EFFECT OF AQUEOUS *FRAGARIA ANANASSA* (STRAWBERRY FRUIT) EXTRACT ON MALE REPRODUCTIVE HORMONES IN ADULT MALE WISTAR RATS

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CERTIFICATION

I certify that this project was written, under my supervision, by **Emeka Ijeoma Blessing** of the Department of Physiology, University of Benin, Benin City, Nigeria.

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DEDICATION

I dedicate this work to God Almighty for his strength and wisdom and grace to carry through.

To my amazing parents, MR AND MRS EMEKA for their constant prayers and encouragements and support in every area to make this project a success, God bless you abundantly.

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ABSTRACT

Strawberry (*Fragaria ananassa*) is an herbaceous plant from the *Fragaria* genus. It is a well-known plant with a widely enjoyed fruit. Strawberry, like other common fruits, contains antioxidants such as ascorbic acid (vitamin C), folic acid, and essential oils It is also high in minerals like iodine, magnesium, copper, iron, and phosphorus, as well as vitamins like thiamine, riboflavin, niacin, vitamin B6, vitamin K, vitamin A, and vitamin E. Hormones have critical role in fertility management and regulation. Endocrine glands produce hormones and release them to target organs in response to stimulation via negative or positive feedback mechanisms. This study was aimed at evaluating the effect of *Fragaria ananassa* (strawberry fruit) extract on male reproductive function by analyzing the male hormones in adult male wistar rat. A total of twenty (20) adult male wistar rats were used for this study. The rats were divided into four (4) experimental groups (A-D) with five (n=5) rats in each group. The rats were acclimatized for two weeks before commencement of administration. Group A was the control group. Group B, C and D were administered 50mg/kg, 100mg/kg and 200mg/kg body weight of *fragaria ananassa* extract orally via gavage respectively. The rats were anesthetized using chloroform vapor and were terminally bled by cardiac puncture. The blood samples were collected using a heparinized tube for hormonal assay. Testosterone was determined by competitive enzyme immunoassay (TYPE 7) and Luteinizing hormone was determined by Immunoenzymometric assay (TYPE 3). The result were statistically analyzed using Graph-Pad prism version 8.0. Comparison within groups were done using one-way ANOVA. The result were presented as mean ±SEM and p-value less than 0.05($P < 0.05$) was considered statistically significant. The result showed non-significant difference in the hormones (Testosterone and Luteinizing hormone) in group B, C and D when compared to the control group (Group A). In conclusion aqueous extract of *Fragaria ananassa* (strawberry fruit) does not have significant effect on the reproductive hormones (Testosterone and Luteinizing hormone) in adult male wistar rats

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

For centuries, humans have used natural products in folk medicine as therapeutic agents and drugs. However, the use of these natural products has increased in recent decades and have been gradually introduced into the pharmaceutical market (Dias et al., 2017). Using laboratory animals, several studies have demonstrated that certain natural products and derivatives might play a protective role against the damaging effects associated with oxidative stress, inflammation, and drugs side effects (Jia et al., 2018; Amin et al., 2008). Moreover, many natural products have already been described as being useful for the treatment of male disorders such as sexual impotence (Mbongue et al., 2012; Soliman et al., 2012). Various natural products have a set of properties that can be beneficial for the functioning of the male reproductive system, both in animals and in humans. These natural products can be an isolated bioactive compound or a product containing a mixture of several bioactive compounds in their composition. Strawberry (Fragariaananassa) is an herbaceous plant from the Fragaria genus. It is a well-known plant with a widely enjoyed fruit. Strawberry, like other common fruits, contains antioxidants such as ascorbic acid (vitamin C), folic

acid, and essential oils (Elkhadragy et al., 2017). It is also high in minerals like iodine, magnesium, copper, iron, and phosphorus, as well as vitamins like thiamine, riboflavin, niacin, vitamin B6, vitamin K, vitamin A, and vitamin E. (Giampieri et al., 2012). Strawberry seeds, on the other hand, are high in necessary polyunsaturated fatty acids that cannot be produced by the human body, in addition to mineral and vitamin content (Pieszka et al., 2013). Flavonoids (primarily anthocyanins with flavonols representing a percentage), tannins (in the form of ellagitannins and gallotannins), and phenolic acids (hydroxybenzoic and hydroxycinnamic acids) have been shown to be abundant in strawberry fruit extract (Proteggente et al., 2002). Hormones have critical role in fertility management and regulation. Endocrine glands create them and release them to target organs in response to stimulation via negative or positive feedback processes (Mclachlan et al., 2002). Male hormones are chemical compounds that govern and control the male reproductive system's activities. The Testicles (which produce Testosterone) or the gonadotropic releasing hormones release these hormones (GnRH). The testes secrete a variety of male sex hormones known as androgens, including testosterone, dihydrotestosterone, and androstenedione. Testosterone is the most abundant and the major testicular hormone in all of these (Dutta et al., 2019).

1.2 Aim

The aim of this study is to evaluate the effect of aqueous Fragariaananassa (strawberry fruit) extract on male reproductive function by analyzing the male hormones in adult male wistar rat.

1.3 Research Questions

- Does aqueous extract of *Fragariaananassa*have effect on the testosterone level in adult male wistar rat?
- Does aqueous extract of *Fragariaananassa*have effect on the Luteinizing Hormone (LH) level in adult male wistar rat?

1.4SpecificObjectives

The objectives of this study are to:

- To determine the effect of *Fragariaananassa* on the testosterone level in adult male wistar rat.
- To determine the effect of *Fragariaananassa* on the Luteinizing Hormone (LH) level in adult male wistar rat.

1.5Scope OfStudy

1. Animal studies: Research involving animal models, such as rats, mice, rabbits, and other animals, to investigate the effects of strawberry extract on male reproductive function.

2. Safety and tolerability studies: Research to investigate the safety and tolerability of strawberry extract in male subjects. This may involve assessing the potential side effects and interactions with other medications or supplements.

1.6 Significance Of Study

The study of the effect of strawberry extract on male reproductive function is significant for several reasons:

1. Promoting male reproductive health: Male infertility is a growing concern worldwide, and natural supplements such as strawberry extract may offer a potential solution for improving male reproductive function. By investigating the effects of strawberry extract on male reproductive health,

this research may contribute to the development of new therapies for male infertility.

2. Potential alternative to conventional treatments: Many conventional treatments for male infertility, such as hormone therapy and assisted reproductive technologies, can be costly and have potential side effects. By exploring the potential benefits of natural supplements such as strawberry extract, this research may provide a more accessible and cost-effective alternative for improving male reproductive health.
3. Understanding the mechanisms of action: By investigating the mechanisms by which strawberry extract exerts its effects on male reproductive function, this research may provide insights into the underlying causes of male infertility and potential targets for future therapies.
4. Promoting the use of natural supplements: By demonstrating the potential benefits of natural supplements such as strawberry extract, this research may promote the use of these products as a safe and effective alternative to conventional therapies for male infertility. This may help reduce the reliance on pharmaceuticals and other treatments with potential side effects.

1.7Justification Of The Study:

Strawberry is among the top 5 fruits with high antioxidant properties in the world. It has been reported to have impacts on the immune system, cardiovascular health, amongst others. A larger percentage of strawberry research focuses on its antioxidants effects but relatively lesser attention is paid to its effects on reproductive hormones. This study is therefore an effort to add to the growing body of knowledge pertaining strawberry's effect on sex hormones, specifically, testosterone and luteinizing hormone

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin Of The Cultivated Strawberry

Fragaria genus (*Rosaceae*), commonly known as strawberry, represents one of the most important food plants all over the world, with a double global production compared with all other fruit berries combined (Liston *et al.,*2014). Their widespread use, primarily because of their flavor, can also lead to considerable benefits to human health. Among other characteristics, nonvisual properties like taste, nutritional values, or aroma make these fruits to be in the top of consumer preferences (Awad *et al.,* 2003). The Rosaceae are a large eudicot family including a rich diversity of crops with major economic importance worldwide, such as nuts (for example, almonds), ornamentals (for example, roses), pome fruits (for example, apples), stone fruits (for example, peaches), and berries (for example, strawberries). Strawberries are prized by consumers, largely because of their complex array of flavors and aromas. The genus *Fragaria* was named by the botanist Carl Linnaeus, on the basis of the Latin word 'fragrans', meaning 'sweet scented', describing its striking, highly aromatic fruit(Edger*et al.,* 2019).The popular garden plant commonly known as cultivated strawberry (*Fragaria×ananassa*) is a homoploid hybrid species with a unique domestication

history spanning less than 300 years. The first *F.×ananassa* individuals originated in western Europe in the early 1700 s as spontaneous hybrids between nonsympatric ecotypes of cross-compatible allo-octoploid (2*n* = 8× = 56) species *Fragaria virginiana* and *Fragaria chiloensis* native to North and South America, respectively. Wild-collected specimens of these species were introduced to Europe in the 1600s and 1700s and became established in common gardens where they freely hybridized. Offspring from these spontaneous hybrids were reported to be phenotypically unique and horticulturally superior to their parents. The interspecific origin of these hybrids, however, went undiscovered for nearly a half century. The original hybrids and their descendants were disseminated and cultivated in Europe for nearly a century, far from the centers of diversity of *F. chiloensis* and *F. virginiana*, before migrating to North America in the early nineteenth century and spreading worldwide. Their horticultural superiority and phenotypic diversity drove the domestication and agricultural ascendancy of *F.×ananassa* over either parent species (Michael*et al.,* 2021). Considering the economic and social importanceof Spanish strawberry production around the world, more research is being done in strawberrybreeding to meet the needs of growers and consumers. In this regard, there are numerous publicand private strawberry breeding programs aimed at developing and releasing cultivars that are well-adapted to the agronomic and environmental conditions of various cultivation areas,

with improved agronomic traits (yield and fruit quality), and harvest periods that are best suited to the farmers' economic interests. Recently, health-related criteria as well as adaptation to diverse culture techniques (such as soil-less and organic culture) have been added into those programs. Climate differences between farming areas and inter-annual volatility may have a negative impact on yield Strawberry cultivars have a strong ability to adapt to microclimates (Bartual *et al.,* 2000),and the employment of various cultural practices and production systems allows for marketable fruit yields and even improved crop quality of strawberry cultivars. Because of the shallow rooting system, high leaf area, and high water content of the fruit, strawberry production necessitates a large amount of water (Klamkowski and Treder, 2006; Grant *et al.,* 2010). Furthermore, growing in polyethylene plastic tunnels, as in Huelva, need irrigation throughout the production cycle (from mid-October to late May/mid-Jun) (Ariza *et al.,* 2011) and at a high frequency, due to the poor soils and low water holding capacity of the soils (sandy soils mostly).

Fig. 2.1: picture of a bunch of strawberry fruits(Michael*et al.*, 2021).

2.1.1 Scientific Classification

Domain: Eukaryotes

Kingdom: Plantae

Division:Magnoliophtya

Class:Magnoliopsida

Subclass: Rosidae

Order: Rosales

Family: Rosaceae

Genus: *Fragaria*

Species: *ananassa*

2.1.2 Botanical Name

Fragaria x ananassa

2.1.3 Common Name

Strawberry

2.1.4 Plant Description

Strawberry is a member of family Rosaceae and genus Fragaria. Botanically, strawberry is not a berry and grows on perennial woody plant. The leaves are present in clusters, generally 3 in number, oval in shape, tooth edged and hairy. The leaves are glossy, smooth and dark green in color on upper side andlight green with small whitish hair on lower side. The plant bears flowers in clusters. The strawberry flower has small green leaf like 5 sepals which enclose white petals. The hermaphrodite flowers are whitish in appearance, bell shaped, and 4–6 mm acrossand are present at the axils of the leaves. As the plant matures and becomes old, therest system changes to woody and the mother crown put forth runners that touch theground and spread, thus increasing the vegetation of plant. The plant shed the leaveswhen old (Sharma & Thakur, 2008). The fruit is 5–10 cm in length, 2–3 cm broad with serrated margins. Strawberryfruit is bright red in color and is heart shaped with rough surface and 1–2 cm indiameter. Strawberry is not a true berry and is known as accessory fruit. Botanicallythe red heart shaped fruit is an enlarged flower system with many seeds embeddedonto the surface. These yellow seeds are actually the true fruit known as achenes. Amedium sized strawberry contains about 200 achenes. The noteworthy thing aboutstrawberries is that the

offspring doesn’t retain any horticultural characteristics ofparent strawberry. The main flesh of strawberry, known by the name cortex, is juicy, reddish white in color and acidic in nature (Hussain *et al.,* 2021).

2.1.5 HabitatAnd Geographical Distribution

Strawberries requirean annual rainfall of 150 mm and prefer soil rich in humus, slightly acidic (pH 5–6.5), sandy loam or light soil with slight acidic nature and good drainage are primerequisite for planting strawberry crops. Soil below 300 mm from surface should befree of heavy clay and rocks as heavy clay soils action water after rains increasingthe risk of diseases and reduce yields (Sharma, 2002). Wild strawberries can growin a wide variety of habitat from open woodlands and meadows to sand dunes andbeaches (Wilson *et al.,*1973)

Strawberries requirean annual rainfall of 150 mm and prefer soil rich in humus, slightly acidic (pH 5–6.5), sandy loam or light soil with slight acidic nature and good drainage are primerequisite for planting strawberry crops. Soil below 300 mm from surface should befree of heavy clay and rocks as heavy clay soils action water after rains increasingthe risk of diseases and reduce yields (Sharma, 2002).

2.1.6Chemical CompositionAnd Nutritional Facts

Strawberry is a low-fat (46 cal/188 kJ) fruit (one cup 240 gr), low in protein and fat, lowglycemic index (GI 25) due to low content of fruit sugar (glucose, fructose), middle fiber-rich, but extremely rich in minerals such as macro elements, particularly essential ones like calcium, magnesium, potassium, and sodium, and microelements such as phosphorus. Strawberries alsoinclude anthocyanins and ellagic acid, which have been linked to health benefits in researchoninflammation, neurological illnesses, aging, and even cancer. Strawberry performs good hydration of the body when the meal represents due to its highwatercontent, which is required for the development of proper metabolic processes in the cell. Potassium, sodium, calcium, magnesium, iron, copper, manganese, fluorine, and iodineareamong the minerals found in this fruit. Manganese is a co-factor for the antioxidant enzymesuperoxide dismutase, as well as copper, which is essential for red blood cell generationandformation with iron content. Strawberry has a very positive relationship between potassium (161mg/100 g) and sodium (18 mg/100 g), which affects the normalization of blood pressureinhumans, hence strawberries are suggested foods for people with hypertension. Strawberryoffersoutstanding nutritional value as a meal due to its low quantity of saturated fat, cholesterol,

andsalt, as well as its small content of vital unsaturated fatty acids and considerable amountsofdietary fiber. The strawberry is high in a variety of vitamins. It is high in B-complex vitamins such as niacin, riboflavin, pantothenic acid, and folic acid, which act as co-factors in the metabolismofcarbohydrates, proteins, and fats. Fisetin (more precisely flavonol) , a strong flavonoid (group of polyphenols) that is rarelyfoundin fruit plants of the Rosaceae family, such as strawberries (Stoner *et al.,*2006) , belongs tothegroup of polyphenols (Stoner *et al.,* 2006)., It's been found in Fabaceae (Acacia spp., Gleditsiaspp.) and Anacardiaceae (Joseph *et al.*, 2009) trees and shrubs, as well as grapes, teas (Sakakibara*et al.,* 2003; Tsuneki *et al.,* 2004), apples, wine (De Santi*et al.,* 2002), and teas (Sakakibara *et al.,* 2003; Tsuneki *et al.,* 2004) produced as infusions. When comparedtoother fruits such as peaches, apricots, apples, and plums, strawberries have 4-10 times theamount of antioxidants. As a result, these compounds are directly responsible for the fruit'sability to symbolize some meal in a condition of illness, tiredness, or fatigue, both acute andchronic, putting strawberries high on the food pyramid. According to some researchonthecombination of antioxidants and anti-inflammatory nutrients, at strawberry (Burton-Freemanet *et al.,* 2010), the focus is directed towards several benefits to human health; here primarilyreferstothe prevention of cardiovascular disease, regulate blood sugar levels, strengthen the immunesystem and in some part even prevents the occurrence of cancer. Both in terms of the content

ofvitamins, strawberry is superior to other fruits (except black currant) and up to seventimescontains more vitamin C and kvercetin,whose physiological effects seen in part inoxidoreductive processes in cellular metabolic reactions (Basu *et al.,* 2010). Strawberries areanexcellent anemia treatment due to their high iron content (anemia). Strawberry has also been widely used in cosmetics and cosmetic goods, particularly thoseforfacial care. It cleans pores and hydrates the skin. Strawberry can cause allergic responsesinsome people, especially youngsters, so it's important to be cautious about howmuch andhowoften you eat it. It is not recommended to take in big numbers until it is determinedthat theperson is not allergic and has no adverse responses (Marzban *et al.,* 2005.)

Fig 2.2: Strawberry Plant, Flower and Fruits(Michael*et al.,* 2021).

2.1.7. Phytochemical Composition

Strawberry (Fragaria x ananassa) is a widely consumed fruit that is rich in various nutrients such as vitamin C, potassium, and dietary fiber. It is also a rich source of phytochemicals such as flavonoids, phenolic acids, and anthocyanins (Giampieri *etal.,* 2012). These phytochemicals have been reported to possess various health benefits such as antioxidant, anti-inflammatory, and anticancer properties.

2.1.7.1 Anthocyanins

Anthocyanins belong to the flavonoids group, with the basic structure of a core flavone,whichconsists of two aromatic rings linked by a three carbon unit. This type of compound is what givescolor to various fruits and can transition from red to blue. In addition to giving fruits their color, anthocyanins protect the plant against the effects of UV radiation and against viral and microbial contamination. These compounds also serve to attract pollinators for subsequent seed dispersal. Pelargonidin-3-glucoside is the major anthocyanin in strawberries independent

from genetic and environmental factors, and the presence of cyanidin-3-glucoside seems to be constant in strawberries, although only in smaller proportions. As mentioned, anthocyanins are mostly responsible for the red, blue, andpurplecolors of flowers, fruits, and vegetables (Koponen *et al.,* 2009) and are known to playakeyrole in the treatment of cardiovascular disease, cancer, diabetes, and others. In addition, they have anti-inflammatory benefits onhumanhealth**.** Anthocyanins in strawberries are the best known polyphenolic compounds and quantitatively the most important. Many studies have determined total anthocyanin content, reporting values from 150 to 600 mg/kg of fresh weight; moreover, investigators have found values of up to 800 mg/kg of fresh weight. More than 25 different anthocyanin pigments have been described in strawberries of different varieties and selections. Furthermore, although glucose seems to be the most common substituting sugar in strawberry anthocyanins, rutinose, arabinose, and rhamnose conjugates have been found in some strawberry cultivars (Giamperi *etal.,*2012).

2.1.7.2Ellagitanins

Ellagitannins, which are ellagic acid derivative complexes (non-flavonoids), belong totheclassof polyphenols known as hydrolyzable tannins. These compounds

have properties similar to those of proanthocyanidins, such as a high molecular weight, water-solubility and the ability to solubilize proteins and alkaloids (Koponen*et al.,* 2007).It isofinterest to investigate the contents of ellagic acid, which has antiviral and antioxidant activityand protects against cancers, including colon, lung and esophageal cancers (Nile*et al.,*2014).Ellagitannins are hydrolyzed in the human digestive system to release ellagic acid, which is a potent antioxidant and has been shown to have anticancer properties. Ellagic acid has been reported to inhibit the growth of cancer cells and induce apoptosis, which is the programmed cell death of damaged or abnormal cells.Several studies have shown that ellagitannins may have potential health benefits. For example, ellagitannins have been reported to possess strong antioxidant properties, which can help to protect against oxidative stress and inflammation. In addition, ellagitannins have been shown to have anticancer properties, which may be due to their ability to inhibit the proliferation of cancer cells and induce apoptosis. Moreover, ellagitannins have been reported to have antimicrobial properties, which may be useful in the development of natural food preservatives (González-Barrio *et al.,* 2010).

2.1.7.3 Flavonols

Flavonols are a group of compounds found in foods in the form of O- and C-glycosides. Thesemay be absorbed in the form of glycosides by humans through the diet, for example,throughtheconsumption of quercetin. It has been shown in studies that these compounds are beneficial tohuman health because they exhibit antioxidant and anticarcinogen characteristics (Hakkinen*et al.,* 1998). The flavonols found in the greatest amounts in strawberry include quercetin3-0-glucoronide. Flavanols are a group of polyphenolic compounds that are present in strawberries and have been found to possess various biological activities such as antioxidant, anti-inflammatory, and cardioprotective properties.Several studies have shown that flavanols may have potential health benefits. For example, flavanols have been reported to possess strong antioxidant properties, which can help to protect against oxidative stress and inflammation (Tangney and Rasmussen, 2013). In addition, flavanols have been shown to have cardioprotective properties, which may be due to their ability to improve vascular function and reduce blood pressure (Tangney and Rasmussen, 2013). Moreover, flavanols have been reported to have anti-inflammatory properties, which may be beneficial for preventing and treating chronic diseases such as cancer and cardiovascular disease

2.1.7.4 Phenolic Acids

In general, phenolic compounds are secondary plant metabolites and are widespreadinall vegetables (Panico *et al.,*2009). Depending on their structure, phenolic compounds aredivided into non-flavonoids and flavonoids, which give rise to other compounds of interest through their antioxidant capacity In strawberries, flavonoids are the most abundant phenoliccompounds (Giampieri *etal.,*2014), and in particular, anthocyanins comprise approximately40%of the total phenolic compounds

2.1.7.5 Cinamic Acids

Cinnamic acid conjugates are natural substances found in fruits and vegetables and are consumedas dietary phenolic compounds. These compounds have various biological activities, includingantioxidant, hepatoprotective, anxiolytic, insect repellent, antidiabetic and anticholesterolemicactivities (Sharma*et al.,* 2002). Giampieri *et al.,*(2014) has identified cinnamicacidconjugates in strawberries

2.1.8 HEALTH BENEFITS OF *FRAGARIA ANANASSA*(STRAWBERRY FRUIT)

2.1.8.1 Cardiovascular Diseases (CVDs)

CVD is the major cause of death worldwide. Studieshave revealed that consumption of strawberries improves cardiovascular health anddecreased risk of heart-related deaths. Anthocyanin from strawberries work againstCVD development. This effect of anthocyanin's is due to the number and positionof OH groups, its chemical structure i.e. conjugated groups, the degree of glycosylation and the presence of donor electron in the ring structure. In vitro studies havereported that berries consumption causes an elevation in levels of HDL cholesteroland reduction in blood pressure while improving the functioning of blood platelets(Tulipani *et al.,* 2011).Anthocyanins from strawberry improve blood antioxidant status and elevateoxidative stress by inhibiting inflammation. Furthermore, intake of strawberries alsoleads to improved vascular function and blood lipid profile while under the oxidationof LDL cholesterol. In a study, supplementation of freeze dried strawberry to CVDpatients resulted in reduction in LDL cholesterol, inflammatory markers (C-reactiveprotein) and oxidized LDL particles—the few risk factors associated with CVD.Anthocyanin help relax blood vessels by improving the flow of blood. Moreover, the fibre along with vitamin C and folate from strawberry reduce cholesterol levelsin arteries and vessels (Chaudhuri *etal.,* 2007).

2.1.8.2 Cancer Prevention

Cancer is the uncontrolled growth of abnormal cells and oxidative stress oftenhastens the process. Animal model studies have shown that administration of straw-berry extract inhibited tumor formation in oral cancer cells and liver cancer cells.Strawberry exerts chemo preventive effect through their ability to fight oxidative stressand inflammation. The protective effect of strawberry is believed to be due to ellagicacid and ellagitannins. Ellagic acid inhibits proliferation of tumor cells by inducingapoptosis and cleaving of DNA bonded with carcinogens (Losso *et al.,* 2004).

2.1.8.3 Oral Disease

Oral health has a significant effect on overall health and quality of life. Conditions such as periodonitis, xerostomia, mucositis, and tooth decay are associated with co-morbidities such as decreased saliva production, difficulty in chewing and swallowing, and loss of taste (Gift and Atchison, 1995). In

addition, new diagnoses of oral cancer are estimated to be in excess of 35,000 in 2010 (Centers for Disease Control and Prevention, 2009). Fruit phenolics have been shown to elicit significant protective effects on oral mucosa when evaluated in numerous pre-clinical animal models (Seeram, 2008) and may be a novel prevention tool instead of costly pharmaceutical agents that may have undesirable side effects. The association of chronic disease and oral health is possibly due to infection, chronic inflammation, genetic predisposition, and potentially nutrition (Ritchie *et al.,* 2002; Ritchie *et al.,* 2003). Seeram *et al.,* (2008) reported a dose-dependent anti-proliferative effectin oralcell lines with a phenolic-enriched strawberry extract.

2.2 MALE REPRODUCTIVE HORMONES

The male reproductive system consists of the internal structures: the testes, epididymis, vas deferens, prostate, and the external structures: the scrotum and penis. These structures are well-vascularized with many glands and ducts to

promote the formation, storage, and ejaculation of sperm for fertilization, and to produce important androgens for male development. The major male androgen is testosterone, which is produced from Leydig cells in the testes. Testosterone can be converted in the periphery to a more active form, dihydrotestosterone via 5-alpha-reductase, or estradiol via aromatase. Other key hormones include inhibin B and Mullerian inhibiting substance (MIS) hormone, both produced by the Sertoli cells in the testes. Important hormones that modulate these include follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are released from the anterior pituitary gland and are regulated by gonadotropin-releasing hormone (GnRH), produced by the hypothalamus(Tiwana and Leslie, 2022).

Male hormones are chemical compounds that govern and control the male reproductive system'sactivities. The Testicles (which produce Testosterone) or the gonadotropic releasing hormonesrelease these hormones (GnRH). The testes secrete a variety of male sex hormones known as androgens, including testosterone, dihydrotestosterone, and androstenedione. Testosterone is the most abundant and the major testicular hormone in all of these. Theinterstitial cells of Leydig cells in the seminiferous tubule produce testosterone, which accounts 47 for around 20% of the mass of the mature testes. The ejaculate is a type of ejaculate that isproduced by the Prostate fluids also aid with sperm nutrition. The urethra goes

throughthemiddle of the prostate gland, carrying the ejaculate to be released at orgasm(Dutta *et al.,* 2019).

2.2.1. Luteinizing Hormone (LH)

Luteinizing Hormone, also known as Lutropin is a glycoprotein hormone that is co-secreted along with follicle-stimulating hormone by the gonadotrophin cells in the adenohypophysis (anterior pituitary). Luteinizing hormone is a part of a neurological pathway comprised of the hypothalamus, the pituitary gland, and gonads (Ilahi and Ilahi, 2022). When testosterone levels are low, GnRH is released, an androgen that exerts both endocrine and intratesticular spermatogenesis, and the Leydig cells produce testosterone (T) under the control of LH (Ramaswamy and Weinbauer, 2014)

2.2.2 Mechanism Of Action

Luteinizing hormone acts by binding to a G-protein coupled receptor, which in turn activates adenylyl cyclase. Adenylyl cyclase, an enzyme, then produces cyclic-AMP, thus increasing its intracellular concentration, which then activates a kinase molecule called protein kinase A (PKA). PKA then phosphorylates specific intracellular proteins that subsequently achieve the end physiological actions of LH like steroid production and ovulation (El sayad*et al.,*2023)

2.2.3 Functions Of Luteinizing Hormone

In men, LH isneeded for androgenization during puberty, sexualdifferentiation, and sexual functioning. Male fertility is closely tied to adequate LH functioningsince it is the precursor to testosterone, which inturn is crucial for sperm production, sperm motility, and energy acquisition by the way ofaugmenting sperm fructolysis and adenylcyclaseactivity (Ramanujam*et al.,*2000)

2.2.4 Control Of Luteinizing hormone

LH secretion from anterior pituitary is suggested to be induced by high-frequency hypothalamic GnRH pulses. When plasma testosterone level is low, hypothalamic

GnRH induces pituitary LH secretion, and when the level of testosterone is adequate, it operates negative feedback inhibition of GnRH and LH release(Gill Sharma, 2009).

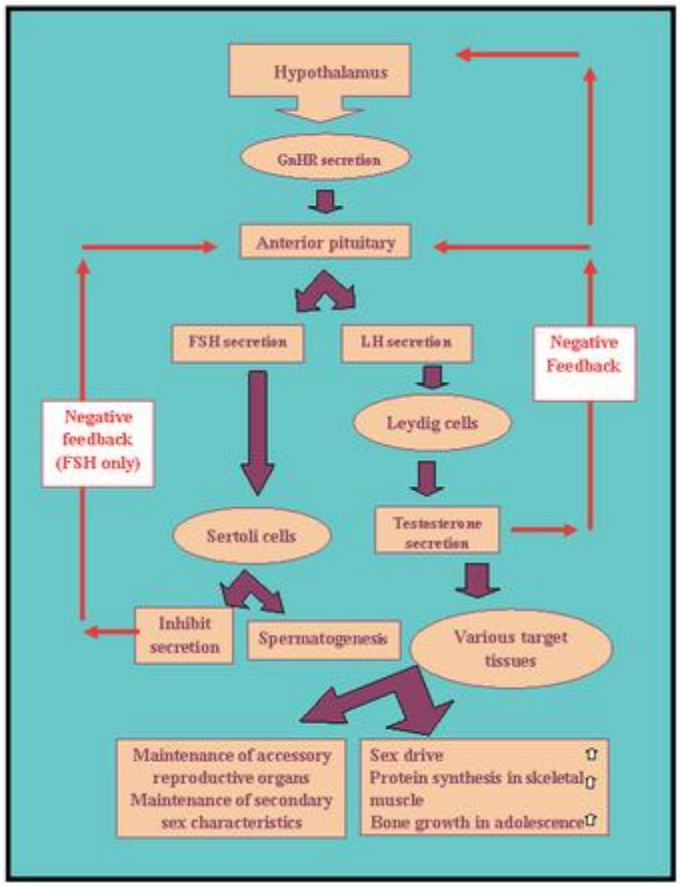


Fig 2.2 DIAGRAM OF HORMONAL REGULATION OF MALE HORMONES

2.2.5Testoterone

Testosterone is the primary male hormone responsible for regulating sex differentiation, producing male sex characteristics, spermatogenesis, and fertility. Testosterone's effects are first seen in the fetus. During the first 6 weeks of development, the reproductive tissues of males and females are identical. At around week 7 in utero, the SRY (sex-related gene on the Y chromosome) initiates the development of the testicles. Sertoli cells from the testis cords (fetal testicles) eventually develop into seminiferous tubules. Sertoli cells produce a Mullerian-inhibiting substance (MIS), which leads to the regression of the Fallopian tubes, uterus, and upper segment of the vagina (Mullerian structures normally present in females). Fetal Leydig cells and endothelial cells migrate into the gonad and produce testosterone, which supports the differentiation of the Wolffian duct (mesonephric duct) structures that go on to become the male urogenital tract(Basaria, 2013).

2.2.6 MechanismOf Action

In puberty, the hypothalamic-pituitary-gonadal axis plays a major role in regulating testosterone levels and gonadal function. The hypothalamus secretes GnRH, which travels down the hypothalamo-hypophyseal portal system to the anterior pituitary,

which secretes luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH are two gonadotropic hormones that travel through the blood and act on receptors in the gonads. LH, in particular, acts on the Leydig cells to increase testosterone production. (Plant and Marshall, 2021).

2.2.7 Testosterone Synthesis

Leydig cells in the testes function to turn cholesterol into testosterone. LH regulates the initial step in this process. Two important intermediates in this process are dehydroepiandrosterone (DHEA) and androstenedione. Androstenedione is converted to testosterone by the enzyme 17-beta-hydroxysteroid dehydrogenase. The majority of testosterone is bound to plasma proteins such as sex-hormone-binding-globulin and albumin. This majority supply of protein-bound testosterone acts as a surplus of testosterone hormone for the body. The small amounts of free testosterone in the blood act at the level of the tissues, primarily the seminal vesicles, bone, muscle, and prostate gland. At the cellular level, testosterone gets converted to dihydrotestosterone by the enzyme 5-alpha-reductase. Testosterone and dihydrotestosterone can bind to cell receptors and regulate protein expression. Both men and women also produce weak acting androgens in the zonareticularis of the adrenal glands. These weak-acting

androgens are known as dehydroepiandrosterone and androstenedione. They bind to testosterone receptors with weaker affinity but can also be converted to testosterone in the peripheral tissues if produced at high amounts (Clark *et al.,* 2018).

2.2.8 Feedback Mechanism

Testosterone limits its own secretion via negative feedback. High levels of testosterone in the blood feedback to the hypothalamus to suppress the secretion of GnRH and also feedback to the anterior pituitary, making it less responsive to GnRH stimuli. The hypothalamic-pituitary axis in the male is controlled by **negative feedback,** which has two paths. In the first path, **testosterone** itself feeds back on both the hypothalamus and the anterior lobe, where it inhibits the secretion of GnRH and LH. At the hypothalamic level, testosterone decreases both the frequency and amplitude of the GnRH pulses. In the second path, the Sertoli cells secrete a substance called**inhibin.** Inhibin is a glycoprotein that is a feedback inhibitor of FSH secretion by the anterior pituitary. Thus, the Sertoli cells, which produce sperm, synthesize their own feedback inhibitor that serves as an "indicator" of the spermatogenic activity of the testes.

Negative feedback control of the hypothalamic-pituitary axis is illustrated when circulating levels of testosterone are decreased (e.g., testes are removed). Under such conditions, the frequency and amplitude of GnRH, FSH, and LH pulses are *increased* because of decreased feedback inhibition by testosterone on the hypothalamus and anterior pituitary (Mega, 2023).

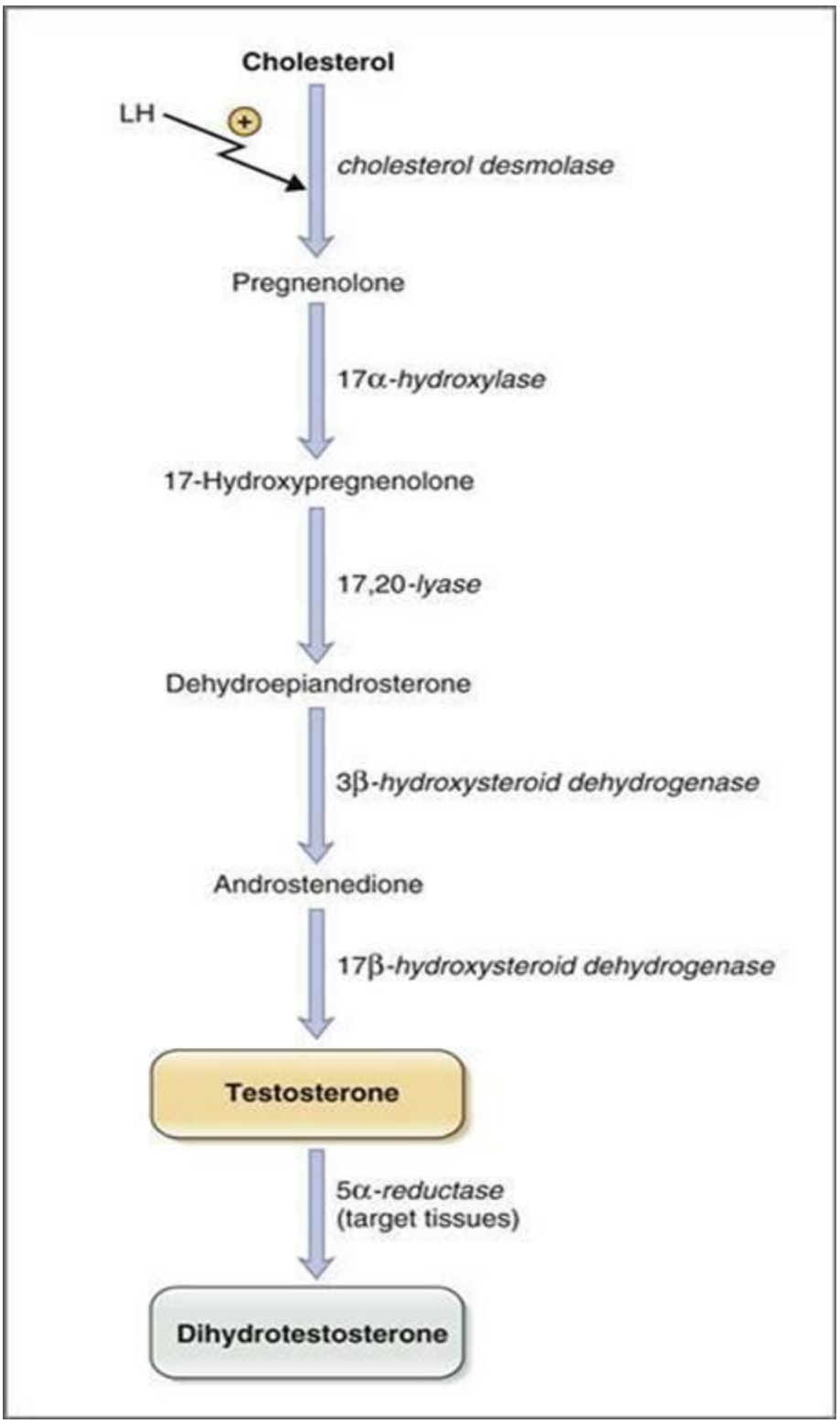


Fig 2.2.8 DIAGRAM OF BIOSYNTHETIC PATHWAY OF TESTOSTERONE

2.2.9 FunctionOf Testosterone

Testosterone is responsible for the development of primary sexual development, which includes testicular descent, spermatogenesis, enlargement of the penis and testes, and increasing libido. The testes usually begin the descent into the scrotum around 7 months of gestation, when the testes begin secreting reasonable quantities of testosterone. If a male child is born with undescended but normal testes that do not descend by 4 to 6 months of age, administration of testosterone can help the testes descend (Kalfa*et al.,* 2019). Secondary sexual traits such as hair distribution around the pubic areas, skin thickness, male pattern baldness, deepened voice, and increased muscle mass are all influenced by testosterone. Testosterone enhances calcium retention and increases bone matrix, both of which are important in the treatment of osteoporosis.Testosterone levels tend to drop with increasing age; because of this, men tend to experience a decrease in testicular size, a drop in libido, lower bone density, muscle mass decline, increased fat production, and decreased erythropoiesis, which leads to possible anemia. (Niechlag*et al.,* 2012).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1Solvents

- Distilled water
- Chloroform

3.1.2Apparatus

- Rotary evaporator (Stuart, England)
- Filter paper (Whatman no. 1)
- Water bath
- Retort stand
- Electrical weighing balance
- Refrigerator (LG, China)

3.1.3Glassware

All glassware used were products of Pyrex (England), Fiolax and Chengdu (China).

- Measuring cylinders (5ml, 1oml, 250ml and 1000ml)

- Conical flasks (250ml)
- Beakers (50ml, 250ml and 500ml)
- Test tubes
- Separating funnel
- Automated pipettes
- Reagent bottles

3.1.4Others

- Cotton wool
- 5ml syringe
- Masking tapes
- Crucible
- Spatulas

3.1.5Experimental Animals

Twenty (20) MaleWistar rats weighing 150-200grams were purchased from the animal house of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin-City, Nigeria. The rats were housed in partitioned wire

meshed cages under standard laboratory condition of humidity, temperature (25± 2°c) and light (12hrs light/dark cycles). All rats were handled according to guiding principles in the care and use of animal's standard care of laboratory animals. They were supplied with feed and water *ad libitum*.The rats were acclimatized for two weeks and housed in separate cages and were randomly divided into four groups of five animals each as follows:

Group A: Control

Group B: were given 50 mg/kg body weight of *Fragariaananassa.*

Group C: were given 100 mg/kg body weight of *Fragariaananassa.*

Group D: were given 200 mg/kg body weight of *Fragariaananassa*.

3.2 METHODS

3.2.1 Plant Collection And Preparation

The *Fragaria ananassa* (strawberry) was procured from Vom, Jos, and Plateau-State, Nigeria. Identification of the plant was carried out by a plant taxonomist at

the Department of Pharmacognocy, Faculty of Pharmacy, University of Benin, Benin-City.

3.2.2 Extraction

The plant taxonomist weighed the fruit and it was actualized 1.4kg. Then it was macerated in a jar with distilled water of 2litres for 24hrs hours, then filtration was involved to separate the filtrate from the residue using filter paper, conical flask and funnel, the filtrate was then concentrated to paste level using crucible and water bath at a very low temperature of 60°, the crude extract was then preserved in a sample bottle.

3.2.3 Experimental Design

Each group containing five animals. Animals in group A was given rat feed and water *ad libitum*, those in test group B received 50 mg/kg body weight of *Fragaria ananassa* (low dose), animals in test group C received 100 mg/kg body weight of *Fragaria ananassa* (normal dose) while animals in test group D received 200 mg/kg body weight of *Fragariaananassa*(high dose)**.**Oral administration of the extract was carried out twice daily throughout the experimental period which lasted

for 4 weeks (28 days). The body weight of the animals was also recorded.Group A represented the control group. Group B, C and D were administered 50mg/kg, 100mg/kg and 200mg/kg body weight of *fragariaananassa* extract orally via gavage respectively.

3.3Hormonal Assay

Laboratory Method for assaying Testosterone.

Testosterone was determined by competitive enzyme immunoassay (TYPE 7).

Reagents used: testosterone calibrators (1ml/vial-Icons A-G), testosterone enzyme reagent (1.0ml/vial), steroid conjugate buffer (7.0ml/vial-Icon), testosterone biotin reagent (6.0ml-Icon), streptavidin coated plate (96 wells-Icon), wash solution concentrate (20ml-Icon), substrate A (7ml/vial-Icon S^A), Substrate B (7ml/vial-Icon S^B), stop solution (8ml/vial-Icon) (Accubind ELISA Microwells Monobind Inc. Lake Forest, CA 92630, USA).

Test Procedure:

- For each serum reference, the microplate's wells, control and specimen to be examined was duplicated and formatted.
- The assigned well contained 0.010ml (10µl) of the appropriate serum reference, control or specimen.
- To all assigned wells, 0.050ml (50µl) of the working testosterone enzyme reagent was added.
- For 20-30 seconds, the microplate was swirled gently for proper mixing.
- For 60 minutes at room temperature, they were covered and allowed to incubate.
- The microplate content was discarded by aspiration and the plate blotted dry.
- 0.350ml (350µl) of wash buffer was added and aspirated; this was done for additional two times.
- To all wells, 0.100ml (100µl) of working substrate solution was added.
- Incubation was done for 15minutes at room temperature.
- To each well, 0.050ml (50µl) of stop solution was added and mixing was done gently for 15-20 seconds.

- In each well, the absorbance was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) using a microplate reader. (Dorfman and Shipley, 1956).

Laboratory Method for assaying Luteinizing Hormone (LH).

Luteinizing hormone was determined by Immunoenzymometric assay (TYPE 3).

Reagents Used:

LH Calibrators (1ml/vial-Icons A-F), LH Enzyme Reagent (13ml/vial-Icon), streptavidin coated plate (96 wells-Icons), wash solution concentrate (20ml/vial-Icon), substrate A (7ML/VIAL-Icon S^A), substrate B (7ml/vial-Icon S^B), stop solution (8ml/vial-Icon) (Accubind ELISA Microwells Monobind Inc. Lake Forest, CA 92630, USA).

Test Procedure:

- For each serum reference, the microplate's wells, control and specimen to be examined was duplicated and formatted.

- The assigned well contained 0.010ml (10µl) of the appropriate serum reference, control or specimen.
- To all assigned wells, 0.050ml (50µl) of the working LH enzyme reagent was added.
- For 20-30 seconds, the microplate was swirled gently for proper mixing.
- For 60 minutes at room temperature, they were covered and allowed to incubate.
- The microplate content was discarded by aspiration and the plate blotted dry.
- 0.350ml (350µl) of wash buffer was added and aspirated; this was done for additional two times.
- To all wells, 0.100ml (100µl) of working substrate solution was added.
- Incubation was done for 15minutes at room temperature.
- To each well, 0.050ml (50µl) of stop solution was added and mixing was done gently for 15-20seconds.

In each well, the absorbance was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) using a microplate reader. (Danzer and Braunstein, 1980; Kosasa, 1981).

3.6 Statistical Analysis

All statistical analyses were carried out using Graph Pad Prism 5.0 version software. The data obtained from all the groups were presented as Mean ± S.E.M (Standard Error of Mean), (n=5) in each group and analyzed for statistical significance by using one-way Analysis of Variance (ANOVA). Values were considered significant at $P < 0.05$.

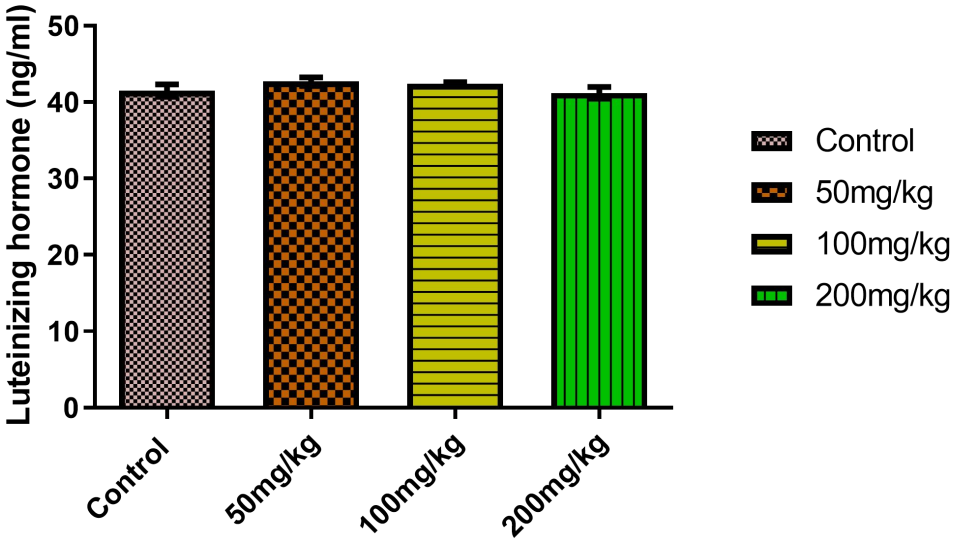
CHAPTER FOUR

4.0 RESULTS

Parameters	Group A	Group B	Group C	Group D
Luteinizing hormone	41.50 ± 0.81	42.67 ± 0.55	42.40 ± 0.22	41.20 ± 0.77
Testosterone level (ng/ml)	36.02 ± 0.71	37.04 ± 0.48	36.80 ± 0.19	35.76 ± 0.66

*P < 0.05 indicates significant difference.

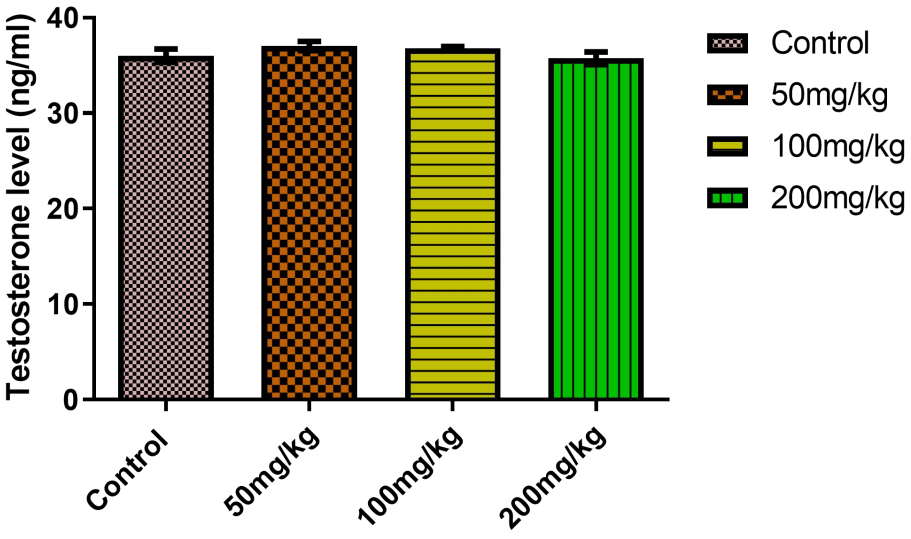
TABLE 1: comparing the mean values of luteinizing hormone and testosterone concentration in wistar rats.



4.1 The effect of *fragariaananassa* on luteinizing hormone levels in wistar rats

Fig I: Bar chart representation of luteinizing hormone actvities

There were no significant changes in Group B (50mg/kg of *fragariaananassa* extract, Group C (100mg/kg of *fragariaananassa* extract), and Group D (200mg/kg of *fragaria ananassa* extract) compared with control respectively



4.2 The effect of *fragariaananassa* on leutinizing hormone levels in wistar rats

Fig II: Bar chart representation of testosterone activites

There were no significant changes in Group B (50mg/kg of *fragaria ananassa* extract), Group C (100mg/kg of *fragaria ananassa* extract) and Group D (200mg/kgof *fragaria ananassa* extract) compared with control respectively.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Strawberry contains antioxidants such as ascorbic acid (vitamin C), folic acid, and essential oils (Elkhadragy*et al.,* 2017) and due to this most of strawberry research has been on its antioxidants effects but relatively lesser attention is paid to its effects on reproductive hormones. The aim of this study is to investigate the effects of aqueous fragariaananassa extract on testosterone and luteinizing hormone levels in adult male wistar rats.

Testosterone is the primary male hormone responsible for regulating sex differentiation, producing male sex characteristics and spermatogenesis (Basaria, 2013). It is also responsible for secondary sexual traits such as hair distribution around the pubic areas, skin thickness, male pattern baldness, deepened voice, and increased muscle mass (Niechlag*et al.,* 2012). The analysis of the hormonal assay results as seen in fig 4.1 revealed that there were no significant changes in the testosterone level of group B, C and D when compared to the control(group A). Luteinizing hormone is produced by the anterior pituitary gland and its main function in males is to stimulate the leydig cells in the testes to produce

testosterone (Ramaswamy and Weinbauer, 2014). The analysis of the hormonal assay results as seen in fig 4.2 revealed that there were no significant changes in the luteinizing hormone level of group B, C and D when compared to the control(group A).

The results disagrees with the study done by Sakpa and Abade (2019) who observed that *fragariaanannassa* extract causes an increase in the luteinizing hormone levels and a decrease in the testosterone levels. The contrast in results may be due to the short duration of study (28 days) and lower dose administered (50mg/kg, 100mg/kg, 200mg/kg) compared to the previous study. These findings have shown that consumption of *fragariaananassa* would not have any significant change in the testosterone level and luteinizing hormone levels. Future research should consider administering a higher dosage and increasing the duration of study

5.2CONCLUSION

Aqueous extract of *fragariaananassa*does not have significant effect on the reproductive hormones (Testosterone and Luteinizing hormone) in adult malewistar rats.

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