

**PREGNANCY OUTCOME STUDY OF JUICE FORMULATED FROM**  
*Cyperus esculentus* (TIGER NUT) ON PREGNANT ALBINO RATS



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**BENIN CITY**

**NOVEMBER, 2025**

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOMEDICAL  
SCIENCES, FACULTY OF SCIENCE LABORATORY TECHNOLOGY,  
UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT THE  
REQUIREMENT FOR THE AWARD OF THE DEGREE OF A  
BACHELOR OF SCIENCE (B.S.c HONOURS) BIOMEDICAL SCIENCES**

**NOVEMBER, 2025**

## CERTIFICATION

This is to certify that this research work was carried out by **Osawoghomwen Sarah OGEDEGBE (Miss)** with matriculation number **LSC2007324** of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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**DEDICATION**

This work is dedicated to God Almighty, whose grace, wisdom, and strength sustained me throughout the course of this research.



## ACKNOWLEDGMENTS

I am deeply grateful to Almighty God for His endless grace, wisdom, and strength that have sustained me throughout the course of this research and my academic journey. My sincere appreciation goes to my project supervisor, Dr. (Mrs.) O.E. Obaro-Onezeyi and beloved husband Dr. P.O. Obaro for their patience, encouragement, and invaluable guidance during the course of this research work. Your constructive criticisms, insightful suggestions, and unwavering support contributed immensely to the success of this study. I would also like to extend my profound gratitude to all the lecturers and laboratory technologists in the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, for their tireless efforts, mentorship, and dedication to ensuring academic excellence. Your knowledge and guidance have helped shape my understanding and practical skills in the field of science. My heartfelt thanks go to my mom, Mrs. Lisa for her moral, spiritual, and financial support. My brother Joshua Ogedegbe the absolute love of my life and my uncles for their financial support. Your prayers, love, and encouragement gave me the strength to keep going even in difficult times.

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#### **ABSTRACT**

Tiger nut (*Cyperus esculentus*) contains bioactive compounds such as polyphenols, resveratrol, flavonoids, and phytoestrogens with antioxidant, anti-inflammatory, and hormone-modulating potentials. Considering the rising prevalence of female infertility and the limitations of conventional therapies, this study explored Tiger nut as a possible natural alternative to enhance reproductive health. This investigation was aimed at evaluating the pregnancy outcome study of juice formulated from *Cyperus esculentus* (tiger nut) on pregnant albino rats. Freshly prepared Tiger nut juice was administered orally to gravid albino Wistar rats at doses of 2 ml/kg and 5 ml/kg across the three trimesters of pregnancy. Key parameters assessed included maternal weight gain, serum progesterone levels, implantation sites, uterine horn development, pregnancy outcomes, fetal biometric indices, and blood glucose levels. The results showed significant improvements in maternal weight, progesterone concentration, implantation success, litter size, and fetal development in the grape-treated groups compared to controls. Additionally, low and higher doses of the Tiger nut juice maintained normoglycemia, within normal ranges. These findings demonstrate that Tiger nut juice positively influences maternal physiology, enhances uterine receptivity, and improves pregnancy outcomes, highlighting its potential role as a nutraceutical for supporting female fertility.





## **CHAPTER ONE**

### **1.0 INTRODUCTION**

#### **1.1 BACKGROUND OF STUDY**

Plant-derived bioactive compounds, present in various parts of plants such as fruits, seeds, bark, peels, and leaves, have long been valued for their therapeutic properties in managing a range of health conditions including diabetes, hypertension, and reproductive disorders. These phytochemicals including carotenoids, polyphenols, flavonoids, and saponins are abundant in fruits, which also provide essential nutrients like fiber, vitamins, and minerals. Their benefits extend beyond nutrition, as they help regulate hormonal activity, improve the quality of gametes, and reduce oxidative stress, all of which are crucial for maintaining reproductive health and pregnancy. Regular consumption of fruits has been linked to better menstrual cycle regulation, enhanced ovulatory function, and lower risks of reproductive pathologies such as polycystic ovary syndrome (PCOS). Although conventional pharmacological treatments for reproductive health issues can be effective, they often carry risks of side effects such as menstrual irregularities, metabolic complications, or liver dysfunction. Given these limitations, there is increasing interest in plant-based bioactive compounds as safer, more accessible, and cost-effective alternatives or adjunct therapies.

These natural products support hormonal balance, improve ovarian and uterine function, enhance libido, and protect reproductive tissues from oxidative damage. As a result, they hold great promise for integrative approaches to reproductive care, particularly in women of reproductive age seeking holistic and well-tolerated options to improve fertility and reproductive outcomes.

## 1.2 TIGERNUT

Tiger nuts, commonly known as "subterranean walnuts," due to their great output and several potential applications are today extensively cultivated all over the world although they were formerly only found in the Mediterranean area (Badejo *et al.*, 2014). The hardy perennial *Cyperus esculentus* plant which has upright, fibrous roots grows 1 to 3 feet tall and reproduces by generating numerous deep and slender rhizomes that produce little tubers at the top of the subterranean stems (Chander, 2018). Tiger nuts are tiny, approximately the size of a peanut (Adenowo and Kazeem, 2020), and are either eaten raw or after soaking for a few hours in water. The tiger nut plant tolerates high soil moisture levels though it is shade-intolerant, it grows best in moist sandy-loam soils but may also thrive even in the hardest clay (Bamishaiye and Bamishaiye, 2011). The typical planting and harvesting dates for *C. esculentus* are between April and November (Das *et al.*, 2014). Tiger nut varieties soil/environmental conditions, cultivation methods and storage conditions all tend to affect the nutritional content of tiger nuts (Dey *et al.*, 2020). For example, while an ambient or elevated storage of tiger nuts showed greater sugar content, their refrigeration rather revealed enhanced activities of  $\alpha$ -amylase and lipase enzymes (Armeli *et al.*, 2021).

Tiger nuts have a rich phytochemical profile that is composed of flavonoids, organic acids, alkaloids, glycosides, (Metsämuuronen and Sirén, 2019), monounsaturated fatty acids, tannins, phytates, and oils. With tiger nut oil having a comparable nutritional profile to olive oil (Codina-Torrella *et al.*, 2015). The nuts also have a significant amount of starch which is an inexpensive and renewable dietary element (Yang *et al.*, 2022). Despite the relatively low protein content, tiger nuts have yet been demonstrated to be effective against diabetes and colon cancer. The fiber content of the nuts also helps alleviate digestive problems and obesity (Ihedioha *et al.*,

2019). Excellent antioxidant qualities of the nuts due to their flavonoid content may be utilized as natural antioxidants against free radicals (Samuel *et al.*,2023). Tiger nuts go by other names as well some of which are; rush nut,earth almonds,chufa, edible rush, "ofio," yellow nutgrass, Zulu nut,crushed almond, "Aya," rush and "imumu," (Djomdi *et al.*, 2020). Many populations in Spain and North Africa include tiger nut in their regular diets (Hassan *et al.*, 2021). In Spain for instance, the nuts are mostly used to make a native beverage called "horchata de chufa" (chufa milk) and in Egypt, they are usually roasted and served as a sweetmeat (Yang *et al.*,2022).C. esculentus been an old crop, has had some advancement made in the analysis of its components, physicochemical qualities and product development however, the breadth and depth of the study is far less compared to that of soybean, peanut and other nut oil crops. This review intends therefore, to add to the scope of available information regarding the nuts with specificity to its bioactive compounds, biological activities,nutritional and health benefits.

**Table 1.1** Scientific Classification Of *Cyperus esculentus*

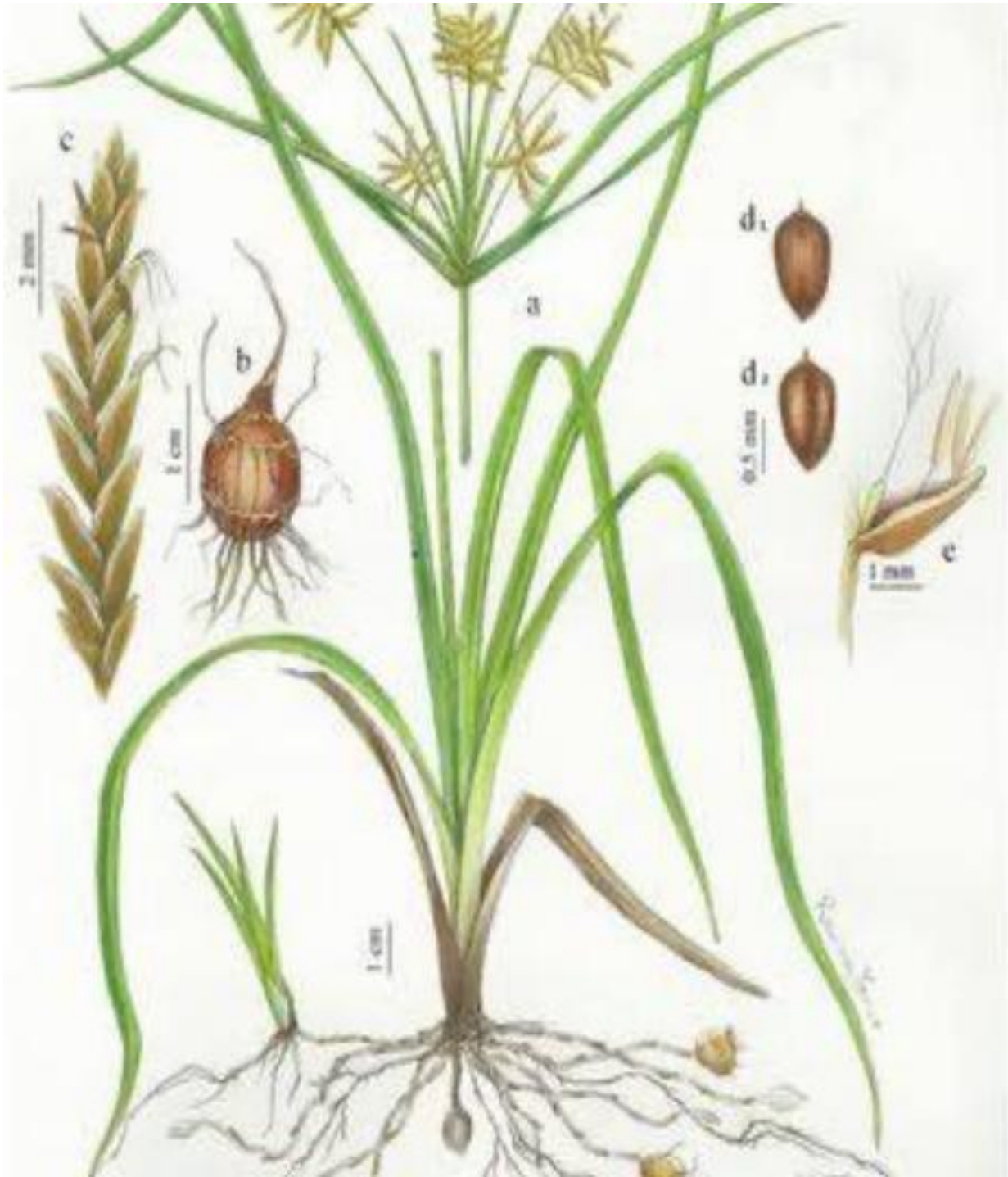
|          |                           |
|----------|---------------------------|
| Kingdom  | Plantae                   |
| Division | Magnoliophyta             |
| Class    | Liliopsida                |
| Order    | Poales                    |
| Family   | Cyperaceae                |
| Genus    | Cyperus                   |
| Species  | <i>Cyperus esculentus</i> |

(Araodion *et al.*, 2020)

### 1.2.1 MORPHOLOGY OF TIGER NUT

*Cyperus esculentus*, commonly called tiger nut or chufa, belongs to the order Graminales, class Angiospermae, subclass Monocotyledonae, and family Cyperaceae, which includes about 75 genera and over 4,000 species. This plant reproduces annually by seeds and perennially by underground corm-like bulbs at the base of leaf clusters, as well as through rhizomes. It is a perennial, grass-like sedge, erect, robust, and smooth in texture (Gene, 2019). The flowering stalk bears a rachis 2–9 cm long, ending in an umbel—a type of inflorescence with golden-brown, flower-bearing rays emerging from the same point. The umbel typically has several short, upright rays and 2–9 longer, ascending rays. Around its base are 3–9 bracts (leaf-like structures) at the same level (Gene, 2023). Each terminal spike contains pinnate spikelets forming golden-brown flower heads. The fruits are small, yellowish, three-sided achenes, many of which are not viable (Wilma and Chester, 2024). The flowers are bisexual, arranged in spikelets along a persistent rachilla with thin, transparent wings 0.3–0.5 mm wide (Follak *et al.*, 2016). Leaves are bright green, with a distinct mid-vein, a waxy surface, and sharply pointed tips.

They are arranged in groups of three, sheathing the stem, and measure 4–9 mm wide by 20–90 cm long. Tiger nut is predominantly wind-pollinated. Mature achenes are yellowish-brown, three-angled, and 1.2–1.5 mm long, enclosed in a thin outer coat. The plant develops white, segmented rhizomes attached to small tubers. As maturity approaches, the tubers turn from white to yellow and increase in size. They are located at the ends of slender rhizomes that become wiry and tough with age. Tubers range from 6–10 mm in length, and a single plant can produce hundreds (Bamishaiye and Bamishaiye, 2011).



**PLATE 1.1:** *Cyperus esculentus* (original drawing by Rosaria Manco): (a) habit of the flowering plant; (b) mature tuber; (c) spikelet; (d1) achene;dorsal view;(d2) achene:ventral view;(e) details of flower and rachilla (Follak *et al.*,2016)



**PLATE 1.2:** Tiger nut in the flowering period (Follak et al.,2016)



**PLATE 1.3:** Tiger nut leaves.

### **1.2.2 ORIGIN AND GEOGRAPHICAL DISTRIBUTION OF TIGER NUT**

*Cyperus esculentus*, commonly known as tiger nut or Chufa, is a plant that thrives as a weed in various temperate, tropical, and subtropical areas across the globe. It can be found in regions such as the Mediterranean, Africa, India, North America, Mexico, Peru, and others (Rebezov *et al.*, 2020). The primary cultivation of tiger nuts is carried out in West African countries like Nigeria, Senegal, and Ghana. Additionally, Spain is recognized as a significant producer and exporter of Chufa, which enjoys popularity within the country (Ezeh *et al.*, 2016). Tiger nut, a crop that was domesticated early on, has been discovered in dry tubers within tombs dating back to predynastic times, approximately 6000 years ago (Kizzie-Hayford *et al.*, 2021).

### **1.2.3 CULTIVATION OF TIGER NUT**

Tiger nut cultivation necessitates a mild climate and sandy soil. Tiger nuts reproduce through seeds, and wind is responsible for pollination. (Bamishaiye *et al.*, 2011). Tiger nuts are commonly found growing as a grass in moist areas. The planting of tiger nuts takes place in April, and the harvest occurs in November. The planting period is divided into two categories: the main period (April-July) and the secondary period (September-November). During the process of harvesting tiger nut tubers from the field, the tubers get mixed with foreign substances such as stones, animal waste, and other unrelated materials. (Ayeh - Kumi *et al.*, 2014).

By literal definition, *Cyperus esculentus* is a tuber rather than a nut, as suggested by the name "tiger nut." The early stages of tuber formation are whitish, and as they mature, a yellow outer membrane envelops them (Bamishaiye *et al.*, 2011). Tiger nuts have been cultivated and used for many millennia in ancient Egypt, but human activity has also effectively transported the plant to other regions of the world for many centuries. Up until the tubers are ready for harvest, tiger nut plants need irrigation practically every week (Amoah *et al.*, 2011). Various studies have

determined that the location and planting period of tiger nut trees impact the mineral content and acceptability of the milk beverage made from them. The impact of various locations and tiger nut varieties on the yield of milk and solids from tiger nut tubers was also examined in a related study by (Asante *et al.*, 2014).

#### **1.2.4 VARIETIES OF TIGER NUT**

There are three different types of tiger nut tubers that can be distinguished by their color: yellow, brown, red, and black. (Sidohounde *et al.*, 2018) However, only two of these varieties, yellow and brown, are commonly found in local markets in Nigeria. The yellow variety can be further classified into two subtypes: large yellow, and small yellow (Bamishaiye *et al.*, 2014).



**PLATE 1.4:** Tiger nut tuber (a) dried tiger nut

(Bamishaiye *et al.*, 2014)



**PLATE 1.5:** Tiger nut tuber (b) fresh tiger nut

(Bamishaiye *et al.*, 2014).

### **1.2.5 PHYTOCHEMICALS OF TIGER NUT**

The results of the phytochemical analysis revealed that tiger nut contains alkaloids, flavonoids, sterols, saponins, and tannins, phenol, phytosterol, glycoside. These compounds have been associated with various biochemical effects of the plant (Yu *et al.*, 2022; Edo *et al.*, 2023).

### **1.2.6 ETHNO-MEDICINAL USE**

Tiger nut tubers were soaked and consumed by the Chinese and Egyptians as a liver tonic. When eaten, they are believed to effectively heal oral, gum, and stomach ulcers. They also help nursing mothers produce more breast milk and encourage regular menstruation, providing additional reproductive health benefits. Among the Zulu, tiger nut tubers are recommended for young girls to initiate menstruation early in life. They are regarded as a good source of improved fertility and are thought to possess aphrodisiac properties (Nwobosi *et al.*, 2013). It has been suggested that eating tiger nut tubers could enhance the human reproductive system and support its maturity (Hassan *et al.*, 2013). In traditional medicine, tiger nut tubers are also utilized as a colon depressant (Wakil *et al.*, 2014) and for treating boils, colds, and poliomyelitis (Okorie *et al.*, 2014). Consumption of the tubers improves the digestive process and can help in the treatment of diarrhea and colitis, an inflammation of the intestines (Nwobosi *et al.*, 2013). Tiger nut extract is also known to stimulate blood circulation and significantly prevent heart disease and thrombosis. Furthermore, eating tiger nut tubers is considered highly beneficial in both preventing and treating bacterial urinary tract infections (Hassan *et al.*, 2013).

### **1.3 PHARMACOLOGICAL POTENTIALS OF TIGER NUT**

The therapeutic properties of tiger nut have been extensively investigated in various research studies, revealing several potential pharmacological benefits across diverse medical conditions.

#### **1.3.1 Anti-diabetic effect**

Tiger nuts (*Cyperus esculentus*) have shown significant potential in the dietary management of diabetes and are considered a beneficial food choice for individuals with elevated blood glucose levels. This benefit is largely attributed to their high content of insoluble dietary fiber, which promotes satiety and helps reduce the intake of starchy foods with a high glycemic index (Achoribo *et al.*, 2017). The fiber also slows gastric emptying and carbohydrate digestion, leading to a gradual release of glucose into the bloodstream. Furthermore, tiger nuts contain bioactive compounds capable of decreasing the absorption of glucose in the intestines following a carbohydrate-rich meal (Ani *et al.*, 2021). Due to their low glycemic index, the carbohydrates in tiger nuts exert minimal impact on postprandial blood sugar levels, thereby avoiding sharp spikes that are often associated with refined carbohydrates (Chukwuma *et al.*, 2010).

In addition to fiber-related effects, tiger nuts are rich in arginine, an amino acid that plays a key role in enhancing insulin sensitivity and improving glucose uptake by body tissues (Owusu *et al.*, 2016). This mechanism supports better blood sugar regulation and may help in preventing long-term diabetic complications.

### **1.3.2 Aphrodisiac effect**

Tiger nut (*Cyperus esculentus*) has been traditionally regarded as a natural aphrodisiac, and scientific investigations have provided evidence supporting its role in enhancing reproductive health. A study by (Nwanegio *et al.*, 2013) evaluated the impact of methanol extracts of tiger nut on reproductive hormones and androgenic factors in adult male Wistar rats. The results revealed a significant, dose-dependent increase in the levels of both male and female reproductive hormones, including testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). In addition, improvements were observed in androgenic parameters such as sperm count, motility, and viability. The aphrodisiac potential of tiger nut is believed to be linked to its rich content of vitamin E, zinc, arginine, and essential fatty acids, which are known to support hormone synthesis, improve blood circulation to reproductive organs, and enhance overall sexual performance. These bioactive nutrients may also contribute to balancing reproductive hormones in females, thus potentially improving libido and fertility. This evidence aligns with ethnomedicinal claims from various cultures, particularly in Africa and the Mediterranean, where tiger nut is consumed regularly to boost sexual drive, improve reproductive function, and address certain fertility-related issues.

### **1.3.3 Hepatic and renal protective effect**

The liver and kidneys are vital organs responsible for detoxification, metabolism, and maintenance of internal homeostasis. Medicinal plants have long been recognized as a crucial source of hepatoprotective and nephroprotective agents, with over 160 phytochemical elements from 101 plant species documented for such purposes. Tiger nut (*Cyperus esculentus*) has shown promising protective effects on these organs.

Research by (Araodion *et al.*,2020) reported that regular consumption of tiger nut tubers has a positive impact on hepatic and renal indices, suggesting their role in maintaining healthy liver and kidney function. These effects are thought to be due to the plant's abundance of antioxidants, essential fatty acids, amino acids (especially arginine), and phytosterols, which help reduce oxidative stress, improve lipid metabolism, and prevent inflammation in liver and kidney tissues. Consequently, dietary inclusion of tiger nut is recommended as part of preventive and supportive strategies for individuals at risk of liver or kidney dysfunction.

#### **1.3.4 Antioxidant effect**

Oxidative stress is a major factor in the pathogenesis of various chronic diseases, including cardiovascular disorders, neurodegenerative conditions, and cancers.

Tiger nut is a rich source of vitamin C, a potent water-soluble antioxidant that not only scavenges harmful free radicals but also enhances iron absorption and sustains optimal vitamin E levels, which are critical for immune function and tissue repair (Roselló-Soto *et al.*, 2019).Furthermore, tiger nut contains high levels of vitamin B1 (thiamine), which supports the proper functioning of the central nervous system and helps the body adapt to physical and mental stress. Its phytochemical profile, including polyphenols, flavonoids, and phenolic acids, contributes to its overall antioxidant capacity. This combined activity helps in cellular protection, slowing down aging processes, and reducing the risk of oxidative damage-related diseases.

#### **1.3.5 HEALTHY SUBSTITUTE FOR LACTOSE INTOLERANCE**

Tiger nut milk, a plant-based beverage extracted from *Cyperus esculentus* tubers, is naturally free from lactose, casein, cholesterol, gluten, added salt, and animal-derived proteins, making it an excellent alternative for individuals with lactose intolerance or dairy allergies. For people who cannot tolerate lactose or gluten present in cow's milk, tiger nut milk provides a safe and

nutritious option .According to (Gambo *et al.*,2014) and (Udezor *et al.*,2012) tiger nut milk is also suitable for diabetics when consumed without added refined sugar. Its high arginine content supports insulin production by stimulating the release of hormones involved in glucose regulation. Additionally, this milk is a preferred choice for athletes, as it provides easily digestible carbohydrates, essential fatty acids, and vitamins that help with energy recovery and muscle repair.

### **1.3.6 TREATMENT OF DIGESTIVE DISORDER**

Tiger nut (*Cyperus esculentus*) has long been utilized in traditional medicine for the management of various gastrointestinal ailments, including indigestion, flatulence, diarrhea, and constipation. Its therapeutic value in digestive health is attributed to the presence of naturally occurring digestive enzymes such as catalase, lipase, and amylase, which aid in the breakdown of carbohydrates, fats, and other food components, thereby enhancing the digestive process (Adejuyitan, 2011). These enzymes promote efficient nutrient assimilation and help regulate bowel function, ensuring the smooth transit of food through the gastrointestinal tract. Furthermore, tiger nut is rich in dietary fiber, which adds bulk to stool, stimulates peristaltic movement, and supports regular bowel movements. This fiber also facilitates improved nutrient absorption from digested food, thereby enhancing overall nutrient utilization and reducing the risk of malnutrition-related complications.

#### **1.4 AIM OF STUDY**

This research work was aimed at studying the pregnancy outcome of juice formulated from *Cyperus esculentus* (tiger nut) on pregnant albino rats

#### **1.5 OBJECTIVE OF THE STUDY**

The objective of the study is to:

1. Review the existing literature, botanical description, and use of *Cyperus esculentus* .
2. Investigate the pregnancy of tiger nut juice on female albino rats.
3. Investigate the properties of juice formulation of tiger nut on albino rats.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 PREGNANCY

Pregnancy in mammals is a highly coordinated and dynamic physiological state in which a fertilized ovum, or zygote, develops within the female reproductive tract until the offspring is born. The process begins with fertilization, where a spermatozoon successfully penetrates the zona pellucida of a mature ovum in the ampulla of the fallopian tube (in most species). This fusion of gametes restores the diploid chromosome number and results in the formation of a zygote—the first cell of a new organism (Sadler, 2020). Following fertilization, the zygote undergoes a series of mitotic divisions known as cleavage, producing smaller cells called blastomeres. As these divisions continue, the developing conceptus transforms into a morula and later a blastocyst. The blastocyst stage is crucial because it possesses two distinct cell populations: the inner cell mass (which forms the embryo proper) and the trophoblast (which will develop into the placenta and supporting membranes) (Gilbert, 2014). Implantation occurs when the blastocyst attaches to and invades the endometrial lining of the uterus. In humans, this typically takes place 6–7 days after fertilization; in mice, implantation occurs around day 4. The trophoblast differentiates into cytotrophoblast and syncytiotrophoblast layers, which facilitate invasion into maternal tissue and establish the early maternal–fetal interface (Norwitz *et al.*, 2015; Suckow *et al.*, 2012).

This implantation event triggers profound maternal physiological adaptations. Endocrine changes—driven by hormones such as progesterone, estrogen, human chorionic gonadotropin (hCG) in humans, and placental lactogens—promote uterine quiescence, immune tolerance to the semi-allogenic fetus, and vascular remodeling to ensure adequate nutrient and oxygen delivery (Aluvihare *et al.*, 2004). From this point onward, pregnancy is maintained by a complex interplay of maternal and fetal signals, involving the endocrine, immune, and cardiovascular systems, until parturition. These changes collectively create a protected and nutritionally rich intrauterine environment, allowing the embryo to develop into a viable fetus and, ultimately, a newborn (Cunningham *et al.*, 2018).

## **2.2 REPRODUCTIVE PHYSIOLOGY IN FEMALES**

The reproductive physiology of females is a complex, tightly regulated system designed to ensure the possibility of fertilization, implantation, and pregnancy. Its central control mechanism is the hypothalamic–pituitary–gonadal (HPG) axis, which coordinates the interaction between the brain and reproductive organs through precisely timed hormonal signals (Plant and Zeleznik, 2015). In this axis, the hypothalamus secretes gonadotropin-releasing hormone (GnRH) in a pulsatile manner, stimulating the anterior pituitary gland to release two key gonadotropins: follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH promotes follicular recruitment and growth in the ovary, leading to the selection of a dominant follicle, while LH supports the maturation of the ovarian follicle and triggers ovulation (Knobil and Neill, 2014).

In humans, the menstrual cycle averages 28 days and consists of three main phases: Follicular Phase – begins on the first day of menstruation and ends with ovulation, during which rising estrogen levels promote proliferation of the endometrial lining, preparing it for possible implantation; Ovulation – a mid-cycle LH surge causes the dominant follicle to rupture, releasing a mature oocyte into the fallopian tube where fertilization can occur; Luteal Phase – the ruptured follicle transforms into the corpus luteum, which secretes progesterone and estrogen to maintain the endometrium, and if fertilization does not occur, the corpus luteum regresses, leading to menstruation (Guyton and Hall, 2021).

### **2.2.1 STAGES IN PREGNANCY**

#### **First Trimester (Weeks 1–12)**

This stage begins at conception, when the sperm fertilizes the ovum to form a zygote, which then undergoes cleavage and develops into a blastocyst. The blastocyst implants into the uterine wall, and the placenta begins to form to support nutrient, waste, and gas exchange between mother and fetus. During this period, organogenesis occurs—major organs such as the heart, brain, and spinal cord begin to develop. The embryo is most vulnerable to teratogens and chromosomal abnormalities at this stage (Norwitz *et al.*, 2001; Moore *et al.*, 2019).

#### **Second Trimester (Weeks 13–26)**

This phase is characterized by rapid fetal growth and structural refinement of organ systems. Functional abilities emerge, such as fetal movement (quickening) felt by the mother, development of hearing, and facial feature formation.

The skeleton begins ossification, and subcutaneous fat starts accumulating. The risk of miscarriage is markedly reduced, and the mother often experiences increased energy (Sadler, 2020; Cunningham *et al.*, 2018).

### **Third Trimester (Weeks 27–40)**

In this final stage, organ systems—especially the lungs and central nervous system—undergo full maturation to prepare the fetus for life outside the womb. The fetus gains significant weight, typically assuming a head-down position in preparation for delivery. Hormonal changes in the mother lead to cervical ripening and increased uterine contractility, initiating labor. The placenta reaches full maturity to sustain the fetus until birth (Guyton and Hall, 2021; Moore *et al.*, 2019).

## **2.2.2 OVULATION AND FERTILIZATION PROCESS**

Ovulation occurs when a surge in luteinizing hormone (LH) causes the dominant follicle in the ovary to rupture, releasing a mature oocyte into the fallopian tube. This process is preceded by follicular development under the influence of follicle-stimulating hormone (FSH) and estrogen. Fertilization takes place in the ampulla of the fallopian tube when a sperm cell successfully penetrates the zona pellucida of the oocyte, resulting in the formation of a diploid zygote (Guyton and Hall, 2021; Knobil and Neill, 2014).

## **2.2.3 ZYGOTE FORMATION AND EARLY EMBRYONIC DEVELOPMENT**

The zygote undergoes multiple mitotic divisions (cleavage) as it travels toward the uterus, forming a morula by day 3–4 post-fertilization. Fluid accumulation within the morula creates a blastocyst by day 5–6. The blastocyst consists of an inner cell mass (which will become the embryo) and a trophoblast layer (which will form part of the placenta) (Moore *et al.*, 2019; Sadler, 2020).

## **2.2.4 IMPLANTATION OF THE BLASTOCYST**

Implantation typically occurs 6–7 days after fertilization. The trophoblast differentiates into the cytotrophoblast and syncytiotrophoblast. The latter invades the endometrial lining, establishing the first maternal–fetal connections. Implantation is facilitated by enzymatic degradation of the endometrium and immune modulation to prevent rejection of the semi-allogenic embryo (Norwitz *et al.*, 2001; Cha *et al.*, 2012).

## **2.3 HORMONAL REGULATION DURING PREGNANCY**

### **2.3.1 Progesterone**

Progesterone is secreted predominantly by the corpus luteum in early pregnancy, under the stimulation of human chorionic gonadotropin (hCG), and later by the placenta after the luteo-placental shift at approximately 8–10 weeks of gestation. It plays a central role in maintaining uterine quiescence by reducing myometrial contractility, maintaining endometrial receptivity, and modulating maternal immune tolerance to prevent fetal rejection (Gellersen and Brosens, 2014).

### **2.3.2 Estrogen**

During pregnancy, estrogen levels rise steadily, primarily as estriol produced by the placenta from fetal adrenal precursors. Estrogen promotes uterine enlargement to accommodate the growing fetus, enhances uteroplacental blood flow, stimulates breast ductal development, and upregulates oxytocin receptors in preparation for labor (Albrecht and Pepe, 1990).

### **2.3.3 Human Chorionic Gonadotropin (hCG)**

hCG is secreted by the syncytiotrophoblast cells of the developing blastocyst soon after implantation. Its primary role is to maintain corpus luteum function during early pregnancy, ensuring continued progesterone and estrogen secretion until the placenta takes over hormone

production. Clinically, hCG serves as a biomarker for pregnancy detection and is also essential for supporting fetal–maternal communication (Cole, 2010).

#### **2.3.4 Prolactin**

Secreted by the maternal anterior pituitary gland, prolactin concentrations increase progressively throughout pregnancy. Prolactin stimulates mammary gland development and lactogenesis, while also influencing maternal metabolism to ensure adequate nutrient availability for the fetus and preparing the breasts for postpartum milk production (Ben-Jonathan *et al.*, 2008).

### **2.4 ANATOMICAL, METABOLIC, AND IMMUNOLOGICAL ADAPTATIONS IN PREGNANCY**

Anatomical – Uterine enlargement, displacement of abdominal organs, and increased breast size due to glandular proliferation.

Metabolic – Increased basal metabolic rate (BMR), enhanced fat storage in early pregnancy, and increased glucose availability for fetal use via insulin resistance in late pregnancy.

Immunological – Modulation of maternal immune responses to tolerate the fetus while maintaining defense against pathogens. This involves a shift toward anti-inflammatory (Th2) cytokine dominance (Mor and Cardenas, 2010; Roberts *et al.*, 2013).

### **2.5 CONCEPTION AND IMPLANTATION**

#### **2.5.1 Requirements for Successful Conception**

Successful conception requires the presence of a healthy, motile sperm and a viable oocyte, along with proper timing within the female reproductive cycle. Fertilization typically occurs within 12–24 hours after ovulation, when the oocyte is still viable. Sperm must undergo capacitation, a biochemical process in the female reproductive tract that enhances their motility and ability to penetrate the oocyte’s zona pellucida (Yanagimachi, 2011). A conducive hormonal

environment, adequate cervical mucus for sperm transport, and unobstructed fallopian tubes are essential (Macklon *et al.*, 2006).

### **2.5.2 Role of Endometrial Receptivity**

The endometrium must be in a receptive state for implantation to occur. This period, often referred to as the “window of implantation”, occurs around days 20–24 of a standard 28-day menstrual cycle. During this time, progesterone and estrogen induce structural and biochemical changes in the endometrium, including increased vascular permeability, stromal edema, and expression of adhesion molecules (such as integrins and selectins) that facilitate embryo attachment (Aplin and Ruane, 2017).

### **2.5.3 Synchronization Between Embryo Development and Uterine Environment**

A precise synchronization is required between the stage of embryo development and endometrial receptivity. By the time the blastocyst reaches the uterus (usually 5–6 days post-fertilization), the endometrium should be fully prepared for implantation. Any delay or mismatch between blastocyst arrival and uterine readiness can result in implantation failure or early pregnancy loss (Wilcox *et al.*, 2020; Achache and Revel, 2006).

## **2.7 FACTORS AFFECTING CONCEPTION SUCCESS**

### **2.7.1 Maternal Age**

Female fertility gradually declines with advancing age, particularly after the mid-30s, due to a progressive reduction in both the quantity and quality of oocytes. Advanced maternal age is also associated with higher rates of chromosomal abnormalities, reduced implantation potential, and increased miscarriage risk (Broekmans *et al.*, 2009).

### **2.7.2 Hormonal Imbalances**

Adequate levels and proper timing of reproductive hormones—such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone, and estrogen—are essential for normal follicular development, ovulation, and endometrial preparation. Disorders such as polycystic ovary syndrome (PCOS), luteal phase defects, and thyroid dysfunction can disrupt hormonal regulation and hinder conception (Azziz *et al.*, 2016).

### **2.7.3 Anatomical Issues**

Structural abnormalities within the female reproductive tract can interfere with gamete transport, fertilization, or implantation. These include blocked fallopian tubes (often due to pelvic inflammatory disease), intrauterine adhesions, uterine fibroids, and congenital malformations such as a septate uterus (Taylor *et al.*, 2010).

### **2.7.4 Lifestyle Factors**

Certain lifestyle behaviors negatively impact fertility. Smoking accelerates ovarian follicle loss and increases oxidative stress, while excessive alcohol intake can impair ovulation. Obesity is linked to hormonal disturbances, anovulation, and poorer pregnancy outcomes, whereas being

underweight can also disrupt menstrual cycles. Chronic stress may alter the hypothalamic–pituitary–gonadal axis, reducing fertility potential (Rich-Edwards *et al.*, 2002).

### **2.7.5 Environmental and Genetic Factors**

Exposure to environmental toxins, endocrine-disrupting chemicals, radiation, or certain infections can damage gamete quality and impair reproductive function. Additionally, inherited genetic abnormalities can affect embryo development, leading to implantation failure or early pregnancy loss (Siristatidis *et al.*, 2016).

## **2.8 PREGNANCY OUTCOMES**

### **Full-Term Delivery**

A pregnancy that reaches between 37 and 42 completed weeks of gestation without significant complications is considered full-term. Full-term delivery is associated with optimal neonatal health, reduced risk of respiratory complications, and improved maternal recovery (Spong, 2013).

### **Healthy Fetal Growth and Development**

When the fetus grows in accordance with gestational age milestones, it reflects adequate placental function, proper nutrient supply, and absence of growth-restricting conditions. This ensures the newborn’s birth weight is within the normal range and supports postnatal adaptation (Lee *et al.*, 2013).

### **Normal Maternal Recovery Postpartum**

Postpartum recovery involves the uterus returning to its pre-pregnancy size, hormonal balance restoration, and recovery from childbirth-related tissue changes. A normal recovery includes minimal complications such as infections or hemorrhage, enabling the mother to resume daily activities

## **Adverse Outcomes**

### **Miscarriage (Spontaneous Abortion)**

Defined as the loss of pregnancy before 20 weeks of gestation, miscarriage is often caused by chromosomal abnormalities, uterine anomalies, or hormonal imbalances. It affects approximately 10–20% of recognized pregnancies (American College of Obstetricians and Gynecologists, 2015).

### **Preterm Delivery**

Birth before 37 completed weeks of gestation is considered preterm. It is associated with increased risks of neonatal respiratory distress, infections, and long-term developmental delays. Causes include maternal infections, cervical incompetence, and multiple pregnancies (Goldenberg *et al.*, 2008).

### **Stillbirth**

The death of a fetus at or after 20 weeks of gestation in utero is termed stillbirth. Common causes include placental insufficiency, hypertensive disorders, and umbilical cord accidents (Lawn *et al.*, 2016).

### **Intrauterine Growth Restriction (IUGR)**

A condition in which the fetus fails to reach its genetically determined growth potential due to placental dysfunction, maternal malnutrition, or chronic diseases. IUGR increases the risk of perinatal morbidity and mortality (Resnik, 2002).

### **Congenital Malformations**

Structural or functional anomalies present at birth may result from genetic defects, environmental teratogens, or maternal infections during pregnancy. These can range from mild (e.g., cleft lip) to life-threatening (e.g., congenital heart defects) (Brent, 2004).

### **2.8.1 FACTORS INFLUENCING PREGNANCY OUTCOMES**

Factors influencing pregnancy outcomes include maternal, environmental, physiological, and nutritional components, each impacting fetal development and maternal health. Maternal factors such as age, health status, nutrition, and stress can either support or jeopardize pregnancy success. Environmental exposures to toxins, infections, and radiation pose risks for fetal growth and congenital anomalies. Physiological factors like hormonal imbalances and uterine abnormalities may impair implantation and fetal development.

Nutritional and phytochemical influences, including antioxidants, certain herbal extracts, and functional foods like tiger nuts, contribute to protecting placental function and supporting reproductive health (Jacobs son *et al.*, 2004; Black *et al.*, 2013; Perera *et al.*, 2019; Poston *et al.*, 2011; Adekanmi *et al.*, 2022).

### **2.8.2 FERTILITY**

Fertility refers to the natural capability of an individual or couple to conceive and produce offspring. It depends on the proper functioning of the reproductive system, including the production of healthy gametes (sperm and eggs), successful fertilization, implantation, and the ability to carry a pregnancy to term. Fertility is influenced by numerous factors such as age, hormonal balance, reproductive anatomy, lifestyle, and environmental exposures. Optimal fertility requires a complex interplay between the endocrine system, reproductive organs, and overall health status (Zegers-Hochschild *et al.*, 2017).

### **2.8.3 INFERTILITY**

Infertility is the inability of a couple to conceive after 12 months of regular unprotected intercourse, classified as primary or secondary, affecting 8–15% of couples globally. It results from female factors (ovulatory disorders, tubal damage, uterine abnormalities, endometriosis,

hormonal issues), male factors (low sperm quality, blockages, hormonal deficiencies), combined causes, or remains unexplained in 10–20% of cases. Risk factors include age, unhealthy lifestyle, stress, and environmental toxins, and management requires addressing both medical and psychosocial aspects ( Zegers-Hochschild *et al.*, 2017; Agarwal *et al.*, 2021; Boivin *et al.*, 2020).

## **2.9 FOLIC ACID**

Folic acid, the synthetic and stable form of vitamin B9, is vital for reproductive health and successful pregnancy outcomes. It is converted in the body to tetrahydrofolate (THF), which acts as a coenzyme in one-carbon metabolism essential for DNA synthesis, repair, methylation, and amino acid metabolism. These processes support rapid cell division, neural tube closure, and healthy fetal development while preventing complications like elevated homocysteine levels. Supplementation before and during early pregnancy significantly reduces the risk of neural tube defects. The mechanism of action involves folic acid's conversion to THF, facilitating nucleotide synthesis and methylation reactions critical for embryonic growth and placental function (Czeizel and Dudas, 2016).

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Collection of Plant sample and Preparation of Tiger Nut milk Juice (TGMJ)

The **Tiger Nut milk Juice (TGMJ)** was purchased in March 2025 from Oba Market in Oredo Local Government area, Benin City, Nigeria. **Tiger Nut** milk fruit (**TGMJ**) was washed and juiced with a food processor freshly prepared daily and kept in an airtight containers for subsequent usage.

#### 3.2 Preparation of stock solution

Daily administration of 2 and 5 mls/kg was calculated on the basis of the weights of the gravid dams and doses that were precisely calculated and measured to be administered to the animals during the experiment (Oshomoh and Obaro-Onezeyi, 2019).

#### 3.3 Chemicals

Chloroform (supplied by Pharmatrends Nigeria Ltd), Sodium Chloride all of analytical standards.

#### 3.4 Drugs

Folic acid, oxytocin, were of pure samples and pharmaceutical standards.

#### 3.5 Experimental Design

#### 3.6 Experimental animals

A total of 75 adult albino Wistar rats consisting of 25 males and 50 females with average weight of 200-280 grams were gotten through the animal house of the Phytomedicine unit in the Department of Plant Biology and Biotechnology, University of Benin. The animals were kept in wooden cages with ambient temperature and kept in typical laboratory circumstances, which include 12-hour cycles of light and darkness. The rats were provided standard pelletized layers mash and clean water for two weeks as acclimatization period prior to the experimental study.

### **3.7 Grouping and mating of animals**

The weights of the female Wistar rats were measured using an electronic weighing balance. The rats were grouped into five (5): I, II, III, IV and V of 5 rats each for the first, second and third trimesters of pregnancy. Male Wistar rats obtained were introduced into each group for mating in a ratio 1:1. Only female rats at pro-estrus after microscopic (UIS450, USA) examination were mated. The female rats were left overnight with their male counterparts.

### **3.8 Examination of vaginal smear**

The next morning after mating, vaginal smear was taken from each female rat in each group and observed by microscopic examination for the presence of sperm. Pipette containing little distilled water was inserted into the vagina of each rat and the vagina fluid was taken, smeared on the glass slides, covered with cover slips and viewed under the light microscope. The presence of sperm cells in the vaginal fluid confirmed a positive sperm test and also a successful mating and was thereby taken as day 0 of pregnancy according to Obaro and Oshomoh (2019).

### **3.9 Administration of extract**

Extract was freshly prepared every morning and administered orally to rats by carefully inserting an orogastric tube into the oral cavity of the rats. The animals were grouped into three trimester groups; first trimester (a), second trimester (b) and third trimester (c), divided into 3 sub-groups (Group I<sup>a-c</sup>, II<sup>a-c</sup>, III<sup>a-c</sup>, IV<sup>a-c</sup> and V<sup>a-c</sup>) consisting of 5 animals each.

Group I (control) – Normal saline (2 ml/kg)

Group II- Positive control (5 mg/kg folic acid)

Group III- Negative control (10 IU of oxytocin)

Group IV and V (2 ml/kg of the extract respectively after acute toxicity study)

Group IV and V (5 ml/kg of the extract respectively after acute toxicity study).

Throughout the period of administration, food and water were given to the rats. For the first trimester group, the extracts were administered to the pregnant dams from day 0 - 7 of pregnancy and the rats were sacrificed on the 8<sup>th</sup> day of pregnancy.

For the second trimester group, the extracts were administered from day 0 - 14 of pregnancy and the rats were sacrificed on the 15<sup>th</sup> day of pregnancy.

For the third trimester group, the extracts were administered from day 0 - 21 and the rats were left to litter.

The weights of the rats in the control as well as treatment groups were recorded on day 0, 7, 14 and day 21 of the gestation period. Examination were carried out every day to look for toxicity indicators which include; salivation, shedding tears, writhing, convulsion, tremors, yellow coloration of fur, hair loss, bleeding, and/or mortality.

### **3.10 Determination of serum progesterone level**

Blood samples was withdrawn from the animals on day 8 and 15 of pregnancy via cardiac puncture, centrifuged at 3000 revolutions per minute for 10 minutes and serum obtained was analyzed using an Enzyme linked immunosorbent assay (ELISA) kit to evaluate serum progesterone levels according to Oshomoh and Obaro (2020).

### **3.11 Pregnancy outcome**

On the 8<sup>th</sup> and 15<sup>th</sup> day of gestation, the female rats were laparotomized under chloroform anesthesia. The lower abdomen was cut open and the uterus was examined for number of implantation sites and width of uterine horns on day 8 and day 15 of pregnancy respectively (Oshomoh and Obaro, 2019).

### **3.12 Parturition**

After parturition, the number of alive and still born pups was recorded. All pups were examined for obvious outward abnormalities including open eyelids and aberrant tails and club foot. The birth weight, gestation length, litter size, crown-rump length and tail length of the pups were recorded according to Oshomoh and Obaro (2019).

### **3.13 Statistical analysis**

Every values presented as Mean  $\pm$  Standard Error of Mean (SEM). Using the UK's Graph Pad Prism 8.2 software, one-way ANOVA was used to analyze the data.  $P \leq 0.05$  was used to define significance for differences.

## CHAPTER FOUR

### 4.0

### RESULTS

**Effect of Tiger Nut milk Juice (TGMJ) on pregnancy in Female Rats at First Second Trimesters and parturition of pregnancy. The results reveal that (TGMJ) juice supported pregnancy development in all the three**

**The Plates shows A, B ,D and E shows normal embryo blastocyst at 2<sup>nd</sup> trimester of pregnancy only that the extract treated groups presented with chronic villi and desidual showing more developed foetal parts than A and B only C which was treated with oxytocin at a dose of 0.5 IU was seen to have embroyblast with Hemorrhage (EBAH).**

**Effect of Tiger Nut milk Juice (TGMJ) on pregnant dam's body and uterine mass index at 1<sup>st</sup> and 2<sup>nd</sup> trimester**

**Results obtained after the administration of extract for 7 and 14 days to pregnancy mice respectively, revealed significant ( $P \leq 0.05$ ) increases in implantation and width of uterine horns gravidity of pregnant dams at first and second trimester in the extract treated gravid dams compared to that of the contro**

**Effect of Tiger Nut milk Juice (TGMJ) on pregnant dam's body and uterine mass index at 2<sup>nd</sup> trimester 1<sup>st</sup> generation**

**The Effect of Tiger Nut milk Juice (TGMJ) on pups at 3rd Trimester parturition**

**The effects of Tiger Nut milk Juice (TGMJ) on fasting blood glucose Level**

## CHAPTER FIVE

### 5.0 DISCUSSION

The present study investigated the effect of tiger nut (*Cyperus esculentus*) juice on pregnancy outcomes in both pregnant and non-pregnant female albino mice. The findings revealed that tiger nut juice positively influenced reproductive performance, as evidenced by improved conception rates, enhanced maternal weight gain during gestation, and healthier litter characteristics (in terms of litter size, viability, and pup weight). These outcomes suggest that the bioactive compounds in tiger nut, including vitamin E, essential fatty acids, and phytosterols, play a crucial role in supporting female reproductive physiology. Tiger nut is known for its rich content of antioxidants, which reduce oxidative stress—a major contributor to infertility and adverse pregnancy outcomes. The presence of vitamin E and polyphenols likely protected maternal tissues and developing embryos from free radical damage, thereby supporting embryonic survival and growth. This aligns with earlier reports indicating that natural antioxidants improve fertility by enhancing gamete quality, implantation, and placental function (Adekanmi *et al.*, 2022). In non-pregnant mice, tiger nut juice consumption appeared to regulate estrous cyclicity and improve reproductive readiness. This effect may be attributed to phytosterols and essential fatty acids, which are known to modulate hormonal balance by influencing estrogen and progesterone synthesis. Similar findings have been reported in studies where plant-derived nutraceuticals enhanced female reproductive function (Olawale *et al.*, 2023).

The improvement in pregnancy outcomes further supports the ethnomedicinal claims that tiger nut enhances fertility and reproductive health. The ability of tiger nut juice to increase litter size and improve pup viability highlights its potential as a functional food supplement for boosting reproductive efficiency. However, while the results are promising, caution should be exercised in extrapolating findings from animal studies to humans, since variations in metabolism, dosage, and physiology may influence outcomes.

## **5.1**

### **CONCLUSION**

This study demonstrated that tiger nut juice exerts beneficial effects on both pregnant and non-pregnant female albino mice. In pregnant mice, supplementation enhanced maternal health, improved litter size, pup viability, and birth weight. In non-pregnant mice, it promoted reproductive readiness and hormonal balance. These findings provide scientific support for the traditional use of tiger nut in enhancing fertility and sustaining pregnancy.

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