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Some Effects of Concomitant Use of Crude Aqueous Extract of Garcinia Kola Seed and Ascorbic Acid on Ovary of Adult Wistar Rat

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Abstract **Original Research**

This study investigated the protective effect of vitamin C against ovarian histological changes induced by aqueous extract of Garcinia kola seed in adult female Wistar rats. Thirty healthy female Wistar rats weighing between 150 and 250 g were randomly assigned to five treatment groups. The control group received distilled water, while the treatment groups received 100 mg/kg or 200 mg/kg of Garcinia kola extract, 200 mg/kg of the extract combined with 100 mg/kg vitamin C, or 100 mg/kg vitamin C alone. A withdrawal subgroup was maintained for two additional weeks after treatment to assess recovery. The extract and vitamin C were administered orally. At the end of the experimental period, the ovaries were excised, weighed and processed for histological examination using Haematoxylin and Eosin staining. Body weight data were analysed using one way Analysis of Variance at a significance level of $p < 0.05$. Histological findings showed that administration of Garcinia kola extract caused follicular degeneration, ruptured follicles and fibrotic changes, with more severe alterations at the higher dose. Animals treated with vitamin C alone maintained normal ovarian architecture, while those that received vitamin C together with the extract showed better preservation of ovarian tissue than animals given the extract alone. The withdrawal group demonstrated evidence of follicular improvement and oocyte regeneration, indicating partial recovery after cessation of treatment. The study concluded that aqueous extract of Garcinia kola adversely affected ovarian histology in a dose dependent manner, whereas vitamin C reduced the severity of these changes and supported recovery of ovarian tissue following withdrawal.

Keywords: Garcinia kola, vitamin C, ovary, ovarian histology, Wistar rats, follicular degeneration, antioxidant.

CHAPTER ONE

1.0 INTRODUCTION

The ovary is a specialised female reproductive organ responsible for the production of oocytes and the secretion of hormones that regulate the female reproductive cycle. It is composed of germ cells and somatic cells, including *granulosa cells*, *theca cells*, and *stromal cells*, which interact to support follicular development, ovulation, and the formation of the corpus luteum after ovulation (JoAnne et al., 2010). Follicular development depends on continuous communication between the oocyte and the surrounding somatic cells, and this

interaction determines whether a follicle progresses to maturity or undergoes degeneration. Recent studies have shown that signalling within the cumulus-oocyte complex is essential for oocyte growth, follicular survival, and normal ovarian function (Xie et al., 2023). In addition, age-related changes in folliculogenesis have been associated with reduced follicular reserve and declining fertility, indicating that the ovary is highly sensitive to both physiological and environmental influences (Esencan et al., 2022).

Because ovarian function depends on coordinated cellular activity, hormonal regulation becomes necessary for maintaining normal reproductive processes. The hypothalamic-pituitary-gonadal axis controls ovarian activity through the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which stimulates the anterior pituitary gland to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones regulate follicular recruitment, maturation, ovulation, and steroid hormone production. Any disturbance within this hormonal pathway or damage to ovarian tissue can interfere with ovulation and reduce fertility (Sunday, 2013). Beyond hormonal control, recent evidence suggests that oxidative stress also influences ovarian physiology. Excessive production of reactive oxygen species can damage granulosa cells, impair follicular development, and contribute to ovulatory dysfunction (Liu et al., 2023; Awonuga et al., 2023).

The relationship between ovarian integrity and reproductive capacity becomes clearer when considering the burden of female infertility. Infertility remains a common reproductive health problem among women of childbearing age, and ovulatory disorders account for approximately 30% of female infertility cases (Gaware et al., 2009). These disorders may arise from hormonal imbalance, abnormal follicular development, ovarian scarring, or premature ovarian failure. Since successful ovulation requires both an intact ovarian structure and effective endocrine regulation, alterations affecting either the ovarian histoarchitecture or the hypothalamic-pituitary-gonadal axis may compromise fertility (Sunday, 2013). Studies on ovarian pathology have further shown that oxidative stress contributes to follicular degeneration, apoptosis of granulosa cells, and reduced oocyte quality, thereby linking cellular injury to impaired reproductive performance (Liu et al., 2023).

Given the sensitivity of the ovary to hormonal and oxidative disturbances, attention has turned to medicinal plants that are commonly consumed for therapeutic purposes. One such plant is *Garcinia kola*, popularly known as bitter kola, which is widely used in West and Central Africa. The plant belongs to the family *Guttiferae* and is commonly called orogbo among the Yoruba people of Nigeria. Traditionally, the seeds are consumed for the treatment of diarrhoea, tuberculosis, fever, bacterial infections, food poisoning, sneezing, and the common cold. Experimental studies have reported several biological activities of *Garcinia kola*, including anti-atherogenic effects (Adaramoye et al., 2005), anti-lipid peroxidative activity (Emerole et al., 2005), aphrodisiac effects (Uko et al., 2001), gastroprotective properties (Olaleye & Farombi, 2006), and antioxidant activity (Farombi et al., 2002). These findings support the widespread traditional use of the plant and suggest that its pharmacological effects extend beyond symptomatic treatment of disease.

While these medicinal properties have attracted considerable interest, the reproductive effects of *Garcinia kola* remain a subject of investigation. Many of its biological activities have been linked to kolaviron, a biflavonoid complex present in high concentration within the seed (Lau, 2001). Kolaviron has been associated with antioxidant and anti-inflammatory actions, yet studies have also reported that prolonged exposure may alter the histological organisation of the hypothalamic-pituitary-gonadal axis in female Wistar rats, leading to impaired reproductive function (Sunday, Adelaja, & Ajayi, 2013). In addition, *Garcinia kola* itself has demonstrated anti-inflammatory activity (Madubunyi, 1995), and previous studies have shown that anti-inflammatory agents can interfere with the ovulatory process (Gaytan et al., 2002). This creates an important question regarding the use of *Garcinia kola* by

women who require its therapeutic benefits but also wish to maintain normal fertility.

The possibility that *Garcinia kola* may affect ovarian function suggests the need to identify agents capable of reducing any adverse reproductive effects associated with its use. One such agent is vitamin C (ascorbic acid), a water-soluble vitamin found in fruits such as oranges, lemons, grapefruit, watermelon, papaya, strawberries, mangoes, pineapples, and cherries, as well as vegetables including tomatoes, broccoli, and green leafy vegetables (Akhilender, 2013). Ascorbic acid functions as an antioxidant and participates in several metabolic processes that protect cells against oxidative damage. Earlier studies showed that vitamin C can regenerate other antioxidants, including α-tocopherol, from their oxidised forms, thereby helping to maintain cellular antioxidant defence mechanisms (Halliwell et al., 1986). More recent evidence has shown that natural antioxidants support follicular growth, oocyte maturation, and embryo development by limiting oxidative stress within reproductive tissues (Vašková et al., 2023; Gomes et al., 2023).

Because oxidative stress has been implicated in ovarian dysfunction, vitamin C may provide protection against tissue changes associated with Garcinia kola administration. Experimental studies on antioxidant supplementation have demonstrated improvements in ovarian follicle survival and reproductive outcomes under conditions of oxidative challenge (Silva et al., 2023). On this basis, the present study investigates whether the simultaneous administration of ascorbic acid and aqueous extract of *Garcinia kola* seed can preserve the histological integrity of the ovary in adult female Wistar rats. The study also examines the effect of vitamin C administered alone in order to determine whether its antioxidant activity can reduce ovarian damage associated with kolaviron exposure.

Significance of the Study

Garcinia kola possesses several medicinal properties and is commonly consumed for the treatment of infectious and inflammatory conditions. However, evidence suggesting that its anti-inflammatory activity may interfere with ovarian function raises concern for women of reproductive age. This study evaluates whether the antioxidant property of vitamin C can reduce the ovarian changes associated with *Garcinia kola* administration. The findings may provide useful information on the interaction between Garcinia kola and vitamin C and may help determine whether both agents can be used together without compromising ovarian histology and fertility.

Aim of the Study

The aim of this study is to investigate whether ascorbic acid can protect the ovarian histology of adult female Wistar rats during the simultaneous administration of aqueous extract of *Garcinia kola* seed. The specific objectives of this study are to:

1. determine the effect of the concurrent administration of vitamin C and aqueous extract of *Garcinia kola* seed on the ovarian histology of adult female Wistar rats;
2. evaluate the effect of different doses of aqueous extract of *Garcinia kola* seed on ovarian histoarchitecture;
3. assess the extent to which vitamin C protects ovarian tissue against changes induced by *Garcinia kola*; and
4. determine the effects of vitamin C on ovarian weight and body weight following the administration of aqueous extract of *Garcinia kola* in adult female Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 SCIENTIFIC CLASSIFICATION AND DIAGRAM OF GARCINIA KOLA

kingdom: Plantae

Phylum: Tracheophyta

Division: Magnoliophyta

Class: Magnoliopsida

Order: Theales

Family: Guttiferae

Genus: Garcinia

Species: Garcinia Kola

Iwu et al., 1990

Fig. 1 Diagram of Garcinia Kola Seed

2.2 MEDICINAL USES OF *GARCINIA KOLA*

Garcinia kola Heckel is a tropical medicinal plant widely distributed across the rainforest regions of West and Central Africa, where it is valued for both its nutritional and therapeutic importance. The seeds, commonly known as bitter kola, are frequently consumed and remain culturally acceptable in many Nigerian communities, where they are traditionally used as a stimulant, general tonic, and remedy for various ailments (Hutchinson et al., 1956). Different parts of the plant, including the seeds, bark, roots, and stems, have long been employed in African traditional medicine for the management of cough, laryngitis, hoarseness of voice, diarrhoea, fever, food poisoning, bacterial infections, and liver disorders (Ayensu, 1978; Iwu, 1982). The stems and roots are also used as chewing sticks because of their antimicrobial activity against oral pathogens (Taiwo et al., 1999). Clinical observations further showed that extracts of *Garcinia kola* improved liver function in patients with chronic hepatitis and cholangitis following herbal treatment (Iwu, 1982). Recent reviews have reaffirmed these traditional applications, attributing them to the rich phytochemical composition of the plant, particularly

flavonoids, biflavonoids, xanthones, benzophenones, and other phenolic compounds with established pharmacological activities (Emmanuel et al., 2022; Tauchen et al., 2023).

2.2.1 Biological Properties of *Garcinia kola*

The medicinal value of *Garcinia kola* is closely associated with its diverse biological activities, most of which have been linked to kolaviron, a biflavonoid complex isolated from the seeds. Experimental studies have demonstrated that *Garcinia kola* possesses antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, anti-diabetic, anti-atherogenic, gastroprotective and aphrodisiac properties (Iwu et al., 1986; Iwu et al., 1990; Adaramoye et al., 2005; Farombi et al., 2009). Braide (1991) reported that dietary administration of powdered *Garcinia kola* seeds reduced gastrointestinal motility and protected experimental animals against castor oil-induced diarrhoea, while Farombi et al. (2000) and Olatunde et al. (2002) demonstrated its anti-atherogenic and anti-lipid peroxidative effects, respectively. Kolaviron has also shown protective effects against chemically induced liver injury caused by aflatoxin, carbon tetrachloride, paracetamol and other hepatotoxic agents (Farombi et al., 2009). Despite these beneficial effects, studies have indicated that its anti-inflammatory activity may interfere with ovulation, a phenomenon previously reported with several anti-inflammatory agents (Gaytan et al., 2002; Iranloye et al., 2008). Recent reviews have confirmed that these biological effects are mediated through antioxidant, anti-inflammatory and cell signalling pathways, although further studies are required to establish its long-term reproductive safety (Dogara, 2022; Tauchen et al., 2023; Nworu & Akah, 2023).

2.2.2 Histology of the Ovary

The ovary is a paired, almond-shaped reproductive organ whose structural organisation is closely related to its reproductive and endocrine functions. It is covered externally by a simple cuboidal epithelium known as the germinal epithelium, beneath which lies the dense connective tissue layer called the tunica albuginea (Anthony, 2005). The cortex forms the outer region of the ovary and contains follicles at different stages of development embedded within a specialised stromal connective tissue composed primarily of spindle-shaped fibroblasts. These follicles undergo continuous growth, maturation, ovulation and regression under hormonal regulation. The inner medulla consists of loose connective tissue containing numerous blood vessels, lymphatics and nerves that provide nutritional and endocrine support to the ovarian tissue (Anthony, 2005). Although the cortical and medullary regions differ structurally, they function as an integrated unit to sustain normal folliculogenesis and steroid hormone production. Recent studies have shown that communication between the oocyte, granulosa cells and theca cells is essential for follicular maturation and maintenance of ovarian integrity, while disruption of this cellular interaction may result in follicular atresia and impaired fertility (Xie et al., 2023; Esencan et al., 2022).

2.2.3 Ovarian Follicle

The ovarian follicle is the fundamental structural and functional unit of the ovary, consisting of an oocyte enclosed by one or more layers of granulosa cells that provide physical support and regulate oocyte growth through continuous metabolic and molecular communication (Kenigsberg et al., 1995; Xie et al., 2023). A specialised basement membrane surrounds the granulosa cell layer, separating the follicle from the surrounding ovarian stroma while maintaining a controlled microenvironment necessary for normal follicular development (Kenigsberg et al., 1995). The earliest follicles, known as primordial follicles, are established during fetal life and are composed of a primary oocyte surrounded by a single layer of flattened follicular cells located within the ovarian cortex (Peters, 1980). These primordial follicles constitute the ovarian reserve, which gradually declines with age owing to follicular atresia and determines the reproductive lifespan of the female (Esencan et al., 2022). During this resting phase, the

oocyte remains arrested in the prophase of the first meiotic division and contains abundant mitochondria, Golgi complexes and endoplasmic reticulum that support future follicular activation and maturation (Gosden, 1995). Functional communication between the oocyte and granulosa cells is maintained through gap junctions and paracrine signalling pathways, which regulate nutrient transfer, follicular survival and acquisition of oocyte developmental competence (Xie et al., 2023).

2.2.4 Development of the Ovary and Its Function

Ovarian development begins during early embryogenesis when primordial germ cells migrate from the yolk sac to the developing gonadal ridge, where they proliferate by mitosis to form oogonia (Gosden, 1995). As fetal development progresses, many oogonia enter the first meiotic division and become primary oocytes, which subsequently arrest at the diplotene stage of prophase I until puberty (Gosden, 1995). Each primary oocyte is enclosed by a layer of flattened follicular cells to form a primordial follicle, thereby establishing the ovarian reserve before birth (Peters, 1980). Although millions of oogonia are produced during fetal life, the majority undergoes physiological degeneration through follicular atresia before birth and throughout reproductive life, leaving only about 300,000 primordial follicles at puberty and approximately 400 to 500 available for ovulation during the reproductive years (Gosden, 1995). More recent evidence indicates that the size and quality of the ovarian reserve are influenced not only by age but also by endocrine regulation, oxidative balance and cellular signalling pathways that preserve follicular viability (Esencan et al., 2022; Liu et al., 2023). Beyond producing mature oocytes, the ovary functions as an endocrine organ by secreting oestrogen, progesterone and other reproductive hormones that regulate the menstrual cycle, ovulation, implantation and the maintenance of pregnancy through coordinated interactions within the hypothalamic-pituitary-gonadal axis (JoAnne et al., 2010).

2.2.5 Follicular Growth

Follicular growth is a continuous physiological process that begins at puberty with the recruitment of a small population of primordial follicles into the growing follicular pool under the influence of follicle-stimulating hormone (FSH) and locally produced growth factors (Kenigsberg et al., 1995; Xie et al., 2023). During the early stages of development, the oocyte enlarges while the surrounding follicular cells change from a single layer of flattened cells to cuboidal granulosa cells, giving rise to the unilaminar primary follicle before continued mitotic proliferation produces the multilaminar or preantral follicle (Gosden, 1995). At the same time, the zona pellucida, a specialised glycoprotein-rich extracellular matrix, is deposited around the oocyte through the combined activities of the oocyte and granulosa cells, allowing cytoplasmic projections and gap junctions to facilitate bidirectional exchange of nutrients, metabolites and regulatory molecules essential for oocyte maturation (Gosden, 1995; Xie et al., 2023). As follicular development progresses, coordinated endocrine signals, antioxidant defence systems and intracellular signalling pathways maintain granulosa cell function and follicular survival, whereas oxidative stress can impair follicular growth, reduce oocyte competence and promote follicular atresia, ultimately compromising female fertility (Liu et al., 2023; Gomes et al., 2023; Vašková et al., 2023).

2.3 FEMALE INFERTILITY

Female infertility is defined as the inability of a woman to achieve pregnancy after 12 months of regular, unprotected sexual intercourse and remains a significant public health concern affecting millions of couples worldwide (World Health Organization, 2023; Practice Committee of the American Society for Reproductive Medicine, 2023). Although infertility may result from abnormalities involving the female reproductive tract, endocrine dysfunction, genetic factors or environmental influences, ovulatory disorders remain one of the leading causes of female infertility, accounting for a substantial proportion of affected women (Unuane et al., 2011;

Practice Committee of the American Society for Reproductive Medicine, 2021). Normal female fertility depends on the coordinated activities of the hypothalamus, pituitary gland and ovaries, and disruption at any level of this hypothalamic-pituitary-gonadal axis may impair follicular development, ovulation and hormone production (JoAnne et al., 2010; Concepción-Zavaleta et al., 2023). Endocrine disorders such as hyperprolactinaemia, thyroid dysfunction, polycystic ovary syndrome and primary ovarian insufficiency are among the most frequently reported hormonal causes of female infertility because they alter gonadotropin secretion and ovarian responsiveness (Unuane et al., 2011; Concepción-Zavaleta et al., 2023). Recent studies further indicate that oxidative stress, chronic inflammation and metabolic disturbances contribute to ovarian dysfunction by impairing granulosa cell function, reducing oocyte quality and disrupting folliculogenesis, thereby lowering the probability of successful conception (Awonuga et al., 2023; Liu et al., 2023; Athar et al., 2024). These findings demonstrate that female infertility is a multifactorial condition requiring an understanding of both endocrine regulation and ovarian physiology to improve reproductive outcomes.

2.4 ANTIOXIDANTS

Antioxidants are substances that protect cells against oxidative damage by neutralising reactive oxygen species (ROS) and other unstable free radicals generated during normal cellular metabolism or in response to environmental and physiological stressors (Sies, 2023). Although oxygen is indispensable for energy production and numerous metabolic processes, its utilisation inevitably leads to the formation of ROS, including superoxide anions, hydrogen peroxide and hydroxyl radicals, which can damage cellular macromolecules when produced in excess (Halliwell et al., 1986; Forman & Zhang, 2021). Under normal physiological conditions, the production of these reactive molecules is balanced by an elaborate antioxidant defence system that preserves cellular homeostasis and maintains redox equilibrium (Valko et al., 2007; Sies, 2023). This defence network comprises both enzymatic antioxidants, such as superoxide dismutase, catalase and glutathione peroxidase, and non-enzymatic antioxidants, including vitamin C, vitamin E, glutathione and naturally occurring polyphenols obtained from dietary sources (Vašková et al., 2023). These antioxidants act through complementary mechanisms by scavenging free radicals, interrupting oxidative chain reactions and limiting lipid peroxidation, protein oxidation and DNA damage before irreversible cellular injury occurs (Sharifi-Rad et al., 2022; Forman & Zhang, 2021). When the generation of reactive oxygen species exceeds the capacity of endogenous antioxidant systems, oxidative stress develops, resulting in impaired cellular function and tissue injury that have been implicated in several pathological conditions, including cardiovascular diseases, neurodegenerative disorders, diabetes, cancer and reproductive dysfunction (Valko et al., 2007; Sies, 2023). Within the female reproductive system, excessive oxidative stress disrupts granulosa cell activity, compromises oocyte maturation and interferes with normal follicular development, thereby reducing fertility potential (Liu et al., 2023). Consequently, antioxidant molecules have attracted considerable attention because of their ability to preserve ovarian function, improve cellular resilience and reduce oxidative injury within reproductive tissues (Vašková et al., 2023; Gomes et al., 2023). This protective role provides a strong scientific basis for investigating antioxidant agents such as vitamin C as possible modulators of ovarian damage induced by bioactive compounds present in *Garcinia kola*.

2.4.1 Vitamin C (Ascorbic Acid) as an Antioxidant

Among the naturally occurring antioxidants, vitamin C (ascorbic acid) is one of the most extensively studied because of its broad physiological functions and its ability to protect cells against oxidative injury. As a water-soluble vitamin, vitamin C is obtained primarily from fruits and vegetables, including oranges, lemons, strawberries,

mangoes, tomatoes, broccoli and green leafy vegetables, since humans cannot synthesise it endogenously (Akhilender, 2013; Vašková et al., 2023). Beyond its nutritional importance, ascorbic acid serves as a potent electron donor that neutralises reactive oxygen species before they initiate oxidative damage to cellular proteins, lipids and nucleic acids (Halliwell et al., 1986; Sies, 2023). It also regenerates other endogenous antioxidants, particularly vitamin E, thereby strengthening the body's overall antioxidant defence system and helping to maintain cellular redox balance under physiological and pathological conditions (Halliwell et al., 1986; Forman & Zhang, 2021). Within the female reproductive system, vitamin C contributes to normal follicular development by protecting granulosa cells from oxidative injury, supporting steroid hormone synthesis and preserving the quality of developing oocytes (Liu et al., 2023). Experimental and clinical evidence further suggests that adequate antioxidant status enhances ovarian function, improves follicular survival and may reduce reproductive disorders associated with excessive oxidative stress (Vašková et al., 2023; Gomes et al., 2023). These protective properties have stimulated interest in the use of vitamin C as a supportive therapeutic agent against reproductive toxicants capable of inducing ovarian degeneration. In the context of the present study, the antioxidant potential of vitamin C provides a plausible biological basis for examining whether its concurrent administration with *Garcinia kola* extract can minimise ovarian histological alterations associated with the bioactive constituents of the plant while preserving normal ovarian structure and function.

2.5 VITAMINS

Vitamins are organic micronutrients required in small quantities to support normal growth, metabolism and physiological functions. Unlike carbohydrates, proteins and fats, they do not serve as direct sources of energy but function primarily as coenzymes, antioxidants and regulatory molecules involved in numerous biochemical reactions essential for maintaining health (National Institutes of Health, 2024). Based on their solubility, vitamins are classified into fat-soluble vitamins (A, D, E and K) and water-soluble vitamins, which include the B-complex vitamins and vitamin C (Naidu, 2003). Since the human body either cannot synthesise most vitamins or produces them in insufficient quantities, they must be obtained regularly through dietary intake to sustain normal cellular activities and prevent deficiency disorders (Carr & Maggini, 2017; National Institutes of Health, 2024). Among the water-soluble vitamins, vitamin C has attracted considerable scientific interest because of its diverse biological functions extending beyond nutrition. Apart from supporting collagen synthesis, wound healing, iron absorption and immune responses, vitamin C plays a central role in protecting cells against oxidative damage through its potent antioxidant activity (Carr & Frei, 1999; Sies, 2023). Recent evidence further demonstrates that adequate vitamin C status contributes to normal ovarian physiology by preserving granulosa cell function, promoting follicular development and reducing oxidative injury within reproductive tissues, thereby supporting female fertility (Vašková et al., 2023; Liu et al., 2023). Given these physiological properties and its established role in cellular protection, vitamin C has become an important subject of investigation in studies evaluating therapeutic strategies against oxidative tissue injury. This makes it particularly relevant to the present study, which seeks to determine whether concomitant administration of vitamin C can protect the ovary against histological alterations induced by aqueous extract of *Garcinia kola*.

2.5.1 Historical Perspective

The history of vitamin C began with attempts to understand and prevent scurvy, a debilitating disease that claimed the lives of thousands of sailors during long sea voyages. In the eighteenth century, the British naval physician James Lind conducted one of the earliest controlled clinical investigations and demonstrated that citrus fruits were highly effective in treating the condition, thereby establishing the relationship between diet and scurvy (Lind, 1753; Carpenter, 2012). Although the specific nutrient responsible was not known at the time,

Lind's observations laid the scientific foundation for subsequent investigations into vitamin C. His findings eventually influenced the British Navy to include lemon or lime juice in sailors' daily rations, resulting in a marked reduction in scurvy among naval personnel (Carpenter, 2012). This development remains one of the earliest examples of nutrition-based disease prevention in medical history.

Scientific understanding of vitamin C advanced considerably during the twentieth century. Albert Szent-Györgyi successfully isolated the compound from natural sources and demonstrated that hexuronic acid possessed antiscorbutic activity, an achievement that later earned him the Nobel Prize in Physiology or Medicine (Szent-Györgyi, 1932). Shortly afterwards, Haworth and colleagues determined its chemical structure and achieved its laboratory synthesis, making large-scale production possible for medical and nutritional use. Since then, vitamin C has become one of the most widely consumed dietary supplements worldwide because of its established physiological functions and favourable safety profile (Naidu, 2003; National Institutes of Health, 2024). Beyond its traditional role in preventing scurvy, current research has expanded knowledge of its involvement in immune regulation, collagen biosynthesis, antioxidant defence and reproductive health, with increasing evidence indicating that adequate vitamin C status supports normal ovarian function and helps reduce oxidative damage associated with female reproductive disorders (Vašková et al., 2023; Carr & Maggini, 2017). These advances have shifted vitamin C from being regarded merely as a deficiency-preventing nutrient to an important bioactive molecule with broad therapeutic potential.

2.5.2 Sources of Ascorbic Acid

Since humans lack the enzyme L-gulonolactone oxidase, they cannot synthesise vitamin C endogenously and must obtain it consistently through dietary intake or supplementation (National Institutes of Health, 2024; EFSA Panel on Dietetic Products, Nutrition and Allergies, 2013). Fresh fruits remain the richest natural sources of ascorbic acid, particularly oranges, lemons, grapefruit, strawberries, guava, kiwi, papaya, pineapple, watermelon, mango and cherries. Significant quantities are also present in vegetables such as broccoli, tomatoes, cauliflower, cabbage, spinach, green leafy vegetables and both green and red peppers (Carr & Maggini, 2017; National Institutes of Health, 2024). The concentration of vitamin C in food, however, is influenced by storage conditions, exposure to oxygen, light and excessive heating during processing or cooking, all of which may substantially reduce its nutritional value (Naidu, 2003).

Where dietary intake is inadequate or physiological demand is increased, vitamin C may also be obtained through pharmaceutical preparations, including tablets, capsules, chewable formulations, powders and effervescent products. Both naturally derived and synthetic vitamin C possess identical chemical structures and biological activity, making them equally effective in meeting physiological requirements (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2013). Regular consumption of vitamin C-rich foods is therefore recommended because the vitamin is water-soluble, has limited body storage and requires continuous replenishment to maintain optimal antioxidant capacity, immune competence and normal reproductive function (Carr & Frei, 1999; Vašková et al., 2023). These characteristics explain why vitamin C has been widely investigated as a protective nutritional agent against oxidative stress-induced tissue damage, including alterations affecting ovarian cells and follicular development.

2.5.3 Chemistry of Ascorbic Acid

Ascorbic acid, commonly referred to as vitamin C, is a six-carbon water-soluble compound with the molecular formula $C_6H_8O_6$. Chemically, it is known as L-ascorbic acid, while its oxidised form, dehydroascorbic acid, also exhibits biological activity because it can be readily converted back to its reduced state within body tissues (Carr & Frei, 1999; Naidu, 2003). This reversible oxidation-

reduction property enables vitamin C to donate electrons to unstable free radicals without becoming permanently damaged, making it one of the body's most effective non-enzymatic antioxidants (Sies, 2023). The vitamin remains highly stable in mildly acidic conditions, particularly between pH 4 and 6, but rapidly loses its activity when exposed to excessive heat, oxygen, light, alkaline conditions or transition metals such as iron and copper, which accelerate its oxidation (Naidu, 2003). These chemical characteristics explain why prolonged storage and excessive cooking often reduce the vitamin C content of fruits and vegetables before consumption.

Apart from its nutritional significance, the unique chemical structure of vitamin C makes it an essential reducing agent in several physiological reactions. It serves as a cofactor for numerous enzymes involved in collagen formation, carnitine biosynthesis and peptide hormone production, while simultaneously maintaining the reduced state of other biologically important molecules (Carr & Maggini, 2017). Recent biochemical studies have further demonstrated that this electron-donating capacity enables vitamin C to preserve cellular integrity by limiting oxidative modifications of proteins, membrane lipids and nucleic acids, particularly in metabolically active tissues such as the ovary where reactive oxygen species are continuously generated during follicular development and ovulation (Liu et al., 2023; Vašková et al., 2023). These properties provide a biochemical basis for investigating vitamin C as a protective agent against ovarian tissue injury induced by pharmacological or plant-derived compounds.

2.5.4 Catabolism of Ascorbic Acid

Following dietary intake, vitamin C is absorbed mainly in the small intestine through specialised sodium-dependent vitamin C transporters, after which it is distributed to virtually all body tissues according to physiological demand (Padayatty et al., 2004). Absorption is highly efficient at moderate dietary levels but gradually declines as intake increases because the transport mechanisms become saturated (National Institutes of Health, 2024). Unlike fat-soluble vitamins, vitamin C is not stored in substantial quantities within the body. Instead, excess amounts circulate briefly in plasma before being metabolised and eliminated through the kidneys, making regular dietary replenishment necessary to maintain adequate tissue concentrations (Lykkesfeldt et al., 2024).

Within cells, ascorbic acid undergoes reversible oxidation to dehydroascorbic acid during antioxidant reactions. When antioxidant demand is excessive, dehydroascorbic acid is further degraded into metabolites such as 2, 3-diketogulonic acid and oxalic acid, which are subsequently excreted in urine (Padayatty et al., 2004). This continuous turnover reflects the active participation of vitamin C in protecting cells against oxidative injury. Although vitamin C has an excellent safety profile, excessive supplementation may occasionally produce mild gastrointestinal discomfort or diarrhoea because unabsorbed quantities remain within the intestinal lumen (National Institutes of Health, 2024). Nevertheless, available evidence indicates that toxicity is uncommon in healthy individuals, while inadequate intake remains a more significant public health concern because persistent deficiency compromises collagen synthesis, immune competence, wound healing and antioxidant defence (Carr & Maggini, 2017). In reproductive tissues, insufficient vitamin C may also increase susceptibility to oxidative damage, thereby affecting follicular development and ovarian function (Vašková et al., 2023).

2.5.5 Vitamin C as an Antioxidant and Co-antioxidant

The biological importance of vitamin C extends far beyond its role as a dietary nutrient because it represents one of the principal antioxidant molecules in extracellular fluids and many intracellular compartments. Reactive oxygen species are continuously generated during normal cellular metabolism, and although moderate concentrations are necessary for physiological signalling, excessive accumulation results in oxidative stress that damages

lipids, proteins and DNA (Sies, 2023). Vitamin C minimises this damage by donating electrons to highly reactive molecules, thereby converting them into more stable compounds before they can initiate destructive chain reactions (Carr & Frei, 1999). Experimental evidence further shows that vitamin C effectively scavenges hydroxyl radicals, superoxide anions, peroxyl radicals and reactive nitrogen species, reducing oxidative injury across a wide range of tissues (Carr & Maggini, 2017).

Its protective function becomes even more significant because vitamin C also acts as a co-antioxidant. During oxidative reactions, antioxidants such as vitamin E become oxidised after neutralising free radicals. Vitamin C regenerates these oxidised molecules back to their active forms, thereby prolonging their antioxidant capacity and strengthening the overall defence system against oxidative stress (Carr & Frei, 1999). This interaction explains why antioxidant molecules rarely function independently but instead operate as an integrated network within biological systems. Recent reviews have shown that this antioxidant network plays a critical role in female reproduction by preserving granulosa cell viability, maintaining mitochondrial function, supporting oocyte maturation and improving follicular survival under conditions of oxidative stress (Gomes et al., 2023; Liu et al., 2023; Vašková et al., 2023). Increasing evidence also indicates that antioxidant supplementation may reduce oxidative damage associated with ovarian dysfunction and improve reproductive outcomes in experimental models (Vašková et al., 2023).

These observations are particularly relevant to the present study because oxidative stress has been implicated in tissue alterations associated with several medicinal plants, including *Garcinia kola*. Since kolaviron, the major bioactive constituent of *Garcinia kola*, possesses diverse pharmacological activities that may influence ovarian histology, evaluating whether vitamin C can counteract possible oxidative injury is scientifically justified. Its well-established antioxidant and co-antioxidant properties therefore provide a strong biological rationale for investigating its protective effect when administered concurrently with aqueous extract of *Garcinia kola* in adult female Wistar rats.

$AH^- + Fe^{3+}$ - - - $A^- + Fe^{2+} + H^+$

$AH^- + Cu^{2+}$ - - - $A^- + Cu^+ + H^+$

$H_2O_2 + Fe^{2+}$ - - - $HO^- + Fe^{3+} + {}^-OH$

$H_2O_2 + Cu^+$ - - - $HO^- + Cu^{2+} + {}^-OH$

$LOOH + Fe^{2+}$ - - - $LO + Fe^{3+} + {}^-OH$

$LOOH + Cu^+$ - - - $LO + Cu^+ + {}^-OH$

HO + LO - - - Lipid peroxidation

Adapted from Carr and Frei (Carr et al., 1999)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

The following were used for this study:

- Thirty-six rats of body weight 150-250g
- Pelletized feed
- Oral cannula
- Dissecting set
- Needles and syringes
- Specimen bottles
- Organ Bottle
- Weighing balance
- Distilled water
- Bouin's Fluid

3.2 METHODOLOGY

3.2.1 Plant Material

3.2.1.1 Plant Collection and Identification

In the first week of March, 2013, Garcinia kola seed were purchased from a local market at Ojaigbo, Ogbomoso Oyo State, Nigeria. This was taken to the University's Agronomy Department for botanical identification.

3.2.1.2 Plant Extraction

Garcinia kola seeds were sliced into thin and

flat sizes, after removing the outer coat. The seeds were dried in room temperature (28±2ºC) for a week, protected from direct sunlight and heat. The seeds were milled to powder using electrical blender, obtaining 250g of Garcinia kola powder which was soaked in 3litres of distilled water for 48hours.The seed was dried before milling because Flavonoids can be degraded by enzyme action when collected plant material is fresh or non-dried before going through extraction processes. The extract obtained was evaporated to dryness on a hot air oven at a temperature of 45degree Celsius to obtain a brownish mass of 12.59g. This was allowed to cool, stored in tight cap-filter container for 1 week before the commencement of the experiment.

3.2.2 Experimental Animals

Thirty healthy adult female Wistar rats, each weighing approximately 150 g, were used for this study. The experimental animals were obtained from a commercial animal breeding facility in Ilorin, Kwara State, Nigeria, and transported to the Animal House of Ladoke Akintola University of Technology (LAUTECH), where the experiment was conducted. Upon arrival, the rats were housed in clean laboratory cages under standard environmental conditions and allowed unrestricted access to clean drinking water and commercially prepared pelletised feed throughout the study.

To minimise stress and ensure physiological stability before treatment, the animals were acclimatised to the laboratory environment for a period of two weeks. During this period, they were maintained under a controlled 12-hour light/12-hour dark photoperiod, ambient temperature of 20 ± 2°C, and relative humidity of 65 ± 5%. Body weights were recorded at the time of procurement, after acclimatisation, before the commencement of treatment, and subsequently at weekly intervals throughout the experimental period using a calibrated weighing balance. Monitoring body weight throughout the study helped assess the general health status of the animals and detect any treatment-related changes.

Following acclimatisation, the rats were randomly assigned into five experimental groups. Four groups consisted of five animals each, while Group T3 comprised ten animals to accommodate both the treatment and withdrawal phases of the study.

All procedures involving the use and handling of experimental animals were carried out in accordance with accepted ethical principles for laboratory animal research. Animal care and experimental protocols complied with institutional regulations and internationally recognised guidelines for the humane use of laboratory animals in biomedical research, including the recommendations of the American Physiological Society (2002) on the care and use of experimental animals. Every effort was made to minimise animal discomfort and ensure their welfare throughout the duration of the experiment.

Table 1 Extract Administration

GROUPS	TREATMENT	
Control	Distilled Water	
T1	100mg/kg of Garcinia kola seed extract (GSKE)	
T2	200mg/kg of Garcinia kola seed extract	
T3	200mg/kg of Garcinia kola seed extract +100mg/kg of Vitamin C	They were sacrificed after two weeks of administration
WT3(WITHDRAWAL		

GROUP)	200mg/kg of Garcinia kola seed extract +100mg/kg of Vitamin C	They were withdrawn from treatment for two weeks after the sacrifice of the other group
T4	100mg/kg of Vitamin C	

3.3 ROUTE OF ADMINISTRATION

Oral route of administration was employed. Precise quantity of *Garcinia kola* extract were suctioned into the syringe and injected orally into the Buccal cavity of the rats using oral cannula. This was also done for vitamin c administration.

3.4 ANIMAL SACRIFICE AND SAMPLE COLLECTION

Following the treatment period, all animals were humanely sacrificed for tissue collection. The control group received distilled water throughout the experiment. Group T1 was administered 100 mg/kg aqueous extract of *Garcinia kola* seed, while Group T2 received 200 mg/kg of the extract. Group T3 received a combined treatment consisting of 200 mg/kg aqueous extract of *Garcinia kola* seed and 100 mg/kg vitamin C. Five animals from this group were sacrificed immediately after the two-week treatment period, whereas the remaining five animals were maintained without further treatment for an additional two weeks to evaluate possible recovery following withdrawal. This subgroup was designated the withdrawal group (WT3). Animals in Group T4 received 100 mg/kg vitamin C only throughout the treatment period.

The experiment lasted four weeks. Treatment for the control, T1, T2, T3 and T4 groups was completed within the first two weeks, while the withdrawal group remained under normal husbandry conditions for an additional two weeks without receiving either *Garcinia kola* extract or vitamin C.

At the end of the respective experimental periods, the animals were sacrificed by cervical dislocation in accordance with accepted laboratory animal welfare procedures. A midline abdominal incision was carefully made to expose the reproductive organs, after which both ovaries were excised. Excess connective tissues were gently removed before the ovaries were weighed using a calibrated electronic analytical balance. The mean weight of the paired ovaries obtained from each animal was recorded and used for subsequent analysis.

3.5 HISTOLOGICAL TISSUE PROCESSING

3.5.1 Tissue Fixation

Immediately after excision, the ovarian tissues were immersed in Bouin's fluid to preserve their microscopic architecture and prevent post-mortem tissue degeneration. Fixation ensured adequate preservation of cellular and nuclear structures prior to histological processing (Weiss et al., 2010).

3.5.2 Tissue Processing

Following fixation, the tissues underwent routine histological processing. The specimens were dehydrated in ascending concentrations of ethanol to remove water, cleared in xylene to eliminate residual alcohol, and infiltrated with molten paraffin wax to provide adequate support for tissue sectioning. This sequence of dehydration, clearing and infiltration followed standard histopathological procedures for paraffin-embedded tissues (Weiss et al., 2010).

3.5.3 Embedding

The infiltrated ovarian tissues were carefully positioned in embedding moulds containing molten paraffin wax. After the wax had solidified, paraffin blocks containing the tissue specimens were obtained for microtomy.

3.5.4 Sectioning

Paraffin-embedded ovarian tissues were sectioned with a rotary microtome to obtain sections of approximately 5-10 µm thickness. The sections were floated on a warm water bath, mounted onto clean glass slides and allowed to dry before staining.

3.5.5 Staining

Prepared tissue sections were stained using the routine Haematoxylin and Eosin (H&E) staining technique. Haematoxylin stained the nuclei blue to purple, whereas eosin imparted varying shades of pink to the cytoplasm and connective tissues, thereby allowing clear differentiation of ovarian cellular structures during microscopic examination (Weiss et al., 2010).

3.5.6 Histological Artifacts

Care was taken throughout tissue handling, processing and staining to minimise the occurrence of processing artifacts that could interfere with microscopic interpretation. Only properly preserved sections with satisfactory staining quality were selected for histological examination and photomicrography.

3.6 STATISTICAL ANALYSIS

The data obtained from the study were summarised and presented as mean ± standard deviation (SD) for each experimental group (n = 5). Differences among the treatment groups were analysed using one-way Analysis of Variance (ANOVA) to determine whether statistically significant variations existed. Where significant differences were detected, appropriate post hoc comparisons were performed in accordance with the procedure described by Duncan (1957). Statistical significance was accepted at $p < 0.05$. All statistical analyses were carried out using GraphPad Prism version 5.00 (GraphPad Software Inc., San Diego, California, USA), while routine calculations were completed using an electronic calculator.

CHAPTER FOUR

4.0 RESULT

4.1 MORPHOLOGICAL FINDINGS

At the first week of administration, mortality was recorded in the group T3 that received 200mg/kg of Garcinia kola with vitamin c. The following week, week two of administration, Animals were observed to suffer from weight loss according to mean values of first and second week discussed under statistical analysis. Mortality was recorded in groups that received 200mg/kg of garcinia kola only, 200mg/kg of garcinia kola and vitamin c, and 100mg/kg. At the first week and second week of withdrawal, no animal was lost.

4.2 HISTOLOGICAL FINDINGS

4.2.1 Control Group

Normal ovary can be observed, with characteristics elongated flattened body, well arranged follicles in their various follicular stages (Plates 1 and 2).

4.2.2 Group T1

Cellular fibrotic and ruptured follicle is seen according to the photomicrograph (plates 3 and 4).

4.2.3 Group T2

Ovary presents with cellular and follicular degeneration (plates 5 and 6).

4.2.4 Group T3

From the photomicrograph, cellular fibrotic and ruptured follicle was observed in ovaries. (plates 7 and 8).

4.2.5 Group Wt3

It shows presence of follicular improvement and oocyte regeneration (plates 9 and 10).

4.2.6 Group T4

From the photomicrograph, ovary appears normal. It is characterized by elongated flattened body, well arranged follicles in their various follicular stages, graafian follicles and corpus luteum, separated by fissures and scares (plates 11 and 12).

4.3 STATISTICAL ANALYSIS

Table 2 Weights of the Wistar Rats during Administraton

GROUP	Average weight of wistar rat week one of administration	Average weight of wistar rat week two of administration
control group	168±11.90	176±27.01
group T1	170±10.80	158±19.24
group T2	156±6.25	136±16.07
group T3	151±11.77	147±17.99
Withdrawal of group T3	147±8.54	165±5.77
group T4	151±11.77	149±24.34

There was increase in the mean value of control group after two weeks of administration.

There was decrease in the mean value of treatment groupT1 after two weeks of administration.

The mean value of treatment group T2, T3 and T4, also decreased after two weeks of administration.

After one week of withdrawal, the mean weight value of treatment group T3 was the same. After two weeks of withdrawal, the mean weight value of of treatment group T3 drastically increased.

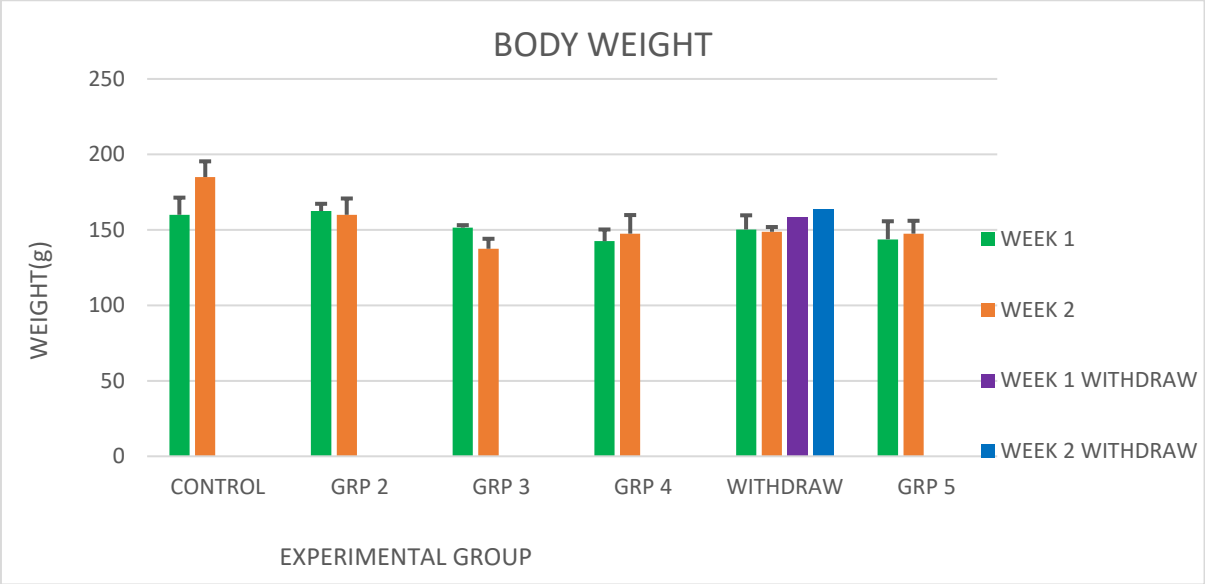
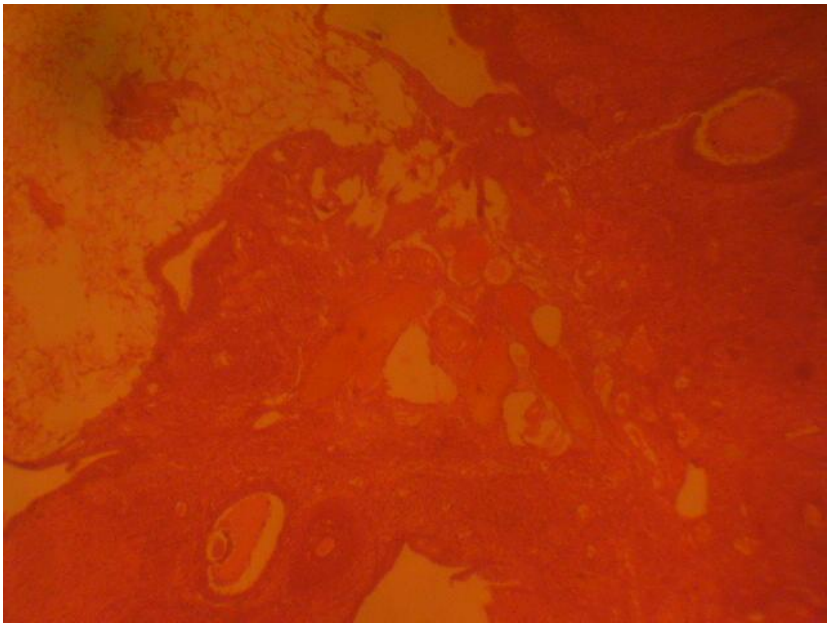


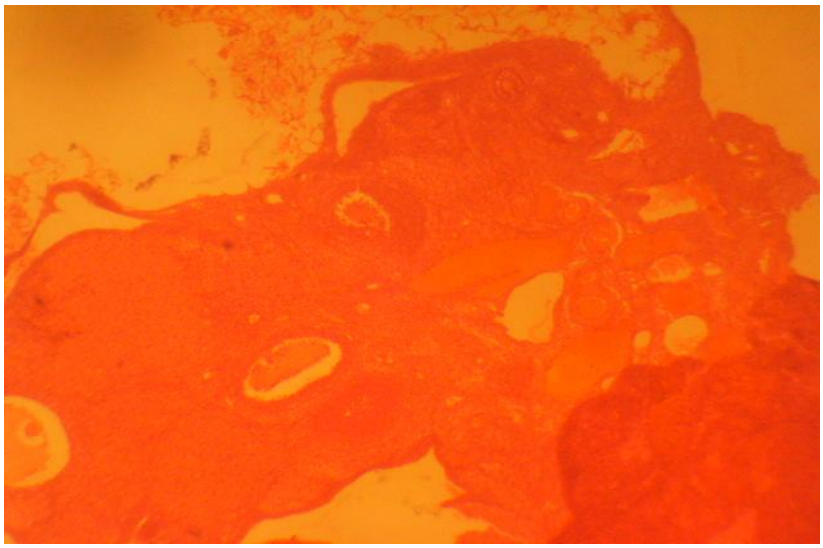
Fig.2: A graph showing the body weights of all the groups.

4.4 PLATES

PHOTOMICROGRAPH OF CONTROL



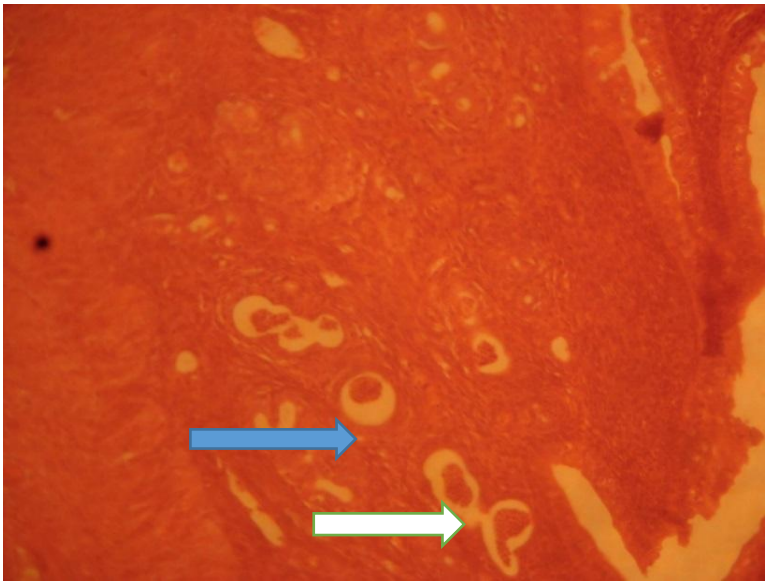
X100



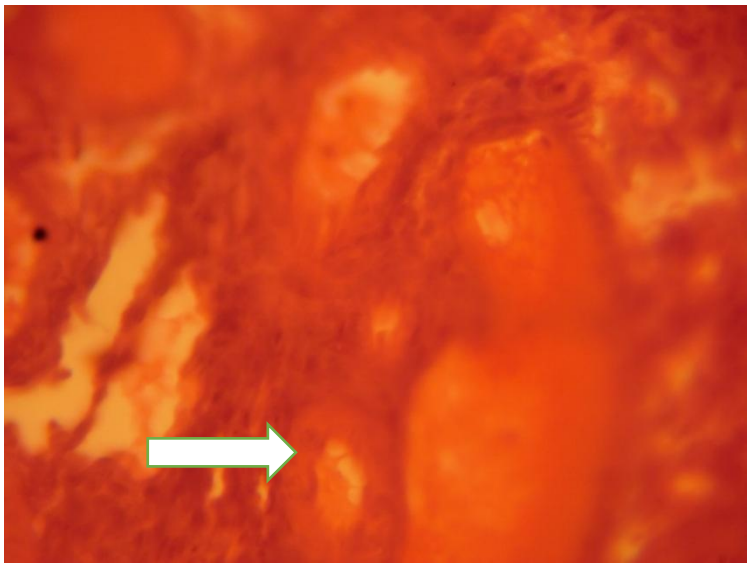
X400

PLATE 1& 2; Photomicrograph (H&E) of Control Group showing normal ovary with elongated flattened body, intact blood vessels and well organized stroma and granulosa.

PHOTOMICROGRAPH OF GROUP T1



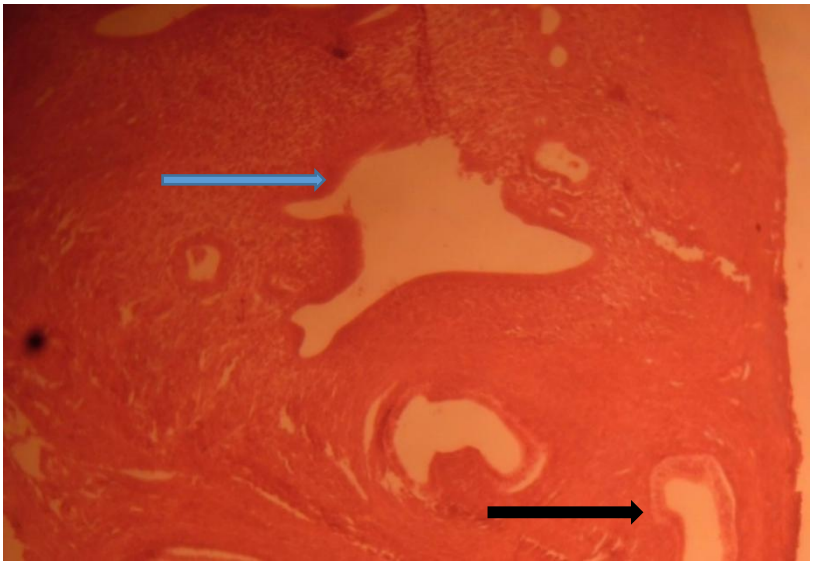
X100



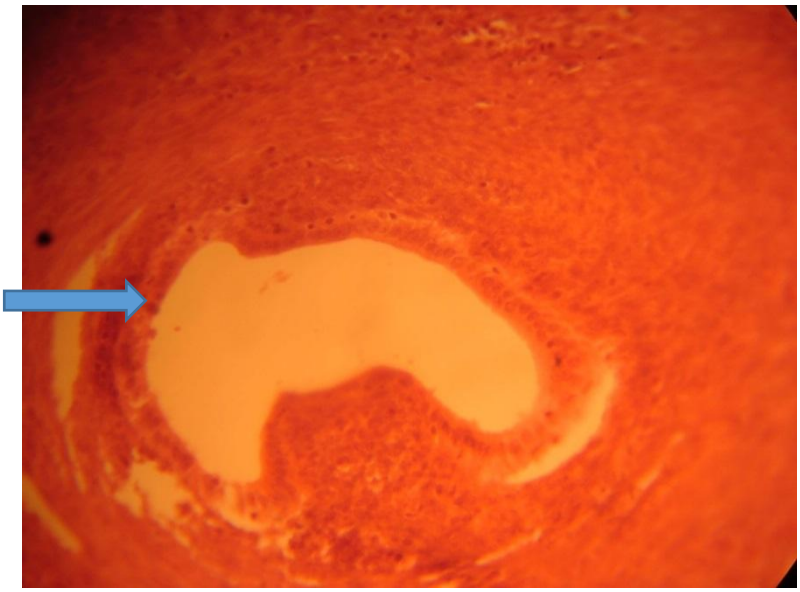
MAGNIFICATION: X400

PLATE 3 &4;Photomicrograph (H&E) of Group T1 showing fibrotic granulosa cells (blue arrow) and ruptured follicle (white arrow)

PHOTOMICROGRAPH OF GROUPT2



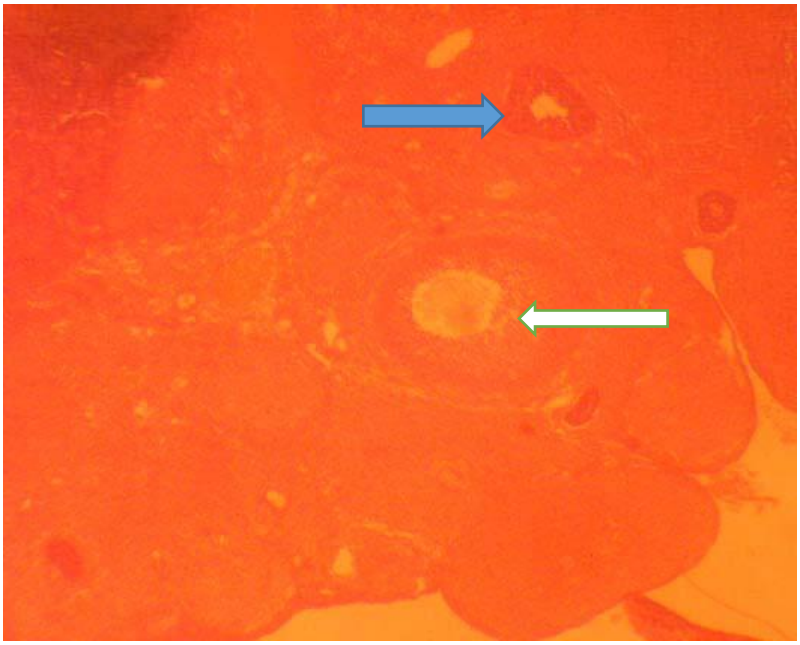
MAGNIFICATION:X100



MAGNIFICATION: X400

PLATE 5&6; Photomicrograph of Group 2 (T2) shows Degenerated follicle (blue arrow), no developing follicles, old degenerated luteum (black arrow).

PHOTOMICROGRAPH OF GROUP T3



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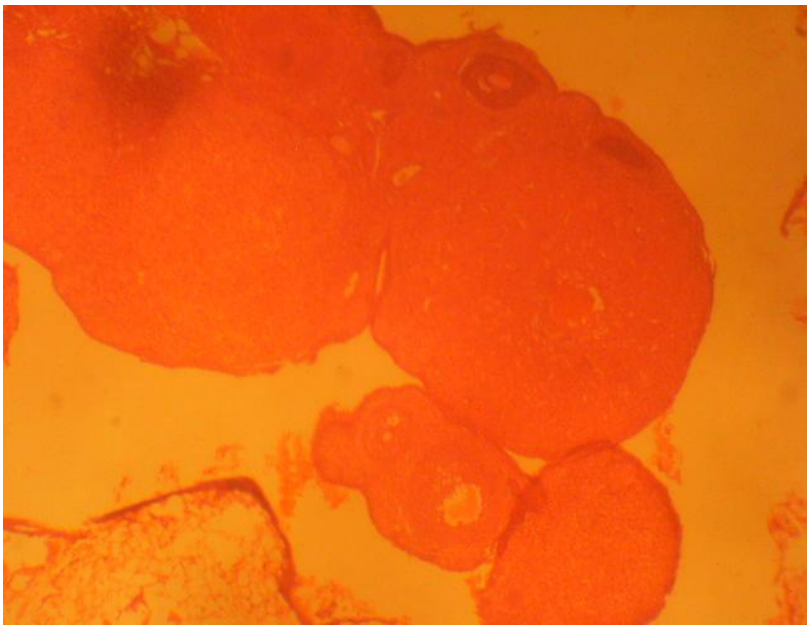
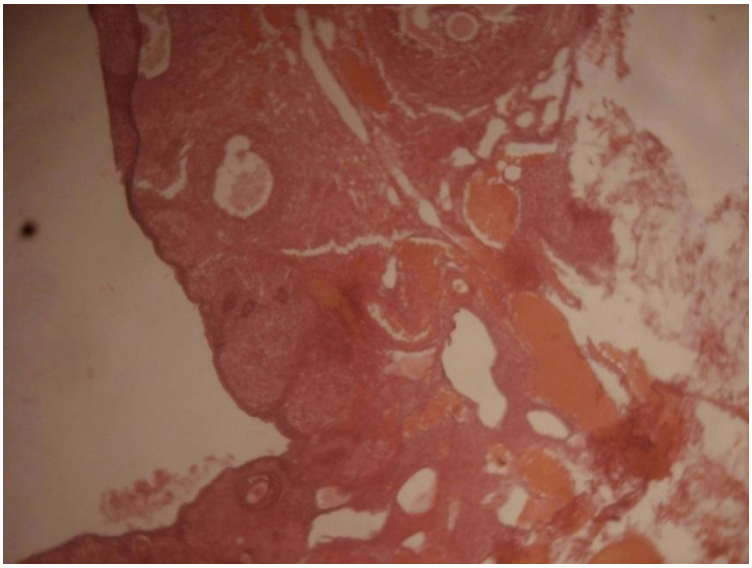


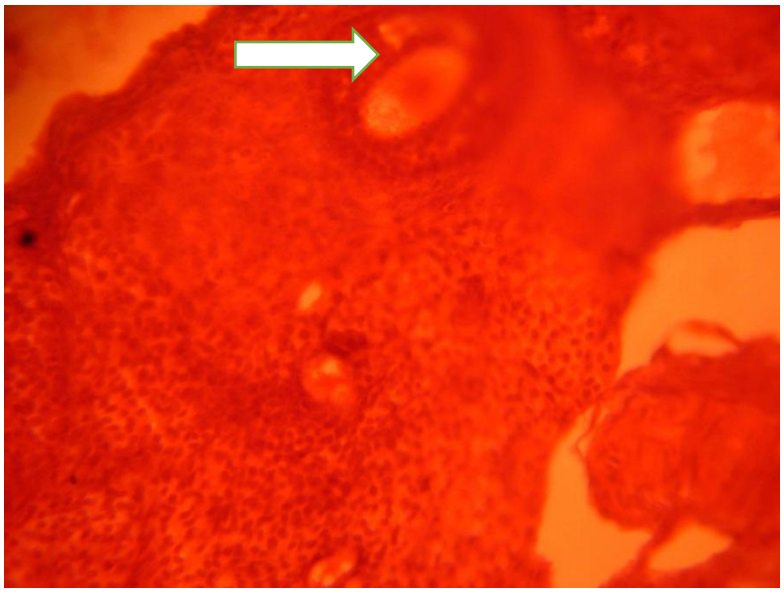
MAGNIFICATION:X400

PLATE 7&8: Photomicrograph (H&E) of Group T3 showing no developing follicles, preserved primordial follicle (blue arrow) and primary follicle (white arrow).

PHOTOMICROGRAPH OF GROUP 4



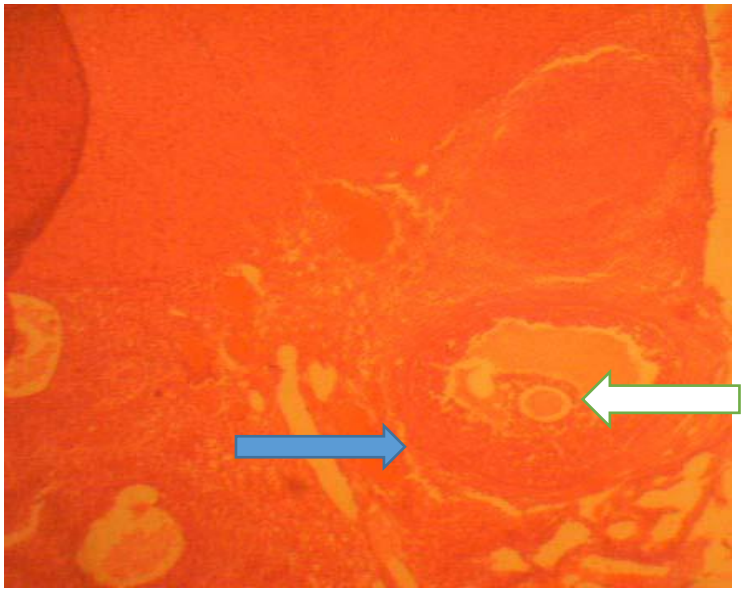
Magnification X100



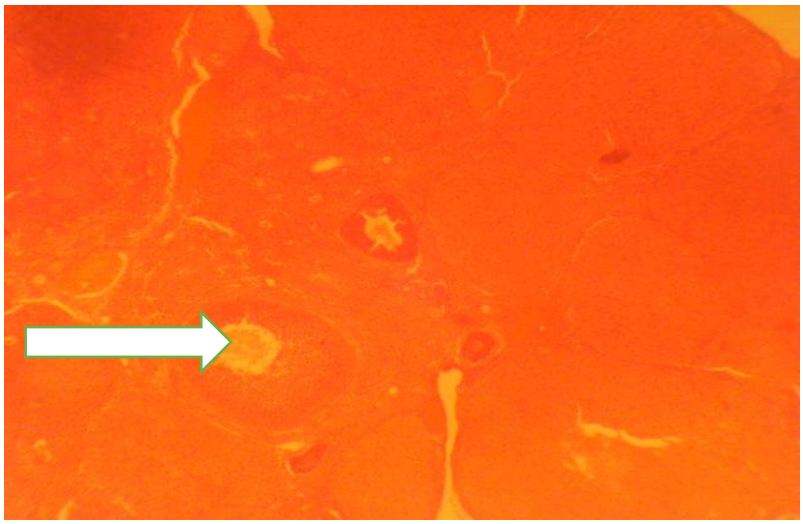
MAGNIFICATION: X400

PLATE 9&10;Photomicrograph (H&E) of Group T4 shows densely distributed oocyte (white arrow), well organized stroma and presence of corpora lutea (blue arrow), separated by fissures and scares.

PHOTOMICROGRAPH OF GROUP WT3



MAGNIFICATION: X100



MAGNIFICATION: X400

PLATE 11&12;Photomicrograph (H&E) of Withdrawal Group (WT3) shows oocyte regeneration (white arrow)and follicular improvement (blue arrow).

CHAPTER FIVE

5.0 DISCUSSION

The present study evaluated the effects of aqueous extract of *Garcinia kola* seed, administered alone and in combination with vitamin C, on the ovarian histology, ovarian weight and body weight of adult female Wistar rats. Histological examination revealed that exposure to *Garcinia kola* seed extract for two weeks altered the normal architecture of the ovary, with the severity of the changes becoming more pronounced as the administered dose increased. Rats treated with 100 mg/kg and 200 mg/kg of the extract exhibited varying degrees of follicular degeneration, including rupture of immature follicles and fibrotic changes within the ovarian tissue. These observations indicate that the extract adversely affected follicular integrity, a process that could interfere with normal follicular maturation and ovulation. Normal ovarian function depends on the coordinated growth of healthy follicles and the preservation of granulosa cell integrity throughout folliculogenesis (JoAnne et al., 2010; Xie et al., 2023). Once these structures are disrupted, the probability of successful ovulation and subsequent conception may be reduced. Similar histological alterations following administration of kolaviron have previously been reported by Sunday et al. (2013), who demonstrated that prolonged exposure to *Garcinia kola* constituents affected the hypothalamo-pituitary-gonadal axis and altered ovarian morphology in experimental animals.

Although ovarian damage was observed in both treatment groups, the lesions appeared more severe among animals that received 200 mg/kg of the extract. Unlike the lower-dose group, where some apparently normal follicles remained, the higher-dose group showed extensive disruption of developing follicles, suggesting a dose-dependent response. Experimental evidence indicates that excessive exposure to bioactive phytochemicals may disturb follicular development by altering cellular signalling pathways, increasing oxidative stress and disrupting granulosa cell function (Liu et al., 2023). Since granulosa cells provide nutritional and hormonal support required for oocyte maturation, structural damage to these cells is likely to compromise follicular survival and reduce reproductive capacity (Esencan et al., 2022). The present findings therefore suggest that increasing the

dosage of *Garcinia kola* extract may progressively reduce the ovary's ability to sustain normal folliculogenesis.

The reduction observed in ovarian weight among animals treated with the higher dose of *Garcinia kola* further supports the histological findings. Ovarian weight is frequently used as an indirect indicator of follicular activity because growing follicles, stromal expansion and increased vascularity contribute to the overall size of the ovary during the reproductive cycle (JoAnne et al., 2010). Consequently, degeneration of follicles and reduced follicular development may result in decreased ovarian mass. The anti-inflammatory activity previously reported for *Garcinia kola* (Madubunyi, 1995) may also contribute to this observation, as inflammatory mediators play essential physiological roles during follicular rupture and ovulation. Earlier studies demonstrated that inhibition of inflammatory pathways can suppress ovulation and impair follicular development (Gaytán et al., 2002). The present findings therefore provide additional evidence that excessive exposure to *Garcinia kola* extract may interfere with normal ovarian physiology.

With respect to body weight, administration of *Garcinia kola* extract did not produce a statistically significant overall reduction throughout the experimental period, although animals receiving the higher dose showed a tendency towards lower body weight compared with the control group. This finding agrees with reports by Akpantah et al. (2003) and Iranloye et al. (2006), who observed no marked changes in body weight following administration of *Garcinia kola* extract. However, it differs from the observations of Braide and Grill (1990), who reported growth retardation after prolonged dietary exposure to powdered *Garcinia kola* seeds. Variations in dosage, method of extract preparation, duration of administration and experimental design may explain these differences across studies.

An important finding of the present study is the apparent protective influence of vitamin C on ovarian tissue when administered concurrently with *Garcinia kola* extract. Although vitamin C did not completely prevent the reduction in ovarian weight or stimulate the formation of numerous new follicles during the treatment period, histological examination indicated better preservation of existing follicles in animals that received both vitamin C and the extract compared with those treated with *Garcinia kola* alone. This observation is biologically plausible because vitamin C serves as one of the major non-enzymatic antioxidants in mammalian tissues, where it neutralises reactive oxygen species and regenerates other antioxidant molecules, including vitamin E (Carr & Frei, 1999). More recent studies have also shown that vitamin C protects granulosa cells against oxidative injury, preserves mitochondrial function and promotes survival of ovarian follicles under conditions of oxidative stress (Vašková et al., 2023; Gomes et al., 2023; Liu et al., 2023). The partial preservation of follicular architecture observed in this study may therefore be attributed to the antioxidant capacity of vitamin C, which possibly reduced oxidative damage induced by *Garcinia kola* constituents.

Animals that received vitamin C alone exhibited well-preserved ovarian architecture with healthy follicles, suggesting that vitamin C supports normal follicular development under physiological conditions. This observation agrees with recent evidence that adequate antioxidant status contributes to maintenance of ovarian homeostasis, protects oocytes from oxidative injury and promotes normal folliculogenesis (Vašková et al., 2023). Since oxidative stress has been implicated in several reproductive disorders, antioxidant supplementation may improve ovarian function by maintaining cellular integrity within the follicular environment (Awonuga et al., 2023).

The most encouraging observation from this study was the regeneration of ovarian follicles following withdrawal of *Garcinia kola* treatment. Animals maintained for an additional two weeks after cessation of treatment demonstrated appreciable recovery of ovarian architecture, including evidence of follicular regeneration. This suggests that the ovarian changes induced by the extract may not be permanent under the conditions investigated. Ovarian tissue possesses a remarkable capacity for repair when the causative insult is removed, provided

that sufficient healthy follicles remain within the ovarian reserve (Esencan et al., 2022). The regenerative changes observed in the withdrawal group therefore indicate that discontinuation of *Garcinia kola* administration, particularly when accompanied by antioxidant support, may allow partial restoration of normal ovarian structure and function.

5.1 CONCLUSION

Garcinia Kola Extract significantly brings about impaired ovulation; therefore, GKSE may be used for family planning with the possible advantage of reversal. Simultaneous consumption of GKSE and Vitamin C poses more possibility for conception as compared to those who do not consume GKSE with vitamin C. Vitamin C have significance in protecting follicles of wistar rat while ingesting Garcinia Kola. The greater the dosage of Garcinia kola being consumed, the lesser the tendency of ovulation

5.2 RECOMMENDATION

From this study, Garcinia Kola may be ingested but stopped few weeks to desired time of conception.

Further studies could be carried out using other kinds of Antioxidants like vitamin E (α-Tocopherol) which also has a positive influence on female's reproductory organ. Also, studies on Hormonal assay could be carried out on Follicular stimulating Hormone (FSH), Luteinizing Hormone (LH), Estrogen and Progesterol in relation to endocrine regulation, when treated with Garcinia kola seed and other Antioxidant.

Vitamin C may be used by adult female of reproductory age who is planning on conceiving as it improves ovulation.

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APPENDIX

Table 3 The Descriptive and Inferential Statistics for Body Weight of Wistar Rats During Administration.

Table Analyzed	BODY WEIGHT		
One-way analysis of variance			
P value	0.0081		
P value summary	**		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	6		
F	3.542		
R squared	0.2616		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	6.092		
P value	0.2974		
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (Between groups)	5112	5	1022
Residual (Within groups)	14430	50	288.7
Total	19540	55	

Tukey's Multiple Comparison Test	Mean Diff.	q		Significant? P < 0.05?	Summary	95% CI of diff
Control vs T1	11.25		1.873	No	ns	-13.96 to 36.46
Control vs T2	27.97		4.656	Yes	*	2.755 to 53.18
Control vs T3	27.5		4.578	Yes	*	2.286 to 52.71
Control vs WT3	17.13		3.292	No	ns	-4.711 to 38.96
Control vs T4	26.88		4.474	Yes	*	1.661 to 52.09
T1 vs T2	16.72		2.783	No	ns	-8.495 to 41.93
T1 vs T3	16.25		2.705	No	ns	-8.964 to 41.46
T1 vs WT3	5.875		1.129	No	ns	-15.96 to 27.71
T1 vs T4	15.63		2.601	No	ns	-9.589 to 40.84
T2 vsT3	-0.4688		0.07804	No	ns	-25.68 to 24.75
T2 vsWT3	-10.84		2.084	No	ns	-32.68 to 10.99
T2 vsT4	-1.094		0.1821	No	ns	-26.31 to 24.12
T3 vs WT3	-10.38		1.994	No	ns	-32.21 to 11.46
T3 vs T4	-0.625		0.104	No	ns	-25.84 to 24.59
WT3 vs T4	9.75		1.874	No	ns	-12.09 to 31.59

Table 4 The Descriptive and Inferential Statistics for Ovary Weight of Wistar Rats During Administration.

Table Analyzed	OVARY WEIGHT
One-way analysis of variance	
P value	0.01
P value summary	**
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	3.897
R squared	0.4481
Bartlett's test for equal variances	
Bartlett's statistic (corrected)	14.21
P value	0.0144

P value summary	*
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (Between groups)	0.4183	5	0.0837
Residual (within groups)	0.5152	24	0.0215
Total	0.9335	29	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Control vs T1	-0.0716	1.093	No	ns	-0.3581 to 0.2149
Control vs T2	0.232	3.541	No	ns	-0.05455 to 0.5185
Control vs T3	0.2636	4.023	No	ns	-0.02295 to 0.5501
Control vs WT3	0.109	1.663	No	ns	-0.1775 to 0.3955
Control vs T4	0.1202	1.834	No	ns	-0.1663 to 0.4067
T1 vs T2	0.3036	4.633	Yes	*	0.01705 to 0.5901
T1 vs T3	0.3352	5.115	Yes	*	0.04865 to 0.6217
T1 vs WT3	0.1806	2.756	No	ns	-0.1059 to 0.4671
T1 vs T4	0.1918	2.927	No	ns	-0.09475 to 0.4783
T2 vsT3	0.0316	0.4822	No	ns	-0.2549 to 0.3181
T2 vs WT3	-0.123	1.877	No	ns	-0.4095 to 0.1635
T2 vs T4	-0.1118	1.706	No	ns	-0.3983 to 0.1747
T3 vs WT3	-0.1546	2.359	No	ns	-0.4411 to 0.1319
T3 vs T4	-0.1434	2.188	No	ns	-0.4299 to 0.1431
WT3 vs T4	0.0112	0.1709	No	ns	-0.2753 to 0.2977