SEXUAL BEHAVIOUR OF FEMALE WISTAR RATS TREATED WITH **HYDROMETHANOLIC FRUIT PULP EXTRACT OF *Azanza garckeana* F. Hoffm.**

By

AISHA AHMAD

DEPARTMENT OF HUMAN PHYSIOLOGY, FACULTY OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA

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BY

Aisha AHMAD (BSc. ABU, 2017)

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DEPARTMENT OF HUMAN PHYSIOLOGY, FACULTY OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA

DECLARATION

I, **Aisha Ahmad** declare that the work in this dissertation, titled: **Sexual Behaviour of Female Wistar Rats treated with Hydromethanolic Fruit Pulp Extract of *Azanza garckeana* F. Hoffm.,** was carried out by me in the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, under the supervision of Prof. Tanko Yusuf and Dr. Ibrahim Suleiman. The information derived from the literature was duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Name of student	Signature	Date

CERTIFICATION

This dissertation, titled: **SEXUAL BEHAVIOUR OF FEMALE WISTAR RATS TREATED WITH HYDROMETHANOLIC FRUIT PULP EXTRACT OF *Azanza garckeana F. Hoffm.*** by **Aisha AHMAD** meets the regulations governing the award of the degree of Master of Science in Human Physiology of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

_____ _____

Prof. Y. Tanko (B.Sc., M.Sc., Ph.D.) Date

Chairman, Supervisory Committee

_____ _____

Dr. I. Suleiman (B.Sc., M.Sc., PhD) Date

Member, Supervisory Committee

_____ _____

Dr. I. G. Bako (B.Sc., M.Sc., Ph.D.) Date

Head, Department of Human Physiology,

Ahmadu Bello University, Zaria

_____ _____

Prof. Muhammad Nasir Shuaibu (B.Sc., M.Sc., Ph.D.) Date

Dean, School of Postgraduate Studies,

Ahmadu Bello University, Zaria

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ABSTRACT

Sexual dysfunction has been estimated to affect 40% of females worldwide. Ripened fruits of *Azanza garckeana* are consumed traditionally as an aphrodisiac without proper scientific investigation. This work was aimed at evaluating the sexual behavior of female Wistar rats treated with hydromethanolic fruit pulp extract of *Azanza garckeana F. Hoffm*. *Azanza garckeana* fruit was gotten from Kaltungo local government of Gombe state. The fruit pulp was air dried after removal of the seeds and pulverized. The pulverized sample was macerated in 70% methanol and 30% water for 72hrs, and then filtered. The filtrate was evaporated to dryness to obtain the extract. Twenty-five female Wistar rats and five (5) male Wistar rats were used for the study, the female animals were randomly divided into 5 groups (n= 5): Group I served as normal negative control and received 1ml/kg distilled water, group II served as positive control and received 100 µg estradiol valerate and 50 µg progesterone 48 hr and 5 hr respectively before sexual behavioural test, while group III, IV and V received 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight of the *Azanza garckeana* extract respectively for 14 days orally before the sexual behavior test. Sexual behavioral tests were performed with sexually experienced male rats 1:1 after the treatment period in a unilevel pacing chamber and the process videotaped for proper scoring. Proceptive behaviors and Lordosis was extrapolated as index for the level of proceptivity and receptivity respectively in the female animals. At the end of the experiment, the rats were anaesthetized with combined doses of ketamine and diazepam injection (50mg/kg/10mg/kg i.p). Blood sample was collected via cardiac puncture for hormonal assays which were done using ELISA kit for estrogen, progesterone and testosterone. The ovaries and uterus were dissected out and fixed in 10% formalin for histological examination. The result showed a significant increase (P<0.05) in proceptivity and receptivity in the treatment (III, IV & V) and positive control (II) groups when compared with the negative control (I) group, indicating enhanced sexual behaviour. The hormonal assay results revealed significant (P<0.05) increase in the serum estrogen (P<0.05) and a significant decrease in serum progesterone in the extract treated groups except at 500 mg/kg dose where it was significantly increased (P<0.05) when compared to all groups. There was significant decrease in testosterone when compared to negative control in the extract treated groups and positive control. The time spent in the male chambers and the number of entries into the male chambers also significantly increased in the extract treated groups when compared to negative control. The histological sections of ovaries and uterus revealed normal uterine and ovarian histoarchitecture, however increased follicular development in the ovary and glandular hyperplasia in the uterus were seen in the treated groups when compared to negative control. In conclusion *Azanza garckeana* hydromethanolic fruit pulp extract demonstrated aphrodisiac property, and may be used for the treatment of women with hypoactive sexual desire.

Keywords: *Azanza garckeana*, sexual behavior, histology, estrogen, progesterone, lordosis quotient, paracopulatory behavior.

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LIST OF ABBREVIATIONS

cGMP - Cyclic guanosine monophosphate

CL - Corpus luteum

CNS - Central nervous system

Df - Darting frequency

Ef - Ear wiggling frequency

FSD - Female sexual dysfunction

FSH - Follicle stimulating hormone

GH - Glandular hyperplasia

GnRH-LH - Gonadotrophin releasing hormone- luteinizing hormone

GTP - Guanosine triphosphate

Hf - Hopping frequency

HSDD - Hypoactive sexual desire disorder

IH - Hyperplasia of the inflammatory cells

LD_{50} - Lethal dose

L_q - Lordosis quotient

N_L - Number of lordosis response

N_m - Total number of mounts

NO - Nitric oxide

PB - Proceptive behavior,

PDE-5 - Phosphodiesterase-5 enzyme

PF - Primary follicle

SD - Sexual dysfunction

SF - Secondary follicle

STI - Sexually transmitted infectionsT -

Duration of test

WHO - World Health Organization

CHAPTER ONE

Introduction

The World Health Organization (WHO) defines sexual health as a state of physical, emotional, mental and social well-being in relation to sexuality, not merely the absence of disease, dysfunction or infirmity (WHO, 2020a). Good sexual health requires a positive approach to sexuality and sexual relationships, as well as having pleasurable and safe sexual experiences (WHO, 2006). Sexual relationship is among the most important social and biological relationships in human life as it has proven to enhance an individual‟s quality of life and well-being (WHO, 2002). One of the main aims of marriage is procreation and this is to ensure the continuity of an individual‟s lineage and more importantly, for sexual fulfillment of both partners (Yakubu *et al*., 2007). Achieving sexual fulfillment requires both partners to be sexually healthy.

Apart from reproduction, sexuality is practiced in humans for experiencing pleasure, boosting self-esteem, cultivating intimacy, and reducing anxiety, stress and tension (Baulieu, 1996). Both males and females typically experience normal sexual activity throughout adult life. Sexual and other physiologic functions diminish with advancing years (Baulieu, 1996). Apart from aged people, healthy young people who are unable to carry out sexual act efficiently or entirely, normally suffer from psychogenic, organic, or mixed etiologies leading to sexual dysfunction (NIH, 1993). Sexual dysfunction refers to difficulties that occur during the sexual response cycle and prevents one from experiencing satisfaction from sexual activity (Laumann *et al*., 1999). Female sexual dysfunction (FSD) may manifest as apparently unrelated emotional disorders that could degrade one‟s quality of life and destroy relationships among family social sphere and even the workplace (Franjić, 2019), these problems make some individuals resort to using sexual enhancing substances.

The term aphrodisiac originated from the Greek word Aphrodite, eulogizing the Greek goddess of love and romance. In modern times, this term has been used for substances that enhance sexual activity and are helpful in treating sexual disorders (Smith, 1974). Over a long period of time, people have devised ways to increase sexual desire and improve sexual health. This has led to the discovery and use of substances called aphrodisiacs (Gundidza *et al*., 2009). Aphrodisiacs are defined as drugs, food, scents or devices that can arouse or increase sexual drive or boost libido. Most aphrodisiacs carry out their function either by altering the level of specific neurotransmitters such as dopamine and serotonin or specific sex hormones such as progesterone, testosterone and estrogen in the body (Alok *et al*., 2013).

Aphrodisiacs can be classified into three categories based on their mechanism of action; (1) potency enhancers (i.e., substances that increase effectiveness of erection in males), (2) sexual pleasure enhancers and (3) libido i.e., sexual desire enhancers. Potency-enhancing substances typically induce vasodilation, often through the nitric oxide (NO) pathway, with some side effects such as inducing hypotension and causing of potentially harmful cardiovascular effects, examples are sildenafil and L-arginine (Sandroni, 2001). Sexual pleasure-enhancing substances cause tumescence and lubrication of the genital mucosa, therefore increasing sensation, example include cantharidin; Libido- enhancing substances alter the concentrations of specific neurotransmitters, example is dopaminergic and serotoninergic pathways or sex hormones example pituitary hormones and testosterone in the central nervous system (CNS) (Sandroni, 2001).

Sexual desire may have many different meanings. It has been equated to desire for sexual activity, desire for sexual intimacy, and the feeling of wanting associated with thinking about an attractive partner. Colloquially, some refer to desire as feeling horny. Some experts favour a

description that does not hinge on sexual behaviour such as the subjective awareness of desire for sexual satisfaction, irrespective of sexual activity (Brotto and Smith, 2014).

Plants constitute an important source of medicines and play a key role in the health of a greater portion of the world‟s population. The use of plants or their products to treat sexual disorders or improve on sexual performance has a long history in most countries, and their investigations in animals have proven that they are effective in improving sexual desire and sexual behaviour (Watcho *et al*., 2007).

Azanza garckeana plant grows naturally in semi-arid areas (Okafor, 1985) and in Nigeria, *Azanza garckeana* is found abundantly in Tula, a town in Kaltungo Local Government Area of Gombe State, Nigeria. It is also found around Kankiya, Katsina State and Daggish Kali hills of Zah district, Michika Local Government area of Adamawa State (Adamu *et al*., 2013). Among the Tula people of North-Eastern Nigeria, ripened fruits are consumed as an aphrodisiac and for treatment of infertility in men (Masila *et al*., 2015). Variously it is called snotappel (Afrikaans), chinga, mukole (Bemba), azanza, tree hibiscus, wild hibiscus, African chewing gum (English), muneko (Lozi), mukole (Lunda); uxhakuxhaku (Ndebele), mkole (Nyanja), mutohwe (Shona), mtobo (Swahili), muneko (Tongan) and morajwa (Tswana) (Orwa *et al*., 2009).

Other parts of the plants have been extensively studied with numerous reports of isolated compounds (Masila *et al*., 2015). In Zimbabwe, an infusion of the roots is dropped into the ear to treat ear ache and it is used orally to treat cough, chest pain, menstruation and as an antiemetic and large doses as an abortifacient (Maroyi, 2011; Mshelia *et al*., 2016). A tea of the stems and leaves is taken to treat liver problems (Ochokwu *et al*., 2015). A poultice of the pounded fruit is applied to abscesses sufficiently thin out inflamed tissue encompassing the infected cavity or draw pus to a head so the abscess may rupture (Ochokwu *et al*., 2015).

1.0 Statement of Research Problem

Reproductive disorders are steadily increasing worldwide due to etiological factors and aging (American Psychiatric Association, 2000). Healthy young people who are unable to carry out sexual act normally suffer from psychogenic, organic, or mixed etiologies leading to sexual disorder (NIH, 1993). Sexual dysfunctions are relatively prevalent in both sexes, with estimates ranging from 10% to 52% of men and 25% to 63% of women. Figures for the prevalence of sexual dysfunctions vary from study to study due to differences in study populations, definitions, and measures (Birbaum, 2009). The most widely cited survey showed that the most common sexual problem is premature ejaculation in men which varies between 28%-32%, depending on age group and a lack of interest in sex in women which is 27%-32%, depending on age group distress (Birbaum, 2009). Although this survey is believed to provide some of the best estimates of sexual problems in the U.S., it neither used the formal clinical diagnostic categories of sexual dysfunctions nor indicated the severity of the problem and related distress (Birbaum, 2009). It is estimated that some types of sexual dysfunction affects 40% of females worldwide, with most being manifest as desire, interest or excitement disorders (McCool *et al*., 2016). In Nigeria the prevalence of female sexual dysfunction is estimated to range between 53.3% and 71.0% (Nwagha *et al*., 2014). Research has reported that there is a close relationship between female sexual dysfunction (FSD) and poor quality of life, which is often associated with psycho-affective problems such as anxiety, melancholy, low self-esteem, or marital problems (Brotto *et al*., 2016).

1.1 Justification of the Study

The diagnostic investigations and treatment opportunities for women with sexual health concerns

are limited, in large part, due to the lack of current global government-approved agents for any sexual health concerns (desire, arousal, orgasmic and sexual pain-related dysfunctions) of pre-menopausal women or for non-sexual pain concerns of post-menopausal women (Goldstein and Wylie, 2015). There are, in contrast, more than 20 US government-approved treatment strategies for men with bother-some male sexual dysfunctions (Franjić, 2019). The use of medicinal plants for health reasons is as old as humanity (Ephrian *et al*., 2008). The wakefulness of the role of medicinal plants in health care delivery in developing countries has led to increasing need for research in traditional medicine, with a prospect of incorporating it into modern medicine (Sofowara, 1993). The rate medicinal plants are used tends to be higher in African countries due to high dependency on medicinal plants for their health care needs (Erhabor *et al*., 2013).

Although various therapeutic options are currently available for treating these disorders, an effective alternative oral therapy that could improve sexual functions and libido without any harmful side-effects will contribute tremendously to the therapeutic armamentarium (Gauthaman *et al*., 2003).

Azanza garckeana has been reportedly used in traditional medicine for treatments or management of more than 20 human diseases and ailments (Alfred, 2017). The plant is used as herbal remedy for diseases like cough, chest pains, infertility in male, menstruation abnormalities, sexually transmitted infections and hepatic impairments (Alfred, 2017). Among the Tula people of North-Eastern Nigeria, ripened fruits of *Azanza Garckeana* are consumed as an aphrodisiac and for treatment of infertility in men (Masila *et al*., 2015). It is also used traditionally to enhance libido as its consumption is claimed to enhance development of a strong desire for copulation in both males and female (Bukar *et al*., 2020). This have not been verified scientifically. The fruit of this important ethno-medicinal plant needs to be investigated with

view of isolating its medicinal principles (Dikko *et al*., 2016). There is dearth of literatures on the aphrodisiac and reproductive effect of the fruit pulp in female.

1.2 Aim of the Study

The aim of this study was to investigate the effect of hydromethanolic fruit pulp extract of *Azanza garckeana* (goron tula) on sexual behavior and some reproductive parameters in female Wistar rats.

1.3 Objectives of the Study

The specific objectives are;

i. To determine the preliminary phytochemical constituents present in the hydromethanolic fruit pulp extract of *Azanza garckeana.*

ii. To evaluate the LD_{50} of the hydromethanolic fruit pulp extract of *Azanza garckeana* via acute toxicity study.

iii. To study the sexual behavior (proceptive and receptive) of female Wistar rats treated with *Azanza garckeana* extract.

iv. To assay for the serum concentration of estrogen, progesterone and testosterone in female Wistar rats after treatment with *Azanza garckeana* extract.

v. To study the histological changes in the uterus and ovaries after treatment with the extract.

1.4 Research Hypothesis

Null Hypothesis (H_o): Hydromethanolic fruit pulp extract of *Azanza garckeana* has no effect on sexual behavior and some reproductive parameters in female Wistar rats.

CHAPTER TWO

2.0 Literature Review

2.1 Azanza Garckeana

Azanza garckeana belongs to the family *Malvaceae*. In Nigeria, it is mostly grown in Tula village of Kaltungo local government area of Gombe State. It has sweet edible fruit and other parts used for multiple purposes in tropical Africa (Ahmed *et al*., 2016). *Azanza garckeana* is one of the indigenous fruit tree species found in Nigeria (Ochokwu *et al.,* 2014). It is found abundantly in Kaltungo and michika local government area of Gombe and Adamawa state in Nigeria and recently, it was discovered at Jimeta town of Yola Adamawa state (Ochokwu *et al*., 2015).

Azanza garckeana has several English names including: tree hibiscus, azanza and snot apple. In Nigeria it is popularly called Goron Tula, in South Africa- *Thespesia garckeana*, in Bostwana-Morojwa, (Ochokwu *et al*., 2014). The plant is also found in Sudan, Kenya, Malawi, Mozambique, Namibia, Tanzania, Zimbabwe, and Zambia. *Azanza garckeana* plant has been discovered to be widely distributed in the eastern, western and southern regions of Africa. It grows naturally in all types of woodlands from sea level to about 1700m above sea level (Mbuya *et al*., 1994). It also grows in semi-arid areas, receiving lowest annual rain fall of 250mm and highest rain fall of 1270mm (Orwa *et al*., 2009). *Azanza garckeana* grows on variety of soils and is found near termite mounds, open land and deserted areas in most parts of Africa while in Nigeria it grows on open woodland in the north-eastern part of the country (Ochokwu *et al*., 2015).

2.1.1 **Taxonomy of *Azanza garckeana***

Azanza garckeana is a member of the *Malvaceae* family. The generic name "*Azanza*" was derived from the word "Azania", a word meaning black and surviving in Zanzibar (Palmer and Pitman, 1972). The specific name "*garckeana*" is in honor of Professor August Garcke (1819 - 1904), a German botanist and plant collector who is a specialist in pharmacognosy (Schmidt *et al*., 2002). Synonyms of *A. garckeana* include *Bupariti garckeana, Rothm., Shantzia garckeana, Lewton and Thespesia garckeana* (Palmer and Pitman, 1972).

The following is the morphological classification of *Azanza garckeana*;

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivision: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Dilleniidae

Order: Malvales

Family: *Malvaceae* – Mallow family

Genus: Azanza

Specie: *Azanza garckeana* (Bioltif *et al.*, 2020).

2.1.2 **Morphological description of *Azanza garckeana***

Azanza garckeana is a deciduous shrub, the tree can grow up to a height of 3-15meters depending on the weather condition. Stem diameter at breast height is up to 25cm. The tree is

multi-stemmed with straight or crooked stem, which is sometimes forking from the base. The bark is rough and greyish-black or brown in color, fibrous with longitudinal fissures. The branches are hairy when young but become smooth as the plant matures. The leaves are distinctively rounded measuring 8 by 12cm on long stalks. They are simple, alternate and roundish with three to five lobes, which are covered in brown star-shaped hairs, and have longitudinal fissures in the midrib. The tip of the leave is usually bluntly pointed or rounded. The base of the leaf is heart-shaped and its 5 to 7 nerved. The young leaves are brown in colour and velvety (Mojeremene and Tshwenyane, 2004). The flowers are large up to 6cm long, solitary on long pedicels in the axils of uppermost leaves, yellow with a purple-brown centre; the petals are globose and capsules are up to 4cm long, the thickness is 3cm. The fruit is globose and has woody capsules of up to 3 to 4cm in diameter, it is divided into 5 segments with each segment containing a seed, the remains of the calyx and epicalyx at the base; the seeds are hemispherical, up to 10 mm long, 7 mm thick, with brownish and woolly floss (Orwa *et al*., 2009). Figure 2.2 shows the shoot, root and leaves of the plant.

Flowering takes place during the raining season, while fruit ripening occurs during the dry season, hence it takes nearly six months from flower fertilization to ripening of the fruit (Orwa *et al*., 2009), in Southern Africa, flowering occurs from December to May and fruiting from February to September while in North Eastern Nigeria flowering occurs from May to October and ripening from November to April (Ochokwu *et al*., 2014). Figure 2.1 and 2.2 describes the fruit and different parts of *Azanza garckeana* plant.



Figure 2.1: Fruit of *Azanza garckeana* plant snapped by candidate (2021).



Figure 2.2: Different parts of the *Azanza garckeana* plant (Bioltif *et al.*, 2020).

2.1.3 Uses of *Azanza garckeana*

The fruits are the most useful and valuable part of the plant. The fruits can be eaten while slightly green or when ripe. Some people dry them and refurbish them later. The fruits can be soaked in small amount of water to make jelly-like syrup, they can also be boiled and used as condiment or made into pottage (Mojeremene and Tshwenyane, 2004). The leaves are used for making relish and can be burned to produce salt. The fruits of *Azanza garckeana* has been found in a number of northern Nigerian states and reported to be used in almost all parts of the country. This is an indication that the plant has economic importance to farmers (Ochokwu *et al*., 2015).

The wood of *Azanza garckeana* has many uses, which are, appreciated by people living in rural area of Tula. The wood serves a source of fuel in areas where there is shortage of firewood, it can also be converted into charcoal (Orwa *et al.,* 2009). The wood has been reported to be used

for construction of materials such as poles, fencing posts, some farm implements, tool handles, domestic utensils and knife sheaths (Orwa *et al*., 2009; Mojeremene and Tshwenyane, 2004 and Ochokwu *et al*., 2014).

The long shoots are traditionally used as whips (Palmer and Pitman, 1972) and the inner bark and pulp is a source of fibre i.e has nutritional value (Ochokwu *et al*., 2015). *Azanza garckeana* is commonly used for ornamental purposes. The roots are medicinal and are taken orally for painful menstruation and to treat respiratory related problems such as cough and chest pain (Orwa *et al.*, 2009). An infusion made from the roots and leaves is dropped into the ear to treat earache or taken orally as an antiemetic (Orwa *et al.*, 2009). The trees provide shade for people during the summer season. *Azanza garckeana* has been used in soil conservation, it is also used as soil protective covering and as green manure (ICRAF, 1992). *Azanza garckeana* is an incidental component of many farming systems. Traditionally many crops (maize, millet sorghum etcetera) are grown under the canopy of *Azanza garckeana* because of its soil conservation property (Mojeremene and Tshwenyane, 2004).

The pulp and seed is an important source of phytochemical and mineral components such as ascorbic acid, Magnesium, Calcium, Sodium, Iron, Potassium, Phosphorus, crude fiber, Carotenoids, Tannins, Saponins, Alkoloids, Flavonoids, Phenols, glucosides and Xanthone (Ochokwu *et al*., 2015).

2.2 Aphrodisiacs

The term aphrodisiac is derived from Greek mythology (Aphrodisiakos- sexual), where Aphrodite was the goddess of love and beauty (Shamloul, 2010). According to the Oxford Learners Dictionary, aphrodisiac means substance or drug that arouse sexual desire (Cowie, 1989), while Encyclopedia of Medicinal plants explained aphrodisiac as the one which excites libido and sexual activity (Mnimh, 1996). The modern definition of aphrodisiac can vary, but is generally regarded as a substance that increases sexual desire (i.e. libido) and/or sexual pleasure. The definition has been extended to include those substances which enhance sexual performance or aid in the proper functioning of the male and female sex organs (Shamloul, 2010).

Food itself is considered as an aphrodisiac in the Ayuerveda because after nourishing different tissues of the body (muscles, fat, nerves), it replenishes the reproductive fluids and promote physical strength and good health. Aphrodisiacs can generally be defined as substances which are consumed, applied topically, smoked, sniffed or delivered through other routes into the body for improving sexual potentials (Mathur, 2012).

The reliance on aphrodisiacs by society has been demonstrated for centuries. In historic Chinese, Indian, Egyptian, Roman, and Greek cultures, such substances were used in traditional remedies for the purpose of enhancing sexual experience (Elferink, 2000). Sexual stimulants are still often called "aphrodisiacs". Aphrodisiacs are the agents which are used extensively by the human beings seeking to improve their sexual life and treat sexual dysfunctions (Alok *et al.*, 2013).

Ayurveda has a whole science of Aphrodisiacs. It is one of the seven branches of its medical system. Ayurvedic aphrodisiacs are more than substances that enhance sexual activity, they are tonics that nurture and give sustenance to the reproductive tissues. Others help to

promote the creative transformation of sexual energy for the benefit of the body and mind. Sexual desire is controlled and regulated by the central nervous system which integrates tactile, olfactory, specific auditory and mental stimuli (Patel *et al*., 2011). The aphrodisiac drugs act by altering the level of specific neurotransmitters acting on the central nervous system or the level of sex hormones in the body (Patel *et al*., 2011). Most of the aphrodisiacs agent act by altering the testosterone and progesterone concentration in the body (Alok *et al.,* 2013).

2.2.1 Classification of aphrodisiacs

Aphrodisiacs can be classified according to their mode of action into two categories. The first type of aphrodisiac acts specifically to increase the libido (i.e., sexual desire) and the second category of aphrodisiac acts by increasing the ability to indulge in sexual activity (i.e., increasing potency). In theory, an aphrodisiac is strictly an agent which arouses or increases sexual desire, but in practice anything which increases the capacity for sexual enjoyment will tend to increase the appetite and can be considered as an aphrodisiac (Zanolari *et al*., 2003).

Aphrodisiacs can have psychological effects, thereby increasing sexual desire and pleasure through hallucinogenic properties or other mood stimulating properties. Aphrodisiacs can also act physiologically by enhancing erection through hormonal changes, increasing blood flow, and relaxing genital smooth muscle (Sandroni, 2001).

The biologically significant aphrodisiac can be categorized into three primary classes;

i. Vigor enhancers: some aphrodisiac simply have high nutritional value thereby improving the immediate health or well-being of the consumer and consequently improving sexual performance and libido.

ii. Purported aphrodisiacs: this group includes substances that have physiological affects but are not psychologically active. They may alter blood flow and increase duration of sexual activity by numbing the genital area.

iii. Psycho-pharmacologically active aphrodisiacs: The third group of aphrodisiac is made up of compounds that can cross the blood brain barrier and stimulates some areas responsible for sexual arousal. This category includes a wide range of neurotransmitters, hormones, pheromones and drugs that can alter the normal function of these molecules. This category is most difficult to study because knowledge of both sexual arousal and the mechanisms of the psychoactive properties of drugs are limited. Only the most general information about sexual arousal and the brain is understood (Bruce *et al.,* 2002).

2.2.2 Mechanism of action of most aphrodisiacs

On sexual stimulation (visual or otherwise) the axons of parasympathetic nerves release nitric oxide (NO). The gas diffuses into the cells of the smooth muscle lining the arteries of the genital tissue and activates the enzyme guanylate cyclase (Chew *et al*., 2000). The activated enzyme acts by converting guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). The cGMP in turn causes relaxation of the genital smooth muscle, leading to dilation and influx of blood into the genital tissue. This blood is essentially trapped and results in an erection. The erection ceases after a while because cGMP is hydrolyzed by phosphodiesterase-5 enzyme (PDE-5) into inactive GMP. PDE-5 enzyme is mainly found in the penile tissues. Aphrodisiac substances enhance sexual activity by inhibiting the hydrolyzing action of PDE-5 enzyme resulting in accumulation of active cGMP and subsequently uninterrupted and prolonged sexual activity through increased blood flow to genitals (Chew *et al*., 2000).

2.2.3 Risks involved with the use of aphrodisiacs

Most aphrodisiac products sold are labeled as "natural" and perceived to be purely from a natural source but usually not true in most cases because majority of the products are either contaminated or mixed with other substances that can be toxic and/or lethal and this may alter the product‟s safety or efficacy. Many of the recognized aphrodisiacs could be safe therapeutic agents or dangerous poisons. As a matter of fact, there is no real distinction between a drug and a poison, many drugs used clinically can be toxic at doses only slightly higher than the recommended therapeutic dose. Believing that a drug of natural origin is most definitely safe and cannot cause any poisoning is not totally true, people often forget that the most deadly venoms are from nature and it is quite likely that many cases of accidental self-poisoning arise from this wrong perception (Zanolari *et al*., 2003).

Increasing cases of poisoning involving individuals who seek to improve their sexual health using chemical and herbal aphrodisiacs are reported worldwide. Upon exposure, the toxicity may vary from mild to severe and may even be life-threatening. Lack of regulation, easy accessibility, contamination, adulteration and toxic potential are some of the dangers deemed to be associated with aphrodisiac poisoning. Contamination, adulteration and natural toxic substances are the three major risk factors associated with aphrodisiac products which could cause poisoning. Herbal aphrodisiacs may be contaminated by pesticides, moulds and/or heavy metals in the farm, and can be adulterated with minerals and/or western medicines, such as steroids, hormones, CNS stimulants *etcetera* (Mitka, 2003).

Since herbal poisoning can result from these factors, qualitative and quantitative evaluation to portray the harmful components of the available herbal remedies should be undertaken.

Scientific research conducted to investigate the mechanism of action, safety (non-lethal dose), and efficacy (effective dose) of aphrodisiacs would be of great benefit to patients and the therapeutic armamentarium (Zanolari *et al*., 2003).

2.3 Sexual Health and Its Importance

The World Health Organization (WHO, 2020a) defines „sexual health as a state of physical, emotional, mental and social well-being in relation to sexuality, not merely the absence of disease, dysfunction or infirmity". Good sexual health requires a positive approach to sexuality and sexual relationships, as well as having pleasurable and safe sexual experiences, free of coercion, discrimination, and violence. For sexual health to be attained and maintained, the sexual rights of all persons must be respected, protected and fulfilled (WHO, 2020a).

Sexual health encompasses reproductive health, which the WHO defines as „a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity, in all matters relating to the reproductive system and to its functions and processes" (WHO, 2020b). The purpose of which is enhancement of one"s quality of life and personal relations and not just counseling and care related to reproduction and STI (sexually transmitted infections). This holistic approach is important in the promotion of gender-sensitive and woman-centered health (Franjić *et al*., 2019).

2.4 Female Sexual Response Cycle

The human sexual response cycle refers to the structured series of changes the body goes through physically, psychologically, and emotionally during arousal and when engaging in sexual activity (Rowland and Gutierrez, 2017). The sexual responses of both sexes begins with desire, this phase is modulated by a balance between dopamine-sensitive excitatory center and the

serotonin sensitive inhibitory center in the brain (Basson, 2001). The arousal phase is characterized by physiological responses which include; increased heart rate, respiration and blood pressure. In addition myotonia, vasocongestion and nipple hardening occur (Rowland and Gutierrez, 2017). The medial pre-optic, anterior hypothalamic region, and some parts of the limbic–hippocampal structures are responsible for sexual arousal in the central nervous system. Upon stimulation, these centers transmit electrical signals via the parasympathetic and sympathetic nervous system (Berman, 2005). The neurogenic mechanisms modulating vaginal and clitoral smooth muscle tone and vaginal and clitoral vascular smooth muscle relaxation are under investigation (Berman, 2005). Testosterone is reported to respond to the modulating of the threshold of these centers. The activation of these centers subsequently initiates the downstream signals, which produce genital sexual responses through the spinal cord and the related reflex centers. During the arousal phase, the parasympathetic nervous system modulates the tumescence of vascular and genital tissues, including the clitoris and peri-vaginal arterioles, the parasympathetic nervous system also stimulates the expansion of the inner two-thirds of the vaginal canal i.e the tenting effect. Estrogen also regulates the process within the vaginal mucosa that results in vaginal lubrication (Tsui *et al*., 2011).

The plateau phase intensifies the responses from the arousal phase, breathing, blood flow and heart rate continues to increase and stabilize. Muscle tension increases and may extend to hands feet, face and other areas of the body. As blood flow increases the vaginal wall darken and the clitoris becomes increasingly sensitive. The vagina expands and the uterus becomes fully elevated. The Bartholin gland produces additional lubrication in and around the vagina (Rowland and Gutierrez, 2017). A series of contractions of the muscles related to the genitals throughout the body occurs at the orgasmic phase. During orgasm, the heart rate, blood pressure, and

respiratory rate increases. At the end of the orgasmic phase, the body returns to the unexcited state, which is termed the refractory phase (Chen *et al*., 2013). Recent studies have suggested that the female sexual response cycle is intrinsically different from the supposed linear four phase model. The new model proposes that the female sexual response cycle consists of independent that overlap in a variable sequence, and may be influenced by various psychological and physical factors (Nappi *et al*., 2003) i.e emotional and physical factors can enhance the motivation and intensity of sexual stimuli, which finally results in sexual satisfaction. Ultimately it means sexual arousal in women is not only affected by the objective extent of sexual excitement but also by subjective emotions (Chen *et al*., 2013). Figure 2.3 shows the traditional model of female sexual response. Figure 2.4 shows the circular model of sexual response.

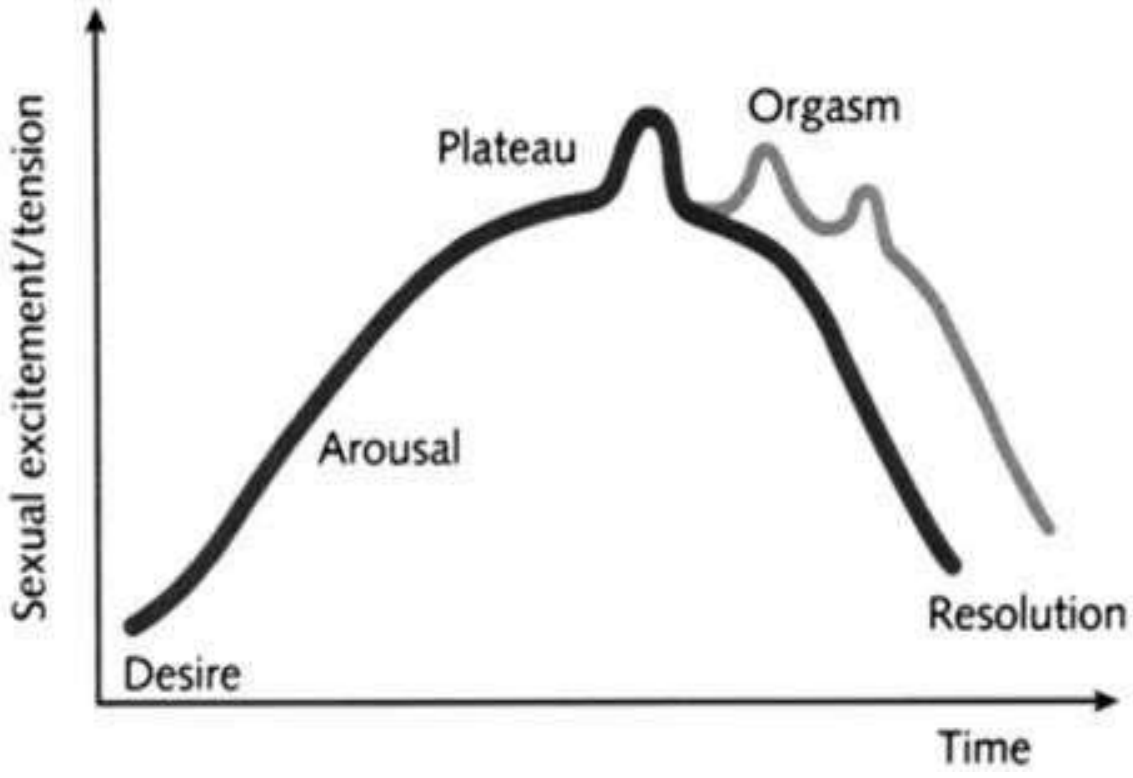


Figure 2.3: The traditional model of female sexual response (Sand and Fisher, 2007).

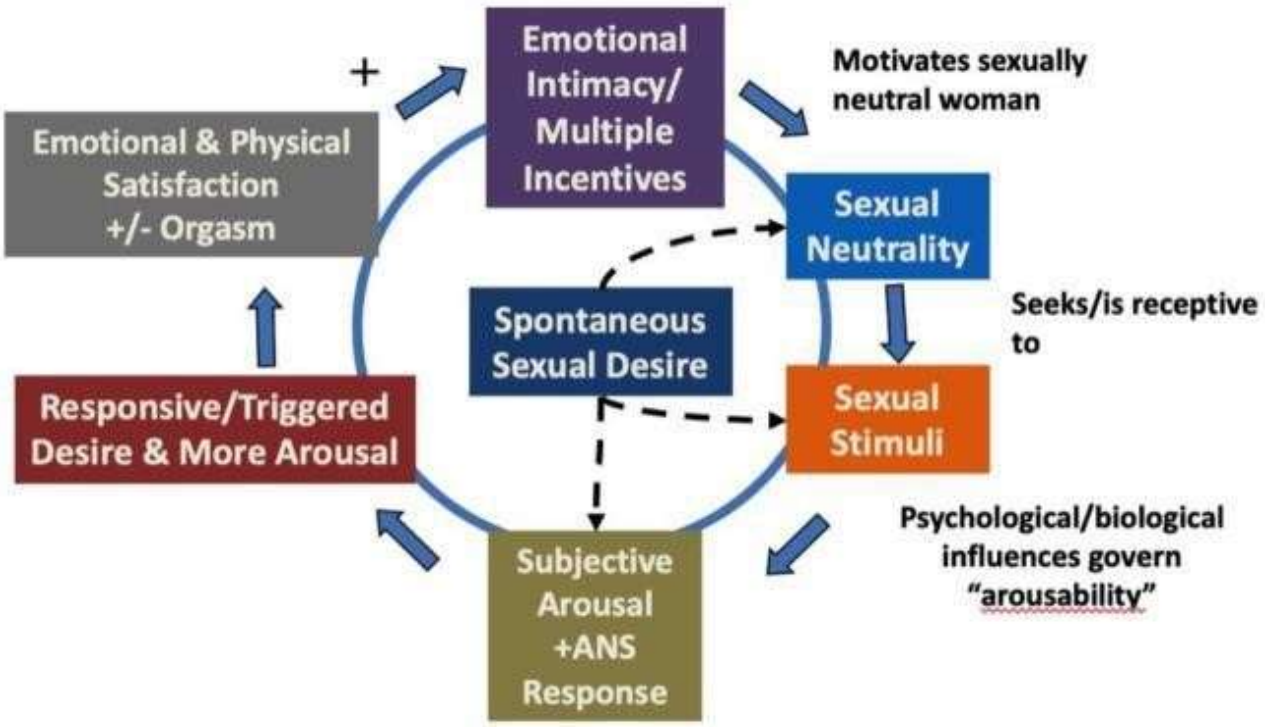


Figure 2.4: Circular model of female sexual response (Basson, 2001).

2.4.1 Impact of ovarian hormones on female sexual function

i. **Estrogen:** Estrogen plays an important role in the regulation of female sexual function. Estradiol which is an active metabolite of the hormone alters nervous transmission during sexual activity in both the peripheral and central nervous system (Shen *et al*., 1999). A depreciation in the serum levels of estrogen results in thinning of vaginal mucosal epithelium and atrophy of vaginal wall smooth muscle. Decreased estrogen levels also result in a less acidic environment in the vaginal canal. This can ultimately lead to vaginal infections, urinary tract infections, and incontinence as well complaints of sexual dysfunction (Shen *et al*., 1999). Estrogen maintains the integrity of the genital vessels and dilates the vessels which result in increased vaginal, clitoral and urethral arterial blood flow resulting in maintenance of the female sexual response by preventing atherosclerotic

compromise to pelvic arteries and arterioles (Sherwin *et al*., 1985).

ii. **Testosterone:** Testosterone, together with its metabolite dihydrotestosterone, are the most potent endogenous androgens in both men and women. It is also the major precursor of estrogens (Davis, 2000). Testosterone effectively increases sexual desire in post-menopausal women that are taking estrogen replacement therapy (Braunstein *et al*., 2005; Davis and Braunstein, 2012), however a lack of understanding of the mechanisms through which female sexual desire is facilitated by co-administration of estradiol and testosterone is one of the primary reasons that it is not FDA-approved (Kingsberg *et al*., 2015). As an aromatizable androgen, it is unclear whether testosterone directly binds to androgen receptors in relevant brain regions to elicit these effects, or whether testosterone leads to an increase in bioavailable estradiol, for example via aromatization and through displacement of estradiol from sex-steroid hormone binding globulins, which bind androgens with a higher affinity than estrogens (Jones *et al*., 2017).

iii. **Progesterone:** Progesterone has a biphasic effect on sexual motivation, initially there is a transient rise in progesterone which stimulates sexual receptivity. Second prolonged secretion of progesterone inhibits sexual receptivity, they exert these actions by pbinding to progesterone receptors. However the precise mechanism of action has not been clearly established (Mani and Portillo, 2010).

2.5 Female Sexual Dysfunction

Female sexual dysfunction is defined as a problem encountered in the sexual response cycle that deviates from a woman‟s normal range of functioning. Defining female sexual dysfunction is not as absolute for women because of the qualitative nature of female sexual function. What may be

abnormal for one woman may be normal for another woman. Sexual dysfunction falls on a continuum with female sexual disorder (Franjić *et al*., 2019). The National Health and Social Life Survey reported that 43% of women experienced sexual problems as compared to 31% of men, this suggests that Sexual dysfunction (SD) may be more commonly related to women than men (Khadabadi and Bhajipale, 2011).

Female sexual dysfunction (FSD) consists of a spectrum of psychological and organic disorders focused on sexual desire with interrelated problems of arousal, orgasm, and sexual pain that degrades the quality of life for many women (Filocamo and Di-Donato, 2017). Female sexual dysfunction (FSD) has been recognized as a medical disorder with increasing prevalence when compared to male dysfunction, therefore there is an urgent need to search for a natural remedy to this problem. A woman is said to have a sexual problem if she lacks or loses sexual desire, feels pains during intercourse, feels nervousness, has difficulty achieving orgasm, and feels a reflex muscle contractions before or during sexual intercourse. Sexual performance problems can be treated or managed to about 70% to 90%, with natural substances called aphrodisiacs (Yadav, 2011). FSD can affect sexually active women of any age group, and its severity worsens with the endocrinology of advancing years. Impact is often subtle. FSD may express as apparently unrelated emotional manifestations that could degrade one‟s quality of life and affect family relationship and ability to socialize with people (Franjić *et al*., 2019). FSD can result from several anatomical conditions such as genital mutilation; pathological conditions such as endometriosis, gynecological cancer, urinary infections, urinary incontinence, endocrine alterations, and degenerative and vascular diseases; and also by psychological problems such as depression and the use of psychoactive drugs (Khajehei *et al*., 2015; Zomkowski *et al*., 2016).

Research has reported that there is a close relationship between female sexual dysfunction (FSD)

and poor quality of life, which is often associated with psycho-affective problems such as anxiety, melancholy, low self-esteem, or marital problems (Brotto *et al*., 2016).

Great progress has been made in both the recognition of female sexual dysfunction as a genuine medical disorder and finding a lasting solution to it. Several drugs from the current treatment strategies target the central control of sexual function through altering levels of dopamine, serotonin, and melanocortin, with the aim of modifying desire and subjective arousal (Rowlans and Tai, 2003). Despite significant breakthrough in sexual medicine over the past 15 years, attraction to natural remedies appears to continue. Because some herbal medicines are also promoted as having not only sexual benefits but for general health improvement, users may not only view them as a more natural way of dealing with their problems, but they may also perceive them as providing health benefits beyond those specifically related to sexual performance (Rowlans and Tai, 2003).

2.5.1 Classification of female sexual dysfunction (FSD)

FSD comprises of four primary domains of dysfunction which includes; hypoactive sexual desire disorder, arousal disorder, orgasmic disorder, and sexual pain disorder (Aydin *et al*., 2015). The etiology of the disease process is complex and women often experience dysfunction across various domains or having a combination of two or more.

(1) Hypoactive sexual desire disorder refers to diminishing or complete lack of sexual fantacies and/or interest in sexual activity through a period of time resulting in distress that is not caused by other mental disorder or medical condition (Angel, 2010). The persistent or recurring deficiency (or absence) of sexual fantasies, and/or receptivity to sexual activity causes this distress. Hypoactive Sexual Desire Disorder may result from psychological/emotional factors or

may occur secondary to physiologic problems such as hormone deficiencies and medical or surgical interventions. Any disruption of the female hormonal ambiance caused by natural menopause, surgically or medically induced menopause, or endocrine disorders, can result in inhibited sexual desire (Berman, 2005).

(2) Sexual arousal disorder is characterized by the lack or absence of desire for sexual activity with sexual stimulation that normally induce sexual arousal, or the inability to maintain sexual responses during sexual arousal. Its causes include; reduced genital sensation, and decreased vaginal smooth muscle relaxation and lubrication. These conditions can occur secondary to psychological factors. However, there is often a medical/physiologic basis such as diminished vaginal/clitoral blood flow, prior pelvic trauma, pelvic surgery, or medications (Berman, 2005).

(3) Orgasmic disorder refers to the inability to reach orgasm even after adequate sexual arousal and stimulation (Angel, 2010). This condition is termed anorgasmia and refers to persistent and recurrent difficulty, delay or absence of attaining orgasm following sufficient sexual stimulation and arousal which mostly results in distress. This may be a primary never achieved orgasm or secondary condition occurring as a result of surgery, trauma, or hormone deficiencies. Primary anorgasmia can be secondary to emotional trauma or sexual abuse (Berman, 2005).

(4) Sexual pain disorder refers to pain in the pelvis or vagina during any stage of normal sexual activity, including desire, arousal, or orgasm. The subcategories of sexual pain disorder include dyspareunia and vaginismus. Dyspareunia is characterized by pain in the pelvic area during or after intercourse. Vaginismus is characterized by involuntary spasm of the vagina related musculature, which results in a painful sexual activity, sexual pain disorder is also usually associated with psychological trauma or physiological pelvic diseases (Angel, 2010).

2.6 Assessing Sexual Behavior (Human versus Rat)

Research on animal sexual behavior generally concerns the investigation of basic behavioral and physiological mechanisms, including neuroendocrine, neuroanatomical, neurochemical and molecular functions. However, such research is also conducted to provide preclinical data on the impact of pharmacological agents, instrumentation, new devices, and/or surgical procedures, prior to clinical trials, or to examine sexual „side effects" of different treatments to sexual dysfunction. Although the study of animal sexual behavior by neuro-endocrinologists has traditionally been concerned with mechanisms of sexual response, more recent use of conditioning and preference paradigms, and a focus on environmental circumstances and experience, has revealed behaviors that are analogous or homologous to human sexual desire (Pfaus, 2006). In all species, sexual behavior is directed by a complex interplay between steroid hormone actions in the brain that give rise to sexual arousability, and experience with sexual reward or pleasure that gives rise to expectations of competent sexual activity, including sexual arousal, desire, and elements of copulatory performance (Pfaus and Scepkowski, 2005).

Like people, rats manifest sexual excitement and desire behaviorally. They will increase their motor output in anticipation of copulation and work for the opportunity to copulate or to obtain primary or secondary (conditioned) sexual rewards associated with copulation. What characterizes many of these behaviors is that they occur before copulation: courtship, operant responses, conditioned locomotion in anticipation of sex, time spent near a particular sexual incentive, or choices made between two or more incentives, can all be considered analogies of anticipatory sexual desire as shown in figure 2.5. Desire can also be inferred from certain appetitive behaviors during copulation, such as solicitation and pacing, in which female rats control the initiation and rate of the copulatory stimulation they receive. A growing body of

evidence indicates that these aspects of sexual behavior are altered in a relatively selective fashion by certain drugs that are known to alter desire in humans (Pfaus, 2006).

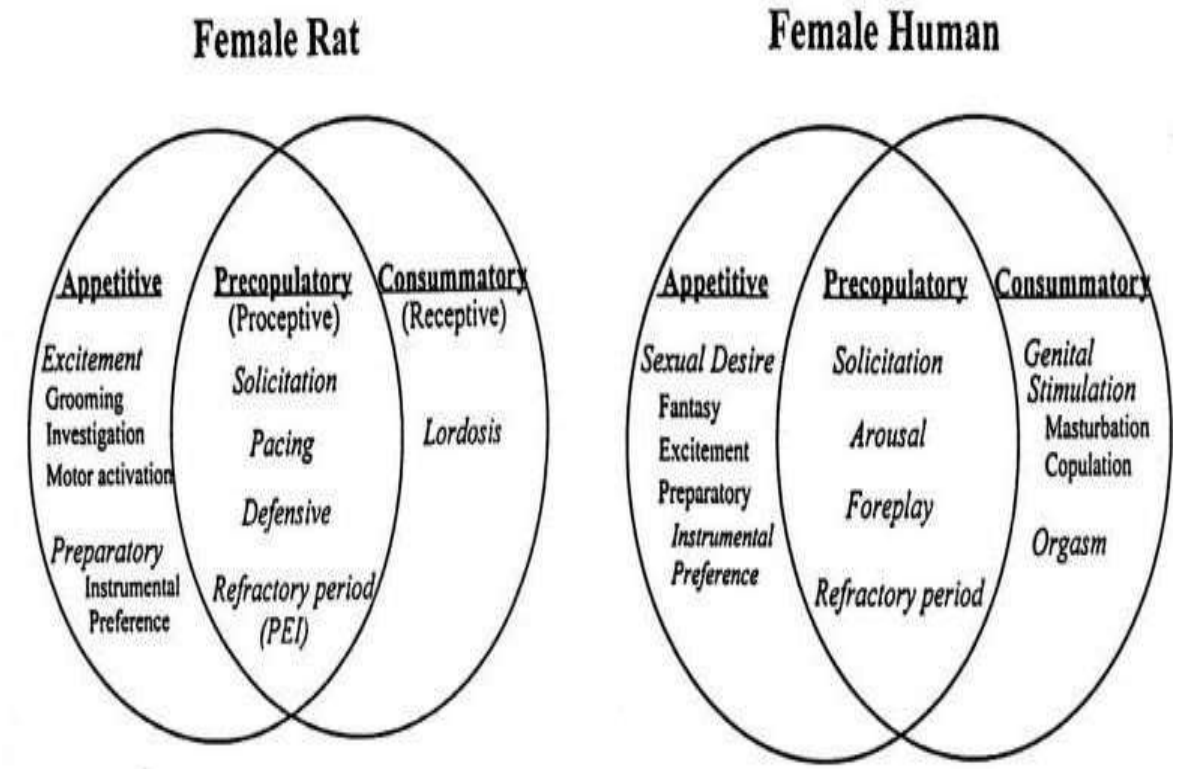


Figure 2.5: Similarities between sexual behavior in rats and humans (Pfaus, 2006)

CHAPTER THREE

3.0 Materials and Method

3.1 Materials

3.1.1 Instruments, apparatus and consumables.

Bowl, cages, clinical hand gloves, camera, cotton wool, drinkers, unilevel pacing chamber, hand towel, cannula, permanent markers, sawdust, stop watch, syringe, water, weighing balance, dettol soap.

3.1.2 Drugs and reagents used

The following reagents and drugs were used: methanol, ketamine 50mg/ml by Rotex Pharmaceuticals Germany, diazepam 10mg/ml Juhel Pharmaceutical Industry Nigeria, Estradiol valerate tablets 2mg Progynova Bayer Pharmaceuticals Germany, progesterone tablets 200mg PRSURG-200, ELISA kits (Fine test rat estradiol, progesterone and testosterone Elisa kit from Wuhan Fine Biotech Co. ltd., China), fruit pulp extract of *Azanza garckeana.*

3.1.3 Ethical approval

Ethical approval was recieved from A.B.U committee on animal use and care with the approval number ABUCAUC/2021/121.

3.1.4 Experimental animals

Thirty-seven (37) female Wistar rats between 4-8weeks old with weight range of 120-140g and five (5) adult male Wistar rats of age range 8-12weeks and weight 250-300g were used for the research. The animals were obtained from animal house Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU), Zaria. The animals were housed in plastic cages and kept at room temperature, they were fed with standard animal feed and water *ad libitum*.

3.2 Methodology

3.2.1 Fruit pulp sourcing and extraction

Azanza garckeana fruit was sought from a farm in Kaltungo Local Government of Gombe state in the month of September, 2021 and identified at the herbarium in the Department of Botany, Ahmadu Bello University Zaria with the voucher number ABU07276. The fruit pulp was air dried after removal of the seeds and pulverized. The pulverized sample was macerated in 70% methanol and 30% water, then allowed to stand for 72 hours and then filtered. The resulting filtrate was concentrated using rotary evaporator and evaporated to dryness on a water bath at 45^{o}C. A yielded percentage of the extract obtained. The dried extract was stored properly in a container before further experiments and analysis.

$$\%\text{yield} = \frac{\text{Weight of dry extract (g)}}{\text{Weight of dry fruit (g)}} \times 100$$

$$\%\text{yield} = \frac{278.5\text{ g}}{1000\text{ g}} \times 100 = 27.8\%$$

3.2.2 Preliminary phytochemical screening tests

Phytochemical screenings of *A. garckeana* was carried out to detect the presence or absence of secondary metabolites using procedures described by Trease and Evans (1983).

3.2.4.1 Test for anthraquinones

Bontragers test: 10 ml of benzene was added in 6 g of the powder sample in a conical flask and soaked for 10 minutes and then filtered. Further 10 ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 seconds. The appearance of pink, violet, or red color indicated the presence of anthraquinones in the ammonia phase.

3.2.4.2 Test for tannins

Bromine Water test: 10 ml of bromine water was added to the 0.5 g aqueous extract. Decolouration of bromine water showed the presence of tannins (Sofowora, 1993).

Ferric chloride test: About 0.5 g of the extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

3.2.4.3 Test for Saponins

Frothing test: 5.0 ml of distilled water was mixed with the fruit pulp extract in a test tube vigorously. The frothing was mixed with few drops of olive oil and mixed. The appearance of foam showed the presence of saponins (Sofowora, 1993).

3.2.4.4 Tests for Flavonoids

Shinoda Test: Pieces of magnesium ribbon and HCl concentrated were mixed with the fruit extract. The appearance of pink color showed the presence of flavonoid

Alkaline Reagent Test: 2 ml of 2.0% NaOH mixture was mixed with the crude extract. The appearance of concentrated yellow color, which becomes colorless when 2 drops of diluted acid was added to the mixture shows the presence of flavonoids.

3.2.4.5 Tests for Cardiac Glycosides

Keller-Kiliani Test: A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% FeCl3 mixture was mixed with 10 ml of extract and 1 ml H_2SO_4 concentrated. The formation of a brown ring between the layers showed the entity of cardiac steroidal glycosides.

3.2.4.6 Test for Terpenoids

Liebermann‟s Test: 2.0 ml of acetic acid and 2 ml of chloroform with whole crude extract were

mixed together. The mixture was then cooled and we added H_2SO_4 concentrated. Green color showed the entity of aglycone, steroidal part of glycosides (Trease and Evans, 1989).

3.2.4.7 Test for Steroids

Salkowski‟s Test: We added 2 ml H_2SO_4 concentrated to the whole crude extract. The formation of reddish brown color indicated the presence of the steroidal aglycone part of the glycoside.

3.2.4.8 Test for Carbohydrates

Molisch test: Few drops of molisch reagent was added to the solution of the extract followed by addition of concentrated H_2SO_4 from the side to form a lower layer. Appearance of a violet ring at the interface indicates the presence of carbohydrates.

3.2.4.9 Test for Phenols

Lead acetate test: Few drops of lead acetate solution was added to the solution of the extract. A yellow coloured precipitate indicates the presence of phenols.

3.2.4.10 Test for Alkaloids

Mayers test: Few drops of mayers reagent was added to the solution of the extract. A cream coloured precipitate indicates the presence of phenols (Trease and Evans, 1989).

3.2.3 Acute toxicity study

The median lethal dose (LD_{50}) of the hydromethanolic fruit pulp extract of *Azanza garckeana* was determined using the method described by Lorke (1983). The experiment was carried out in two phases. In phase I (trial phase) nine female rats were selected randomly and divided into three groups of three rats each (n= 3). Groups 1, 2 and 3 were given *Azanza garckeana* fruit pulp extract orally at doses of 10, 100 and 1000 mg/kg respectively. The animals were observed for 24 hours for any sign of toxicity or mortality. In the second phase, three rats were placed at

random into three groups of one rat each (n =1). The groups were individually treated with 1600, 2900 and 5000 mg/kg doses of *Azanza garckeana* fruit pulp extract based on the outcome of the initial trial. The median lethal dose (LD_{50}) of *Azanza garckeana* fruit pulp extract was determined by taking the geometric mean of the maximum dose producing 0% mortality and the minimum dose producing 100% mortality.

$$LD_{50} = \sqrt{(D0 \times D100)}$$

D0= Highest dose that gave no mortality. D100= lowest dose that produced mortality

3.2.4 Determination of estrous cycle

Prior to the start of administration, the estrous cycle of each rat was monitored to determine the estrous cycle phases. This was carried out according to the method of Long and Evans (1922), based on cytology of vaginal smears obtained daily in the morning (9–10am). Vaginal secretions were collected using a 1 ml syringe filled with 0.02 ml of normal saline (0.9% NaCl) by inserting the tip into the rat vagina but not deeply, the content of the syringe was emptied into the vaginal canal then aspirated back immediately to collect the vaginal sample. The vaginal fluid was placed on glass slides, a different glass slide for each cage. One drop was collected with a clean tip from each rat and viewed unstained under a light microscope with 10 and 40 x objective lenses (Long and Evans, 1992). Appendix I shows the image of the vaginal secretions placed on glass slides. Appendix II shows the vaginal secretion viewed with x40 magnification of the microscope.

The estrous phases were identified based on the predominant cell type present on the glass slides. Pro-estrous (1^{st} phase of the cycle) was identified by the presence of predominately nucleated epithelial cells, estrous phase (2nd phase of the cycle), by presence of predominantly anucleated

`cornified cells with irregular shapes, meta-estrus (3rd phase of the cycle) by presence of same proportion on leucocytes, cornified and anucleated epithelial cells; and the last phase of the cycle, di-estrous by predominance of leucocytes.

3.2.5 Experimental design

Twenty-five (25) adult female Wistar rats were randomly divided into 5 groups of five (5) animals each (n = 5).

i. Group I served as the negative control group and received distilled water 1ml/kg.

ii. Group II (positive control) received Estradiol valerate 100µg given 48hours before testing and 50µg progesterone five (5) hours before testing orally (Mokhtari and Zanboori, 2011).

iii. Group III received 250 mg/kg of the hydromethanolic fruit pulp extract of *A. garckeana*.

iv. Group IV received 500 mg/kg.

v. Group V received 1000 mg/kg.

The extract was administered orally for a period of 14 days. Assessment of sexual behaviour was carried out after the treatment period.

3.2.6 Assessment of sexual behavior

A unilevel pacing chamber (60 L × 40 W × 40 H cm) was used. The chamber was bisected by a transparent Plexiglas wall containing 3 small holes permitting the female to enter or exit the half of the cage in which the male was confined. The holes were too small for the male rats to pass

through, thus allowing the female to control or “pace” the sexual interaction. During the first two weeks of the lab experiment the animals (female and male) were habituated separately to the pacing chamber twice a week for two weeks. On the day of the study, experimental female rat (that are in their estrous phase) were placed in one half of the chamber and a stimulus male was introduced into the other half after 5 minutes. Appetitive and consummatory behaviors were evaluated for a period of 30 minutes per female animal. For each test, the presence or absence of hops and darts displayed by the female was noted. Solicitations was scored as a head-wise orientation to the male, followed by an abrupt run away regardless of whether the female remained in the male's side of the chamber (Meyerson and Lindstrom, 1983). Each group of female animals have their experimental male i.e ratio of 5:1 was used.

3.2.6.1 Determination of lordosis quotient (Test for receptivity)

The female rats were expected to solicit sexual contact by entering the male chambers and receive mounts from the male. The whole event was videotaped for 30 minutes and the lordosis quotient was calculated as a measure of sexual receptivity. Lordosis is defined as dorsiflexion of the back in response to a mount by the male. Lordosis quotient was calculated by dividing the number of lordosis responses by the total number of mounts and multiplied by 100 (Telleria *et al*., 1997).

$$Lq = \frac{N_L}{N_m} \times 100$$

Lq: lordosis quotient N_L: number of lordosis response N_m: total number of mounts



Figure 3.1: Female rat exhibiting lordosis (McCarthy, 2012).



Figure 3.2: Female rat mounted by the male (Barnett, 1963).

3.2.6.2 *Test for proceptivity***:** In the presence of a male partner, the estrous female exhibits a variety of courting or soliciting gestures, collectively termed "Proceptive behavior". This was observed and recorded during the period the female was in the apparatus.

Hop: A short leap with the animal landing on all four paws followed by the assumption of crouching posture. The hopping frequency was determined.

Dart: A run consisting of several steps abruptly terminated by the adoption of the crouching posture. The darting frequency was determined.

Ear wiggling: Rapid lateral shaking of the head causing the appearance of distinctive anterior-posterior ear vibrations usually accompanied by an upward toss of the head. The frequency of occurrence was also determined.

All frequencies of hopping, darting, and ear wiggling during the test was added and divided by the length of the test to establish a standard measure of proceptive behavior (Tennent *et al*., 1980). The time spent in each compartment was also determined.

$$PB = \frac{H_f + D_f + E_f}{T}$$

PB: proceptive behavior, D_f: darting frequency, H_f : Hopping frequency, E_f: Ear wiggling frequency T: Duration of test

3.2.6.3 Preparation of the male animals used for mating

For the habituation phase, male rats received 4 training test sessions (twice a week for 2weeks) in the unilevel copulatory pacing chamber with non-experimental receptive females i.e. the females used for the training of the males were not used for the rest of the study. Only males

displaying active sexual behavior during the 4 training test sessions were included in the final experiment. Immediately prior to the mating test with the experimental females, males were given brief access to fully receptive, non-experimental females (each given 100µg Estrogen 48 hours and 50µg Progesterone 5 hours prior to the mating test to ensure full receptivity) (Watcho *et al*., 2004). Only males displaying active mounting behavior were used with the experimental females.

3.2.7 Animal sacrifice and preparation of samples

After oral administration of the extract for fourteen (14) days, rats were euthanized with combined ketamine and diazepam anaesthesia (50mg/kg/10mg/kg) given intra-peritoneally (Miaffo *et al*., 2019). Each rat was dissected to expose the heart and blood samples were collected in plain bottles by cardiac puncture. The blood sample was centrifuged and the sera obtained was used for hormonal analyses (Adebayo, 2015).

3.2.8 Hormonal assay

The serum level of estradiol, progesterone and testosterone were analyzed using enzyme linked immunosorbent assay (Elisa) kits by the method of Tietz (1995) following the manufacturer‟s manual.

i. **Progesterone:** The principle of this immunoassay follows the typical competitive binding scenario. During incubation, a fixed amount of HRP-labeled progesterone competes with the endogenous progesterone in the standard sample, or quality control serum for a fixed number of binding sites of the specific progesterone antibody.

ii. **Estradiol:** The principle of this immunoassay also follows the typical competitive binding scenario. Competition occurs between unlabeled estradiol (present in standards,

controls and samples) and an enzyme-labelled estradiol (conjugate) for a limited number of antibody binding sites on the microwell plate.

iii. **Testosterone:** Testosterone elisa kit applies the competitive enzyme immuno-assay technique. Standard or samples are added to the appropriate microtiter plate wells with an antibody specific for testosterone and an enzyme-labelled testosterone (conjugate). The labeled testosterone and the testosterone in the sample to be analyzed compete for a limited number of antibody binding sites on the microwell plate.

The assays were carried out according to the procedure described by the manufacturers; FineTest Rat Progesterone, Estradiol and Testosterone ELISA kit with a sensitivity of 0.188ng/ml, 7.5pg/ml and 0.188ng/ml respectively. Assay procedure: The plate 2 was washed before adding standard, sample and control wells. 50 µl of sample was added to each well and immediately after 50 µl of biotin-labeled antibody was added into each well; the plate was gently tapped for 1 minute to ensure thorough mixing then it was incubated for 45 minutes at 37^{o}c. The plate was then washed three times with the wash buffer, immersing it for 1 minute each time. 100 µl HRP-streptavidin conjugate was then added to each wells, the plate was then sealed and was incubated for 30 minutes at 37^{o}c. The plate was then washed five times with the wash buffer, immersing it for 1 minute each time. 90 µl TMB substrate solution was then added to each wells, the plate was then sealed and was incubated for 20 minutes at 37^{o}c. After which 50 µl stop solution was added, and the value for absorbance was read immediately at 450nm. The concentration was extrapolated by plotting a graph of mean absorbance value at 450nm after subtraction the blank value against concentration (ng/ml) on the x-axis.

3.2.9 Histological studies

At the end of the behavioral study, the ovaries and uterus were carefully dissected out. The uterine and ovarian tissues were fixed in 10% formalin for about 24 hours at room temperature, after 24 hours the uterine and ovarian tissue were trimmed to obtain 3-5mm thick sample. The samples were then placed in a tissue processing cassette. To dehydrate the tissue, the tissues were immersed in increasing percentages of alcohol in solution 50%, 70%, 100% alcohol, with the 100% solution changed twice for an average time of 45minutes in every solution. The tissues were then cleared for clearer imagery by immersion for 1 hour in xylene. The tissue while still enclosed in the processing cassette was impregnated to provide support and replace the clearing agent using molten paraffin and then embedded in the same molten paraffin to form a mold, the mold was then submerged in cold water to avoid crystallization (Slaoui and Fiette, 2011). After the mold had hardened, the mold was carefully sectioned using a rotary microtome and placed on glass slides. After which the samples were stained using hematoxylin and eosin dye. After staining, it was immersed in xylene and rinsed using different percentage alcohol. Two drops of resin medium was dispensed on the specimen and a cover glass was placed to keep specimen in place. The histological architecture examined under the microscope and photomicrographs obtained (Heidger *et al*., 2002).

3.3 Statistical Analysis

The data obtained from the study was expressed as mean ± standard error of mean (SEM). The significant differences among the groups was assessed using one-way analysis of variance (ANOVA) followed by Tukey‟s *post hoc* test. *P* values < 0.05 was considered statistically significant. All data were analyzed using IBM SPSS Version 23.0.

CHAPTER FOUR

4.0 Results

4.1 Phytochemical constituents of hydromethanolic fruit pulp extract of *Azanza garckeana*

The phytochemical analysis of the hydromethanolic fruit pulp extract of *A. garckeana* revealed the presence of cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids and terpeniods, but anthraquinones and alkaloids were not detected (Table 4.1).

Table 4.1: Phytochemical constituents of hydromethanolic fruit pulp extract of *Azanza garckeana* fruit

S/N	Phytoconstituents	Test	Inferences
1	Alkaloids	Dragendorff test	-
2	Cardiac Glycosides	Keller-Kiliani test	+
3	Saponins	Frothing test	+
4	Phenolic compounds	Lead acetate test	+
5	Tannins	Ferric Chloride test	+
6	Steroids	Salkowiski test	+
7	Carbohydrates	Molisch test	+
8	Flavonoids	Shinoda test	+
9	Terpeniods	Liebermann Burchard test	+
10	Anthraquinones	Bontragers test	-

Keys: + PRESENT - ABSENT

4.2 Acute toxicity study of the hydromethanolic fruit pulp extract of *Azanza garckeana*

As shown in table 4.2, no deaths were observed during the first and second phase of the acute toxicity studies carried out on the hydromethanolic extract of *Azanza garckeana*. From the result obtained it is concluded that the LD50 is greater than 5000 mg/kg, indicating that doses below 5000 mg/kg are acutely non-toxic and doses above 5000mg/kg are considered practically non-toxic according to the Hodge and Sterner scale.

Table 4.2 Acute toxicity study of hydromethanolic fruit pulp extract of *Azanza garckeana*

PHASE ONE

Group (n=3)	Dose (mg/kg body weight)	No. of Deaths	Percentage mortality (%)
1	10	0/3	0
2	100	0/3	0
3	1000	0/3	0

PHASE TWO

Group (n=1)	Dose (mg/kg body weight)	No. of Deaths	Percentage mortality (%)
1	1600	0/1	0
2	2900	0/1	0
3	5000	0/1	0

$LD_{50} = \sqrt{(D0 \times D100)}$

D0= Highest dose that gave no mortality. D100= lowest dose that produced mortality.

4.3 Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on the proceptive behavior of female Wistar rats

A significant ($P < 0.05$) increase in the proceptive behavior was observed in the groups administered standard drug, 250 mg/kg extract (2.34 ± 0.07), 500 mg/kg (2.22 ± 0.12) extract and 1000 mg/kg (2.02 ± 0.11) dose of extract when compared to the negative control group (0.64 ± 0.02) that received distilled water. There was no significant difference noted amongst the treated groups as shown in figure 4.1.

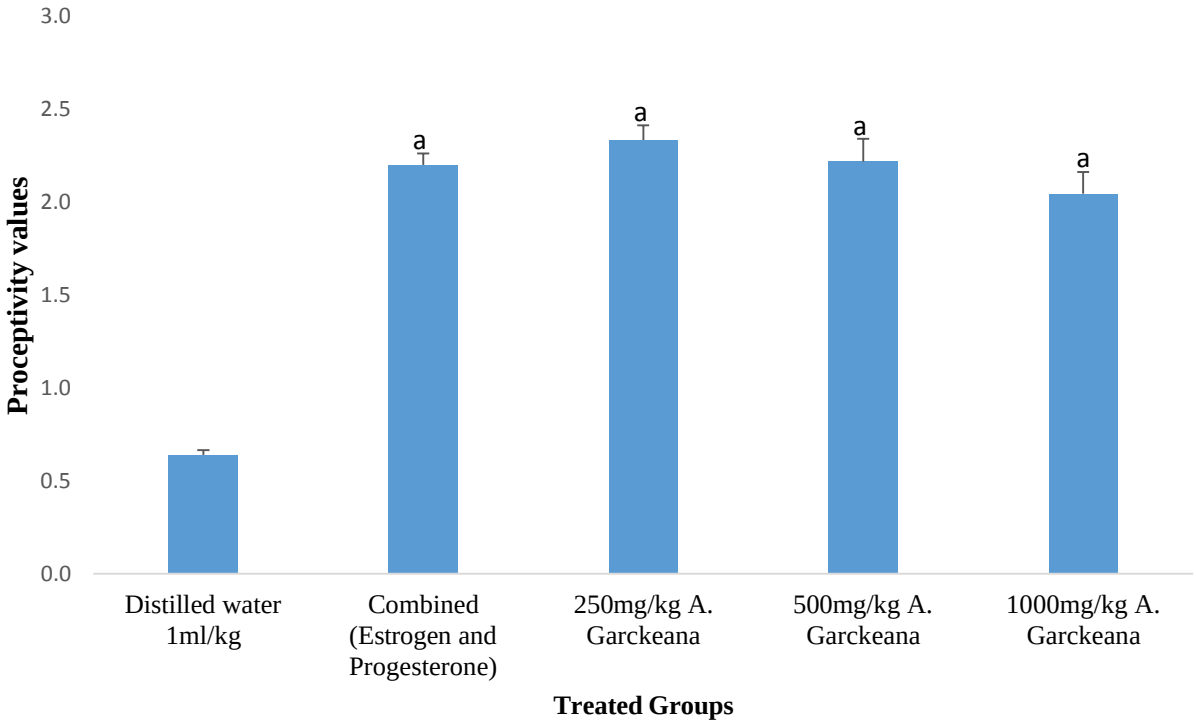


Figure 4.1: Effect of hydromethanolic fruit pulp extract of *A. garckeana* on proceptive behavior of female Wistar rats.

Data represented as mean ± SEM, values with error bars having superscript letter [a] = $p < 0.05$ are significantly different when compared to distilled water. Combined estrogen and progesterone (Estradiol valerate 100 µg and progesterone 50 µg).

4.4 Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on the receptivity of female Wistar rats.

A significant ($P < 0.05$) increase in the receptivity was observed in the positive control (14.80 ± 0.96), 250 mg/kg (31.20 ± 1.93), 500 mg/kg (36.20 ± 2.41) and 1000 mg/kg (41.80 ± 2.95) extract treated group when compared with the normal control (0.00 ± 0.00) as shown in figure 4.2. The significant ($P < 0.05$) increase in the receptivity observed in the group administered 1000 mg/kg dose of *A. garckeana* was significantly higher than the positive control group, 250 mg/kg and 500 mg/kg *A. garckeana* extract treated group. A significant ($P < 0.05$) increase in receptivity was also observed in the 250 mg/kg and 500 mg/kg extract treated group when compared with the positive control group.

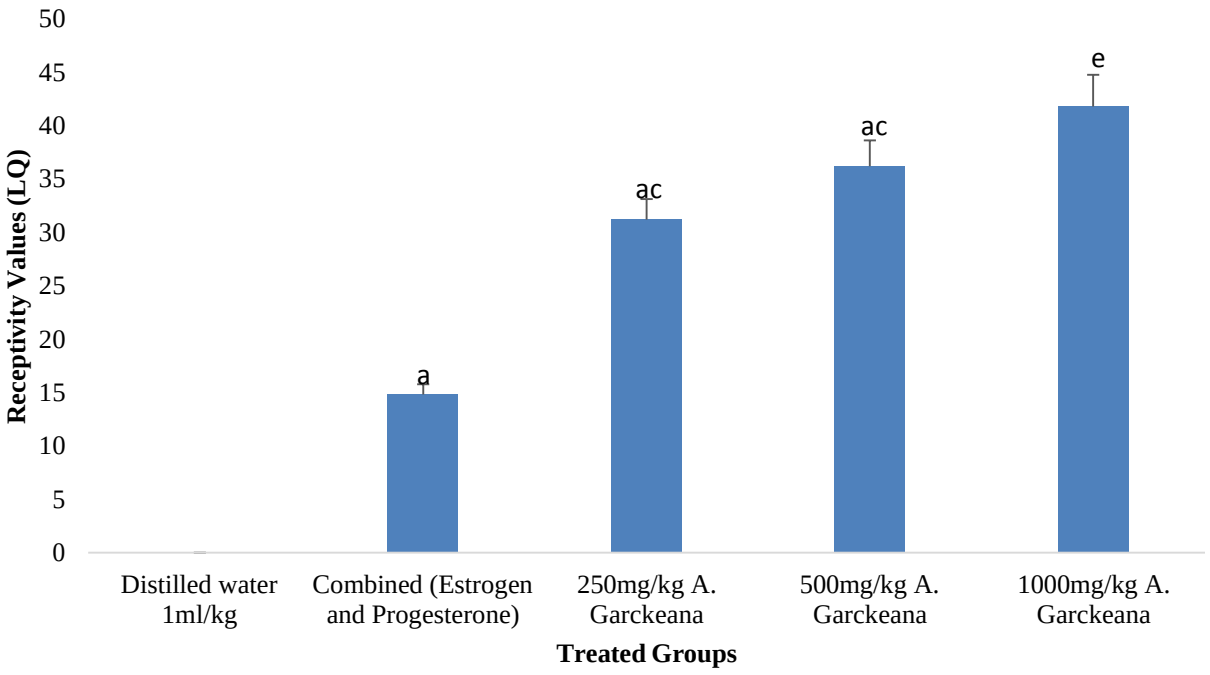


Figure 4.2: Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on receptivity of female Wistar rats.

Data represented mean ± SEM, values with error bars having different super superscript letters [e, a, c] = $p < 0.05$ are significantly different when compared to all groups, distilled water and positive control respectively. Combined estrogen and progesterone (Estradiol valerate 100 µg and progesterone 50 µg).

4.5 Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on the time spent and number of entries into the male chambers by the female Wistar rats.

The result obtained showed a significant increase in both the number of entries and time spent in the male chamber in the groups administered the standard drug, 250 mg/kg dose of extract and 1000 mg/kg dose of extract when compared to the negative (normal) control group that received distilled water. However, no significant difference in number of entries and time spent in the 500 m`g/kg treated groups when compared to the normal control (Table 4.3).

Table 4.3: Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on the time spent and number entries into the male chambers by the female Wistar rats

Groups chamber	Number of entries	Time spent in the male chamber (s)
Negative control	6.60±0.55	171.60±4.72
Positive control	14.80±1.30[a]	271.80±41.35[e]
250mg/kg Extract	13.40±2.07[ad]	318.80±10.52[e]
500mg/kg Extract	6.00±1.00[cb]	202.40±4.39[bcd]
1000mg/kg Extract	12.60±1.34[a]	357.60±6.58[e]

Data represented mean ± SEM, values with different super superscript letters [a,b,c,d, e] = $p < 0.05$ show the level of significance when compared to negative control, 1000 mg/kg extract, positive control, 250 mg/kg and all groups respectively. Positive control= Combined estrogen and progesterone (Estradiol valerate 100 µg and progesterone 50 µg).

4.6 Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on serum estradiol concentration in female Wistar rats.

There is a significant increase ($p < 0.05$) in the level of estradiol hormone in the groups administered the standard drug (positive control) (108.40 ± 1.74 pg/ml), 250 mg/kg extract (104.40 ±1.02 pg/ml), 500 mg/kg extract (112.40 ± 2.11 pg/ml) and 1000 mg/kg (70.00 ± 0.71 pg/ml) extract when compared to the control group that received distilled water (41.80 ± 2.95 pg/ml). However, the level of estradiol at the highest dose of 1000 mg/kg is significantly lower than positive control, 250 mg/kg and 500 mg/kg of the extract.

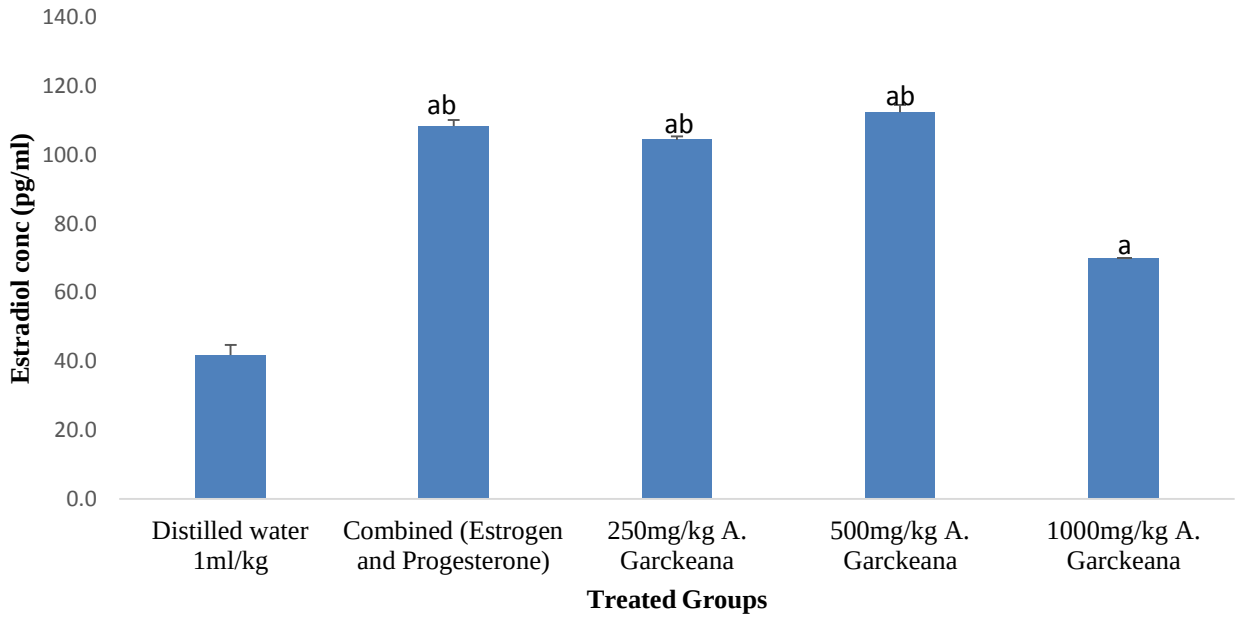


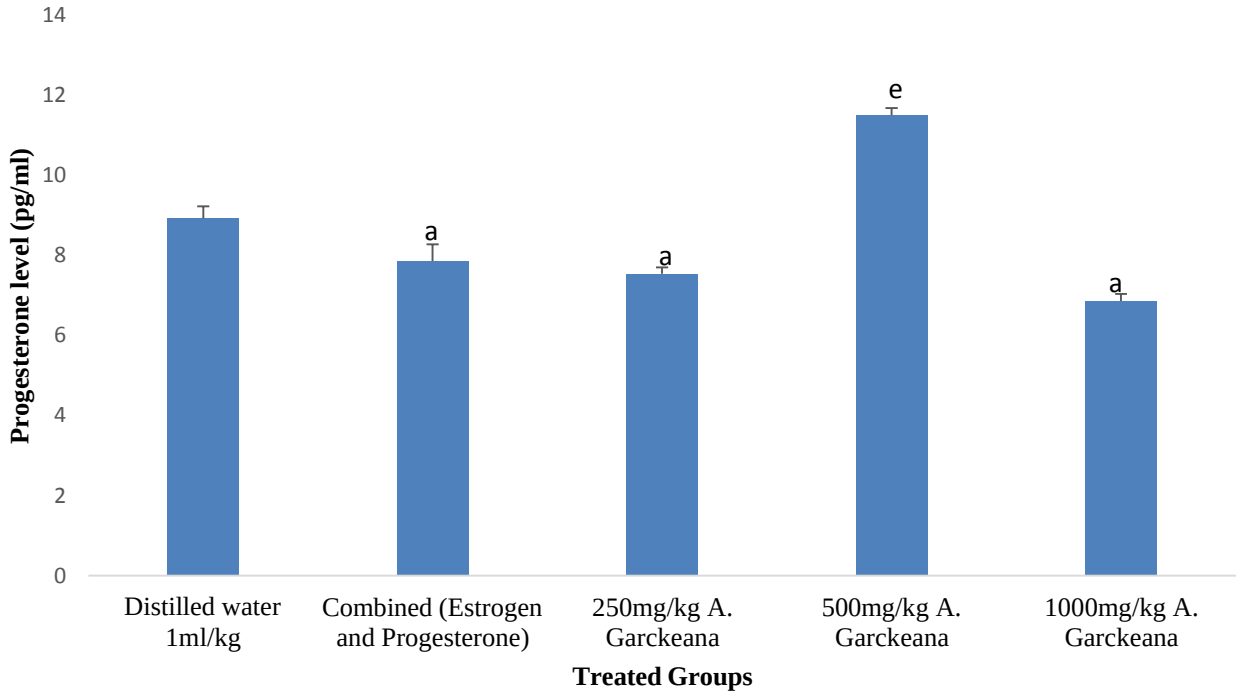
Figure 4.3: Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on serum estradiol concentration in female Wistar rats.

Data is represented mean ± SEM, values with error bars having superscript letters ^{a,b} = $p < 0.05$ are significantly different when compared to distilled water and 1000 mg/kg respectively. Combined estrogen and progesterone (Estradiol valerate 100 µg and progesterone 50 µg).

4.7 Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on serum progesterone concentration in female Wistar rats.

The level of progesterone hormone in the groups administered the standard drug (7.86 ± 0.41 pg/ml), 250 mg/kg (7.54 ± 0.15 pg/ml) and 1000 mg/kg (6.86 ± 0.16 pg/ml) of the extract is significantly lower than the negative control group (8.92 ± 0.30 pg/ml) (figure 4.4). However, there was a significant increase in the group administered 500 mg/kg extract (11.50 ± 0.17 pg/ml) when compared to the negative control group, positive control, 250 mg/kg and 1000 mg/kg extract treated groups.

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Figure 4.4: Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on serum progesterone concentration in female Wistar rats.

Data represented mean ± SEM, values with error bars, having different super superscript letters ^{e,} ^{a} = $p < 0.05$ are significantly different when compared to all groups and distilled water respectively. Combined estrogen and progesterone (Estradiol valerate 100 µg and progesterone 50 µg).

Figure 4.8: Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on serum testosterone concentration in female Wistar rats.

The testosterone hormone concentration in the groups administered the standard drug (1.42 ± 0.12 pg/ml), 250 mg/kg (1.70 ± 0.11 pg/ml), 500 mg/kg (0.92± 0.13 pg/ml) and 1000 mg/kg (1.40 ± 0.16 pg/ml) of the extract was significantly lower than the level of testosterone hormone in the negative control group (2.18 ± 0.09 pg/ml). The significant decrease ($p < 0.05$) in the level of testosterone observed in the group administered 500 mg/kg extract was significantly lower when compared to the positive control, 250 mg/kg and 1000 mg/kg extract treated groups.

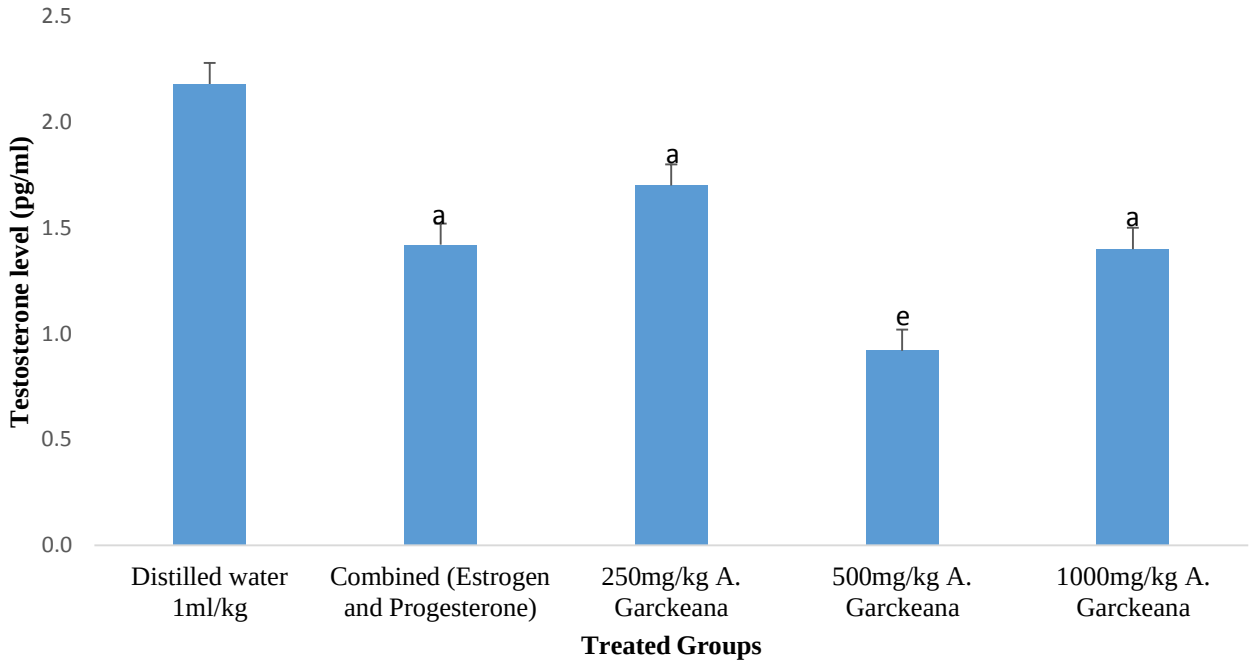
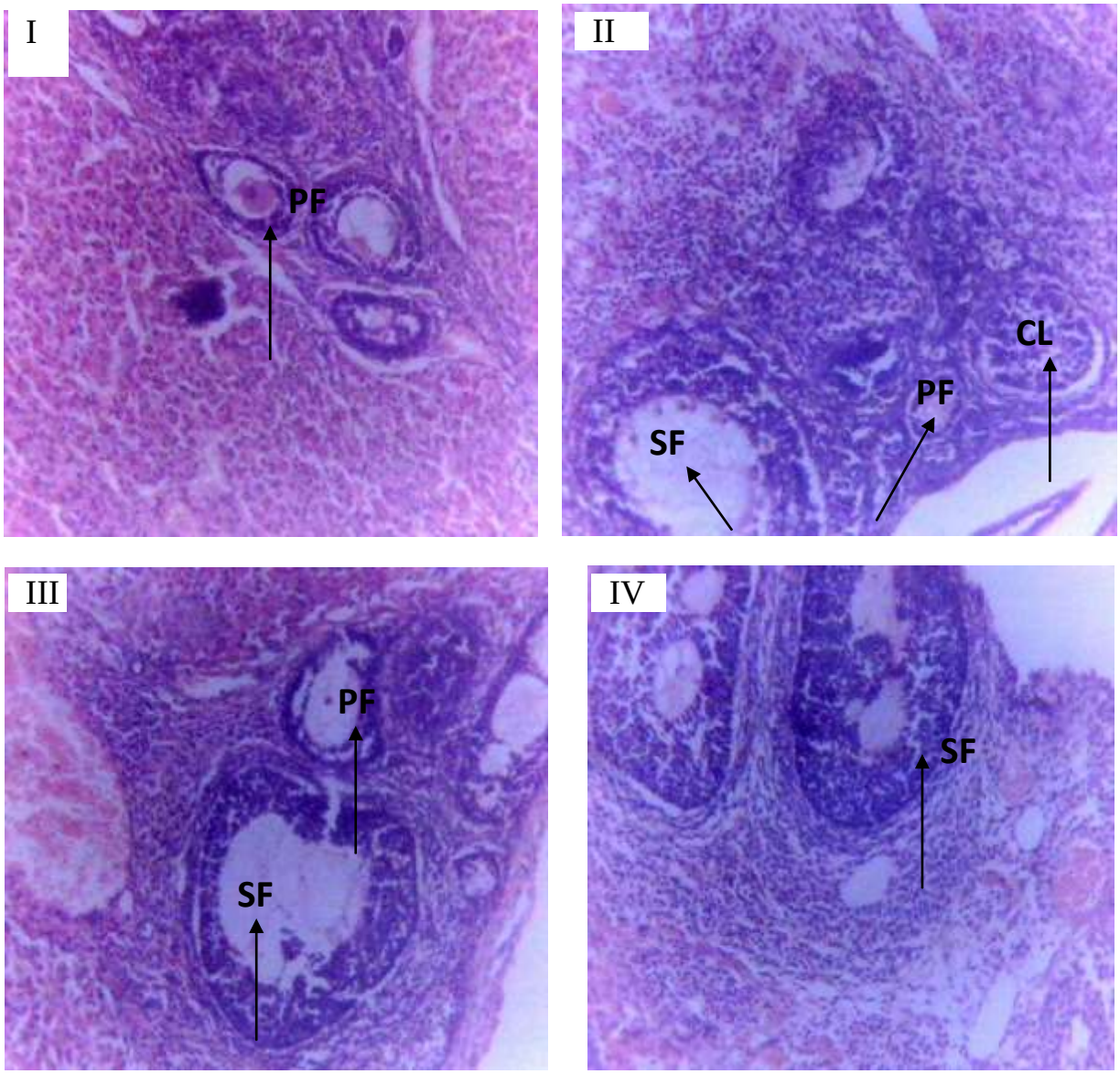


Figure 4.5: Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on serum testosterone concentration in female Wistar rats.

Data represented mean ± SEM, values having different super superscript letters $^{e, a}$ = $p < 0.05$ are significantly different when compared to all groups and distilled water respectively. Combined estrogen and progesterone (Estradiol valerate 100 µg and progesterone 50 µg).

4.9 Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on histo-architecture of the ovary in female Wistar rats

As shown on plate I, there was slight hyperplasia of the inflammatory cells (IH), 65% primary follicle (PF), 20% secondary follicle (SF) and 15% corpus luteum (CL) in the negative control group, Plate II showed slight hyperplasia of the inflammatory cells, 40% primary follicle (PF), 35% secondary follicle (SF) and 15% corpus luteum (CL) in the positive control group, the group administered 250 mg/kg dose of extract showed no hyperplasia of the inflammatory cells, 50% primary follicle (PF), 30% secondary follicle(SF) and 20% corpus luteum (CL) as shown on plate III, the 500 mg/kg dose of extract group showed normal ovary with 60% primary follicle (PF), 25% secondary follicle (SF) and 25% corpus luteum (CL) on plate IV. The 1000 mg/kg dose of extract group result showed a normal ovary with 55% primary follicle (PF), 35% secondary follicle (SF) and 10% corpus luteum (CL) as shown on plate V. The percentages signify the number of a type of cell compared to the total number of all the cells present in the ovary.



Photomicrograph sections of the ovary using H & E X250 magnification

Plate I: Normal control

Plate II: Positive control (combined estrogen and progesterone)

Plate III: 250 mg/kg extract treated group

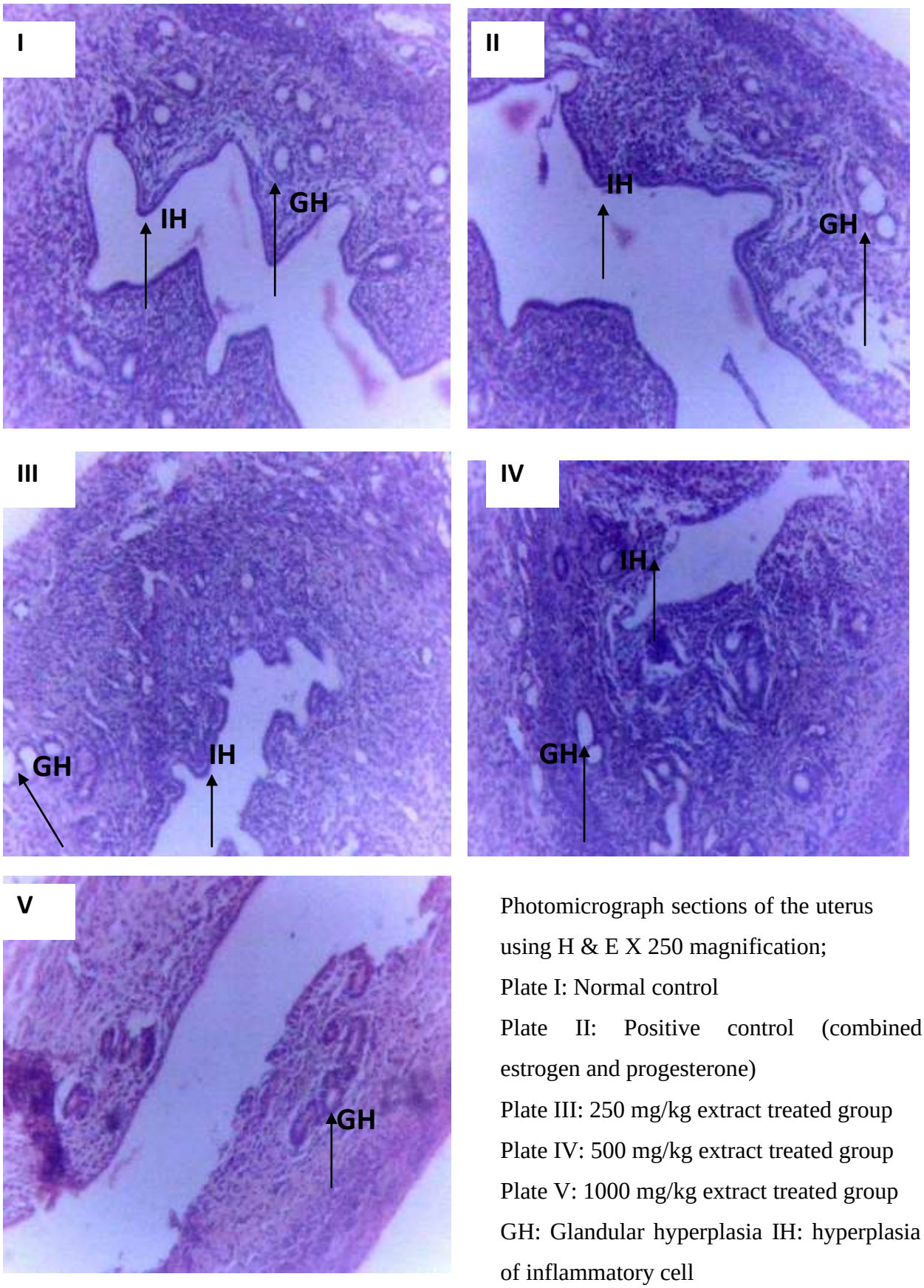
Plate IV: 500 mg/kg extract treated group

Plate V: 1000 mg/kg extract treated group

IH: hyperplasia of the inflammatory cells, PF: primary follicle, SF: secondary follicle CL: corpus luteum

4.10 Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on histo-architecture of the uterus in female Wistar rats

The result show slight glandular hyperplasia (GH) and slight hyperplasia of inflammatory cell (IH) of the uterine tissue in the negative control group as shown on plate I, there is moderate glandular hyperplasia in the positive control group as shown on plate II, the group administered 250 mg/kg dose of extract showed moderate GH and slight IH as shown on plate III, the group administered 500 mg/kg dose of *A. garckeana* extract showed moderate GH and slight IH of the uterus as shown on plate IV, the group administered 1000 mg/kg dose of *A. garckeana* extract showed moderate GH with no IH of the uterus shown on plate V.



Photomicrograph sections of the uterus using H & E X 250 magnification;

Plate I: Normal control

Plate II: Positive control (combined estrogen and progesterone)

Plate III: 250 mg/kg extract treated group

Plate IV: 500 mg/kg extract treated group

Plate V: 1000 mg/kg extract treated group

GH: Glandular hyperplasia IH: hyperplasia of inflammatory cell

CHAPTER FIVE

5.0 Discussion

The phytochemical analysis of the hydromethanolic fruit pulp extract of *A. garckeana* revealed the presence of cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids and terpeniods. Similar result to this was reported by Ochokwu *et al*., (2015) but anthraquinones and alkaloids were not detected in this study as in contrast with the earlier report. The presence of flavonoid and saponin in the fruit extract could be the reason for the high aphrodisiac activity i.e increased sexual behavior and sex hormone levels observed in this study. Steroidal saponins have been recorded to play a role in enhancing sexual behavior by either binding to hormone receptors, which may result in conformational change that will enhance the physiological function of the sex hormones or bind to enzymes that are involved in the synthesis of such hormones and thus enhance its production (Gauthaman and Adaikan, 2008). Saponins found primarily in the leaf *Tribulis terrestris* L. have been used as an aphrodisiac agent in rats (Goel and Maurya, 2020). These phytochemical contents of *A. garckeana* may justify the claim for their remarkable aphrodisiac activities, and its usage in treatment of diseases.

Toxicity is one of the most important aspects of any medication because it can be used to govern the extent of therapeutic utility. The acute toxicity study provides information on the adverse effects of a single dose of a drug or substance within 24 hrs following oral or dermal administration (Lorke, 1983). The result of acute toxicity of *A. garckeana extract* in this study revealed that the LD_{50} is more than 5000 mg/kg and it is acutely non-toxic below that dose.

The results on the effect of *Azanza garckeana* fruit pulp extract on proceptive behaviour shows a

significant increase in the level of proceptive behavior in the positive control and extract treated groups when compared to the normal control group. The level proceptivity noted in the three doses of the extract were not significantly different. Generally, elevated sex hormone level enhances sexual behavior in humans (Wilson and Hunters 1987). This finding maybe as a result of the increase in the level of estrogen in the groups treated with the extract as compared to control obtained in this study. This in turn may be due to the steroidal saponin content of *A. garckeana,* the regulation of female sexual motivation in rats by ovarian hormones has been demonstrated previously in numerous experiments (Wilson and Hunters 1987). This result is in agreement with the study carried out by Khadabadi and Bhajipale (2011) on the effect of *Abutilon indicum* extract on female libido in rats who reported a significant increase in proceptive behavior after 11 and 21days pretreatment with 200 and 400mg/kg of the extract. The result also agrees with Atuadu *et al.* (2018) on the effect of ethanolic seed extract of *citrullius lanatus* on hypothalamic-pituitary-gonadal (hpg) axis and sexual behavior of female Wistar rats who reported a significant increase in the proceptive behavior of the female Wistar rats after week 1 and 2 treatment with high dose of the seed.

During copulation, female rats display lordosis, a distinct spinal reflex posture with flexion of the back, extension of the neck, and elevation of the hindquarters and rump. This posture usually lasts 0.5 – 1.5 s. A female displaying this behavior in response to male mounting is frequently described as sexually receptive. The period of receptivity is termed “behavioral estrus” (Long and Evans, 1922). Lordosis quotient is the most important indicator of female receptivity (Kagathara *et al.,* 2015) and the most frequently used parameter for assessing the effect of hormones and drugs on female sexual behavior in rodent (Uphouse, 2000; Uphouse and Guptarak, 2010).

This findings obtained in this study on the effect of *Azanza garckeana* on Lordosis quotient are similar with the work of Nwafor *et al.* (2013) who reported that lordosis quotient significantly increased with a corresponding decrease in lordosis latency which might be as a result of the estrogenic property or presence of phytochemical constituents (flavonoids and saponin) in the methanolic extract of *smilax kraussiana*. Studies have shown that female rodent reproductive behaviors are strictly dependent on ovarian hormones acting within the central nervous system (Blaustein and Erskine, 2002), it has been established that estrogen modulates sexual motivation by its action on the ventromedial nucleus of the hypothalamus (VMN) (Pfaff, 1980).

The results on the effect of *A. garckenana* fruit pulp on the level of estrogen obtained from this study revealed a significant increase in the level of estrogen in the three doses of the extract tested when compared to normal control, the significant increase of estrogen may be as a result of increase in gonadotropin released by direct stimulation of accessory sex organs of the female animals during the copulatory act. These findings can be corroborated with the result obtained from the histological section of this study, which showed overall increased folliculogenesis in the treated groups when compared to control. Folliculogenesis refers to increase in growth and development of follicles with resultant increase in estrogen secretion from follicular cells. Estrogen is known to increase sexual desire (Thornton and Finn, 1999), and also acts in a feedback mechanism, influencing the production of follicle stimulating hormones (FSH) from the pituitary gland. FSH in turn promotes the development of the immature ovarian follicles, which increases the production of estrogen from the ovary (Yakubu *et al.,* 2008). Increase in estradiol levels could also be related to the saponin present in the extract which is believed to be an estrogen precursor (Gauthamen *et al.,* 2000). At the higher dose of 1000 mg/kg the level of estrogen hormone significantly dropped when compare to the level at lower doses of 250 mg/kg

and 500 mg/kg, this could be due to a negative feedback mechanism elicited by the higher dose of the extract on the ovary.

The results on the effect of *Azanza garckenana* fruit pulp on the level of progesterone obtained from this study agrees with the report of Yakubu *et al.* (2008), who observed that extract of *C. aconitifolius* caused a decrease in serum progesterone level of female rats. The increase in progesterone level observed at 500 mg/kg at estrus phase could be due to potent positive feedback mechanism on the pituitary to release gonadotropic hormones which stimulate granulosa cells to increase production of progesterone. It could also be as a result of an increase in follicular numbers observed at that dose (500 mg/kg) from the histological study. Zemlan and Adler (1978) observed both lordosis and proceptive behaviour in ovariectomized, adrenalectomized rats treated with very large doses of estrogen, although the behaviour was not measured quantitatively. These authors suggest that both behavioural components are primarily regulated by estrogen, but that proceptivity is much less sensitive to the hormone and requires higher levels for expression. Progesterone, while not essential, was presumed merely to amplify the effect of low estrogen doses on proceptive and receptive behaviour.

The results on the effect of *Azanza garckenana* fruit pulp on the level of serum testosterone obtained from this depicts that the extract has a testosterone depleting action which is more potent at the 500 mg/kg dose of extract, this could be an indication that GnRH-LH signaling was activated by the extract, because testosterone is converted to estrogen in females through this GnRH-LH signaling pathway resulting in the high levels of estrogen obtained (Douma *et al.*, 2007). This finding agrees with the report of Meyerson and Lindstrom (1983) who reported depletion in the level of testosterone after treatment with estradiol benzoate. Research has shown that increased levels of estrogen result in rising levels of sex hormone-binding globulin, which

then leads to the reduction in the level of free testosterone (Clayton, 2007). Contrariwise the study reported by Bukar *et al*., (2020) on the ameliorative and fecundity potentials of aqueous extract of *Azanza garckeana* fruit pulp in formalin induced toxicity on male albino mice revealed a significant increase in testosterone levels at all doses when compared to the control.

The increase in time spent in the male chamber by the female rat could be as a result of the high level of the sex steroid hormone (estradiol) which increased the ability of the female rat to stay in close proximity to the male rats and subsequently promote the copulatory act. This result is in agreement with the study carried out by Khadabadi and Bhajipale (2011), on the effect of *Abutilon indicum* extract on female libido in rats who reported that pretreatment with *Abutilo indicum* at 100, 200 and 400 mg/kg doses significantly improved the core proximity time for male target as compared to control rats.

The results from the histological studies on the effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on the histo-achitecture of the ovary showed an increase in the proliferation and growth of the ovarian follicle from primary follicle to secondary follicle then subsequently to corpus luteum (shown by the percentages of each type of follicle) in all three doses of the extract when compared to control. This corroborates with the significant increase in estrogen level in the extract treated groups when compared to control. Estrogen acts in a feedback mechanism where it influences the production of follicle stimulating hormones (FSH) from the pituitary gland. FSH in turn promotes the development of the immature ovarian follicles which increases the production of estrogen from the ovary (Yakubu *et al.,* 2008). This result is similar to the result obtained by Tijjani *et al.* (2018) on the aphrodisiac effect of aqueous stem bark extract of *Ficus sycomorus* on female Wistar rats, where they reported an increase in the number of ovarian follicles in the high dose seed extract treated group when compared to control.

The results from the histological studies on the effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on the uterine tissue showed increased uterine gland hyperplasia in the positive control and extract treated groups except at 1000 mg/kg where no significant change was observed. Increased gland hyperplasia is noted by the widening of the uterine glands and the increase in the number of secretory glands. This could be due to the effect of the active phytochemicals in the extract like saponin that promote the action of estrogen. Follicle stimulating hormone (FSH) released during the first days of the reproductive cycle, stimulates ovarian follicles to mature and as they mature, the follicles secrete increasing amounts of estradiol, an estrogen (Weschler, 2002). Estradiol has multiple effects on the female reproductive tract of which includes the initiation of the growth of endometrial glands (Senger, 2007), under the control of estrogen secreted by the ovarian (graafian) follicle the endometrium proliferates, increases in thickness and its lining becomes cuboidal. The uterine glands also become straight, long and widely separated (Haroun, 2016). Endometrial growth is accompanied by increased blood flow, especially through the spiral arteries, which grow rapidly under the influence of estrogens (Johnson, 2003). The insignificant change at 1000 mg/kg can be correlated with the result obtained on estrogen level which showed a decreased level of estrogen in this group and this could in turn be due to a negative feedback on the estrogen levels elicited by higher concentration of the extract.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

1. Phytocehmical constihtuents of hydromethanolic fruit pulp of *Azanza garckeana* include cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids and terpeniods. The presence of saponins and flavonoids could be the reason for the aphrodisiac activity of the extract.
2. The medial lethal dose ld_{50} of the extract is greater than 5000 mg/kg, indicating that doses below 5000 mg/kg are acutely non-toxic and non-lethal.
3. The extract significantly increased the sexual motivation shown by the increased receptivity [F (4, 24)= 76.2, p = 0.000], proceptivity [F (4, 24)= 25.3, p = 0.000] and proximity time [F (4, 24)= 79.1, p = 0.000] of the female Wistar rats towards the male Wistar rats.
4. The serum concentration of estradiol was significantly increased [F (4, 24)= 262.1, p = 0.000] after treatment with the extract, estradiol is a sex hormone that increases sexual motivation. The serum concentration of testosterone significantly decreased [F (4, 24)= 4.5, p = 0.010] after treatment with the extract which could be due to increased conversion of testosterone to estrogen. The level of progesterone significantly reduced [F (4, 24)= 47.5, p = 0.000] at 250 and 1000 mg/kg and significantly increased at 500mg/kg, this could be attributed to the biphasic nature of progesterone secretion and folliculogenesis.
5. The ovarian follicle showed increased proliferation and growth and the uterus showed increased endometrial growth after treatment with the extract.

6.2 Conclusion

This study has demonstrated that hydromethanolic fruit pulp extract of *Azanza garckeana* possesses significant aphrodisiac potentials in female Wistar rats evidenced by the increased sexual behavior, serum estradiol concentration, histological changes in the uterus and ovaries, and increased proximity time of the extract treated animals, possibly due to presence of phytochemical constituents flavonoids and saponin which have been documented to have aphrodisiac activity.

6.3 Recommendations

Based on the findings of this study, it is recommended that:

1. The ethanolic and aqueous extract of *Azanza garckeana* should be studied to determine the most effective extract in enhancing sexual behavior.
2. A study should be carried out to determine the precise active components in the extract of *Azanza garckeana* responsible for enhancing sexual behavior and reproductive parameters.
3. A research should be carried out on the effect of *Azanza garckeana* on fertility, to establish if it has a fertility enhancing property.
4. The effects of lower doses (below 250mg/kg) of the extract of *Azanza garckeana* should be established at different phases of the estrus cycle, pregnancy and lactation.
5. A research should be carried out on the effect of *Azanza garckeana* on sexual behavior of male Wistar rats after treatment with the extract.

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APPENDIX I

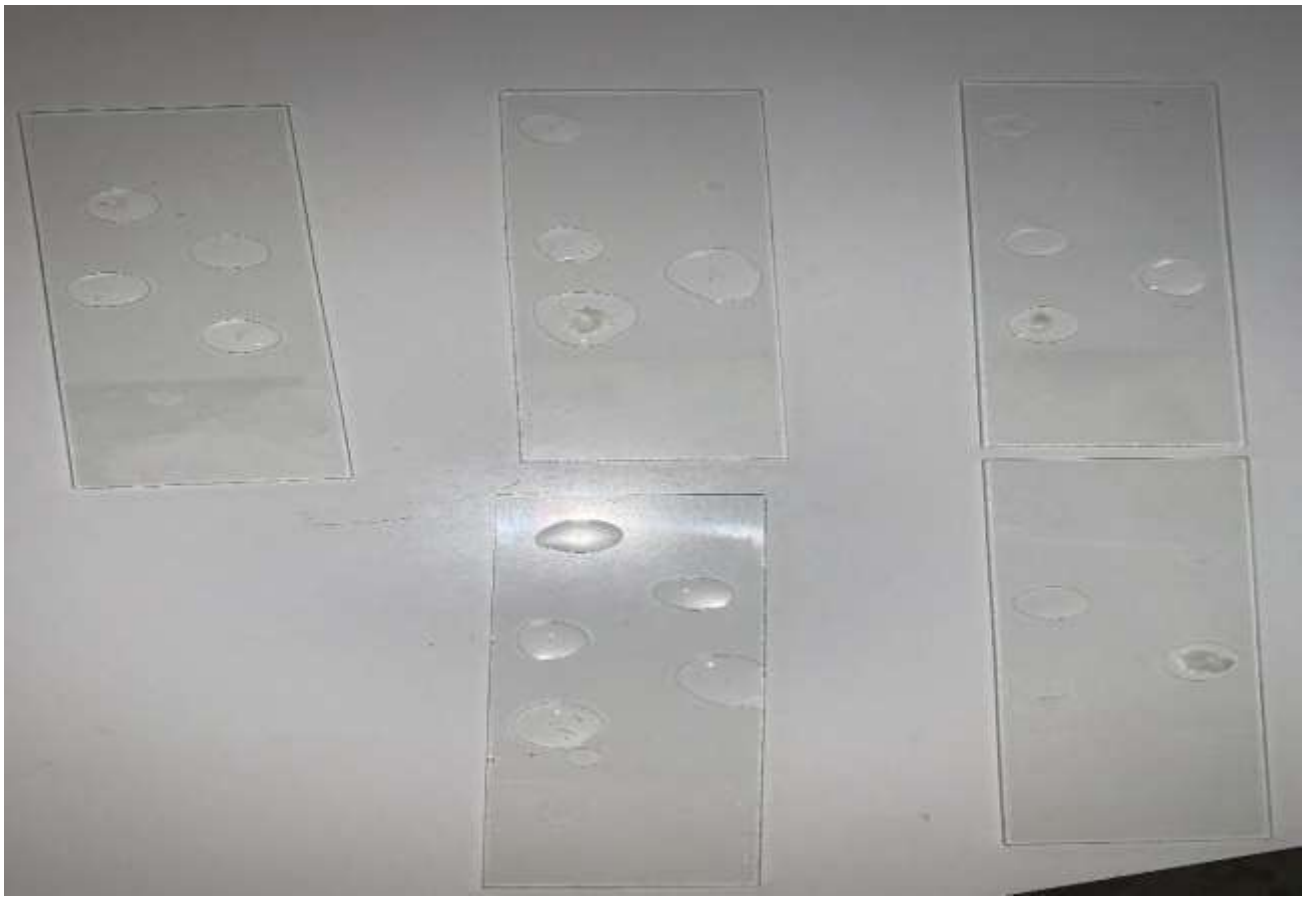
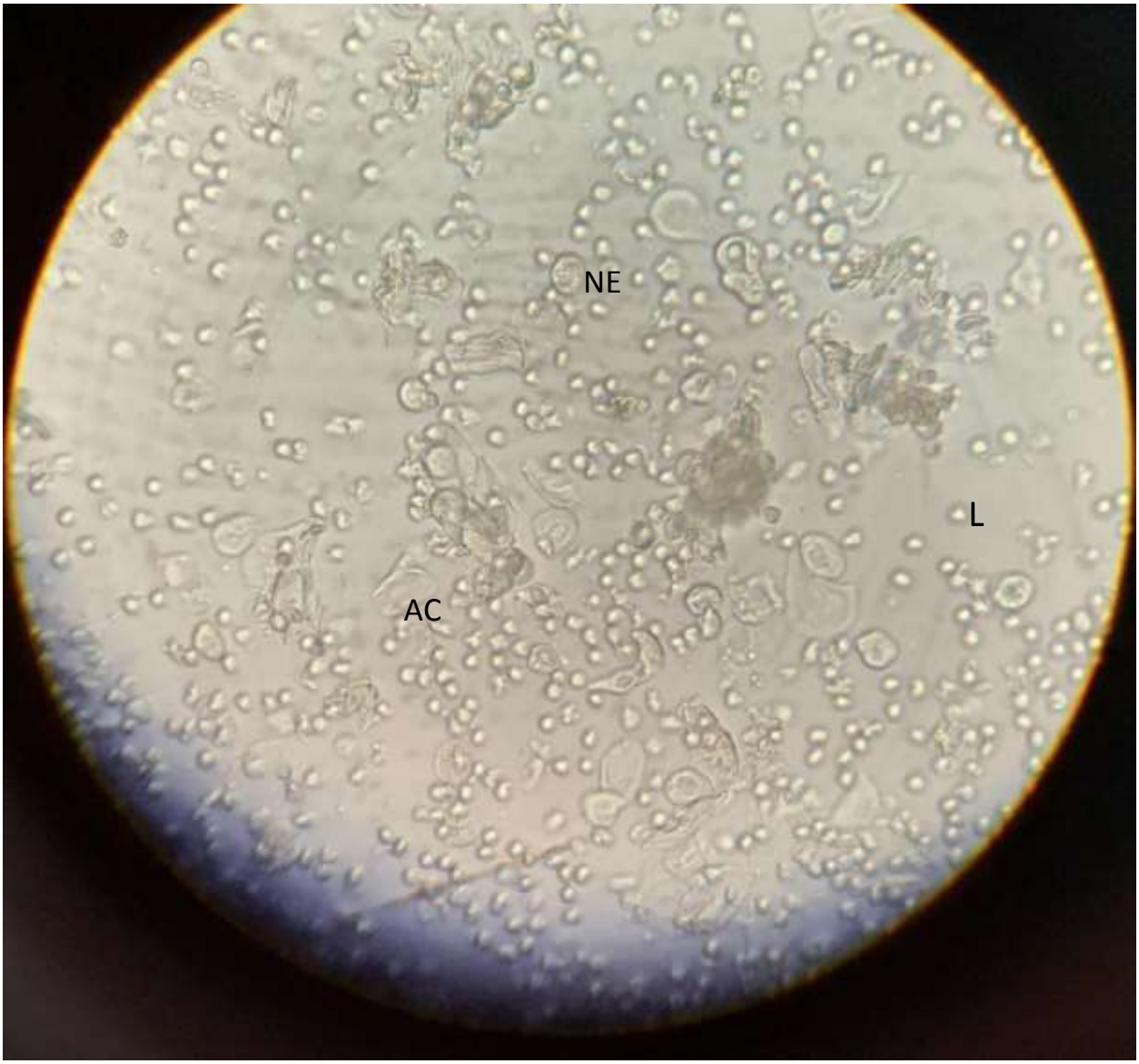


Image of vaginal secretions on microscopic glass slide.

APPENDIX II



Vaginal secretion viewed with x40 magnification showing all cell types indicating Metestrus.

Keys: NE= nucleated epithelial cells AC= anucleated cornified cell L= leucocytes.

APPENDIX III



Copulatory unilevel pacing chamber.

APPENDIX IV



Candidate placing the experimental female Wistar rat in the unilevel pacing chamber for the behavioural study

APPENDIX V

Effect of hydromethanolic fruit pulp extract of *A. garckeana* on sexual behavior in `female Wistar rats.

Groups	Proceptivity	Receptivity
Negative control	0.64 ± 0.02	0.00±0.00
Positive control	2.20 ± 0.06[a]	14.80 ± 0.96[a]
250 mg/kg Extract	2.34 ± 0.07[a]	31.20 ± 1.93[a,c]
500 mg/kg Extract	2.22 ± 0.12[a]	36.20 ± 2.41[a,c]
1000 mg/kg Extract	2.02 ± 0.11[a]	41.80 ± 2.95[e]

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APPENDIX VI

Effect of hydromethanolic fruit pulp extract of *Azanza garckeana* on sex hormone levels in female Wistar rats

Groups	Estrogen level	Progesterone level	Testosterone level
Negative control Positive	41.80 ± 2.95	8.92 ± 0.30	2.18 ± 0.09
control	108.40 ± 1.74^{ab}	7.86 ± 0.41 ^{a}	1.42 ± 0.12 ^{a}
250mg/kg Extract	104.40 ±1.02^{ab}	7.54 ± 0.15^{a}	1.70 ± 0.11 ^{a}
500mg/kg Extract	112.40 ± 2.11^{a,b}	11.50 ± 0.17^{e}	0.92± 0.13^{e}
1000mg/kg Extract	70.00 ± 0.71^{a}	6.86 ± 0.16^{a}	1.40 ± 0.16^{a}

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APPENDIX VII: Ethical Approval



Committee On Animal Use And Care
Directorate of Academic Planning & Monitoring
Ahmadu Bello University, Zaria

Appl No: ABUCAUC/2021/Human Physiology/121
Approval No: ABUCAUC/2021/121

23rd September, 2021

Dr.Yusuf Tanko,
Department of Human Physiology,
Faculty of Basic Medical Sciences,
Ahmadu Bello University,
Zaria.

Dear Sir,

APPROVAL OF RESEARCH STUDY'APHRODISIAC AND SOME REPRODUCTIVE EFFECTS OF METHANOLIC FRUIT PULP EXTRACT OF AZANZA GARCKEANA (GORON TULA) IN FEMALE WISTAR RAT'

This is to convey the approval of the ABUCAUC to you for the aforestated study domiciled in the Department of Human Physiology. The approval is predicated on the assumption that you shall maintain and care for the Experimental Animals as approved after the visitation of the Committee.

Monitoring of the Research by spot checks, invitations or any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCAUC Page on the University's website.
Note upon completion of the research, ethical clearance certificate will be issued.

A.B. Abdulkadir
For: Chairman, ABUCAUC.

Cc. Director, IAIICT
Dean, Faculty of Basic Medical Sciences
HOD, Human Phy ology
Prof. C. A. Kudi, Chairman, ABUCAUC

Chairman: **Prof C. A. Kudi** *DVM, M.Sc, PhD (ABU), PhD (Lel.) PGDE (PLY)* 08065978003 | S [redacted]