Androgenic side effects, Mood and Libido changes, prostatic effects, liver and metabolic risks. (Adeniji *et al*., 2010).

2.3.2.4 CONTRAINDICATIONS OF PROVIRON

Contraindications of proviron include prostrate cancer, Male breast cancer (Androgen-Dependent Carcinoma), Severe liver Disease, Nephrotic Syndrome, Hypersensitivity to Mesterolone or excipients (Adeniji *et al*., 2010).

2.4 Relevance to Fertility Studies

The use of medicinal plants for fertility enhancement has gained research attention due to their potential to improve reproductive function without the adverse effects associated with synthetic

drugs. Pineapple contains bromelain, Vitamin C and manganese, which may enhance sperm motility and protect against oxidative stress. Coconut milk is rich in medium- Chain fatty acids, minerals and antioxidants, which may contribute to hormonal balance and improved sperm parameters. Both are promising candidates for further investigation in animal fertility studies.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1 STUDY DESIGN

The study was a preclinical laboratory based experimental study. It was carried out at Science laboratory Technology (SLT) laboratory, Faculty of Life Sciences, University of Benin.

3.2 COLLECTION OF RESEARCH MATERIALS

The *Ananas comosus* fruit and *Cocos nucifera* (ACCN) was purchased from fruit vendors in Oba market in Oredo Local Government Area of Edo State Nigeria.

3.3 EXTRACTION OF PINEAPPLE JUICE AND COCONUT MILK

200g of both Coconut and pineapple were weighed (1:1) and cut into smaller pieces and grounded in an electric blender. The blended sample was poured into a cheesecloth contained in a bowl and then after the milk was squeezed out of the cheesecloth and poured in an airtight container for further test. Calculated doses in ml/kg were carried out using the weight of the animals.

3.4 TOXICITY STUDY.

3.4.1 Acute Toxicity Study

Acute toxicity study was carried out by methods of (OECD, 2008) Organization of economic co-operation development guidelines Using up and down method. Three (3) male mice and 3 female mice each were administered 2000 ml/kg of the *Ananas comosus* Juice and *cocos nucifera milk* observed for 24 hours for possible signs of toxicity, mortality or morbidity.

3.4.2 Reproductive Toxicity

Reproductive toxicity was carried out on male Wistar rats administered *Ananas Comosus* juice and *Cocos Nucifera* milk.The male rats were treated for 28 days (Leko *et al.,* 2021).

3.5 GROUPING OF ANIMALS

Animals were classified into four groups and were administered based on their groupings.

GROUP 1: Control group received 10ml/kg of distilled water.

GROUP 2: Positive control group received 25mg/kg of Standard drug Proviron..

GROUP 3: Received 50ml/Kg of pineapple juice.

GROUP 4: Received 250ml/kg of Coconut Milk.

3.6 Solvents/chemicals

3.7 Male Reproductive Study

Male rats weighing 180-200g was randomly allocated into 4 experimental groups. Group 1-control (distilled water 10ml/kg), group 2: positive-control (standard drug proviron 25 mg/kg) and group 3-4 extract treated groups administered 50 and 250ml/kg/bwt/p.o *Ananas Comosus* juice and *Cocos Nucifera* milk for 28 days. Twenty-four hours after the last administrations, the animals were sacrificed, organ weights changes were evaluated, sperm count and morphology assessment were evaluated along with levels of male reproductive hormones as testosterone were measured in serum (Showky *et al.,* 2021).

3.8 Sperm Cell Morphology and Count

3.8.1 Isolation of Sperm Cells

Sperm cells were collected from the vas- deferens of the sacrificed rats; the rats were sacrificed and the vas-deferens located and ligated with a minimum of 36 mm length, both extremities of the vas deferens was ligated, cut and placed in a sterile petri dish. To the petri dish, 6 µl of normal

saline already adjusted to 37±2°C was added. The Vas deferens was teased to allow the sperm cell diffuse out of it. A drop of the sperm cell from the petri dish was placed on a grease free clean slide and covered with a transparent cover slip.

3.8.2 Motility of Spermatozoa

The sperm cell motility was determined with the correlation between progressively motile sperm cells after ejaculation and the fertility. The motility was evaluated with regards to three variables: Progressively motile, Non-progressive motility and immotile spermatozoa and it us usually expressed in percentile. Spermatozoa can show good motility and viability in the seminal plasma 24 hours after ejaculation but in some semen samples the motility may decline much faster (Showky *et al*., 2021).

A drop of the sperm cell was taking from the petri dish and dispensed on a clean grease free slide and further covered with a transparent cover slip. The slide was placed on the microscope and viewed with the x20 and x40 objective magnification lens. The motility was scored in percentage according to their nature of motility as; Progressive, Non-progressive and Immotile sperm cells.

3.8.3 Vitality Testing

One volume of semen (a drop) was milted into two volumes of eosin solution (1% diluted water). After 30 seconds three volumes of nigrosine solution (10% nigrosine) was added and the sample homogenized. A thin smear was then made immediately and air dried. The Stained slide was examined under the oil immersion objective lens (x100). Live spermatozoa were unstained (white) and the dead ones were red.

3.8.4 Morphology

The sperm cell morphology was assessed by staining the slide with the Improved Eosin and Leishman stain (Ibeh *et al*., 2018).

A drop of the sperm cells was dispensed on grease free clean slides and a smear was made; the slide was left to air dry. The slide was flooded with the Improved Eosin and Leishman stain for 15 mins. The stain was rinsed and the back was blotted dry with cotton wool and left to air dry. The slide was placed in a microscope with the magnification lens at x100. The slide was viewed with at least 30 magnification fields, the normal and abnormal sperm cells were spotted and scored in percentage.

3.8.5 Hormonal assay

Testosterone (TST), progesterone (PGST), prolactin hormone, follicle stimulating hormone (FSH), leuthenizing hormone (LH), Estrogen 2 hormone (E2), were quantitatively analysed using Eliser kit USA. The total TST, PGST, FSH, Lu and E2 in the sera of male and female Wistar rats were determined.

3.9 Data Analysis

The results from the studies were expressed as mean ± SEM. Statistical analysis were carried out using graph pad prism 6 version software (UK). Comparisms between the control and treated groups were analysed using one-way ANOVA and, Dunnett's multiple comparisms test. * = $P < 0.05$, was regarded as indicating significant difference.

CHAPTER FOUR

4.0 RESULTS

Table 4.1: Effect of extract *Ananas Comosus* Juice and *Cocos Nucifera* milk on sperm cell quality of adult male Wistar rats

STUDY GROUP BY TREATMENT	TOTAL SPERM CELL COUNT X106cells/mm3	PROGRESSIVE MOTILITY (%)	NON PROGRESSIVE MOTILITY (%)	IMMOTLE (%)	NORMAL MORPHOLOGY (%)	ABNORMAL MORPHOLOGY (%)
Control(Distilled Water)10ml/Kg	690 ±1.2	81±0.2	12±0.1	07±0. 2	85 ±0.4	15±0.1
Proviron 25mg/Kg	685 ±5.0[b]	84 ±0.5	09±0.4	07 ±0.4[d]	91 ±0.2[a]	09±0.6
ACCMJ 50ml/kg	812 ±4.0[d]	88 ±0.1[d]	08±0.2	04±0.5[C]	95 ±0.8[d]	05±0.3
ACCMJ 250ml/Kg	732 ±7.2[b]	89±0.4 [d]	09 ±0.3	02±0.6[d]	98 ±0.4[d]	02±0.2

significantly increased at p< a, b ,and d [a =] P<0.05 in comparison with the control group. [b =] P<0.01, [c =] P <0.001 , [d =] P<0.0001

(n=5).

Key: ACCMJ = *Ananas Comosus* Juice and *Cocos Nucifera* milk

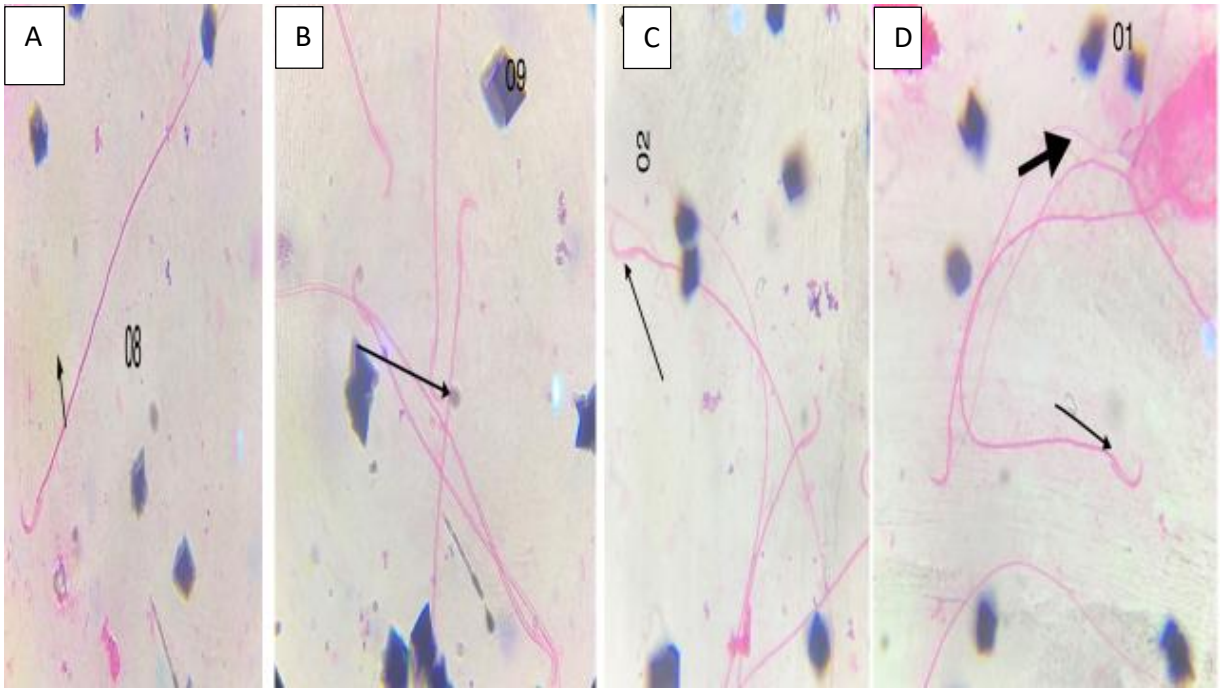

Plate 4.1: Effect of **Table 1:** Effect of *Ananas Comosus* Juice and *Cocos Nucifera milk* on sperm cell quality of adult male Wistar rats

On sperm characterization of adult male Wistar, the male rats treated for 28 days (X100 objective lense). A= Normal Sperm morphology, with well formed head, body and tail, which was control, B= Normal head, body and tail proviron 25 mg/kg group which was positive control. C= ACCMJ 50ml/ and D = ACCMJ 250 ml/kg, (n=5).

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Table 4.2: Effect of extract *Ananas Comosus* Juice and *Cocos Nucifera* milk on Body and reproductive organ weight index

Parameter	**Control(Distilled Water) 10ml/Kg**	**Proviron 25 mg/kg**	**ACCMJ 50ml/kg**	**ACCMJ 250ml/kg**
Body weight(g)	209±1.5	201.3^{d}±7.2	188.5^{a}±0.22	227.3^{d}±0.3
Male reproductive organ (mg)	113.±0.1	123^{a}±0.1	110±0.3	125^{b}±0.3

significantly increased at p< a, b ,and d ^{a} = P<0.05 in comparison with the control group. ^{b} = P<0.01, ^{c} = P <0.001 , ^{d} = P<0.0001 .

Table 4.3:Effect of extract *Ananas Comosus* Juice and *Cocos Nucifera* Milk on Male Hormonal assay

Key: **LH** =Leuthenizing hormone, **FSH**= Follicle stimulating hjormones and **EST2 = Estrogen 2 hormones**

Treatment	Testosterone	LH	FSH	EST2
Control (Distilled Water) 10ml/Kg	20.0±0.51	3.75±0.12	1.98±0.15	0.20±0.51
Proviron 25mg/kg	20.9±0.40	9.60±0.15^{d}	0.80±0.41 ^{d}	0.7±0.40
ACCMJ 50ml/kg	23.7±0.38^{d}	0.90±0.03^{d}	0.30±0.06 ^{d}	0.7±0.38^{d}
ACCMJ 250ml/kg	22.9±0.18^{d}	0.10±0.05^{d}	0.40±0.92^{d}	2.9±0.18^{d}

The unit of Hormones in MIU/ML, Results are expressed as mean ± SEM (n= 6) , d = $p<0.0001$.

CHAPTER FIVE

5.0 DISCUSSION

5.1 EFFECT OF EXTRACT *Ananas Comosus* JUICE AND *Cocos nucifera* MILK ON SPERM CELL QUALITY OF ADULT MALE WISTAR RATS (n=5)

The administration of the extract of *Ananas Comosus* Juice and *Cocos nucifera* Milk (**ACCMJ**) improved several Parameters of sperm quality in adult male Wistar rats in table 1 when compared to the control and Proviron groups.

5.1.1 Total Sperm cell Count

The total sperm count increased significantly in the ACCMJ 50ml/Kg group ($812 \pm 4.0 \times 10^6$ cells/mm^3) and ACCMJ 250ml/kg group (732

$\pm 7.2 \times 10^6$ cells/mm^3) compared to the control ($690 \pm 1.2 \times 10^6$ cells/mm^3) and Proviron ($685 \pm 5.0 \times 10^6$ cells/mm^3). The study showed that the enhancement in sperm concentration suggests that the extracts stimulate spermatogenesis, possibly through improved testicular function and Hormonal modulation (Showky *et al*., 2021).

5.1.2 Progressive Motility

Progressive motility, a key determinant of fertilizing potential, was

Markedly increased with the 250ml/kg group showing the highest value

(89 ±.0.4%), followed closely by the 50ml/kg group (88 ± 0.1%)

Compared to the control (81 ± 0.2%) and proviron (84 ± 0.5%). This indicates enhanced sperm Viability and potential fertilizing capacity

(Showky *et al*., 2021).

5.1.3 Non-progressive and Immotile Sperm

Non- progressive motility and immotility decreased in the extract-treated groups. Immotile sperm percentage dropped from 7 ±. 0.2% in the control group to 0.2 ± 0.6% in ACCMJ 50 ml/kg and 250 ml/kg, this study indicates marked improvement in motility (Leko *et al*., 2021).

5.1.4 Sperm Morphology

Sperm morphology analysis revealed a substantial increase in normal Sperm forms in ACCMJ 50ml/kg (95 ± 0.8%) and 250ml/kg (98 ± 0.4%) compared to the control (85 ± 0.4%) and proviron (91 ± 0.2). Abnormal sperm morphology decreased sharply in treated groups with ACCMJ 250ml/kg showing only 2 ± 0.2% abnormalities versus 15± 0.1% in the control. These results are consistent with reports that *Ananas Comosus* Juice rich in bromelain and antioxidants, and *Cocos nucifera* Milk Containing Lauric acid and essential micronutrients, protect spermatozoa from Oxidative damage and structural deformities (Leko *et al*., 2021).

5.2 EFFECT OF EXTRACT *Ananas Comosus* JUICE AND *Cocos Nucifera* MILK ON SPERM CHARACTERIZATION OF ADULT MALE WISTAR RATS TREATED FOR 28 DAYS (X100 Objective lense)

Plate A (control): Sperm cells were normal in appearance with intact heads, midpieces and tails, indicating no degenerative changes in untreated animals. Plate B (proviron 25mg/kg): Sperm morphology was also normal, consistent with proviron's Known androgenic properties that enhance spermatogenesis and Sperm structure. Plate C (ACCMJ 50ml/kg): Sperm cells retained normal structure comparable to the Control group, showing that the lower dose of extract maintained sperm integrity. Plate D (ACCMJ 250ml/kg): Sperm morphology was normal with intact heads and tails, suggesting that the higher dose of extract not only preserved but possibly enhanced spermatogenesis. Normal Sperm morphology in extract treated groups

(especially at 250ml/kg) indicates improved development of sperm and spermatogenesis, epididymial maturation including Structural integrity (normal head and tail) implies lower levels of developmental defects and membrane damage (Showky *et al*., 2021).

5.3 EFFECT OF EXTRACT *Ananas Comosus* JUICE AND *Cocos nucifera* MILK ON BODY AND REPRODUCTIVE ORGAN WEIGHT INDEX

The ACCMJ 50 ml/kg juice and the proviron group showed increased reproductive organ (testes/combined reproductive organ) mass relative to control and the 250 ml/kg group. Increased Organ weight often reflects stimulated spermatogenic tissue (spermatogenic cell proliferation), leydig/Sertoli cell hypertrophy or increased intestinal tissue. The lower organ Weight at 50ml/kg suggests either no effect at that dose or a slight Negative/neutral effect compared with control (Showky *et al*., 2021).

5.4 EFFECT OF EXTRACT *Ananas Comosus* JUICE AND *Cocos nucifera* MILK ON MALE HORMONAL ASSAY

Testosterone increased in extract treated groups, was highest in ACCMJ 50ml/kg compared to control. Elevated testosterone levels are consistent with enhanced spermatogenesis and libido. Luteinizing hormone (LH) was marked increased in proviron compared to control, but significantly reduced in ACCMJ treated groups, suggesting different modes of hormonal modulation. Both ACCMJ doses lowered FSH significantly compared to control. Since FSH is essential for spermatogenesis, its reduction alongside increased testosterone may indicate a feedback inhibition mechanism. EST2 (Estrogen 2) notably increased in ACCMJ 250ml/kg compared to Control. While moderate estrogen is essential for male fertility, excessive levels can have inhibitory effects, however, in this case, it may suggest balanced steroidogenesis (Showky *et al*., 2021).

CONCLUSION

This study showed that extract of *Ananas Comosus* Juice and *Cocos nucifera* Milk especially at 50 and 250 ml/kg, improved male reproductive indices in Wistar rats. Sperm morphology remained normal across treatment groups, with notable increases in body weight and reproductive organ mass at higher doses. These enhancements may be attributed to antioxidant and androgenic compounds that protect testicular cells and stimulate spermatogenesis. The results are consistent with previous research, indicating that pineapple and coconut extracts have potential as natural fertility enhancers.

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