EVALUATION OF THE ANTI-FERTILITY PROPERTIES OF *Cissus rotundifolia* (Forssk.) Vahl. EXTRACT IN FEMALE WISTAR RATS (*Rattus norvegicus*)

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Animal Physiology of Egerton University

EGERTON UNIVERSITY

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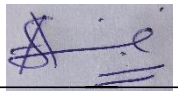
DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted or presented for examination in any institution.

Signature:  Date: 18/9/2020

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Recommendation

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations.

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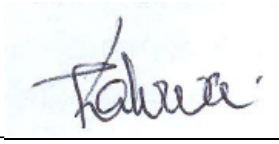
Signature:  Date: 21/9/2020

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DEDICATION

To my siblings Oliver and Rozinah and my mother, Ruth Wambua Kallberg, who has done all she can to establish the foundation in my education;

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ABSTRACT

Sexual and reproductive ill-health is responsible for over one-third of the global burden of disease among women of reproductive age (15-45 years). Approximately 200 million women in the developing world have an unmet contraceptive need. Consequently, about fifty-one million unintended pregnancies occur each year due to contraceptive none use and has led to unsafe abortions being carried out. In Kenya, 465 thousand abortions were carried out in 2012. Conventional steroid and non-steroid based compound contraceptives are effective when used according to directions but cause considerable side effects. Therefore, the search for novel, efficacious, affordable, reversible and safe anti-fertility agents with minimal side effects is imperative. In Tana River County of Kenya, the plant *Cissus rotundifolia* (Forssk.) Vahl., commonly known as Arabian wax leaf, has been traditionally used as a fertility regulator. The aim of this study was to validate the anti-fertility efficacy of the crude extract using Wistar rat model. The plant was obtained from Tana River County and transported to the University of Nairobi, where it was dried and an aqueous extract prepared. The acute oral toxicity on the aqueous plant extract was done in accordance to the OECD 423 guidelines. The phytochemical compounds present in *Cissus rotundifolia* aqueous extract were determined. The effect of the plant extract on the oestrus cycle was determined by administering 400, 800mg/kg of the extract and 0.5 ml physiological saline to three groups of five rats each respectively for 14 days. Vaginal smear cytological features from all rats were monitored daily between 9 and 10 am. The effect of *C. rotundifolia* aqueous extract on other reproductive parameters such as mating success, fertility index, litter size, and on ovarian and uterine histomorphology of Wistar rats was also determined. The plant extract caused no significant gross pathological changes in all the vital organs observed at dose level 300, 2000 and 5000mg/kg in the acute oral studies. The phytochemical studies showed that *Cissus rotundifolia* extract had alkaloids, tannins, saponins, phenols and glycosides. The plant extract caused a dose-dependent significant increase in mean frequency of proestrus and metestrus phases and a significant reduction in estrus and diestrus phases ($p<0.05$). The plant extract did not cause a significant effect on mating success. It also caused a dose dependant reduction in fertility index in pre-mating, post-mating and pre-post treatment groups ($p<0.05$) compared to the control. The structural integrity of the pre-antral follicles, antral follicles, corpora lutea and uterine endothelial lining was compromised by *Cissus rotundifolia* extract at both doses. Based on the findings of this study, this plant extract has anti-ovulatory and anti-implantation properties and is a potential anti-fertility agent. Future studies should consider evaluating the reversible anti-fertility properties in laboratory animals.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AIDS	Acquired Immunodeficiency Syndrome
CL	Corpus luteum
FSH	Follicle Stimulating Hormone
HIV	Human Immunodeficiency Virus
IUDs	Intrauterine devices
LARC	Long Acting Reversible Contraceptive
LD	Lethal Dose
LH	Luteinizing Hormone
MOH	Ministry of Health
OECD	Organisation for Economic Co-operation and Development
PGCs	Primordial Germ Cells
SDGs	Sustainable Development Goals
SEM	Standard Error of the Mean
SPSS	Statistical Package for the Social Sciences
TFR	Total Fertility Rate
TMPs	Traditional Medicine Practitioners
UoN	University of Nairobi
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION

1.1 Background Information

The achievement of reproductive health is crucial for individual and family well-being and it is important for economic and social development. The United Nations Sustainable Development Goals (SDGs) adopted in 2016, emphasize the eradication of extreme poverty, promoting gender equality and empowering women, good health and well-being for people among other goals (Mazza *et al*., 2017; Pradhan *et al.*, 2017).

Although several key elements of sexual and reproductive health are justified in goal three of the SDGs, a measure of women's capacity to regulate their fertility safely and effectively is missing. It is also widely recognized that universal access to reproductive health care, including family planning and sexual health, is essential for the achievement of all of the SDGs (Marston & Cleland, 2003). Sexual and reproductive ill-health is responsible for over one-third of the global burden of disease among women of reproductive age (15-49 years) (Lule *et al*., 2007; Ochako *et al*., 2015). This figure represents about 18 % of the overall disease burden. There has been a significant rise in access to contraceptives globally. However, despite the increase, an estimated 137 million women in the developing countries still have unmet contraceptive need. The strong desire to regulate fertility combined with lack of access to effective contraceptive results in the largest number of unintended pregnancies. Consequently, about fifty-one million unintended pregnancies occur yearly (Mazza *et al*., 2017).

An additional 25 million unwanted pregnancies occur due to inconsistent or incorrect use of contraceptive methods or failure of the method (Lule *et al*., 2007). Failure to prevent pregnancies leads to induced abortion in some women. Abortion carried out in back streets and under unhygienic conditions results in high mortality and morbidity rates. Approximately 25% of maternal mortality is caused by unsafe abortion (Ochako *et al*., 2015). Insufficiently spaced pregnancies bear significant health and economic consequences for both the child and the mother (Sedgh *et al*., 2007).

In Africa, nearly half of the population is under 25 years (Sedgh & Hussain, 2014). Most live in low resource countries where reproductive health services are not readily accessible. Thus, obstetric complications such as obstructed labour, eclampsia and fistula are quite common (Kaingu *et al*., 2017). The risks to unmarried women and adolescents living in resource-poor

settings are further compounded by unintended pregnancies and 'backstreet' abortion options (Rutstein, 2005).

In Kenya, 75% of health facilities and personnel are concentrated in urban areas (Kaingu *et al*., 2013). Unmet need for family planning occurs when couples or individuals are currently not using any form of contraceptive yet they wish to postpone or avoid pregnancy (Sedgh & Hussain, 2014). Factors contributing to the unmet need include inadequate access to contraceptive services and correct information, myths about modern contraception, lack of knowledge about contraceptives and social disapproval (Sedgh & Hussain, 2014). Poor couples are less likely to have access to family planning and other reproductive health services (Kaingu *et al*., 2013; Medicinal, 2005). Fertility regulation and the demand for contraception correlate to the women's educational level, husband's occupation, employment status, degree of autonomy and place of residence (Lule *et al*., 2007). Persistent inadequate funding in family planning by the Ministry of Health (MOH) has also impacted to inadequate distribution and inaccessibility of contraceptives (Ross & Winfrey, 2001). It is thus evident why many women in rural parts of sub-Saharan Africa have an unmet need for contraception (Ernd, 2005).

There is a considerable gap between the actual and desired family sizes as a result of the traditional knowledge related to the health of humans and animals. According to Shinwari and Khan (1998), 30% of pharmaceutical preparations used for conventional medicine preparation are based on plants. Steroid and non-steroid based compounds used as contraceptives are effective when used according to directions but carry considerable side effects (Campbell & Graham, 2006). Inadequate access and skills gap in certified health officers has contributed to some women seeking traditional medicine practitioners in fertility regulation (Sedgh *et al*., 2007). There is renewed interest, spearheaded by the World Health Organization (WHO), in the use of medicinal plants for primary healthcare needs. This has prompted research into the potential of such plants in fertility regulation. To alleviate the unmet contraceptive need in Tana River, this study was carried out to validate the anti-fertility potential of *Cissus rotundifolia* plant traditionally consumed by women in Tana River County, Kenya.

1.2 Statement of the Problem

Fertility regulation is a necessary determinant of economic and social development. The use of contraceptives among women in Kenya and other developing countries has been on the rise despite considerable barriers to their use. These barriers include myths, side effects,

health concerns, social opposition, inadequate or total lack of reproductive health services or information, disapproval from family members and religious beliefs. The stringent regulatory measures and inadequate access to contraceptives culminate in unintended pregnancies that might result in unsafe abortions, high morbidity and poor maternal health. Abortions result in 25% maternal mortality rates. Unsafe abortions are a leading cause of maternal morbidity and mortality in Kenya. Therefore, unsafe abortions and their associated complications will continue to be a constant feature with the continued inadequate access to contraceptives. The use of the current hormonal contraceptives is associated with a myriad of side effects. The contraceptives are also not readily available to all women at all times due to funding; thereby supply challenges. Therefore, there is need to explore safe, efficacious reversible medicinal alternatives that would address the current unmet contraceptive need in rural areas.

1.3 Objectives

1.3.1 General objective

To contribute to contraception through use of plant extracts.

1.3.2 Specific objectives

i) To determine the acute oral toxicity of *C. rotundifolia* aqueous extract in female Wistar rats.

ii) To determine the phytochemical compounds present in *C. rotundifolia* aqueous extract.

iii) To determine the effect of *C. rotundifolia* aqueous extract on oestrus cyclicity in Wistar rats.

iv) To determine the effect of *C. rotundifolia* aqueous extract on mating success, fertility index, gestation length and litter size in Wistar rats.

v) To determine the effect of *C. rotundifolia* aqueous extract on Wistar rat ovarian and uterine histomorphology.

1.4 Hypotheses

H_{01}: There is no significant difference in the acute oral toxicity of *Cissus rotundifolia* aqueous extract in female Wistar rats.

H_{02}: There are no phytochemical compounds present in *Cissus rotundifolia* aqueous extract.

H_{03}: *Cissus rotundifolia* aqueous extract has no significant effect on oestrus cyclicity in Wistar rats.

H_{04}: *Cissus rotundifolia* aqueous extract has no significant effect on mating success, fertility index, gestation length, and litter size in Wistar rats.

H_{05}: *Cissus rotundifolia* aqueous extract has no significant effect on Wistar rat ovarian and uterine histomorphology.

1.5 Justification

Global maternal mortality rate remains high. Some of the reproductive issues that women face globally range from pregnancy and associated complications, fertility issues, and menstrual complications. This constitutes 18% of the global burden of disease for women of reproductive age. Traditional medicine practitioners (TMPs) indigenous knowledge is mostly passed on verbally from generation to generation. Almost none is recorded. It is thus crucial to verify this information by studying the properties of the plants to ascertain the credibility of their use in reproductive health as claimed by herbalists. In Tana River County, TMPs are the community resource persons and are routinely consulted because of their wide indigenous medicinal knowledge base. This tradition has persisted in many rural communities due to inequitable health provision. Of the 20 million unsafe abortions performed each year, over 90% are carried out in developing countries. Illegal abortions in rural areas are mostly due to unmet contraceptive need. The unmet need contributes to the need to validate the efficacy and safety of alternative methods that are readily accessible to women in rural areas. Some conventional contraceptives have undesirable side effects; others are abhorred and are yet to be embraced by cultural societies. Consequently, research into the anti-fertility properties of medicinal plants is necessary to explore alternative forms of contraceptive technologies. Therefore, this study evaluated the effect of *C. rotundifolia* plant extract on various reproductive parameters to ascertain its anti-fertility properties.

1.6 Scope and Limitations of the Study

1.6.1 Scope

This study focused on the overall evaluation of the anti-fertility properties of *Cissus rotundifolia* using female Wistar rat model. The study only used the roots of the plant; which were collected from Tana river County. To investigate the anti-fertility properties of the plant reproductive parameters such as oestrus cyclicity, mating success, fertility index, gestation length and litter size in Wistar rats were determined. The study also covered the acute oral toxicity, qualitative analysis of the phytochemical compounds and histomorphology of the ovary and uterus of the Wistar rats. However, this study did not cover the quantitative phytochemical screening and hormonal profile on the effect of the plant extract on reproductive hormones.

1.6.2 Limitations

Sourcing a large number of rats at once to be used for the specific objectives was a challenge hence delaying data collection. There were sudden deaths of some experimental rats during the study hence affecting the data collected. The hormonal assay kits were very expensive hence limiting the determination of the how the reproductive hormones were affected by plant extract.

CHAPTER TWO
LITERATURE REVIEW

2.1 An overview of the mammalian female reproductive process and physiology

A mammalian female reproductive system is a group of organs that are well-coordinated to undertake processes such as oogenesis, ovulation, fertilization, implantation, and gestation once fertilization occurs.

2.1.1 Oogenesis and ovulation in mammalian females

Reproductive success in females is dependent on the dual function of the ovary; oogenesis (growth and development of the oocyte) and steroidogenesis (production of steroid hormones). These ovarian processes ensure the delivery of viable ova for fertilization and play a key role in establishing the appropriate environment for maintenance and development of the fertilized ovum (Ahman & Shah, 2007). During the development of the oocyte (oogenesis), the primordial germ cells (PGCs) give rise to oogonia after their migration to the genital ridge through mitosis. The migration, proliferation and colonisation of the PGCs depend on their interaction with associated somatic cells. Before the formation of the follicle, mitotic divisions cease and meiosis is initiated by the germ cells to form primary oocytes in a process called oocytogenesis. Germ cell nests breakdown to form somatic pre-granulosa cells that surround oocytes, which initiates the formation of primordial follicles during meiotic arrest (Ankush *et al*., 2011).

During follicular development, the follicles containing the oocytes undergo both morphological as well as biochemical alteration. Such changes include steroid responsiveness and production, enzyme activation, enhanced sensitivity to gonadotropins and receptor expression. Alteration of any of these variables can result in impaired or complete reproduction failure. Progression in all the antral stages and ovulation is reliant on the support of the pituitary-secreted gonadotropins which are follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Ahman & Shah, 2007).

Follicle stimulating hormone (FSH) mediates antral development. Under its influence, granulosa cells produce oestrogen. The oestrogen, in turn, promotes further differentiation of the granulosa cells itself. Oestrogen essential for the increase of crucial enzyme activities triggers the onset of a pre-ovulatory luteinizing hormone (LH) surge and prevents premature biochemical luteinization. In response to luteinizing hormone, the selected Graafian follicle ruptures releasing the ovum into the fallopian tube (ovulation). Following ovulation, the

remaining cellular components of the ruptured follicle, involutes and vascularity increases (Piyali *et al*., 2012). They are then transformed into luteal cells under the influence of LH. The luteal cells form a transient endocrine structure referred to as corpus luteum (CL) which secretes progesterone hormone. Progesterone hormone is responsible for the growth and development of the uterine endometrium layer in preparation for implantation of the blastocyst (Dugoua, 2011).

2.1.2 Fertilization

Successful fertilization is characterized by a complex interaction between spermatozoa and ovum. The initial step in fertilization is the binding of spermatozoa to the zona pellucida which is a glycoprotein-rich thick, transparent extracellular coat that surrounds the plasma membrane of an oocyte (ovum). The binding of the spermatozoa to the zona pellucida is then followed by the acrosome reaction and penetration of spermatozoa through the zona pellucida. The latter is crucial in fertilization and oocyte development. The final step involves the fusion of the egg and sperm pronuclei (Ankush *et al*., 2011).

The zona pellucida is vital for viable oocyte development in preparation for fertilization (e.g., in gamete recognition at the zona pellucida), in the prevention of polyspermia and the protection of early embryos. For the blastocyst to be competent, it has to shed its zona pellucida. Shedding of the zona pellucida or interference with its structural integrity tampers with nutrient exchange and other molecular substances through the zona pellucida (Ahman & Shah, 2007).

2.1.3 Implantation and pregnancy establishment

The integrity of the blastocyst determines the success of implantation and the synchronization that occurs between the developmental stages of the embryo. The complex implantation process includes factors such as the paracrine, autocrine and endocrine processes and it is modulated by features that are not clearly known and which are still under research. Molecular mechanisms that occur during implantation of the embryo have been demonstrated using animal models. Despite the well-documented implantation research, early pregnancy loss still occurs before or during implantation (Ankush *et al*., 2011).

For implantation to be successful it depends on the achievement of proper development of the embryo to the blastocyst stage and at the same time, the development of a receptive endometrium that will accept embryo implantation and establishment (Kubota *et al*., 2016). The endometrium is receptive only for short periods in rodents and humans. Beyond this

"window of implantation" period, the embryo is unable to successfully establish contact with a refractive endometrium (Rodrigues *et al*., 2015).

2.1.4 Overview of hormonal regulation

The hypothalamus, through its secretion of gonadotropin-releasing hormone, stimulates anterior pituitary gland to produce the gonadotropins FSH and LH (Beshay & Carr, 2012). The two gonadotropins act on the ovaries to promote follicle development, ovulation, corpus luteum formation and ovarian steroid hormones secretion. Nuclear oestrogen and progesterone receptors mediate many of the actions of oestrogen and progesterone hormones (Kubota *et al*., 2016). Mice lacking β oestrogen receptor (ERβ) exhibit a lack of progression from pre to antral follicles (Emmen *et al*., 2005). Steroid synthesis inhibitors decrease the survival and growth of antrum follicles and reduce estradiol production by secondary follicles (Rodrigues *et al*., 2015). Oestrogen is required for normal follicle health; it inhibits granulosa cell apoptosis, diminishes follicle atresia in rats (Piyali *et al*., 2012) and increases the number and size of rodent and bovine follicles (Rodrigues, 2007).

Progesterone is also anti-apoptotic but at high doses inhibits follicle growth. Indeed, studies in the rat suggest that progesterone is not necessary for antral follicle growth (Piyali *et al*., 2012). Rats possessing null mutations at the progesterone receptor (null female) exhibit failure in ovulation and ovulatory processes and are thereby infertile (Kubota *et al*., 2016). Under the influence of oestrogen and progesterone, the endometrium undergoes a transition and acquires an appropriate morphological and functional state; referred to as the "window of implantation" during which the blastocyst attaches to the endometrium (Beshay & Carr, 2012). Oestradiol is critical for the growth and thickening of the endometrium; while progesterone is responsible for endometrial gland proliferation and secretion which has nutritive value to the embryo and also essential for implantation (Kubota *et al*., 2016).

The establishment of pregnancy requires the presence of a functional corpus luteum (CL) that is able to produce sufficient progesterone. A viable conceptus sends specific signals to a "pregnancy-ready" uterus; these signals rescue the corpus luteum from luteolysis. Maternal recognition of pregnancy in rodents involves activation of the non-functional CL of the estrous cycle into the functional CL of pregnancy. The formation and maintenance of CL and production of progesterone require two events. First, mating induces the release of prolactin from the anterior pituitary, which increases LH receptors on luteal cells to form the CL and suppress 20 α-hydroxysteroid dehydrogenase activity; this transition prevents the conversion

of progesterone to 20 α-hydroxyprogesterone, which does not support pregnancy (Ahman & Shah, 2007). Second, the lactogenic hormones that are produced by the uterine decidua and placenta act through prolactin receptors on the luteal cells to maintain their function and the production of progesterone throughout gestation. Luteinizing hormone (LH) is key in luteolization of the granulosa cell remnants following ovulation. A disruption of LH release will, therefore, compromise CL formation, lead to inadequate levels of progesterone which in-turn interferes with the decidualization of the endometrium leading to failed implantation (Pang *et al*., 2011).

2.2 Medicinal plants phytochemistry

Phytochemicals, also called bio-nutrients, are a wide variety of bioactive compounds that occur naturally in plants. They are termed as bioactive because of their ability to interact with one or more components of living tissue causing a wide range of effects. Medicinal plants are known to be a rich source of phytochemicals (Mamta *et al.*, 2013).

Phytochemicals are of two types; primary and secondary compounds. The primary phytochemicals include chlorophyll, proteins, sugars and amino acids. While secondary phytochemical compounds include terpenoids, alkaloids, saponins, phenolics, tannins, flavonoids and glycosides (Roy *et al.*, 2018). The phytochemical compounds play an important role in plants by protecting the plant cells from pathogenic attack and environmental hazards such as pollution, stress, drought and damage. They also contribute to the plant's colour, aroma and flavour. Phytochemical compounds accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds. Their levels vary from species to species depending on the variety (Lu *et al*., 2010).

Phytochemical compounds are also known to play a role in human beings such as anti-inflammatory, antioxidant, lipid-lowering, anti-hypertensive and regulating the expression of a great variety of genes (Yahia, 2011). Thus, they offer several health benefits, including anti-aging, cardiovascular disease prevention and protection against chronic diseases such as diabetes mellitus, cancer and neurodegenerative diseases. Studies have also shown that these phytochemical compounds affect reproduction in several ways. For instance, *Aspilia africana* leaf extract was shown to contain saponins, tannins, flavonoids and glycoside. These compounds caused alteration of oestrus cycle in female Wistar rats; resulting in prolonged proestrus and a reduced diestrus and estrus (Sharma *et al*., 2014). *Hibiscus rosa-sinensis* leaf extract was also shown to contain steroid, tannins, saponins and flavonoids (Roy *et al.*, 2018).

These phytochemical compounds had anti-implantation activity in female Wistar rats. Other plants with anti- implantation properties include *Ocimum sanctum*, *Lawsonia inermis* leaves, *Ricinus communis* seeds and *Terminalia belerica* bark (Lu *et al*., 2010; Roy *et al*., 2018). All these contained tannins, saponins, flavonoids and alkaloids. Sharma *et al*. (2014), has shown that saponins lower serum androgens and 17β-oestradiol, but elevates progesterone levels, suggesting that saponins modulate steroidogenesis in the ovary. *Cassia fistula* seeds, *Bougainvillea spectabilis* leaves and *Piper betle* petiole have shown anti-oestrogenic and anti-fertility activity attributed to flavonoids and phenolic compounds (Egamberdieva *et al*, 2016). Secondary metabolites of *C. rotundifolia* should, therefore, be exploited as potential novel contraceptive compounds.

2.3 *Cissus rotundifolia* (Forssk.) Vahl.

Cissus rotundifolia, commonly known as the Arabian Wax Leaf, Peruvian Grape Ivy or Venezuelan Treebine, belongs to the family Vitaceae. In Tana River county, this plant is locally known as *Mkwembe*, *Maneke*, *Neke* in Pokomo and *Arma* in Orma (Kaingu *et al*., 2013).

The plant is easy to grow, attractive and has succulent vines that require very little care once established. It can be grown outdoors or as a houseplant. It has stems with almost round leaves. The leaves are fleshy, waxy, cupped and of a deep green colour (Figure 1). The dorsal portion of the leaves feels soft and velvety to touch. The fruits are berries which turn red when ripe. The plant is widely distributed in Kenya; being found from the high-water mark at the coast to Lake Victoria, but only at an average altitude below 1,524 meters. It is also common in seasonally arid savannah areas (Ernd, 2005).

Cissus rotundifolia has been used in various parts of the world for different purposes; for example, it is used as a food thickener in rural Nigeria and has anti-bacterial activity (Dugoua, 2010). In the southern region of Saudi Arabia, its leaves are widely consumed after cooking by local people as leafy vegetables. It is commonly used to prepare various dishes according to traditional dietary culture; however, its nutritional potential has not been assessed (Ahman & Shah, 2007). In Tana River county, Kenya, this plant is known to have some anti-fertility potential and it has been used traditionally by boiling its roots and the decoction taken orally for 30 days as a contraceptive (Kaingu *et al*., 2013).

Figure 1:Arabian Wax leaf plant, *Cissus rotundifolia* (Forssk.) Vahl. (Source: Author).

2.4 The Wistar rat

The Wistar rat (*Rattus norvegicus*) is an outbred albino rat. This breed was developed at the Wistar Institute in 1906 for use in biological and medical research and is notably the first rat developed to serve as a model organism at a time when laboratories primarily used the common house mouse (*Mus musculus*) (Clause, 1998) . The Wistar rat is currently one of the most popular rats used for laboratory research. It is characterized by its long ears and a tail that is less than its body length (Figure 2).

Figure 2: Wistar rat, *Rattus norvegicus* (Source. Author).

2.4.1 Rat oestrus cycle, gestation period and litter size

The oestrus cycle is a rhythmic reproductive cycle in sexually mature female mammals and is influenced by the release of gonadotropins from the pituitary gland and sex hormones from the gonads. The rat oestrus cycle (just like any mammal) is characterised by the vaginal epithelial change which is an index of good functioning of the neuroendocrine reproductive system and ovarian activity (Deshmukh *et al*., 2014; Marcondes *et al*., 2002). The oestrus cycle of a rat is made up of proestrus phase (nucleated epithelial cells and leukocytes are absent), estrus phase (anucleated epithelial cells), metestrus phase (leukocytes, cornified and nucleated epithelial cells in equal proportions) and diestrus phase (mainly leukocytes) (Figure 3) within the vagina and lasts an average of 4-5 days (Hamid & Zakaria, 2013; Paccola *et al*., 2013).

Varying levels of hormones such as 17β -estradiol and progesterone in the various stages of the oestrus cycle stimulate cyclic changes in the cell morphology and histology of the uterine epithelium and ovaries of the rat (Ajay & Prakash 2012). During proestrus, the endometrium is lined by tall columnar epithelium with regular mitosis. Little or no epithelial cell degeneration of the epithelial or gland occurs. In the ovaries, degeneration of the corpora lutea proceeds under mediation from central fibrous tissue formation (Deshmukh *et al*., 2014). At diestrus, the uterus is small and inactive with a slit-like lumen lined with low cuboidal or columnar epithelium showing occasional degenerate cells. Within the ovaries, the

corpora lutea attains maximum size with vacuoles at the centre and the presence of early formation of fibrous tissue. During estrus, cellular degeneration occurs in the gland and the epithelial lining of the uterus. Mitotic activity and leucocyte infiltration also occurs. In metestrus, vacuolar degeneration occurs in the uterine epithelium and a rejuvenated mitotic activity is also present (Kubota *et al*., 2016).

The uterine endometrial morphological changes are driven by ovarian steroids. Therefore, disruption or interference in oestrogen and progesterone levels compromises these changes hence compromising fertilization and implantation. The rat has a short gestation period (21± 2 days) and is, therefore, an ideal model for studying the effects of chemicals on reproductive parameters (Ankush *et al*., 2011). The litter size of a rat is 9±3. According to Hamid and Zakaria (2013), they reported lengthening of rat gestation period due to the effects of endocrine disruptors while the litter size is not significantly affected. The present study aimed at studying the effect of fertility regulating plant extracts on gestation length and litter size.

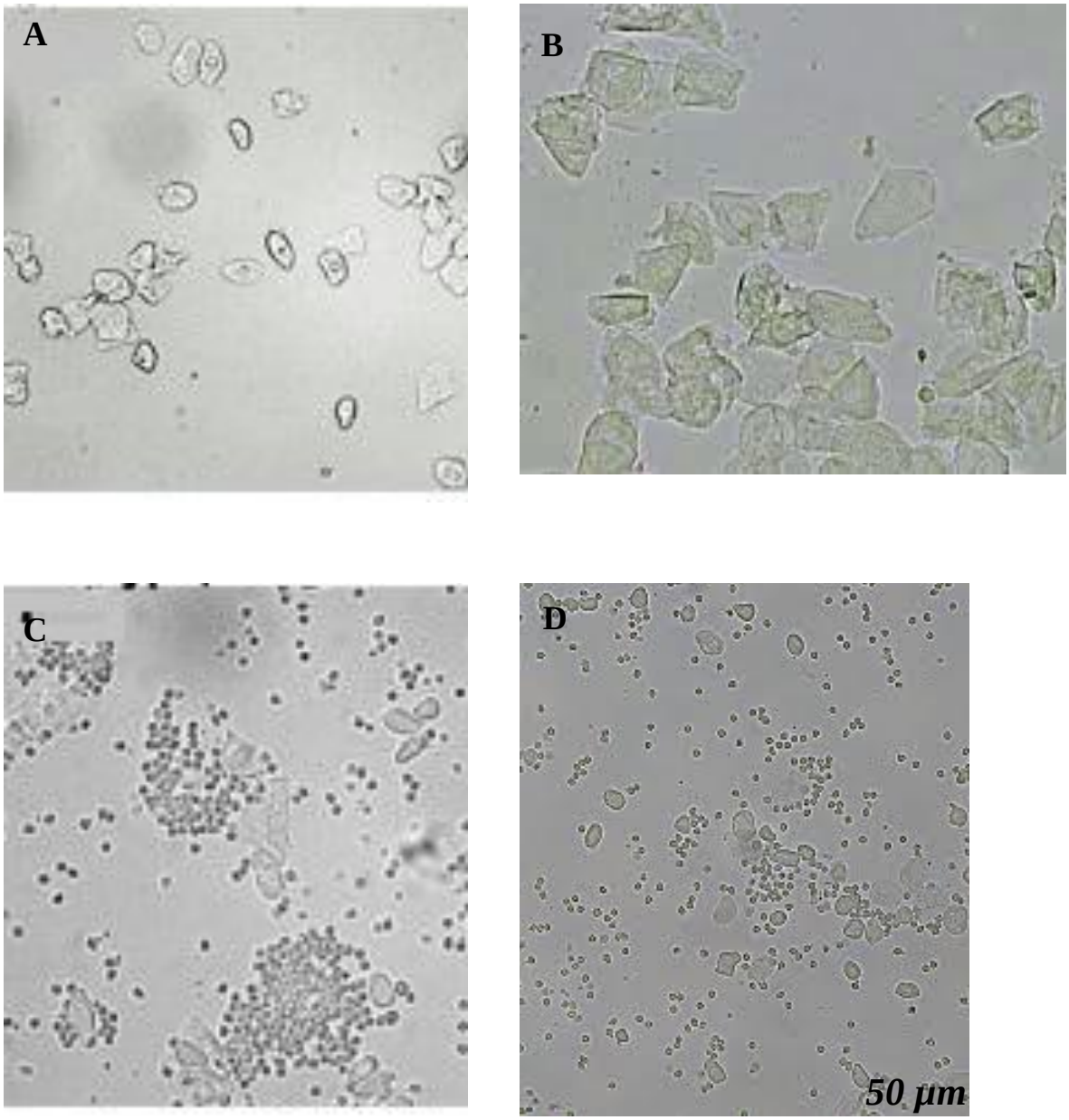


Figure 3: Unstained vaginal smears from rats showing the four phases of oestrus cycle.

A: Proestrus phase, which has nucleated epithelial cells and leukocytes are absent. **B**: Estrus phase, which is made up of large anucleated epithelial cells. **C:** Metestrus phase, which has leukocytes, cornified and nucleated epithelial cells in equal proportions. **D:** Diestrus phase, which is mainly made up of small leukocytes (*Scale bar*: *50 µm*), Mg 400×. (Source: Paccola *et al*., 2013).

2.5 Female reproductive health management using herbal remedies

Herbal medicines have been used for the treatment of human ailments for thousands of years (Yakubu *et al*., 2007). In developing countries, 80% of the population still relies on traditional medicine to meet their healthcare needs (WHO, 2003). It is no wonder therefore that the World Health Organization is pushing for renewed research interest on medicinal plants used by traditional medicine practitioners (TMPs) the world over. The importance of herbal remedies is further emphasized by the fact that globally, 30% of the pharmaceutical preparations used for conventional medicine preparation are based on plants (Shinwari *et al*., 2006; Yakubu *et al*., 2007).

Reproductive issues and ailments constitute 18% of the global burden of disease for women of reproductive age and are the number one cause of maternal mortality in developing countries (WHO, 2003). Female reproductive ailments range from pregnancy and related complications, fertility issues, and menstrual complications. TMPs by their nature do not keep records and most of the knowledge they have is passed on verbally from generation to generation (Giday *et al*., 2010; Mills *et al*., 2006). There is thus, need not only to capture this indigenous knowledge but also to study the plants in order to provide credible evidence to support therapeutic efficacy claims by herbalists (Sofowora, 1993). In Tana River County, TMPs are routinely consulted because of their wide indigenous medicinal knowledge base a tradition that has persisted in many rural communities due to inequitable health provision (Kaingu *et al*., 2011).

In Kenya, 75% of health facilities and personnel are concentrated in urban areas (WHO, 2003). The national doctor to patient ratio is 1: 20,000 but in Tana River County with only 57 health facilities, the doctor: patient ratio is 1: 95,500 (WHO, 2003); emphasizing a serious shortage of both health facilities and staff in the County. On the other hand, the ratio of TMP to patients is 1: 987 (KNBS, 2009), suggesting that the TMPs are more readily accessible.

In general, reproductive health faces a number of challenges such as unsafe motherhood, high maternal mortality rates, and inadequate family planning services in rural areas due to poor funding by the ministry of health. This study evaluated the anti-fertility properties of the plant *C. rotundifolia* through *in vivo* physiological tests done in female Wistar rats.

2.5.1 The progress of contraceptive use to date

By 1960 the world's population had grown to around 3 billion people, having taken just 33 years to increase from 2 billion (Hannaford & Belfield, 2009). Rapid population growth is one of the major challenges facing developing countries like Kenya, with its inevitable consequences on all aspects of development, especially employment, education, housing, health care, sanitation and environment (Ochako *et al*., 2015).

Family planning services in Kenya began in 1976. Before then, there were few reversible contraceptive choices: mainly barrier methods, spermicides and a few plastic-only and metal-based intrauterine devices (IUDs) (Mills *et al*., 2006). Many relied on 'withdrawal' method. By 1996, family planning services were still considered weak because of lack of demand. Despite the low demand, the total fertility rate (TFR) reduced from 8 in the 1970s to 7 in 1980s. The TFR decline in Kenya has been one of the highest in Africa (Kenya Demographic Health Survey 2008/2009) (Hannaford & Belfield, 2009). By comparison, however, the use of family planning services in Africa is still the lowest globally. Some of the contributing factors are inadequate number of trained health care providers, inadequate client counselling and unreliable supplies of contraceptive options (Muanda *et al*., 2016). Access to high quality family planning and reproductive health services is thus a growing concern in sub-Saharan Africa (Tsui *et al*., 2010) which also has the highest population growth rate (3% per annum). Governments are becoming increasingly concerned about the adverse effects of such rapid population growth on development efforts (Tsui *et al*., 2010). Women in Africa start having children early and in large numbers and yet an estimated 22 million in the region have unmet contraceptive need (are currently not using any fertility regulating method even though they would wish to delay or avoid future pregnancies) (Mills *et al*., 2006).

Forty percent (40%) of the annual 215,000 maternal deaths occur in Africa due to reproductive health dysfunction stemming from unsafe abortions and increased sexual activity by adolescents (Darroch & Singh, 2013). This has enhanced a growing interest in and response to family planning and reproductive health programs (Adams & Garcia, 2006; Ahmed *et al*., 2012; Tsui *et al*., 2010;). Despite the unmet contraceptive need, those family planning services that do exist are often underutilized especially in rural parts of the countries; probably due to poor service delivery (Darroch & Singh, 2013). It might also be due to low levels of motivation for women to avoid pregnancies in certain communities (Ochako *et al*., 2015). Several studies have reported on barriers to contraceptive uptake/continuation being disapproval from family members especially husbands/partners (Adetunji, 2011; Ochako *et*

al., 2015); religious beliefs (Shraboni & Singh, 2015); culturally unacceptability and/or undesirable side effects (Darroch & Singh, 2013; Ochako *et al*., 2015). It is therefore imperative for Government and Non-governmental organizations to collaborate and come up with viable solutions on how to improve access and supply of high demand contraceptives in rural parts of Kenya. At the same time increase funding for research exploring novel contraceptive options (Shraboni & Singh, 2015).

2.5.2 Review of current female contraceptive methods

The oral pills and injectable hormones are the most common reversible contraceptive options in Kenya for preventing unintended pregnancies. Frequent intercycle bleeding which is culturally unacceptable in some communities and health fears that the method could cause irreversible infertility are some of the existing barriers to this method (Darroch, 2013). Inadequate supply, overburdened health system, inadequate client counselling and follow-ups, are some of the factors that lead to non-uptake or discontinuation of the method (Adetunji, 2011) in rural communities despite the high demand. Darroch and Singh (2013) report on failure of the method as partly being due to the refusal by some health care providers to give hormonal methods to non-menstruating women; others turn away those who are late for injection. Some discontinuations are due to family disapproval (Parohit *et al.,* 2008). All these factors play in a field where women do not always have control over the use of contraceptives.

Other methods are the long-acting reversible contraceptives (LARC)**.** These are ideal for people who want to delay, space or limit the number of pregnancies (Adetunji, 2011). These include intrauterine devices (IUD) and hormonal implants. The IUDs are considered to be effective long-lasting barriers; however, it is risky to the client and/or health provider especially when dealing with HIV positive persons. The method requires a health provider to physically insert the device into the reproductive tract and exposes one to the risk of HIV infection (Darroch, 2013). The hormonal implants are highly effective and more convenient, and with unmatched effectiveness compared to other contraceptive methods (Ochako *et al*., 2015). Despite this, they are not easily accessible in rural communities (Adetunji, 2011). Globally, access is also lower among poorer, less educated, rural, and younger women (Ochako *et al*., 2015). These implants release ultra-low amounts of progestin into the bloodstream continuously (Jacobstein & Harriet, 2013). Currently, three implants are available, namely Implanon, Jadelle, and Sino-implant II. They are convenient, immediately

effective and offer 3 to 5 years of extremely reliable contraceptive protection (Bradley *et al*., 2011).

The barrier method is also a commonly used form of contraceptive. It includes male and female condoms, diaphragms, sponge and cervical cap. These methods are used each time during sexual intercourse. The barrier methods are also effective when used accordingly and also protect against sexually transmitted infections including HIV (Ochako *et al*., 2015). Other contraceptive options are the use of topical spermicidal compounds that kill or disable sperms, emergency pills, and birth control patch. The birth control patch is worn on the skin and releases oestrogen and progesterone directly into the skin. Each patch contains a 1-week supply of hormones. It releases a constant daily dose of hormones equivalent to the lowest-dose oral contraceptive pill formulation (Ochako *et al*., 2015).

There is also the natural method which includes withdrawal or pulling out method which involves withdrawal of the entire penis from the vagina before ejaculation. Fertilization is prevented because sperm does not contact the female partner's egg. This method remains a significant means of fertility control in most rural areas (Jacobstein & Harriet, 2013). The other natural method is the calendar or rhythm method, which is preferred by women from North Eastern Kenya who have resisted modern contraceptives due to religious affiliation. The method advocates for the avoidance of sex during unsafe days of the cycle (day 8 to 18^{th}) after the commencement of menses (Ochako *et al*., 2015).

Permanent fertility regulating methods include; tubal ligation (fallopian tubes are cut or blocked) and hysterectomy (removal of the uterus and sometimes the ovaries) in females; and vasectomy in males (cutting of the vas deferens) whose popularity is gradually increasing (Bretveld *et al*., 2007). As the Government and other nongovernmental agencies undertake situation analysis to identify and address weaknesses and challenges in the current contraceptive supplies and services in order to improve program delivery, majority of women in rural parts of Kenya continue to suffer unintended pregnancies, unsafe abortions, and untimely death. It is therefore important to explore fertility regulating potential of plants as alternative contraceptive options to the millions of women in rural parts of Kenya who lack access to modern contraceptives (Bradley *et al*., 2011).

2.5.3 Medicinal plants used as contraceptives

Much advancement has been made in the field of chemically formulated contraceptive methods. However, many of these techniques have been known to have great side effects and

are also not easy to access by women from rural areas. The solution has been to use herbal or medicinal plants as contraceptives. Plants have been instrumental in screening many ailments worldwide and it is no surprise that several of these have found application in contraception and even during pregnancy (Bretveld *et al.*, 2007). Effective fertility regulation nevertheless has remained a crucial need due to the growing global population. Research is on-going regarding orally active non-steroidal methods. More research, under the stewardship of the WHO, is being carried out to legitimise the use of traditional practices, which apply medicinal plants as contraceptives. The move as previously stated is aimed at providing alternatives to the use of synthetic oral contraceptives that have been known to cause hormonal imbalance, hypertension, weight gain and increased risk factor for cancer and other complications (Dugoua, 2010).

Plants hold great promise to the discovery of effective anti-fertility agents as several global studies have shown. Several plants have demonstrated their contraceptive properties. These include *Hibiscus rosa-sinensis, Embelia ribes, Daucus carota, Butea monosperma, Sapindus trifoliatus, Mentha arvensis, Ferula jaeschkeana, Gossypium herbaceum, Tripterygium wildfordii* that have been reported to contain contraceptive properties in India (Osonuga *et al*., 2014).

In Africa, plants such as *Leonotis ocymifolia* in Ethiopia (Geremerev *et al*., 2005), *Abrus precatorius L., Carica papaya, Senna alata, Citrullus lanatus, Citrus limon, Curculigo pilosa, Macrosphyla longistyla, Ricinus comunis* and *Sorghum bicolor* in Nigeria (Bablola, 2009; Sofowora, 1993), *Markhamia zanzibarica, Combretum illairii, Ricinus communis, Croton menyharthii, Suregada zanzibariensis, Plectranthus barbatus, Ficus natalensis, Ximenia americana, Citrus sinensis, Harrisonia abyssinica, Grewia villosa willd* and *Cissus rotundifolia* in Kenya, have been reported to contain contraceptive properties (Kaingu *et al*., 2013). From animal model studies, these plants are known to have reversible contraceptive, anti-implantation and abortifacient properties.

Interference with sperm activity can also be used to achieve fertility regulation. Plants such as *Piper nigrum* and *Azadirachta indica* have been reported to have anti- spermatogenic effects and cause a significant reduction of spermatozoa motility (Ajay & Prakash, 2012). Other plants function by disrupting the endocrine regulation of the reproductive process. *Mormodica chaaraantia* is one such plant and it causes a reduction of the oestrogen and progesterone hormones (Osonuga *et al*., 2014). *Juniperis communis* had significant anti-progestational properties whereas *Quassia amara* reduced FSH, LH, testosterone and

epididymal sperm counts in rats (Raji & Bolarinwa, 1997; Sandhya *et al*., 1990). Medicinal plants have numerous possibilities in improving reproductive health as research has demonstrated thus far.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

This study was carried out in Tana River County, which is located in the south-east part of the coast region, Kenya. The plant *Cissus rotundifolia* (CK030) was collected from Garsen, Itsowe and Ngao subdivisions of Tana River County which have a widespread use of herbal medicine (Kaingu *et al*., 2013).

Tana River County is bordered by Kitui county to the West, Mwingi to the North West, Garissa to the North East, Ijara to the East, Meru North and Isiolo to the North, Lamu to the South East and Malindi to the South West. It also borders the Indian Ocean to the South with a coastal strip of 35 km. The county lies between latitude 1° 30′ 0″ S and longitude 40° 0′ 0″ E (Figure 3), and covers a total land size area of 38,782 km^2 (Dugoua, 2010). The land in Tana River county generally slopes south-eastwards with an altitude that ranges between 20m to 200m above sea level (Tana River county, n.d). According to the Kenya population and housing census report (2019), the county has a population of 315,943 persons. Ninety-six percent (96%) of Tana River County lies in the coastal lowland zone six which is characterized by low, erratic rainfall and high temperature. The rainfall is low, bimodal and erratic with a mean annual range of 300-500mm (Ochako *et al*., 2015). Long rains occur in the months of April and May while short rains occur in the months of October and November.

The average annual temperature is approximately 30˚C together with humid conditions that occur along the coastal line. The coastal region receives up to 1200mm of rain annually although it varies and is highly unreliable. The level of poverty is very high. It is estimated that 72% of the total county population lives below the poverty line (Omosa, 2005). Acute droughts often accompanied by destitution and ethnic conflict revolving around sharing of natural resources are partly responsible for the high poverty level. Both drought and ethnic conflict retard development, consequently entrenching poverty. The county is inhabited by various ethnic groups; the main ones being, Munyoyaya, Wata, Bajuni and Mijikenda. The Pokomo, Munyoyaya, Malakote and Mijikenda are involved in farming activities while the Orma, Wardei and Somali are mainly cattle keepers. Most villages are found along the river Tana where farming is favourable. The pastoralists live in *manyattas* concentrated around watering points like dams, wells and boreholes (Omosa, 2005).

All the laboratory experiments in this study were done at the University of Nairobi, Chiromo campus in the Department of Veterinary Anatomy and Physiology laboratories. The University is situated in Nairobi which is the capital city of Kenya. It is a fast-growing city with a population of over 3.5 million. The city centre has an area of over 700 square kilometres and stands at an altitude of 1,675 meters above sea level (Ochako *et al*., 2015). It is 140 kilometres south of the equator and about 480 kilometres west of the Indian Ocean. This city experiences no real winter or summer because it is near the equator and at a high altitude. The days in Nairobi are warm and the nights cool for the greater part of the year. with the temperature rarely rising above 27˚C in the middle of the day or falling below 10˚C at night. The average annual rainfall is about 2000 mm (Ernest *et al*., 2013).

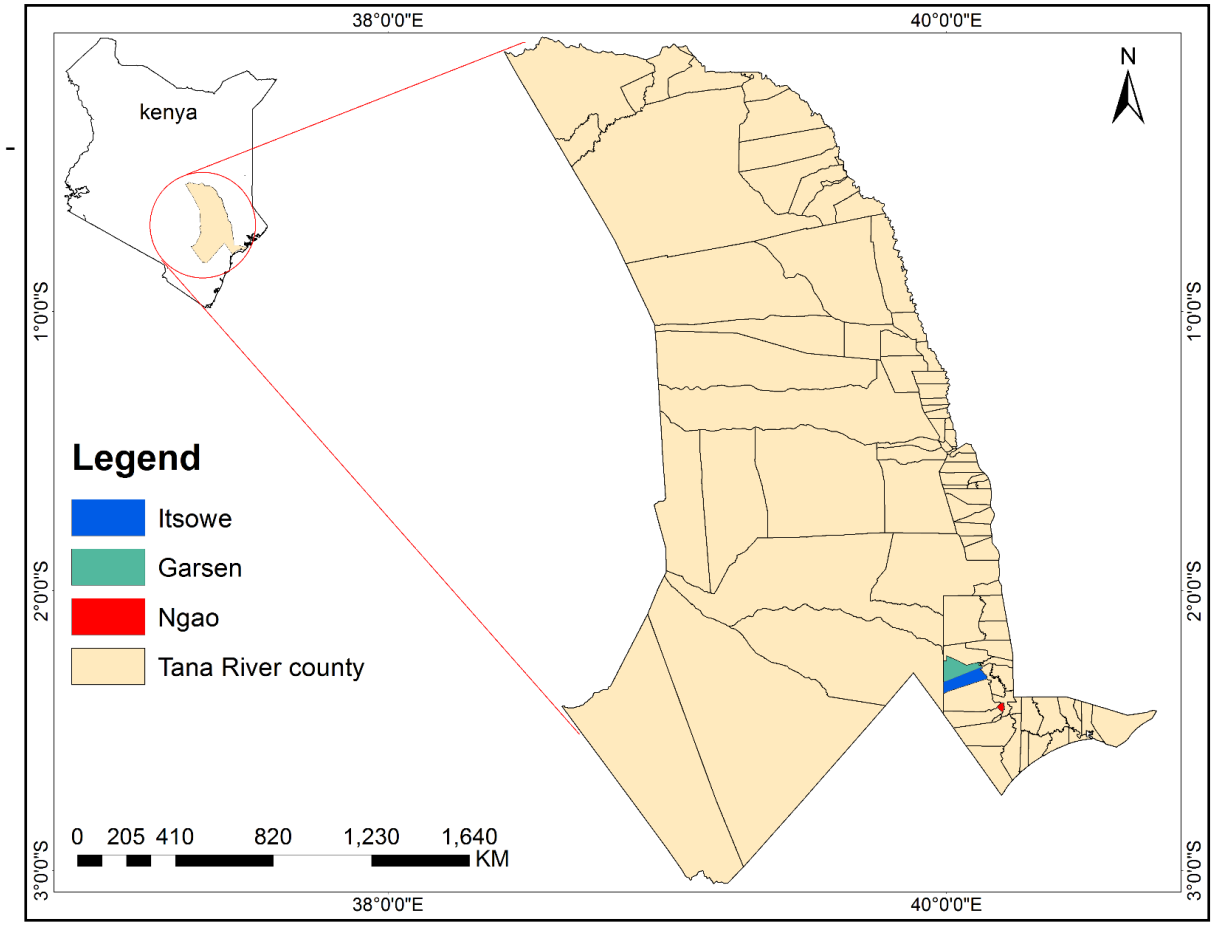


Figure 4: Tana River County showing the three subdivisions from where the plant was collected. (Source: DIVA-GIS shapefiles modified using ArcMap 10.3).

3.2 Study design

The experimental design adopted was Randomized Block design (RBD) with five replicates of rats per treatment. The rats were randomly selected in each treatment group.

3.3 Plant collection and preparation

The roots of *C. rotundifolia* were collected from Garsen, Itsowe and Ngao subdivisions of Tana River County, Kenya. The roots of the plant were obtained from the soil using a garden knife and a hoe and transported in sterile plastic bags to the University of Nairobi, Department of Veterinary Anatomy and Physiology laboratories in Chiromo campus. To remove the soil, the roots were then thoroughly washed using tap water. The roots were cut into small pieces using a knife, after which they were kept under shade and dried for two weeks (Kaingu *et al*., 2018). The dried roots were then ground into powder using a Cunningham grinder (Artsan Manufacturing, Massachusetts, USA) as described by Gakuya (2001). The obtained *C. rotundifolia* root powder was weighed using a Lark digital weighing balance (Gold Member Manufacturers, China) and packed in 300g sachets that were properly labelled and stored in cool and aerated cupboards away from direct sunlight.

3.3.1 Preparation of *C. rotundifolia* aqueous extract

The root powder stored in the 300g sachets was macerated in distilled water at a ratio of 1 to 6 (w/v) in a conical flask. The conical flask containing the suspension was then immersed into a water bath and heated at 60^{o} C for one hour. Thereafter, filtration was carried out using Whatman filter paper (number 4). The filtrate was then transferred into a round-bottomed flask and was slowly rotated in a bowl containing acetone and dry ice. This helps to coat the filtrate onto the inner surface of the round-bottomed flask by freezing out excess water. The dry ice-coated round-bottomed flask was then fixed in a freeze drier machine and lyophilised for 48 hours until the extract was completely dry (Chan *et al*., 2011; Kaingu *et al.*, 2017). The extract was then kept in labelled sterile vials and stored in the refrigerator until use.

3.3.2 Experimental animals

Mature and normal cyclic female Wistar rats, aged 3-4 months and weighing between 160-250 grams were used in the study. The rats were purchased from the Department of Biochemistry, University of Nairobi, Kenya and kept in the animal house, Department of Veterinary Anatomy and Physiology. The female rats were randomly caged in groups of five and they were maintained under standard environmental conditions of 12 hours light and 12 hours darkness at 24-25^{o}C and relative humidity of 30-70 %. They were fed on commercially obtained diet pellets (Unga Limited, Kenya) and tap water was provided *ad libitum.* The bedding material used was wood shavings that was changed every other day to prevent the accumulation of urine and faecal waste (Al-Shemary *et al*., 2018). The experimental protocol

was approved by the Faculty of Veterinary Medicine,University of Nairobi, Animal Care and Use Ethics Committee with reference number FVM BAUEC/2019/191.

Male Wistar rats were also kept in the same room but in different cages. Proven fertile male rats were randomly introduced into female cages at the ratio of 2:5 at the appropriate time (Kaingu *et al.*, 2017).

3.4 Determination of acute oral toxicity of *C. rotundifolia* aqueous extract

The acute oral toxicity test was determined using the acute oral class method as described by Organisation for Economic Co-operation and Development, OECD 423 (2004). In this test, female rats were caged in groups of three and kept in their cages for 7 days, prior to dosing, to acclimatize. After which the doses were prepared shortly prior to administration by dissolving the specific dose extract in 0.5 ml of distilled water. The fixed dose levels were 5, 50, 300, 2000 and 5000mg/kg body weight. Three animals were used at each of the specified dose levels. Depending on the mortality rate, the result of each step determined if further testing was needed for 3 additional animals at the same dose level or 3 additional at the next lower or higher dose level.

In this study, the starting dose was 300 mg/kg as per OECD 423 guideline. The body weight of each rat was taken a day before extract administration. Food was then withheld overnight but water was provided *ad libitum*. The animals were weighed just before extract was administered through intra-abdominal lavage (Day 0) between 9 and 10 am. The control group was administered with 0.5 ml physiological saline. Food was further withheld for 4 hours after extract administration.

After extract administration, the animals were observed individually. Clinical signs were physically observed and recorded during the first 4 hours, 24 hours, daily thereafter up to the 14^{th} day. The observations included behaviour pattern changes, skin and fur changes, eyes (pupil size), rhinorrhoea (nasal discharge), respiratory rate, tremors, convulsions, salivation, diarrhoea, sleep, coma, lethargy and death.

At 300mg/kg body weight, no mortality was recorded. Therefore 3 additional rats were administered with 2000mg/kg. These rats did not die. A limit dose of 5000mg/kg was then administered to 3 additional rats. These too did not die. The body weights of the test animals were recorded individually, immediately prior to dose administration (day 0), and on day 1, day 7 and on day 14. After 14 days of post-treatment observation, all the animals were euthanized and subjected to gross necropsy and examined pathologically. The pathology was

based on macroscopic examination of organs of the test animals in comparison to the control group.

3.5 Phytochemical screening of *C. rotundifolia* aqueous extract

Phytochemical screening of *C. rotundifolia* aqueous extract was carried out as per the method described by Prashant *et al.* (2011) and Sahira and Catherine (2015) to decipher the presence of alkaloids, flavonoids, phenols, terpenoids, tannins, saponins and glycosides.

3.5.1 Alkaloids

The protocol was carried out as per the method described by Sahira and Catherine (2015) to decipher the presence of alkaloids. Ten (10) mg of *C. rotundifolia* aqueous extract was dissolved in 5 ml of hydrochloric acid (1.5% v/v) and filtered. The filtrate was used to test for alkaloids using Mayer's test**.** Two ml of Mayer's reagent (Potassium Mercuric Iodide) was added to 2ml of the filtrate. The presence of alkaloids was indicated by the formation of a yellow-coloured precipitate.

3.5.2 Flavonoids

The protocol was carried out as per the method and alkaline reagent test described by Prashant *et al*. (2011) to decipher the presence of flavonoids. Two millilitres of dilute ammonia and 2ml of concentrated sulphuric acid were added to 3ml of *C. rotundifolia* aqueous extract and vigorously shaken. The presence of flavonoids was indicated by the formation of intense yellow colour.

3.5.3 Phenols

The protocol was carried out as per the method and Ferric Chloride test described by Prashant *et al*. (2011) and Sahira and Catherine (2015) to decipher the presence of phenols. Two millilitres of distilled water was added to a test tube containing 1ml of *C. rotundifolia* aqueous extract followed by four drops of 10% ferric chloride ($FeCl_3$) solution. The appearance of a blue-black colour indicated the presence of phenols.

3.5.4 Terpenoids

The protocol was carried out as per the method described by Sahira and Catherine (2015). Two millilitres of chloroform was added to 1 ml of *C. rotundifolia* aqueous extract in a test tube and it was vigorously shaken. It was then followed by 3ml of concentrated sulphuric acid (H_2SO_4) then heated for two minutes. The presence of terpenoids was indicated by the formation of a grey colour.

3.5.5 Tannins

The protocol was carried out as per the method described by Prashant *et al*. (2011) and Sahira and Catherine (2015) to decipher the presence of tannins. Five millilitres of distilled water was added to two millilitres of *C. rotundifolia* aqueous extract and heated to boil. One millilitre of 2% Iron Chloride ($FeCl_2$) was then added. The formation of a blue-black colour indicated the presence of tannins.

3.5.6 Saponins

The protocol was carried out according to the method and foam test described by Prashant *et al*. (2011) to decipher the presence of saponins. Five millilitres of the *C. rotundifolia* aqueous extract was diluted to 20ml by distilled water and shaken for 10 minutes. Formation of a persistent layer of foam indicated the presence of saponins.

3.5.7 Glycosides

To test for glycosides, the protocol was carried out as per the method described by Prashant *et al*. (2011) and Sahira and Catherine (2015). One millilitre of glacial acetic acid was added to 0.5 ml of *C. rotundifolia* extract. One drop of 2% Iron Chloride ($FeCl_2$) was added and the mixture was shaken. One millilitre of sulphuric acid (H_2SO_4) was added to the mixture. The formation of a brown ring indicated the presence of glycosides.

3.6 The effect of *C. rotundifolia* aqueous extract on oestrus cycle

The effect of *C. rotundifolia* aqueous extract on oestrus cycle was evaluated using fifteen normocyclic female Wistar rats divided into 3 groups of 5 rats each. These animals were not mated even though male rats were kept in the same room but in different cages. They were monitored daily for the first 10 days to ensure cyclicity through specific cytological features that distinguished the four stages of the oestrus cycle (Diestrus, Proestrus, Estrus and Metestrus). Only rats with regular oestrus cycles were used for the study (Karateke *et al*., 2018).

Rats in Group 1 (control) received 0.5 ml of physiological saline through intra-abdominal lavage for 14 days. Group 2 and 3 rats received *C. rotundifolia* extract at 400 and 800mg/kg respectively, through intra-abdominal lavage daily for 14 days. Vaginal smears (done by flushing cells from the vaginal lining by introducing a small amount of physiological saline into the vagina using a pipette) were collected daily from all the rats between 8 and 10 am and examined for oestrus cycle cytological features (Kaingu *et al.*, 2018).

3.7 The effect of *C. rotundifolia* aqueous extract on mating success, fertility index, gestation length and litter size in Wistar rats

The anti-fertility efficacy of *C. rotundifolia* on mating success, fertility index, gestation length, litter size and body weight were evaluated using three treatment regimes on normocyclic female Wistar rats. The three treatment regimes were pre-mating extract administration, post-mating extract administration and pre- and post- mating extract administration. The positive control rats were treated with oestrogen/ progesterone injection while the negative control were administered with 0.5 ml physiological saline.

3.7.1 Pre-mating extract administration

Fifteen rats were randomly divided into 3 subgroups (A, B and C) of 5 rats each. Sub group A (control) received 0.5 ml of physiological saline. Sub group B and C received 400 and 800mg/kg of *C. rotundifolia* aqueous extract respectively. These doses were administered daily for 14 days through intra-abdominal lavage after which the rats were mated. Vaginal smears were taken from the rats by swabbing daily until pregnancy was proven. The first day of gestation was taken to be the day spermatozoa were detected in the vaginal smear under the light microscope. This indicated mating success.

The females were monitored daily by taking their weights until they littered (Ahman & Shah, 2007; Kaingu *et al.,* 2018). The litter size, as well as the gestation length, was recorded.

3.7.2 Post-mating extract administration

Fifteen rats randomly were divided into 3 subgroups (D, E and F) with 5 rats each and allowed water and pellets *ad libitum*. All rats were mated. After a successful mating; which was shown by presence of spermatozoa under microscope. Sub group D (control) received 0.5 ml of physiological saline, sub groups E and F received 400 and 800mg/kg of *C. rotundifolia* aqueous extract respectively]. These doses were administered daily through intra-abdominal lavage throughout the gestation length. The females were monitored daily by weighing until they littered (Kaingu *et al.,* 2017). The litter size, as well as the gestation length was recorded.

3.7.3 Pre- and post-mating extract administration

This treatment was carried out according to the method described by Kaingu *et al.* (2017)**.** Fifteen rats were treated in a similar manner as described in section 3.7.1 above except that the administration of aqueous extract continued after mating until the end of gestation. Control animals were treated in a similar manner but received 0.5 ml physiological saline

(Kaingu *et al*., 2018). The females were monitored daily by weighing until they littered. The litter size, as well as the gestation length was recorded.

3.7.4 Positive control

Five normocyclic female rats were used as positive control animals. They received oestrogen/ progesterone (15µg oestradiol/0.15mg progestrin) subcutaneous injection once. They were then mated and vaginal smear was monitored daily to check for presence of spermatozoa (Ankush *et al.*, 2011). Gestation length, litter size as well as body weights of all animals was recorded.

The fertility index was calculated as the number of pregnant rats divided by the total number of animals successfully mated multiplied by 100 (Osonuga *et al.*, 2014).

$$Fertility\ index\ (\%) = \frac{number\ of\ \ pregnant\ rats}{total\ number\ of\ succefully\ mated} \times 100$$

3.8 The effect of *C. rotundifolia* aqueous extract on ovarian and uterine histomorphology

Fifteen mature normocyclic female Wistar rats were used in this study. The rats were randomly divided into 3 groups of 5 rats each. Group 1 received 0.5 ml physiological saline, Group 2 and 3 received 400 and 800mg/kg of *C. rotundifolia* aqueous extract respectively, daily for 28 days through intra-abdominal lavage (Kaingu *et al*., 2017). On the 29th day of treatment, all the rats were humanely sacrificed using carbon dioxide gas. The animals were kept in a glass chamber and carbon dioxide gas pumped into chamber for a duration of 5 minutes (Nagao *et al*., 2001).

The body of all the rats was flushed using physiological saline and immediately thereafter, left ovaries and uterine horns were harvested and preserved in 10% buffered neutral formalin for 24 hours. Later on, the ovaries and uterine horns were processed histologically as per the protocol described by Hanneia *et al*. (2013).

The tissues were cut into 8-micron thickness sections using a rotary microtome (Jinhua Craftek Instrument Co., Ltd, Zhejiang, China). They were stained with Haematoxylin and Eosin. The number and the structural integrity of the follicles and corpora lutea and the transverse and longitudinal sections of uteri were observed under a light microscope (Kaingu *et al*., 2017). Photomicrographs of the different sections of the test animals were taken and compared to control group sections with Leica LAS EZ software version 1.8.0. After the experiment, all carcasses were incinerated.

3.9 Data analysis

Statistical analysis of the data was done using SPSS Statistical Software version 22. The data was tested for homogeneity of variance before the application of parametric tests. One-way Analysis of Variance (ANOVA) was used to test for significant difference in the mean weights of the test animals and control animals at different dose levels of acute oral toxicity (300, 2000 and 5000mg/kg) at $p < 0.05$. One- way ANOVA was also used to test for any significant difference in the mean of frequency appearance of stages of oestrus cycle (proestrus, estrus, metestrus, diestrus) at 400 and 800mg/kg of *C. rotundifolia* aqueous extract. The same test was used to test for any significant difference in the mean litter size, gestation length, and body weight at 400 and 800mg/kg for pre- mating, post- mating and pre-post mating regimes of extract administration. The fertility index in all the three regimes was calculated as the number of pregnant rats divided by the total number of animals successfully mated multiplied by 100 before comparing them using one-way ANOVA. In all the above cases, a post hoc test (Multiple comparison Dunnett t-test) was used to separate the means specifically against the control. In all the statistical tests, the significance threshold was set at $\alpha = 0.05$ and significance level at 95 % i.e. $p < 0.05$. All the physiological test result values were expressed as mean ± standard error of the mean.

The histological sections were observed under a light microscope at ×400 magnification and photomicrographs of the ovaries and uteri of the test animals and control were compared using Leica LAS EZ software version 1.8.0.

CHAPTER FOUR

RESULTS

4.1 Acute oral toxicity test of *C. rotundifolia* aqueous extract

4.1.1 Clinical observations

The rats showed minimal activity for the first 3 hours after administering *Cissus rotundifolia* aqueous extract at 300, 2000 and 5000mg/kg. The test animals then recovered and showed normal behaviour like their control counterparts throughout the 14 days of post-treatment period. No mortality was reported in all the doses. At the limit dose (5000mg/kg), however, the rats showed increased breathing rate, and the fur became rough, compared to the control rats.

4.1.2 Body weight of the test animals

The mean body weights (g) of the test animals from fasting day (before extract administration) day 0 (day of extract administration), day 1, day 7 and day 14 of post-treatment are presented in Table 1. The results show that the control animals and the test animals at 300, 2000 and 5000mg/kg showed a reduction in mean weight between fasting day and day 0 (Table 1). From day 1 to day 14 of post-treatment, the control and the test animals at the three dose levels showed a significant increase in mean body weights at $P<0.05$ as shown in Table 1.

The results of One-way ANOVA also revealed that there was no significant difference in mean weights at the three dose levels (300, 2000, 5000mg/kg) compared to the control group at $P>0.05$ over the study period as presented in Table 2. It was therefore concluded that *C. rotundifolia* aqueous extract is safe and not toxic. Based on the LD_{50} studies, 400 and 800mg/kg doses were selected for the experiment.

Table 1: The mean body weights (g) (Mean ± SEM) of treated animals at different dose levels before extract administration (fasting day) and day 0, day 1, day 7 and day 14 of post-treatment.

	Control	**300mg/kg**	**2000mg/kg**	**5000mg/kg**
Fasting day	179.90 ± 11.36	170.07 ± 2.65	183.55 ± 3.76	175.13 ± 2.60
Day 0	163.60 ± 10.41	150.40 ± 2.40	173.43 ± 4.04	157.89 ± 3.67
Day 1	179.62 ± 11.05	169.28 ± 7.00	187.72 ± 5.13	175.32 ± 2.33
Day 7	195.30 ± 11.57	183.67 ± 10.84	198.28 ± 0.88	185.56 ± 3.18
Day 14	208.11 ± 11.93	196.14 ± 9.45	203.83 ± 5.36	197.74 ± 3.52

Table 2: The mean weights (g) of the treated animals at different dose levels (300, 2000, 5000mg/kg) compared to the control group over the study period.

Doses	**Mean ± SEM**
Control group	185.31 ± 4.89
300 mg/kg	173.90 ± 4.62
2000 mg/kg	189.31 ± 4.29
5000 mg/kg	178.33 ± 4.52
All the means were not significantly different ($p>0.05$)	

4.1.3 Pathological examination

After the 14 days of treatment period, the test animals showed no significant gross pathological changes in all the vital organs observed, between the three dose levels (300, 2000, 5000mg/kg) compared to the control. In all the test animals the vital organs such as the heart was normal with a smooth consistency, both the right and the left lungs were normal, the spleen and the liver had a smooth consistency and had a normal size, the stomach, intestines and uterus were all normal with no macroscopic alteration.

4.2 Phytochemical compounds of *C. rotundifolia*

Phytochemical screening of *C. rotundifolia* aqueous extract revealed the presence of alkaloids, phenols, tannins, saponins and glycosides as shown in Table 3. However, flavonoids and terpenoids were absent.

Table 3: Phytochemical compounds of *C. rotundifolia* aqueous extract.

Plant Extract	Alkaloids	Phenols	Flavonoids	Tannins	Saponins	Terpenoids	Glycosides
C. rotundifolia	+	+	-	+	+	-	+

4.3 Effect of *Cissus rotundifolia* aqueous extract on oestrus cycle

At 400mg/kg *C. rotundifolia* aqueous extract disrupted the oestrus cycle of the rats. Both the proestrus and metestrus phases increased to 4.00 ±0.45 and 5.80 ±0.6, respectively. However, the increase was not significant ($p>0.05$) compared to the control group (3.40 ±0.66 and 3.20 ±0.20, respectively), over the 14 days of extract administration (Figure 5). On the contrary, there was a significant ($p<0.05$) decrease in both the estrus and diestrus phases (2.80 ±0.58 and 1.20 ±0.37, respectively), compared to the control group (3.80 ±0.20 and 3.40 ±0.66, respectively), over the 14-day treatment period (Figure 5).

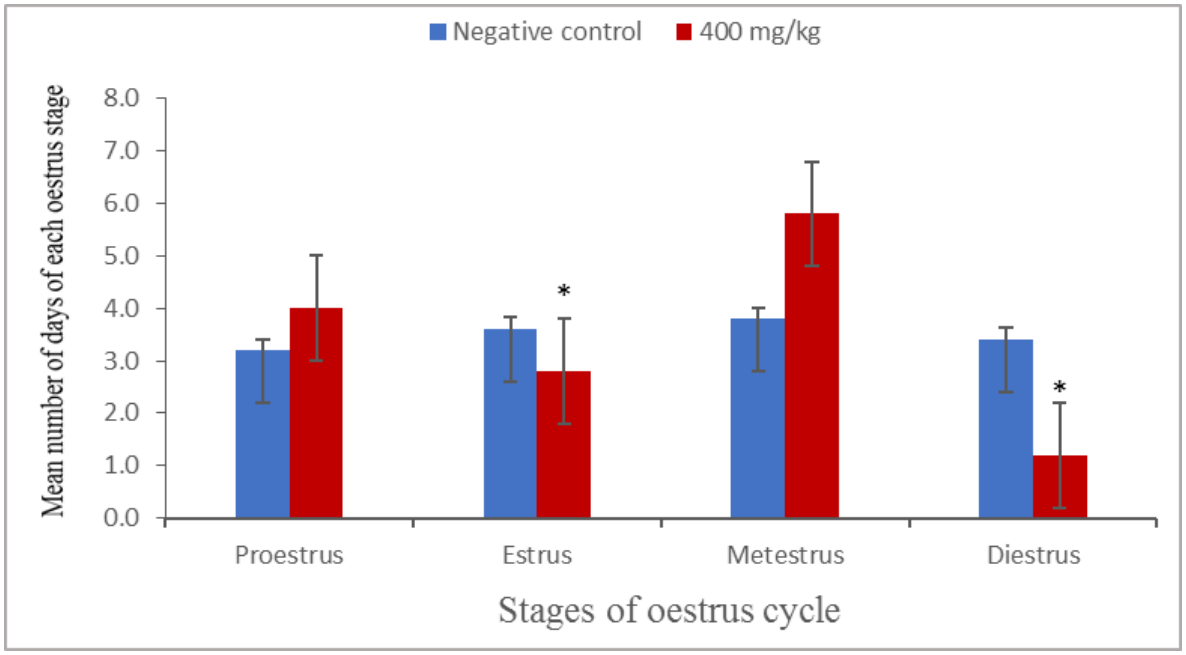


Figure 5: The mean number of days of each oestrus cycle stage over the 14-day *Cissus rotundifolia* extract administration (400mg/kg). Note: The horizontal bars are ± SEM, * represents P<0.05.

Likewise, *C. rotundifolia* aqueous extract at the dose of 800mg/kg disrupted the oestrus cycle of the rats too. There was a significant ($p<0.05$) increase in both proestrus and metestrus stages (4.50 ±0.58 and 6.80 ±0.66, respectively), compared to the control group (3.20 ±0.20 and 3.40 ±0.66, respectively). There was also a significant ($p<0.05$) decrease in both estrus and diestrus stages (2.00 ±0.71 and 1.80 ±0.45, respectively) compared to the control group (3.8 ±0.2 and 3.4 ±0.66, respectively) as presented in Figure 6, over the period of 14 days of extract administration. The summary of the effect of both 400 and 800mg/kg *C. rotundifolia* on oestrus cycle is graphically represented in Figure 7.

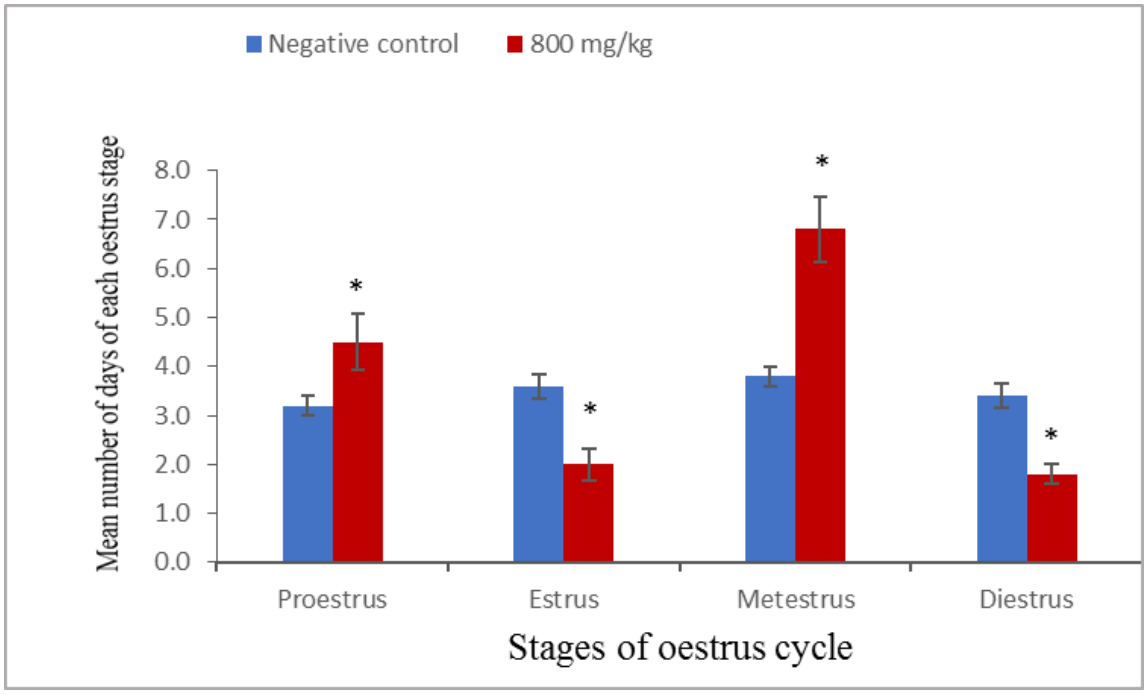


Figure 6: The mean number of days of each oestrus cycle stage over the 14-day extract *Cissus rotundifolia* administration (800mg/kg). Note: The horizontal bars are ± SEM, * represents P<0.05.

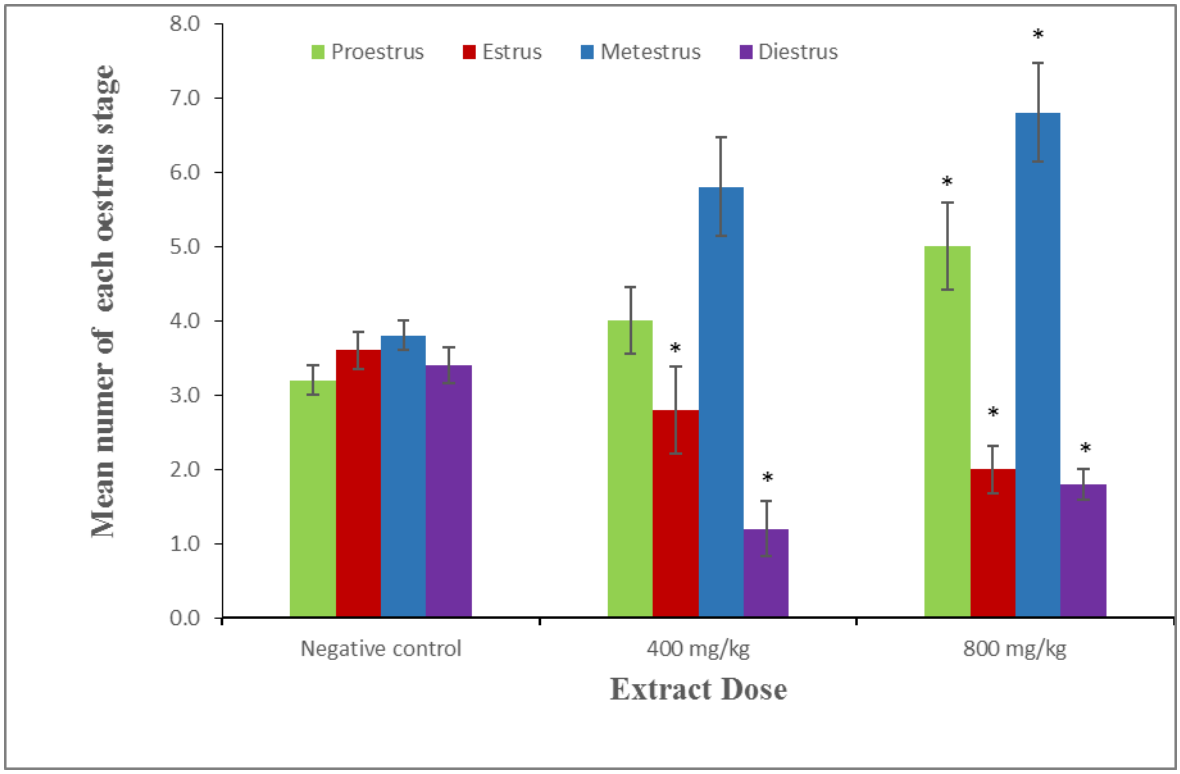


Figure 7: Summary of the effect of 400 and 800mg/kg *Cissus rotundifolia* aqueous extract on the mean appearance frequency of oestrus cycle stages. Note: The vertical bars are ± SEM, * represents P <0.05.

4.4 The effect of *Cissus rotundifolia* aqueous extract on mating success, fertility index, gestation length, and litter size in Wistar rats

4.4.1 Effect of *C. rotundifolia* extract administered before mating (pre-mating)

Cissus rotundifolia aqueous extract at both 400 and 800mg/kg doses had no significant effect ($p>0.05$) on mating success, compared to the control group. There was also no significant effect ($p>0.05$) of the extract at 400mg/kg dose on fertility index compared to the negative control. However, at 800mg/kg, the extract caused a significant reduction ($p<0.01$) in fertility index compared to the negative control (Table 4 and Figure 8).

At both 400 and 800mg/kg doses, the *C. rotundifolia* extract caused a significant increase ($p<0.05$) in gestation length (23.8 ±0.21 and 26.0 ±0.36, respectively) compared to the control (21.0 ±1.00) (Table 4 and Figure 8).

The litter size was significantly reduced ($p<0.05$) at both 400 and 800mg/kg (9.00 ±0.71 and 5.00 ±0.04, respectively), compared to negative control (12.10 ± 0.27). The rats gained weight during the 14 days of premating extract administration (Table 4).

Table 4: The effect of *Cissus rotundifolia* before mating treatment regime on mating success, fertility index, gestation length, litter size, and body weight. Note: * represents P< 0.05 ** represents P< 0.01.

	Mating success (%)	**Fertility index (%)**	**Gestation length (days)**	**Litter size**	**Body weight (grams)**
Negative control	100	80	21.0 ±1.00	12.00 ±0.27	243.21 ±0.11
Positive control	0	0	0	0	252.36 ±0.23
400mg/kg	100	80	23.8 ±0.21*	9.00 ±0.71*	275.6 ±0.15
800mg/kg	100	20 **	26.0 ±0.36*	5.00 ±0.04*	269.4 ±0.02

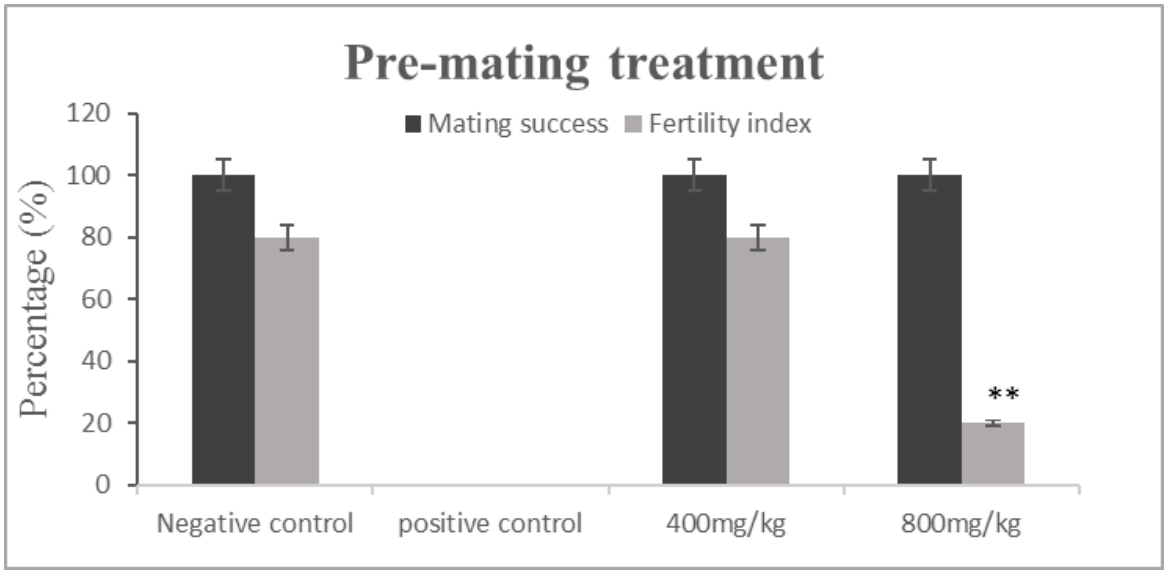


Figure 8: The effect of *C. rotundifolia* extract administered before mating on fertility index and mating success. Note: ** represents P<0.01.

4.4.2 Effect of *C. rotundifolia* extract administered after mating (post-mating)

Cissus rotundifolia extract at 400 and 800mg/kg had no significant effect (p>0.05) on mating success compared to the control. At 400mg/kg, the plant extract caused a significant reduction (p<0.05) in fertility index of 60% compared to 100% of negative control. At 800mg/kg *C. rotundifolia* extract caused a significant reduction (p<0.01) in fertility index of 20% compared to 100% of negative control as shown in Table 5 and Figure 9.

Cissus rotundifolia extract at 400 and 800mg/kg significantly prolonged the gestation length (p<0.05) to 24.0 ±0.12 and 25.0 ±0.31, respectively, compared to the control group (21 ±1.00) (Table 5).

The litter size was also significantly reduced (p<0.05) at both 400 and 800mg/kg (8.00 ±0.53 and 4.00 ±0.20) respectively compared to the negative control (12.10 ±0.27) (Table 5). The rats gained weight throughout the study period.

Table 5: The effect of *Cissus rotundifolia* aqueous extract administered after mating on mating success, fertility index, gestation length, litter size, and body weight. Note: * represents P< 0.05, ** represents P< 0.01.

	Mating success (%)	**Fertility index (%)**	**Gestation length (days)**	**Litter size**	**Body weight (grams)**
Negative control	100	100	21.0 ±1.00	12.00 ±0.27	253.21 ±0.34
Positive control	0	0	0	0	265.36 ±0.29
400mg/kg	100	60 *	24.0 ±0.12*	8.00 ±0.53*	279.6 ±0.15
800mg/kg	100	20 **	25.0 ±0.31*	4.00 ±0.20*	263.4 ±0.02

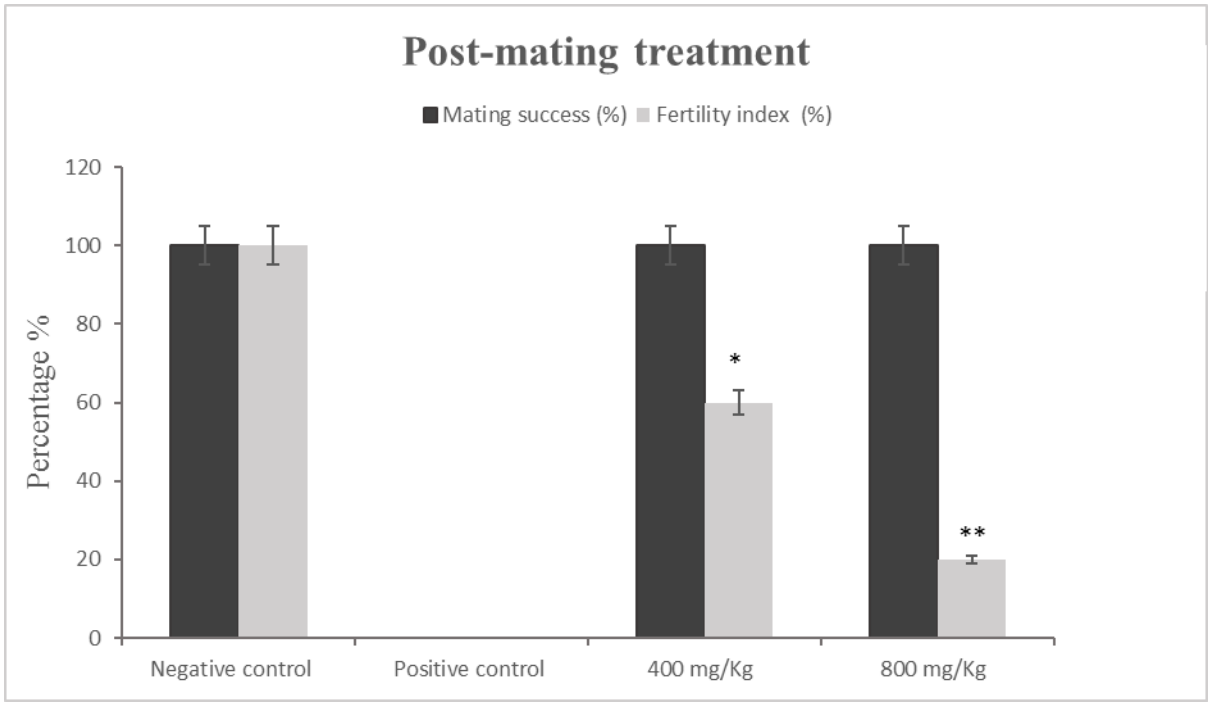


Figure 9: The effect of *C. rotundifolia* extract administered after mating on fertility index and mating success. Note: * represents P< 0.05, ** represents *P*<0.01.

4.4.3 Effect of *C. rotundifolia* extract administered before and after mating (Pre-post mating)

Cissus rotundifolia at 400 and 800mg/kg had no significant effect ($p>0.05$) on mating success compared to control. At 400mg/kg, the plant extract caused a significant reduction ($p<0.05$) in fertility index to 20% compared to 100% of negative control. At 800mg/kg *C. rotundifolia* caused a significant reduction ($p<0.05$) in fertility index of 0% compared to 100% of negative control, as shown in Table 6 and Figure 10.

Cissus rotundifolia at 400mg/kg significantly prolonged ($p<0.05$) the gestation period to 26.0 ±0.11 compared to the control group (21.0 ±1.00) (Table 6). At 800mg/kg, none of the rats littered because the fertility index was significantly reduced to 0%.

The litter size was significantly reduced ($p<0.05$) at 400mg/kg (5.00 ±0.36) compared to negative control (11.00 ±0.43). At 800mg/kg, none of the rats littered. The rats gained weight throughout the period (Table 6).

Table 6: The effect of administering *Cissus rotundifolia* extract "before and after mating" on mating success, fertility index, gestation length, litter size, and body weight. Note: * represents P< 0.05, ** represents P< 0.01.

	Mating success (%)	**Fertility index (%)**	**Gestation length (days)**	**Litter size**	**Body weight (grams)**
Negative control	100	100	21.0 ±1.00	11.00 ±0.43	260.21 ±0.39
Positive control	0	0	0	0	275.30 ±0.23
400mg/kg	100	20 *	26.0 ±0.11 *	5.00 ±0.36 *	280.25 ±0.76
800mg/kg	100	0	0	0	245.98 ±0.023

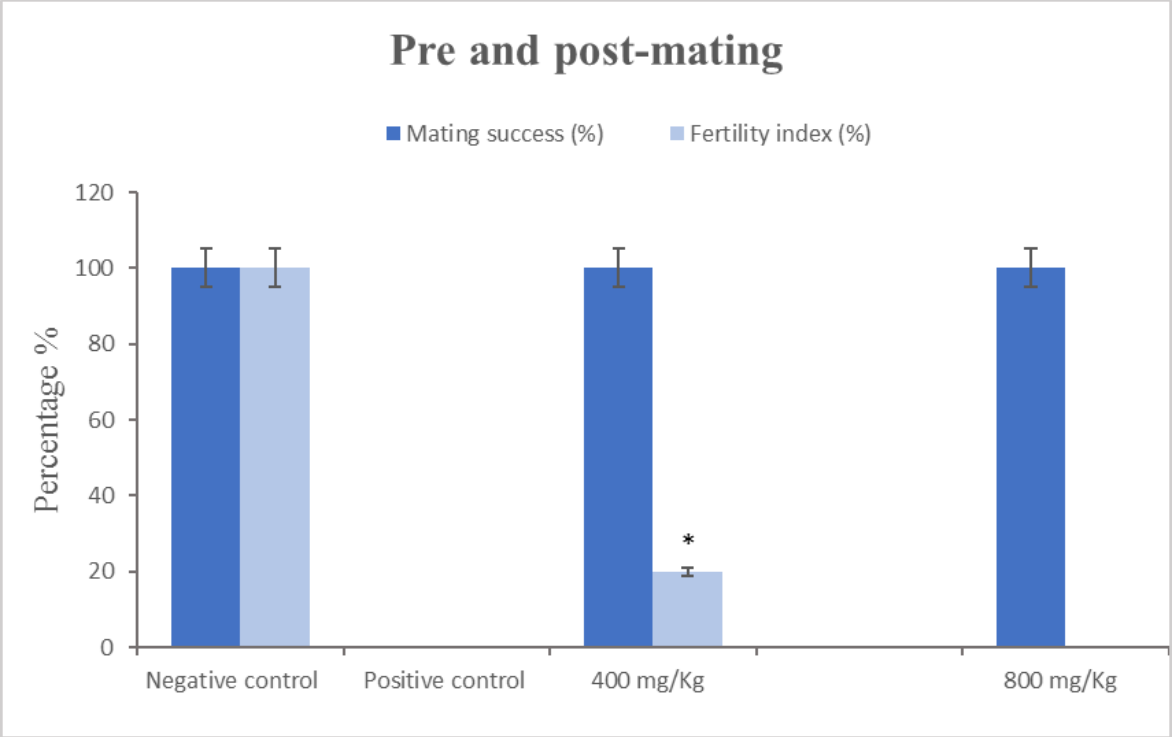


Figure 10: The effect of *C. rotundifolia* extract administered before and after mating on fertility index and mating success. Note: * represents P< 0.05.

4.5 The effects of *Cissus rotundifolia* extract on ovarian and uterine histomorphology

4.5.1 Effect of 400mg/kg *C. rotundifolia* aqueous extract on the ovarian follicles

Cissus rotundifolia aqueous extract at 400mg/kg caused a reduction in the number of the preantral follicles (Figures 11-14). The pre-antral follicles were affected with many being atretic and had degenerated compared to control (Figure 11). The zona pellucida and the oocyte were missing in the pre-antral follicles, and the structural integrity of the granulosa cell layer and the theca cell layer was also disrupted (Figure 12). The antral follicle was also affected with the oocyte and zona pellucida missing (Figure13B). The granulosa cell layer had pyknotic cells and its structural integrity was compromised. The theca cell layer was also disrupted (Figure 13B) compared to the control that had a well-demarcated granulosa cell layer, zona pellucida and viable oocyte (Figure 13A).

The principle observation in the ovaries of treated rats was degeneration of ova in all the follicles, which were in different stages of their development. The first sign noticed in an atretic follicle was pyknosis and fragmentation of the inner granulosa cells.

The corpora lutea structure was also affected. Some were atretic showing signs of vacuolation (Figure 14B). The number of corpora lutea were significantly reduced compared to the control (Figure 14A). The ovarian stroma was mostly occupied by corpora lutea and some atretic ovarian follicles compared to the control.

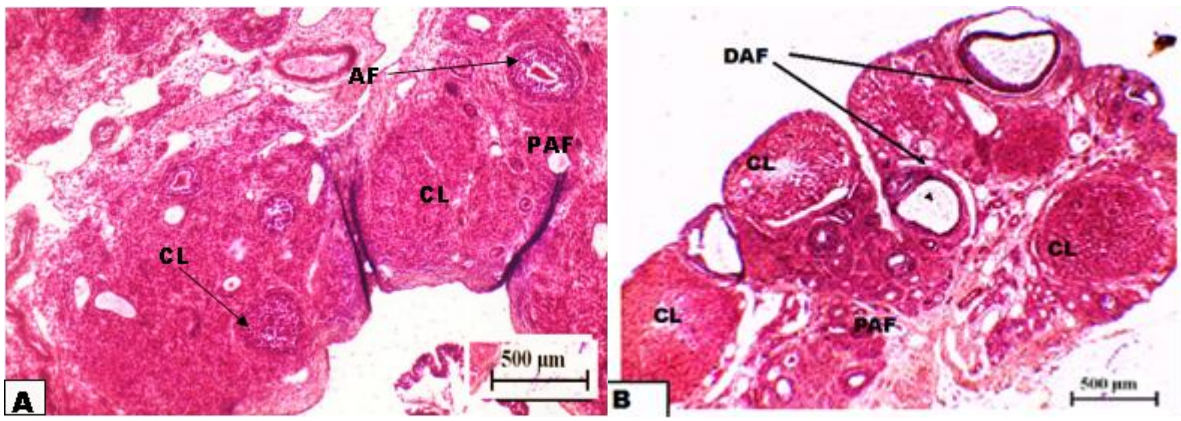


Figure 11: The effect of 400mg/kg *Cissus rotundifolia* extract on ovaries. A: Control, notice the normal preantral and antral follicles with their structural integrity intact. B: Experimental, degenerating antral follicles and reduction in the number of preantral follicles. Mag.100x (Key: AF-antral follicle; CL-corpus luteum; DAF-degenerative antral follicle; PAF–preantral follicle).

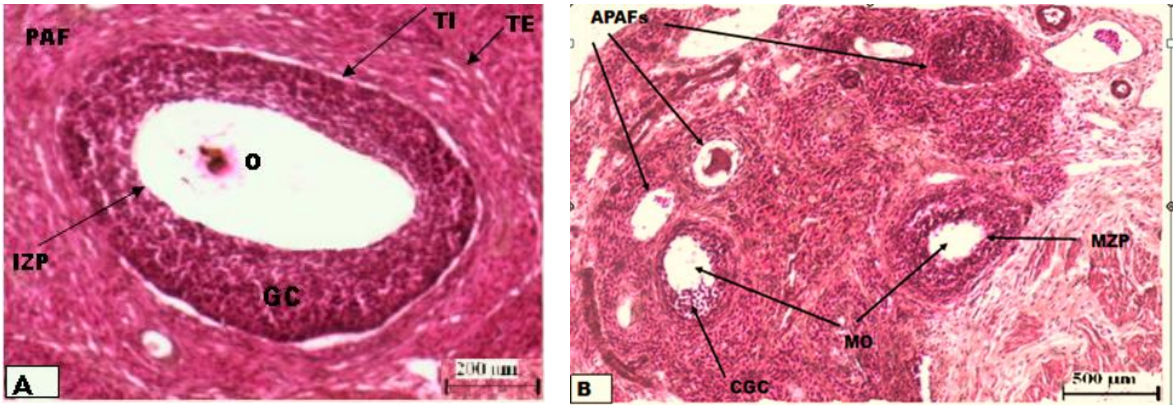


Figure 12: The effect of 400 mg/kg *Cissus rotundifolia* extract on pre-antral follicles. A: Control, normal pre-antral follicle with viable intact oocyte and zona pellucida, intact structural integrity of internal and external theca cells and granulosa cells. B: Experimental, many atretic preantral follicles and lack of oocyte and zona pellucida. Structural integrity of the granulosa cell layer was compromised compared to the control. Mg 400x (Key: APAFs-atretic pre-antral follicles; CGC-compromised granulosa cells; GC-granulosa cells; O-intact oocyte; IZP-intact zona pellucida; MO-missing oocyte; MZP-missing zona pellucida; PAF–preantral follicle).

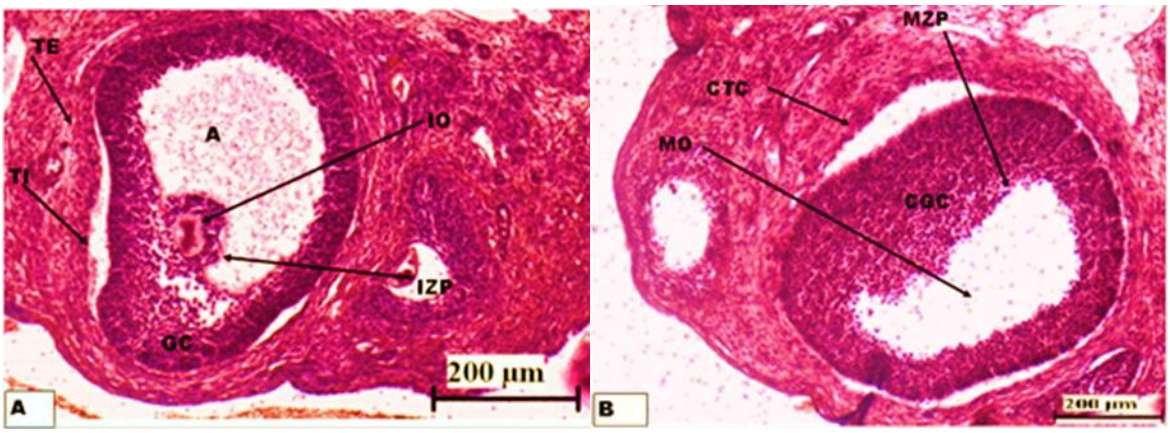


Figure 13: The effect of 400mg/kg *Cissus rotundifolia* extract on antral follicle. A: Control, normal antral follicle with intact oocyte, well-defined zona pellucida, granulosa cell layer, theca interna and theca externa. B: Experimental, missing oocyte and zona pellucida. Pyknotic cells in granulosa cell layer. The structural integrity of the theca cell layer and granulosa cell layer is compromised compared to the control. Mg 100x. (Key: A-antrum; CGC-compromised granulosa cells; CTC-compromised theca cell layer; GC- granulosa cells; IO-intact oocyte; IZP-intact zona pellucida; MO-missing oocyte MZP-missing zona pellucida; TE-theca externa; TI- theca interna

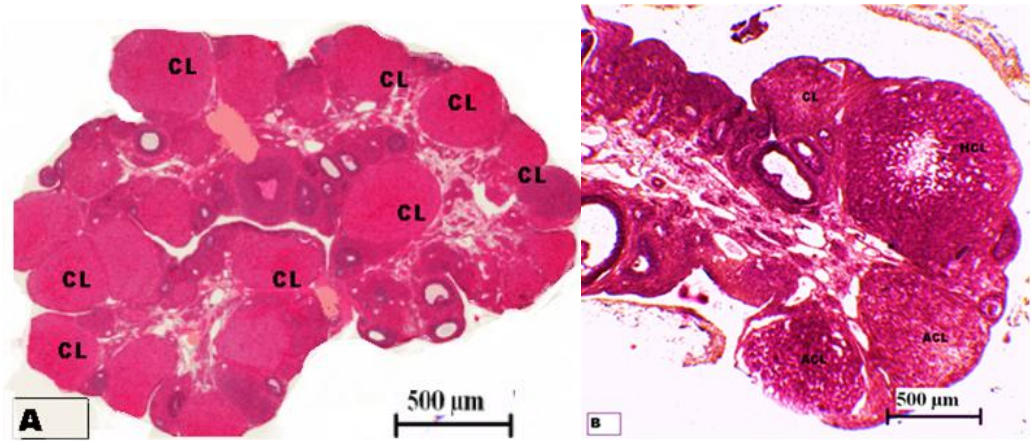


Figure 14: The effect of 400mg/kg *Cissus rotundifolia* aqueous extract on corpus luteum. A: Control, normal corpora lutea with intact structural integrity and with no signs of vacuoles. B: Experimental, disrupted corpora lutea structure where some are atretic showing signs of vacuolation. One of the corpus luteum on the right side of photomicrograph has undergone hypertrophy. The number of corpora lutea has reduced compared to the control. Mg 400× (Key: CL- corpus luteum; ACL-atretic corpus luteum; HCL-hypertrophied corpus luteum).

4.5.2 Effect of 800mg/kg *Cissus rotundifolia* aqueous extract on ovarian follicles

Cissus rotundifolia aqueous extract at 800mg/kg caused changes in the various stages of ovarian follicles compared to the control (Figure 15). The structural integrity of the antral follicle was compromised with presence of pyknotic cells in the granulosa cell layer. The theca cell layer was also disrupted. Corpora lutea was also compromised (Figure 15B). The extract caused degeneration of pre-antral follicles with the structural integrity of the granulosa cell layer compromised. The zona pellucida and oocyte of the pre-antral follicle was also missing. The ooplasm shrunk and theca cell layer was disrupted compared to the control (Figure 16). The antral follicles were degenerating with their oocyte compromised due to the presence of a shrunken ooplasm (Figure17B). There was no zona pellucida and the structural integrity of the granulosa cells was compromised and showed the presence of the pyknotic cells. The theca cell layer was disrupted compared to the control (Figure 17A).

Cissus rotundifolia aqueous extract at 800mg/kg caused degeneration of the corpora lutea structures compared to the control (Figure18). The cytoplasm was condensed and there was presence of pyknotic cells within the ovarian stroma compared to the control. The ovarian stroma structure was disrupted and calcified, and the number of corpora lutea was reduced compared to the control (Figure18).

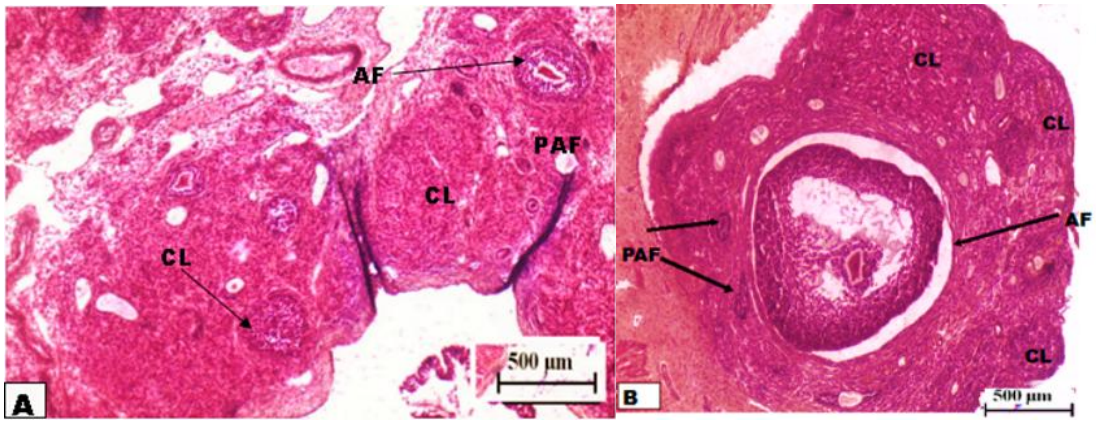


Figure 15: The effect of 800mg/kg *Cissus rotundifolia* aqueous extract on ovaries. A: Control, normal pre-antral, antral follicles and corpus luteum with intact structural integrity. B: Experimental, degenerating pre- antral follicles. The structural integrity of the antral follicle is compromised with pyknotic cells available in the granulosa cell layer. The theca cell layer is also compromised. Corpora lutea is also compromised. Mg 400x. (Key: AF-antral follicle; PAF-preantral follicle; CL-corpus luteum).

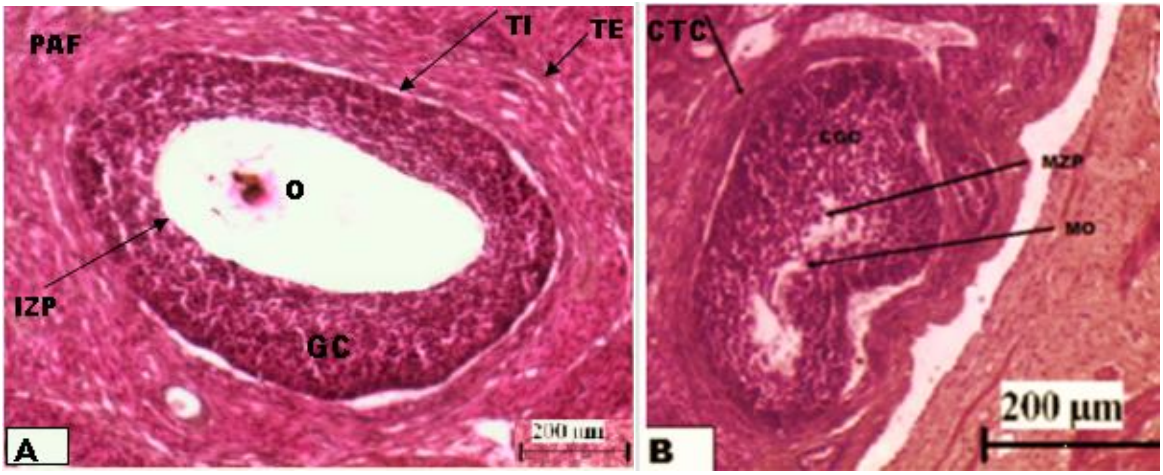


Figure 16: The effect of 800mg/kg *Cissus rotundifolia* aqueous extract on pre-antral follicle. A: Control, normal pre-antral follicle with intact oocyte and zona pellucida. The structural integrity of the granulosa cells and theca cells is intact. B: Experimental, a degenerating pre-antral follicle with the structural integrity of the granulosa cell layer compromised. Oocyte and zona pellucida are missing, ooplasm has shrunk and theca cell layer disrupted. Mg 100×. (Key: CGC-compromised granulosa cell; CTC-compromised theca cell; GC-granulosa cell; PAF-pre-antral follicle; O-oocyte; MO-missing oocyte; MZP-missing zona pellucida).

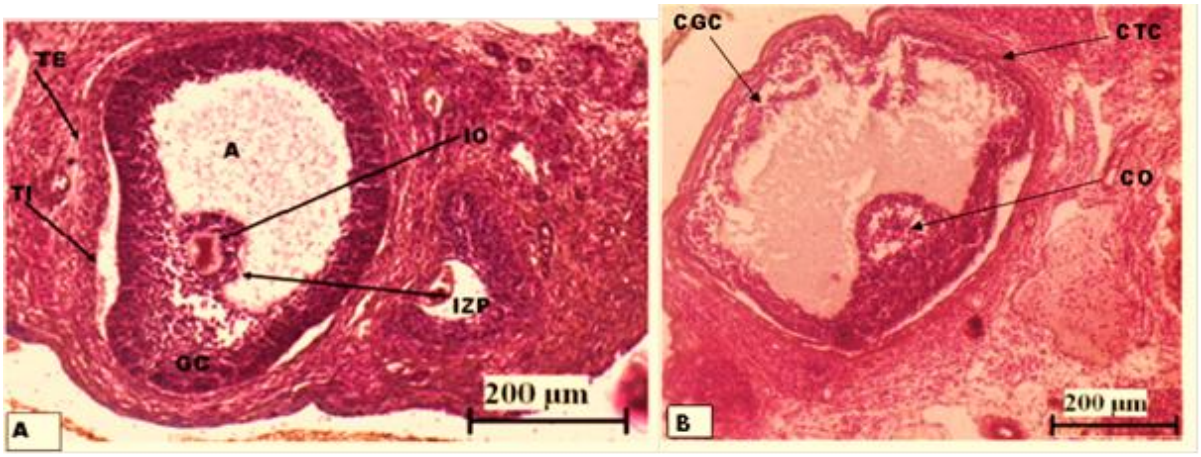


Figure 17: The effect of 800mg/kg *Cissus rotundifolia* aqueous extract on antral follicle. A: Control, normal antral follicle with intact oocyte, well defined zona pellucida. The structural integrity of the granulosa cell layer, theca interna and theca externa is intact. B: Experimental, a degenerating antral follicle, with a compromised oocyte, shrunken ooplasm, loss of zona pellucida, disrupted theca cell layer and compromised granulosa cell layer that shows presence of the pyknotic cells. Mg 100×. (Key: A-antrum; GC- granulosa cells; IO-intact oocyte; IZP-intact zona pellucida; TI-theca interna; TE-theca externa; CO-compromised oocyte; CGC-compromised granulosa cells; CTC-compromised theca cell layer).

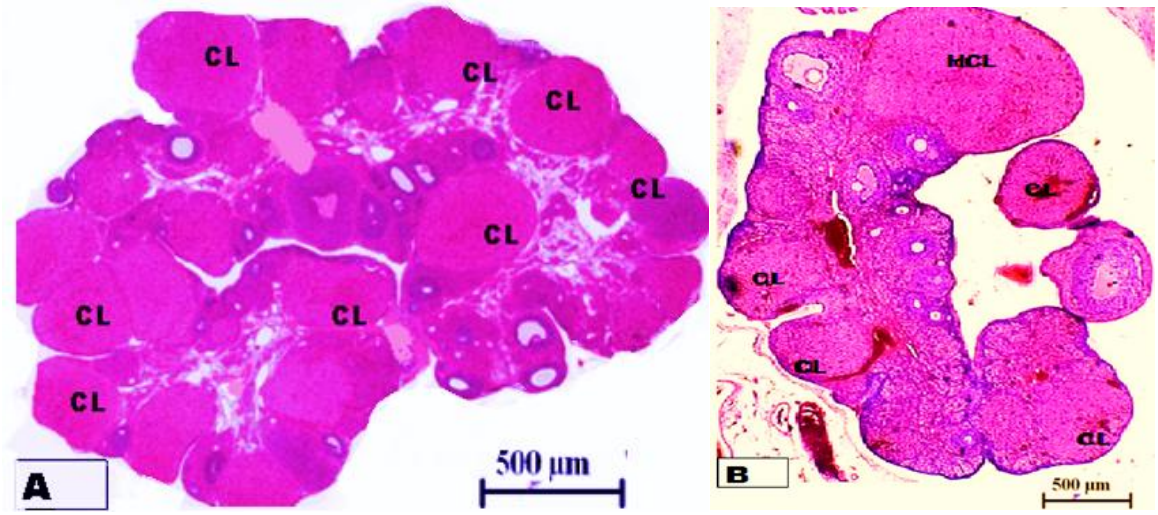


Figure 18: The effect of 800mg/kg *Cissus rotundifolia* aqueous extract on corpus luteum. A: Control, intact structural integrity of the corpora lutea with no signs of vacuoles. B: Experimental, degenerative corpora lutea structures with condensed cytoplasm and presence

of pyknotic cells compared to the control. One of the corpus luteum on the top right side of photomicrograph has undergone hypertrophy. The ovarian stroma structure is disrupted and calcified. The number of corpora lutea has reduced compared to the control. Mg 400×. (Key: CL- corpus luteum; HCL-hypertrophied corpus luteum).

4.6 The effect of *Cissus rotundifolia* extract on uterine histomorphology

At both 400 and 800mg/kg, *Cissus rotundifolia* extract caused a disruption of endometrial structural integrity and showed the presence of pyknotic cells within the uterine stroma. The structural integrity of the uterine glands was slightly disrupted with minimal signs of vacuolation compared to the control that had intact endothelial lining and uterine glands structural integrity (Figure 19 and Figure 20).

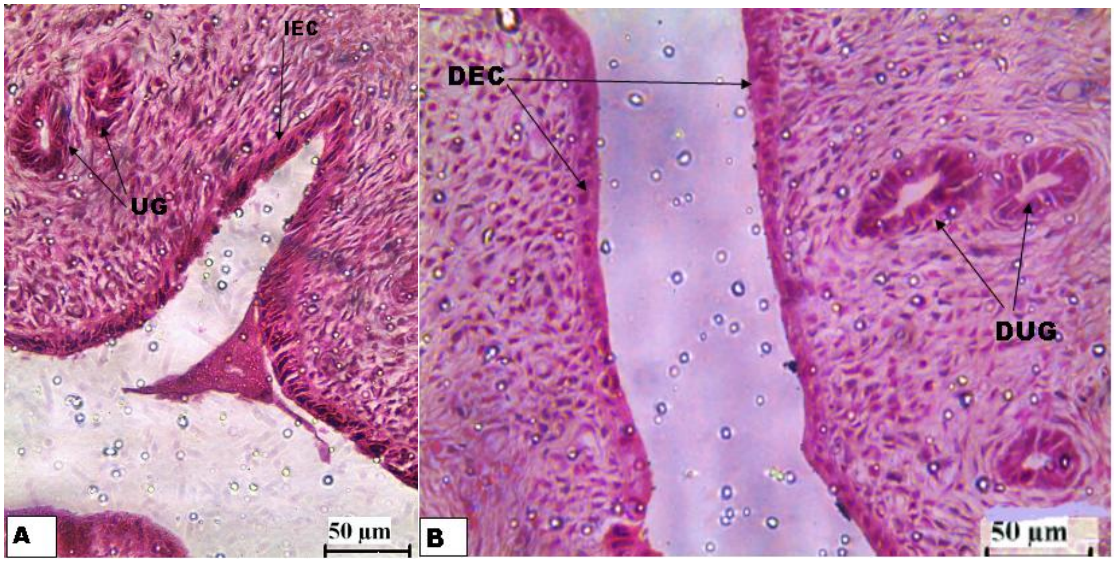


Figure 19: The effect of 400mg/kg *Cissus rotundifolia* aqueous extract on uterine endometrium. A: Control, normal uterine endometrium showing intact simple columnar endothelial cells and uterine glands. B: Experimental, the structural integrity of endothelial cells is disrupted and there is presence of pyknotic cells within uterine stroma. The structural integrity of the uterine glands is slightly disrupted. Mg 400x. (Key: IEC- intact endothelial cell layer; DEC- disrupted endothelial cell layer; DUG-disrupted uterine glands).

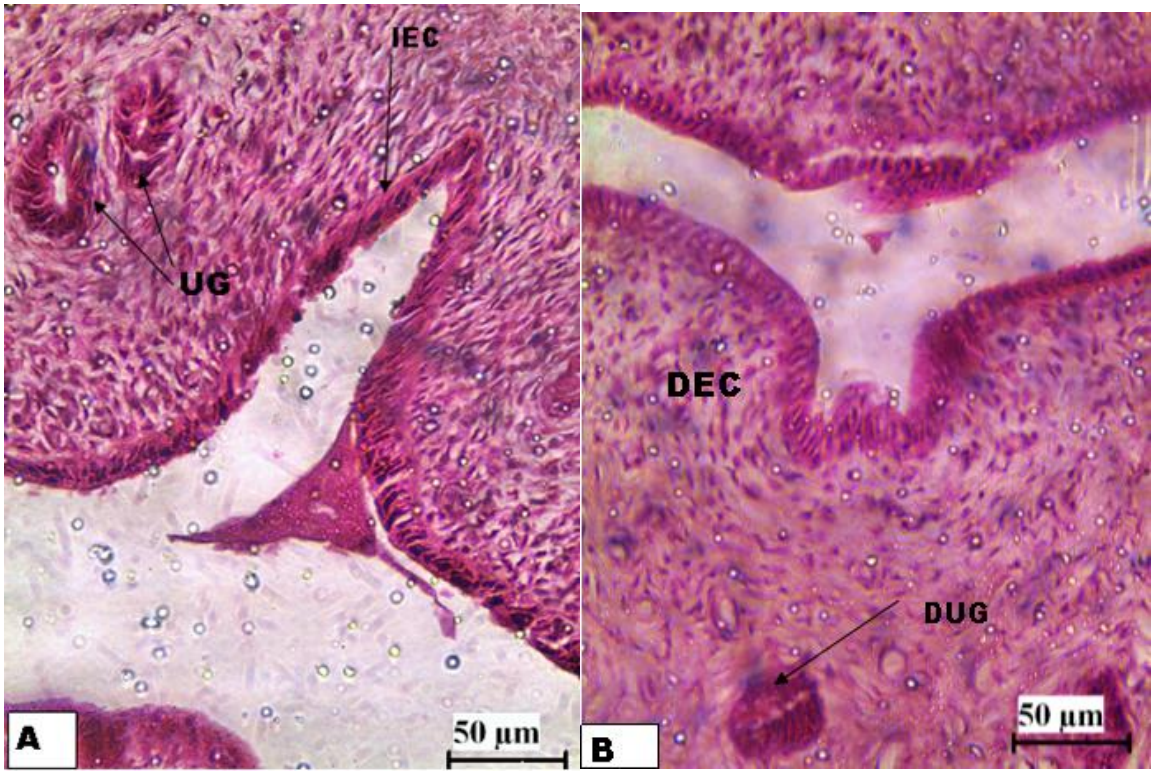


Figure 20: The effect of 800mg/kg *Cissus rotundifolia* aqueous extract on uterine endometrium. A: Control, intact simple columnar endothelial cells and uterine glands with no signs of vacuolation: B*:* Experimental, the structural integrity of the endothelial cells is disrupted. There is presence of pyknotic cells within uterine stroma. The extract caused vacuolation of uterine glands within the stroma. Mg 400×. (Key: DEC- disrupted endothelial cell layer; IEC- intact endothelial cell layer; DUG-disrupted uterine glands).

CHAPTER FIVE

DISCUSSION

5.1 Acute oral toxicity of *C. rotundifolia* aqueous extract

Cissus rotundifolia is indigenous in Tana River County and readily available. The crude extract is traditionally used as a fertility regulator in Tana River County, Kenya. In this study, acute toxicity studies carried out using *C. rotundifolia* aqueous extract did not cause any rat mortality even at the highest dose of 5000mg/kg. There was a general decrease in body weights in the experimental animals which occurred in both control and treated groups between fasting day and the first 24 hours of post-treatment (Table 1).

These results are in agreement with the OECD 423 guideline (2004), where the reduction in the weights of experimental rats cannot be taken as an effect of test compound but as a result of the routine 24-hour starvation of the method. Similar observations were made by Egamberdieva *et al*. (2016), on acute oral studies done on some medicinal plants in Uzbekistan where the test animals and the control group showed a reduction in weight loss between the fasting day and the first 24 hours after extract administration. The authors also concluded that some of the medicinal plants were not toxic even at the highest dose of 5000mg/kg. This finding concurs with that of Mustapha *et al.* (2011), who reported on non-toxic effects of *Rhynchosia subblobata* plant extract on experimental rats at 5000mg/kg. Similarly, Musila *et al*. (2017), reported no toxic effects on rats treated with *Caesalpinia volkensii* plant extract at the limit dose of 5000mg/kg.

The results of the present study showed no pathological changes on the vital organs after 14 days of treatment with *C. rotundifolia* aqueous extract. This finding is similar to that of Egamberdieva *et al.* (2016), who reported that *Saccharum officinarum L.* and *Ferula varia* extracts, respectively, did not cause any pathological changes on vital organs of the experimental animals. Therefore, these plants were non-toxic and safe for use in the treatment of gastrointestinal disorders and as anti-inflammatory drugs.

According to Musila *et al*. (2017), *Caesalpinia volkensii* had no pathological effect on the vital organs of the test animals. The extract from *Moringa oleifera* seeds which were being tested for anti-tumour activity had no significant effect on the body weight and vital organs (Jaafaru *et al*., 2018). Other plants like *Cordyline fruticose* (Naher *et al*., 2019); *Gladiolus segetum* (Marref *et al.,* 2018) and *Alternanthera brasiliana* (Kasthuri & Ramesh, 2018), have also been reported to have no effect on the test animals from the acute oral toxicity studies.

However, the results of this study are in contrast to those of Manzo *et al*. (2019), who reported that the stem bark extract of *Acacia nilotica L*. caused clinical signs of toxicity such as reduced physical activity, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma at 5000mg/kg. There was also a significant reduction in body weight of the test animals, and vital body organs such as the liver and the kidney were affected. Ghorani- Azam *et al*. (2018), too, reported that *Datura stramonium* caused clinical signs of toxicity, and eventually death to all the experimental rats at a dose of 350mg/kg. Nevertheless, this study has shown that *C. rotundifolia* aqueous extract is safe to use even at high doses and causes no pathological changes to vital body organs. This could explain its widespread use in Tana River county.

5.2 Phytochemical compounds of *C. rotundifolia*

Phytochemical screening of *C. rotundifolia* aqueous extract established the presence of alkaloids, phenols, tannins, saponins and glycosides (Table 3). However, flavonoids and terpenoids were absent. Plant phytochemicals are known to cause anti-fertility in females by acting as oestrus cycle disruptors, anti-estrogenic agents, anti-implantation agents or abortifacient agents (Kurniati, *et al*., 2017). Studies have shown that alkaloids, present in several plants, have an anti-ovulatory property, and consequently caused disruption of the oestrus cycle in the females (Circosta *et al*., 2001; Malviya *et al*., 2011; Sharma *et al*., 2014). Alkaloids, saponins, phenols and tannins have been reported to have an anti-implantation effect in rats (Ankush *et al*., 2011; Panda *et al*., 2011; Saravaran & Renuka, 2012). A study by Francis *et al*. (2002) attributed the anti-fertility effect of *Coriandrum sativum* and *Guaiacum officinale* in female rats to the presence of saponins and tannins in the extract. The authors further reported on the saponins as having abortifacient, anti-zygotic and anti-implantation effects. Londonkar *et al*. (2009), working on crude *Sida acuta* extract, attributed its anti-fertility effect on rat to the presence of phenols which had anti-zygotic, blastocytotoxic and anti-implantation activity.

This study has established the presence of phytochemicals in *C. rotundifolia* aqueous extract. These phytochemicals, especially alkaloids, saponins and tannins, might be responsible for the anti-fertility effects of the plant as claimed by traditional medicine practitioners in Tana River County.

5.3 The effect of *Cissus rotundifolia* aqueous extract on oestrus cycle

This study has shown that at 400 and 800mg/kg *C. rotundifolia* extract significantly disrupted the oestrus cycle. The plant extract caused a significant increase in the frequency of proestrus and metestrus and a significant reduction in the frequency of estrus and diestrus phases in the experimental rats, compared to the control (Figures 5 and 6). Oestrus cycle is a rhythmic reproductive cycle in sexually mature female mammals and is influenced by the release of gonadotropins from the pituitary gland and sex hormones from the gonads. While the female cyclicity is characterised by vaginal changes as observed in oestrus cycle it also is an index of good functioning of the neuroendocrine reproductive system and ovarian activity (Deshmukh *et al*., 2014; Marcondes *et al*., 2002).

The disruption of the normal oestrus cycle in this study also indicates the disruption of ovarian progesterone and oestrogen balance whose levels are controlled by hypothalamus releasing hormones and pituitary gonadotropins (Namulindwa *et al*., 2015). Oestrogen and progesterone are the hormones responsible for the normal histology and functioning of female genital tract (Pattanayak & Mazumdar, 2009). The *C. rotundifolia* extract might have interfered with hormone synthesis which might have brought about the changes observed in the experimental rat's oestrus cycles. The findings of this study are consistent with those of Kaingu *et al*. (2018), who reported a reduction in estrus phase and a prolonged metestrus phase in female rats treated with aqueous extract of *Croton menyharthii* and *Uvariodendron kirkii*. Obinna and Kagbo (2018), too, reported that *Costus lucanuscianus* leaf extract caused an increase in proestrus and metestrus phases, and a reduction in diestrus and estrus phases in experimental rats.

However, the results of this study are in contrast to those of Kage *et al*. (2009), who reported that *Trichosanthes cucumerina* aqueous extract caused a significant increase in estrus and diestrus. Monsefi *et al*. (2015), too, reported that *Anethum graveolens* caused a significant increase in estrus and diestrus phases and the total time of oestrus cycle in female Wistar rats.

In general, the oestrus cycle serves as a surrogate marker and has been frequently used to evaluate the impact of any anti-fertility agent (Deshmukh *et al*., 2014; Kurniati *et al.*, 2017). Thus, *Cissus rotundifolia* aqueous extract disrupted the oestrus cycle by significantly increasing the proestrus and metestrus and significantly reducing estrus and diestrus phases, respectively. This makes the plant extract a potential anti-fertility agent.

5.4 The effect of *Cissus rotundifolia* aqueous extract on mating success, fertility index, gestation length, and litter size

5.4.1 Effects of extract on mating success and fertility index

In this study, mating was considered successful once a vaginal plug was established or the presence of spermatozoa in a vaginal smear was microscopically observed. *Cissus rotundifolia* extract at both doses of 400 and 800mg/kg had no significant effect on mating success. Rats ovulate between late proestrus and estrus phase of the oestrus cycle, and it is during this time that the female rats are receptive to the males. During folliculogenesis, the rising levels of oestradiol stimulates the secretion of follicle stimulating hormone (FSH) which initiates the growth and the maturation of primary follicles to secondary follicles. The mature secondary follicles then release ova (ovulation). At the start of folliculogenesis, many primary follicles are recruited but only a few of them mature to release the ova; the rest undergo degeneration (atresia) (Varshney *et al*., 2016). In this study, 80% of the rats administered with the extracts before mating (pre-mating), mated successfully. This was an indication that the females were receptive to the males, and thus, estrus, and hence ovulation, occurred in these rats.

Cissus rotundifolia extract administered before mating (pre-mating), at 400mg/kg had no significant effect on fertility index, unlike at 800mg/kg, which had a significant effect (Table 4 and Figure 8). This finding is similar to that of Dinesh *et al*. (2012), who reported a significant reduction in fertility index and no significant effect on mating success in female rats treated with *Bambusa vulgaris* aqueous leaf extract. Kaingu *et al*. (2018), too, reported similar results in female Wistar rats treated with aqueous extract of *Croton menyharthii* and *Uvariodendron kirkii* leaf extract.

It is possible that fertilization and/or implantation was disrupted by the high dose (800mg/kg) of the *C. rotundifolia* extract. Fertility in females is determined by the developmental competence of an oocyte, the ability of the oocyte to be fertilized and give rise to a viable embryo and for that embryo to successfully implant in the uterus. Anything interfering with any of these processes could lead to fertility failure. *Cissus rotundifolia* at 400mg/kg, did not cause a significant effect on fertility index probably because of the low dose hence low quantities of the active ingredient affecting the targeted reproductive process (Daniyal & Akram, 2015).

On the other hand, fertility index was significantly affected in the rats treated with both doses (400 and 800mg/kg) of *C. rotundifolia* extract after mating (post-mating) (Table 5 and Figure

9). This suggests that implantation was disrupted and was not successful in the uterus of these rats. This finding corroborates that of Azamthulla *et al*. (2015) and Hu *et al.* (1985), who reported on anti-implantation effects of *Acalypha indica*, *Ocimum sanctum* and *Butea monosperma,* extracts administered to rats.

For the pre-post mating extract administration treatment, fertility index was significantly reduced to 20% and 0% in the rats treated with 400 and 800mg/kg of *C. rotundifolia* aqueous extract, respectively (Table 6 and Figure 10). Since a few rats littered at the end of the gestation period, this suggests that pre-post mating extract administration regime could have had a significant effect on folliculogenesis, oogenesis, ovulation or implantation. These results are similar to those of Kaingu *et al*. (2018), who reported on a significant reduction in fertility index in female Wistar rats treated with aqueous extract of *Croton menyharthii* and *Uvariodendron kirkii* on pre-post regime. Dinesh *et al*. (2012) and Sharma *et al*. (2014), too, reported on *Ricinus communis* and *Punica granatum* plant extracts which significantly affected folliculogenesis, ovulation, and implantation in Wistar rats.

In general, contraceptive properties of drug compounds could be anti-ovulatory, anti-fertilization, disrupt embryo implantation or cause abortion. A disruption of pituitary gonadotropins or ovarian steroids compromises folliculogenesis and oogenesis and leads to infertility. Thus, this study has established that *Cissus rotundifolia* aqueous extract has anti-ovulatory and anti-implantation properties.

5.4.2 Effect of *C. rotundifolia* on gestation length, litter size and body weight

Cissus rotundifolia aqueous extract at 400 mg/kg and 800 mg/kg significantly prolonged the gestation length in the test animals compared to the control in all the treatment regimens (Tables 4, 5 and 6). The extract also caused a significant reduction in litter size compared to the control group in all the treatment regimens (Tables 4, 5 and 6). These findings are similar to those of Kaingu *et al*. (2018), who reported aqueous extracts of both *Uvariodendron kirkii* and *Croton menyharthii* significantly prolonged the gestation length and reduced the litter size of female Wistar rat. Dare *et al*. (2011), too, reported a significant reduction of litter size in rats treated with aqueous extracts of *Anacardium occidentale* and *Allium sativum.*

However, these findings are in contrast to those of Iyare & Adegoke (2014), who reported no significant change in gestation length of Wistar rats treated with *Hibiscus sabdariffa* aqueous extract. Kamita *et al*. (2014), also reported no significant change in litter size in rats treated with *Bridelia micrantha* and *Ximenia americana* aqueous leaf extract. Nevertheless, the

significant reduction in litter size also supports the anti-implantation property of *C. rotundifolia* extract as discussed previously. In the present study, the experimental rats in pre-mating, post-mating and pre-post mating showed an increase in body weights. There was no observed lack of appetite, no signs of sickness, and the experimental rats were as active as the control. This suggests that *Cissus rotundifolia* extract did not affect the physiological functions within the rat's body. This is similar to Okoyea *et al*. (2015), who reported on leaf extract of *Telfairia occidentalis* which did not affect the physiological functioning of the experimental rats.

5.5 The effect of *Cissus rotundifolia* extract on ovarian and uterine histomorphology

Histological studies of ovaries in the control group showed the various types of follicles at all stages of folliculogenesis (primordial, primary, secondary, and mature follicles) (Figures 11A and 15A). *Cissus rotundifolia* aqueous extract at 400 and 800mg/kg increased atretic pre-antral follicles (Figures 11B, 12B, 15B and 16B) and a reduction in the number of primary follicles. This suggests a compromised folliculogenesis and may explain the anti-fertility effect of *C. rotundifolia* extract.

The consistent reduction in the number of follicles in the presence of *C. rotundifolia* at 400 and 800mg/kg supports the findings of objectives 3 and 4 of this study on the effect of the plant extract on oestrus cycle and fertility index; where disruption of oestrus cycle and reduction in fertility index was observed. Oestrus cycle is driven by both pituitary gonadotropins and ovarian steroid hormones. A disruption or disturbance of hormonal balance especially oestradiol disrupts the oestrus cycle (Varshney *et al.*, 2016). Levels of oestradiol start increasing as follicle stimulating hormone secretion gradually increases and initiates antral follicular growth and maturation. From the histomorphology results, the antral follicular growth was affected (Figures 13B and 17B), which therefore, implies that the production of follicle stimulating hormone (FSH) and oestradiol was affected. The affected production of oestradiol could be due to the compromised granulosa and theca cellular layers, as exemplified in Figures 12B,13B, 15B,16B and 17B, which play an active role in androgen and oestradiol synthesis. It could also be due to a direct effect of the plant extract on the pituitary gland (Devi *et al*., 2015).

An optimal blood level of FSH is a prerequisite for the initiation and maintenance of normal ovarian folliculogenesis. Therefore, the present histological finding suggests a hypothalamic-pituitary gonadal axis dysfunction after treatment with *C. rotundifolia*

aqueous extract. Similar studies have reported corroborating results (Daniyal & Akram, 2015; Devi *et al*., 2015; Dinesh *et al*., 2012) thereby leading to a reduction in ovulated oocytes and litter size. This supports the findings of objective 4 on the effect of *C. rotundifolia* on litter size where the litter size was significantly reduced. Fully grown oocytes and ovulated eggs from rats without mZP2 and mZP3 glycoprotein lack a zona pellucida and are infertile (Sakila *et al*., 2009). According to the histological findings, the zona pellucida was compromised (Figure 16B) and in some cases it was completely missing (Figure 12B, 13B and 17B) compared to the control which had an intact zona pellucida.

As stated in the introduction, it is possible that a disruption of this bi-directional communication between oocyte and surrounding granulosa cells is responsible for compromised folliculogenesis and oogenesis especially where the structural integrity of theca cells and granulosa cells was compromised thereby compromising fertility (Kaingu *et al.*, 2017). This finding corroborates with that of Okoyea *et al*. (2015), who reported that *Telfairia occidentalis* seed oil compromised the theca cells, granulosa cell layer and zona pellucida. The results are also in line with those of Devi *et al*. (2015), who found out that *Rhynchosia subblobata* plant extract compromised zona pellucida and granulosa cell layer hence being a cause of infertility by the plant extract.

Cissus rotundifolia aqueous extract at both 400 and 800mg/kg caused degeneration of corpora lutea and hence a reduction in the number of corpora lutea (Figures 14B and 18B). These findings concur with those of Hossein *et al*. (2016), who reported that palm pollen extract caused a reduction in the number of corpora lutea in rats. Odirichukwu (2015), too, reported that extract of unripe *Carica papaya* fruit caused a dose-dependent reduction in the number of corpora lutea in rats. Kaingu *et al.* (2017), also reported that *Uvariodendron kirkii* caused a dose-dependent hypertrophy of the corpora lutea in rats.

However, the results of this study are in contrast to those of Monsefi *et al*. (2015), who reported that *Anethum graveolens* increased the number of corpora lutea in rats. *Alchornea cordifolia* extract was also reported to have no effect on the number of corpora lutea in mice (Ebenyi *et al*., 2016). Kaingu *et al*. (2017), too, reported that *Croton menyharthii* caused an increase in the number of corpora lutea in rats.

The corpus luteum secretes progesterone hormone. Progesterone in turn plays a crucial role in preparing the endometrium for possible implantation. A decrease in the number of corpora lutea possibly leads to disrupted oestradiol progesterone ratio therefore possibly

compromising the window of implantation. Progesterone is key in pregnancy maintenance. Near term levels of progesterone go down triggering other hormonal and molecular changes that terminates gestation (Kennedy *et al*., 2014). This supports the results of objective 4 of this study; where *C. rotundifolia* extract at 800mg/kg caused the rats not to litter at all and hence the fertility index reduced to 0%; this was due to the window of implantation being compromised.

The structural integrity of uterine endothelial lining is important for implantation to be successful and for gestation to be established. The results of this study showed that the endometrium of rats treated with *C. rotundifolia* extract was compromised (Figures 19 and 20). This finding is similar to that of Suhaimi *et al*. (2016), where *Ficus deltoidea* leaf extract caused vacuolation of the uterine glands and disruption of the uterine endothelial lining. The growth and differentiation of endothelium during each oestrus cycle is controlled by oestradiol and progesterone. A disruption of the hormonal balance affects implantation (Neetesh *et al*., 2016; Vijay *et al*., 2004). The disruption of the granulosa cell layer, theca cell layer and corpus luteum supports the uterine lining histomorphology results. This further supports the objective 4 results on the significant reduction in fertility index. It is therefore, possible that endometrial receptivity was compromised leading to failed implantation.

This histomorphology study has revealed a disruption of the structural integrity of the follicular cells, theca cells, granulosa cells, and a disruption of zona pellucida at both dose levels of *C. rotundifolia* aqueous extract. This strongly points to interference with folliculogenesis and oogenesis that would lead to compromised fertility of the female. This might, therefore, explain the traditional consumption of crude plant extract to cause infertility as a method of contraception desired by the women in Tana River County.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i) *Cissus rotundifolia* aqueous extract has no effect on body weights at the fixed OECD dose levels (300, 2000, 5000 mg/kg).

ii) *Cissus rotundifolia* has alkaloids, tannins, saponins, phenols and glycosides phytochemicals. The presence of these phytochemicals is responsible for the anti-fertility activity of *Cissus rotundifolia* aqueous extract.

iii) The plant aqueous extract disrupts the oestrus cycle causing an increased metestrus and proestrus phases and a reduction in estrus and diestrus phases. This makes the plant extract a potential anti-fertility agent.

iv) *Cissus rotundifolia* extract has an effect on reproductive parameters such fertility index, gestation length, and litter size. It can therefore, be concluded that *Cissus rotundifolia* extract has anti-ovulatory and anti-implantation properties.

v) *Cissus rotundifolia* extract affected the ovarian and uterine lining histomorphology of the Wistar rats. It disrupted the structural integrity of the follicular cells, granulosa cells, theca cells, zona pellucida and vacuolation of uterine glands. This strongly points to interference with folliculogenesis and oogenesis that would lead to a compromised fertility of the female.

6.2 Recommendations

i) Further studies should consider evaluating the anti-implantation activity of *Cissus rotundifolia* through the assessment of the implantation sites on opened uteri and effect of the extract on histomorphology of pituitary gland, liver and kidney.

ii) Hormonal profile on the effect of *Cissus rotundifolia* on reproductive hormones should be incorporated in the evaluation of the plant extract as an anti-fertility agent.

iii) Evaluation on the reversible fertility regulating effects of *Cissus rotundifolia* extract in female Wistar rats should be considered.

REFERENCES

Adams, J. D., & Garcia, C. (2006). Women's health among the Chumash. *Evidenced Based on Complementary and Alternative Medicine*, **3**(75), 125-131.

Adetunji, J. A. (2011). Rising Popularity of Injectable Contraceptives in sub-Saharan Africa. *African Population Studies*, **25**(2), 587-604.

Ahman, E., & Shah, I. (2007). *Unsafe abortion: Global and Regional Estimates of the Incidence of Unsafe Abortion and Associated Mortality in 2003.* Geneva: World Health Organization.

Ahmed, S., Li, Q., Liu, L., & Tsui, A. O. (2012). Maternal deaths averted by contraceptive use: An analysis of 172 countries. *Lancet,* **380**(9837), 111-125.

Ajay, K., & Prakash, O. (2012). Potential antifertility agents from plants: A comprehensive review. *Journal of Ethnopharmacology*, **14**(6), 1-32.

Al-Shemary, N., Mousa, S., & Muslim, Z. (2018). Histological and Hormonal Study about the Effect of Aqueous Extract of *Ocimum gratissmum* on Female Reproductive System in Albino Mice. *Journal of Pharmaceutical Sciences and Research,* **10**(4), 765-767.

Ankush, R., Singh, A., Sharma, A., & Bhatia, V. (2011). Antifertility activity of medicinal plants on reproductive system of female rats. *International Journal of Bio-engineering Science and Technology*, **2**(3), 44-50.

Azamthulla, M., Rajkapoor, B., & Kavimani, S. (2015). A Review on medicinal plants exhibiting antifertility activity. *World Journal of Pharmacy and Pharmaceutical Sciences,* **4**(3), 243-272.

Bablola, K. (2009). An examination of the usage of herbal contraceptives and abortifacients in Lagos State, Nigeria. *Ethnobotanical Leaflets*, **13**(2), 140-146.

Beshay, V. E., & Carr, B. R. (2012). *Hypothalamic-pituitary-ovarian axis and control of the menstrual cycle. Clinical Reproductive Medicine and Surgery: A Practical Guide*, eds Falcone T, Hurd WW (Springer, New York), 31–42.

Bradley, S. E., Croft, T. N., & Rutstein, S. O. (2011). The Impact of Contraceptive Failure on Unintended Births and Induced Abortions*: Estimates and Strategies for Reduction.* Calveton, Maryland, USA: ICF.Macro.

Bretveld, R., Brouwers, M., Ebisch, I., & Roeleveld , N. (2007). Influence of pesticides on male fertility. *Scandinavian Journal of Work Environment and Health,* **33**(1), 13-28.

Campbell, O., & Graham, W. (2006). Strategies for Reducing Maternal Mortality: Getting on With What Works. *Lancet*, **368**(9543), 1284-1299.

Chan, K.W ., Iqbal, S., & Nicholas, M. H. (2011). Preparation of deodorized antioxidant rich extracts from 15 selected spices through optimized aqueous extraction. *Journal of Medicinal Plants Research,* **5**(25), 6067-6075.

Circosta, C., Sanogo, R., & Occhiuto, F. (2001). Effects of *Calotropis procera* on estrous cycle and on estrogenic functionality in rats. *Farmaco,* **56**(7), 373-378.

Clause, B.T. (1998) The Wistar Institute Archives: rats (not mice) and history. http://www.amphilsoc.org/mendel/1998.htm#Clause. Accessed 9th June 2018.

Daniyal, M., & Akram, M. (2015). Antifertility activity of medicinal plants. *Journal of Chinese Medical Association,* **78**(7), 382-388.

Dare, S. S., Hamman, W. O., Musa, S. T., Goji, D. T., & Oyewale, A. A. (2011). Effects of aquoeus Extract of *Anarcadium occidantale* (Cashew) leaf on Pregnancy outcome on Wistar rats. *International Journal of Animals and Veterinary Advances,* **3**(2), 77-82.

Darroch, J. E. (2013). Trends in Contraceptive Use. *Contraception,* **87**(3), 259-263.

Darroch, J. E., & Singh, S. (2013). Trends in Contraceptive Need and Use in Developing Countries in 2003, 2008, and 2012. An Analysis of National Surveys. *The Lancet,* **381** (9879), 1756-1762.

Deshmukh, V., Zade, V., & Aluta, T. (2014). Antifertility effects of aqueous, ether and chloroform extracts of *Caesalpinia pulcherrima* on female albino rats. *International Journal of Research Studies in Biosciences,* **2**(11), 13-20.

Devi, P., Kumar, P., Nidhi, L., & Dhamija, I. (2015). Antifertility Activity of Medicinal Plants on Male and Female Reproduction. *International Journal of Pharmaceutical Sciences and Research,* **6**(3), 988-1001.

Dinesh, K., Ajay, K., & Prakash, O.M. (2012). Potential antifertility agents from plants: A comprehensive review. *Journal of Ethnopharmacology*, **10** (55), 1-32.

Dugoua, J. J. (2010). Herbal Medicines and Pregnancy. *Drugs in Pregnancy and Lactation Symposium*, (pp. 1-9). Toronto, Canada.

Dugoua, J. J. (2011). *Natural Health Products (NHPs) in Pregnancy and Lactation: A review of the landscape and blueprint for change.* Toronto, Canada: University of Toronto.

Ebenyi, L. N., Akubugwo, E. L., Ogbanshi, M. E., Agbafor, K. N., & Inya-Agha, O. R. (2016). Fertility Enhancing Potentials of *Alchornea cordifolia* on Albino Rats. *Middle-East Journal of Scientific Research,* **24**(5), 1802-1808.

Egamberdieva, D., Mamedov, N., Ovidi, E., Tiezzi, A., & Craker, L. (2016). Phytochemical and Pharmacological Properties of Medicinal Plants from Uzbekistan. *Journal of Medicinally Active Plants,* **5**(2), 59-75.

Emmen, J. M., Couse, J. F., Elmore, S. A., Yates, M. M., Kissling, G, E., & Korach, K. S. (2005). *In vitro* growth and ovulation of follicles from ovaries of oestrogen receptor (ER){alpha} and ER {beta} null mice indicate a role for ER {beta} in follicular maturation. *Endocrinology*, **6** (5), 2817–2826.

Ernd, O. (2005). The Impact of Water Conflicts on Pastoral Livelihoods. In *The case of Wajir District in Kenya.* (pp. 50-52). Winnipeg: International Institute for Sustainable Development.

Ernest, M., Jean-Jacques, D., Daniel, P., & Gideon, K. (2013). *Herbal Medicines in Pregnancy and Lactation: An Evidence-Based Approach.* Taylor & Francis.

Francis, G., Zohar, K., Harinder, P., & Klaus, B. (2002). The biological action of saponins in animal systems: A review. *British Journal of Nutrition,* **88**(25), 587-605.

Gakuya, D.W. (2001). *Pharmacological and Clinical evaluation of antihelminthic activity of Albiza anthelmintica Brogn, Maeruaedulis De Wolf and Maerua subcordata De wolf plant extracts in sheep and mice*. PhD thesis. University of Nairobi, Department of Veterinary Clinal Studies. (pp. 50-52).

Geremerev, T., Yalemtsehay, M., & Eyasu, M. (2005). *In vivo* and *in vitro* anti-fertility and anti-implantation properties of *Leonotis ocymifolia* in rats. *African Journal of Traditional, Complementary and Alternative Medicines*, **2(**2), 50-60.

Ghorani-Azam, A., Sepahi, S., Riahi-Zanjani, B., Alizadeh-ghamsari, A., Mohajeri, S., & Balali-Mood, M. (2018). Plant toxins and acute medicinal plant poisoning in children: A systematic literature review. *Journal Research of Medical Science,* **1**(4), 23-26.

Giday, M., Asfaw, Z., & Woldu, Z. (2010). Medicinal plants of Melnit ethnic group of Ethiopia: an ethnobotanical study. *Journal of Ethnopharmacology,* **124** (10), 513-521.

Hamid, H.Y., & Zakaria, Z.A. (2013). Reproductive characteristics of the female laboratory rat. *African Journal of Biotechnology*, **12**(19), 2510-2514.

Hannaford, P., & Belfield, T. (2009). The contraceptive revolution: some excellent progress but work still to be done. *British Journal of General Practice*, **59**(558), 60-75.

Hanneia, I. M., Hasan, A., Abdel, L., Reda, H. E., Wessam, M. A., & Mona, I. S. (2013). Effect of methomyl on fertility, embryotoxicity and physiological parameters in female rats. *Journal of Applied Pharmaceutical Science,* **3**(12), 109-119.

Hossein, K. J., Hojatollah, K. J., & Zahra, B. (2016). The Effect of Palm Pollen Extract on Polycystic Ovary Syndrome (POS) in Rats. *International Journal of Medical Research and Health Sciences,* **5**(5), 317-321.

Hu, B. H., Sha, H., Wang, C. R., Yu, D. E., & Wu, W. C. (1985). Studies on the anti-fertility constituent of the flower Yuan Hua: isolation and structure of Yuanhuatine. *Acta Chimica Sinica,* **43**(15), 460-462.

Iyare, E. E., & Adegoke, O. E. (2014). Gestational Outcome in Rats That Consumed Aqueous Extract of *Hibiscus sabdariffa* During Pregnancy. *Pakistan Journal of Nutrition,* **10**(4), 350-354.

Jaafaru, M. S., Abdi- Karim, N. A., Eliaser, E. M., Waziri, P. M., Ahmed, H., Kong, L., & Abdul, F. (2018). Nontoxic Glucomoringin-Isothiocyanate (GMG-ITC) Rich Soluble Extract Induces Apoptosis and Inhibits Proliferation of Human Prostate Adenocarcinoma Cells (PC-3). *Journal of Nutrients,* **10**(1174), 2-16.

Jacobstein, A. R., & Harriet , S. (2013). Contraceptive implants: providing better choice to meet growing family planning demand. *Global Health Science and Practice,* **1**(1), 11-17.

Kage, D. N., Vijaykumar, B. M., Seetharam, Y. N., Suresh, P., & Saraswati, B. P. (2009). Effect of Ethanol Extract of Whole Plant of *Trichosanthes cucumerina* and *Cucumerina Linnaeus* on Gonadotropins, Ovarian Follicular Kinetics and Estrous Cycle for screening of Anti-fertility Activity in Albino rats. *International Journal of Morphology*, **27**(1), 173-182.

Kaingu, C. K., Oduma, J. A. & Kanui, T. (2011). Practices of Traditional Birth Attendants In Machakos District, Kenya. *Journal of Ethnopharmacology,* **142**(2012), 495-502.

Kaingu, C. K., Oduma, J. A., Mbaria, J., & Kiama, S. (2013). Medicinal plants traditionally used for the management of female reproductive dysfuncton in Tana River County, Kenya. *International Journal of Genuine Traditional Medicine*, **3**(2), 17-20.

Kaingu, C. K., Oduma, J., Mbaria, J., & Kiama, S. (2017). Effects of *Croton menyharthii* and *Uvariodendron kirkii* extracts on ovarian corpora lutea and reproductive hormones. *Discovery Phytomedicine,* **4**(1), 21-25.

Kaingu, C. K., Oduma, J., Mbaria, J., & Kiama, S. (2018). Anti-fertility and anti-implantation potential of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract in female Winstar rats. *International Journal of Medicinal Plants*, **112**(2018), 844-855.

Kamita, M. K., Matu, E. N., Njenga, E., Wanga, J., Amalemba, G., & Kingondu, E. V. (2014). *In vivo* antifertility activity and phytochemical screening of selected Kenyan medicinal plants. *African Journal of Pharmacology and Therapeutics,* **3**(3), 85-94.

Karateke, A., Dokuyucu, R., Dogan, H., Ozgur, T., Tas, Z. A., Tutuk, O., Agturk, G., & Tumer, C. (2018). Investigation of Therapeutic Effects of Erdosteine on Polycystic Ovary Syndrome in a Rat Model. *Medical Principles and Practice*, **27**(6), 515-522.

Kasthuri, O. R., & Ramesh, B. (2018). Toxicity Studies on Leaf Extracts of *Alternanthera brasiliana* (L.) Kuntze and *Alternanthera bettzickiana* (Regel) Voss. *Journal of Applied Pharmaceutical Science*, **8**(10), 082-089.

Kennedy, D., Lupattelli, A., Koren, G., & Nordeng, H. (2016). Safety classification of herbal medicines used in pregnancy in a multinational study. *BMC Complementary and Alternative Medicine*, **16**(102), 36-41.

Kenya National Bureau of Statistics (KNBS). (2009). *Analytical Report on Projections of Special Population Groups, 2010-2030.* Nairobi: Kenya National Bureau of Statistics, Ministry of Planning, National Development and Vision 2030.

Kenya Population and Housing Census report (2019). Kenya National Bureau of Statistics (pp. 11-13). Kenya.

Kubota, K., Wei, C., Pramod, B., Michael, A., Karim, R., Jay, L., Vivian, R., & Michael, S. (2016). Rethinking progesterone regulation of female reproductive cyclicity. *Journal of the National Academy of Sciences*, **113**(15), 4212–4217.

Kurniati, R., Dewi, H., & Hendra, M. (2017). Effect of Water Decoction of Langsat Bark (*Lansium domesticum Corr*.) on Estrous Cycle and Uterus Weight in Mice (*Mus musculus* L.) The 7th International Conference on Global Resource Conservation. *The 7th International Conference on Global Resource Conservation.* **44**. AIP publishing.

Londonkar, R. L., Sharangouda, J. P., & Saraswati, B. P. (2009). Phytochemical and contraceptive property of *Sida acuta burm*. in Albino rats. *International Journal of PharmTech Research*, **1**(4), 1260-1266.

Lu, A., Beehner. J., Czekala. N., Koenig. A., Larney. E., & Borries, C. (2010). Phytochemicals and reproductive function in wild female Phayre's leaf monkeys (*Trachypithecus phayrei crepusculus*). *Hormones and Behavior*, **59**(2011), 28-36.

Lule, E., Singh, S., & Chowdhury, S. A. (2007). *Fertility Regulation Behaviors and Their Costs: Contraception and Unintended Pregnancies in Africa and Eastern Europe & Central Asia.* Washington, DC: The International Bank for Reconstruction and Development / The World Bank.

Malviya, N., Sanjay J., Vipin, B.G., & Savita, V. (2011). Recent Studies on Aphrodisiac Herbs for the Management of Male sexual Dysfunction- A Review. *Acta Poloniae Phramaceutica. Drug Research*, **68** (1), 3-8.

Mamta, S., Jyoti, S., Rajeev, N., & Abhishek, G. (2013). Phytochemistry of Medicinal Plants. *Journal of Pharmacology and Phytochemicals*, **1**(6), 168-182.

Manzo, L. M., Moussa, I., Ikhiri, K., & Yu, L. (2019). Toxicity studies of *Acacia nilotica* (L.):A review of the published scientific literature. *Journal of Herbmed Pharmacology*, **8**(3), 1-12.

Marcondes, F. K., Bianchi, F. I., & Tanno, A. P. (2002). Determination of the oestrus cycle phases of rats: some helpful considerations. *Brazil Journal of Biology*, **62**(13) 609-614.

Marref, S. E., Benkiki, N., Melakhessou, M. A., & Bouzidi, S. (2018). Acute Toxicity, Anti-ulcer and Anti-inflammatory Effects of Methanol Extract of *Gladiolus segetum* in Rats. *Pharmacognosy Journal*, **10**(4), 758-762.

Marston, C. C., & Cleland, J. (2003). Relationships Between Contraception and Abortion: A Review of the Evidence. *International Family Planning Perspectives*, **29**(1), 7-13.

Mazza, D., Bateson, D., Frearson, M., Goldstone, P., Kovacs, G., & Baber, R. (2017). Current barriers and potential strategies to increase the use of long-acting reversible contraception (LARC) to reduce the rate of unintended pregnancies in Australia: An expert roundtable discussion. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, **57**(2), 15-16.

Medicinal, N. P. (2005). Indigenous use of medicinal trees and shrubs of Margalla Hills National Park, Islamabad. *Pakistan Journal of Forest*, **30**(4), 63-90.

Mills, E., Duguoa, J. J., Perri, D., & Koren, G. (2006). *Herbal Medicines in Pregnancy and Lactation: An Evidence-Based Approach.* London, UK.: Taylor & Francis.

Monsefi, M. M., Mojaba, M., Elham, H., Farnaz, G., & Ramin, M. (2015). Anti-fertility effects of different fractions of *Anethum graveolens L*. extracts on female rats. *African Journal of Traditional Complementary and Alternative Medicine*, **9**(3), 333-341.

Muanda, M., Ndongo, P. G., Taub, L. D., & Bertrand, J. T. (2016). Barriers to Modern Contraceptive Use in Kinshasa, DRC. *PLoS One*, **11**(12),167-170.

Musila, M. N., Ngai, D. N., Mbiri, J. W., Njagi, S. M., Mbinda, W. M., & Ngugi, M. P. (2017). Acute and Sub-Chronic Oral Toxicity Study of Methanolic Extract of *Caesalpinia volkensii* (Harms). *Journal of Drug Metabolism and Toxicology*, **8**(1), 2-9.

Mustapha, A. R., Bawa, E. K., Ogwu, D., Abdullahi, U. S., Kaikabo, A. A., & Diarra, S. S. (2011). Effect of ethanoic extract of *Rhynchosia sublobata* (Schumac) Meikle on the

oestrus cycle in Wistar rats. *International Journal of Medicinal and Aromatic Plants*, **1**(2), 122-127.

Nagao, T., Yoshimura, S., Saito, Y. N., Usumi, K., & Ono, H. (2001). Reproductive effects in male and female rats of neonatal exposure to genistein. *Reproductive Toxicology*, **15**(4), 399-411.

Naher, S., Aziz, M. A., Akter, M. I., Rahman, S. M., & Sajon, S. R. (2019). Analgesic, anti-inflammatory and anti-pyretic activities of methanolic extract of *Cordyline fruticosa* (L.) A. Chev. leaves. *Journal of Research in Pharmacy*, **23**(2), 198-207.

Namulindwa, A., Nkwangu, D., & Oloro, J. (2015). Determination of the abortifacient activity of the aqueous extract of Phytolaccadodecandra (L'Her) leaf in Wistar rats. *African Journal of Pharmacology* , **9**(3), 43-47.

Neetesh, K. J., Suman, J., Mehta, S. C., & Tonpay, S. D. (2016). Anti-implantation and Anti-estrogenic Activity of *Boerrhavia diffusa* root extract in Female albino rats. *American Journal of Pharmacology Sciences*, **4**(2), 15-19.

Obinna, V. C., & Kagbo, H. D. (2018). Evaluation of *Costus lucanuscianus* leaf extract for anti-fertility effect in female albino rats. *International Journal of Advanced Research in Biological Sciences*, **5**(1), 153-158.

Ochako, R., Mbondo, M., Aloo, S., Kaimenyi, S., Thompson, R., Temmerman, M., & Kays, M. (2015). Barriers to modern contraceptive methods uptake among young women in Kenya: a qualitative study. *BMC Public Health*, **15**(118), 126-145.

Odirichukwu, E. O. (2015). Effects of Aqueous Methanolic Extract of Unripe *Carica papaya* Fruit on Midterm Pregnancy in Wistar Albino Rats. *Journal of Veterinary Advances*, **5**(6), 962-967.

OECD 423. (2004). Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No 24.

Okoyea, C. N., Ogwub, D. I., Ochiogua, J. I., Abiaezuted, L. S., & Mbegbue, C. N. (2015). Effects of methanolic leaf extract of *Telfairia occidentalis* (hook f.) On the reproductive indices of female albino rats. *Scientific Journal of Veterinary Advances*, **4**(4), 25.

Omosa, E. K. (2005) The Impact of Water Conflicts on Pastoral Livelihoods; In *The case of Wajir District in Kenya. International Institute for Sustainable Development*, **6**(15), 52.

Osonuga, O., Gasanoka, I., & Osonuga, A. (2014). Oral administration of leaf extracts of *Mormodica charantia* affect reproductive hormone of adult Wistar rats. *Asian Pacific Journal of Tropical Biomedicine*, **4**(1),521-S524.

Paccola, C. C., Resende, C. G., Stumpp, T., Miraglia, S. M., & Cipriano, I. (2013). The rat estrous cycle revisited: a quantitative and qualitative analysis. *Animal Reproduction,* **10**(4), 677-683.

Panda, S. K., Padhi, L. P., & Mohanty, G. (2011). Antibacterial activities and phytochemical analysis of *Cassia fistula (Linn.)* leaf. *Journal of Advanced Pharmaceutical Technology and Research,* **2**(1), 62-67.

Pang, P, C., Chiu, P. C., & Lee, C, L. (2011). Human sperm binding is mediated by the sialyl-Lewis(x) oligosaccharide on the zona pellucida. *Science*, **333**(8), 1761–1764.

Parohit, A., Surendra, K., & Keshav, B. (2008). Contraceptive efficacy of *Plumbago zeylanica* root extract in male albino rats with special emphasis on testicular cell population dynamics. *Ancient Science Life*, **27**(80), 31-35.

Pattanayak, S. P., & Mazumdar, P. M. (2009). Effect of *Dendrophthoe falcate* on female reproductive system of Wistar rats. *Contraception,* **80**(3), 314-320.

Piyali, S., Ghungroo, S., Pratip, C., Sayani, B., Bikas, C, P., & Syed, N, K. (2012). Puerarin, selective oestrogen receptor modulator, disrupts pregnancy in rats at preimplantation stage. *Reproduction*, **144** (10), 633–645.

Pradhan, P., Costa, L., Rybski, D., Lucht, W., & Ropp, J. (2017). Development Goal (SDG) Interactions. *Earth's Future, 5*(10), 1169–1179.

Prashant, T., Bimlesh, K., Mandeep, K., & Gurpreet, K. (2011). Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia*, **1**(1),98-106.

Raji, Y., & Bolarinwa, A. (1997). Antifertility activity of *Quassia amara* in male rats *in vivo* study. *Life Sciences*, **61**(120), 1067-1074.

Rodrigues, E. (2007). Plants of restricted use indicated by three cultures in Brazil (Cabocloriver dweller, Indian and Quilombola). *Journal of Ethnopharmacology*, **111** (10), 295– 302.

Rodrigues, J. K., Navarro, P. A., Zelinski, M. B., Stouffler, R. L., & Xu, J. (2015). Direct actions of androgens on the survival, growth and secretion of steroids and anti-Mullerian hormone by individual macaque follicles during a three-dimensional culture. *Human Reproduction,* **3**(10), 664-667.

Ross, J., & Winfrey, W. (2001). Contraceptive Use, Intention to Use, and Unmet Need during the Extended Postpartum Period. *Interntional Family Planning Perspectives,* **27**(1), 20-27.

Roy, P., Mandal, P., Panda, S., Mitra, S., & Subba, A. (2018). Pharmacognosy and Phytochemical Screening of some Plant Derived Medicine to Treat Dysmenorrheal Pain by the Rajbanshi Community. *Pharmacology Journal,* **10**(4), 738-746.

Rutstein, S. (2005). Effects of Preceding Birth Intervals on Neonatal, Infant and Under-Five Years Mortality and Nutritional Status in Developing Countries: Evidence from Demographic and Health Surveys. *International Journal of Gynaecology and Obstetrics*, **89**(11), 7-24.

Sahira, B., & Catherine, L. (2015). General Techniques Involved in Phytochemical Analysis. *International Journal of Advanced Research in Chemical Science (IJARCS),* **2**(4), 25-32.

Sakila, S., Begum, N., Kawsar, S., Begum, Z. A., & Zoh, M. S. (2009). Relationship of antifertility effects of *Andrographis paniculata* and hormonal assay in female rats. *Bangladesh Journal of Medical Science,* **8**(2), 10-14.

Sandhya, P., Tewari, R., & Prakash, A. (1990). Hormonal properties of ethanolic extract of *Juniperus communis Linn. Ancient Science of Life*, **10**(25), 106-113.

Saravanan, P., & Renuka, C. (2012). Medicinal plants with potential antifertility activity-A Review of sixteen years of herbal medicine research (1994-2010). *International Journal of Physiotherapy and Research*, **1**(1), 481-494.

Sedgh, G., & Hussain, R. (2014). Reasons for contraceptive non-use among women having unmet need for contraception in developing countries. *Studies in Family Planning*, **45**(2), 151-169.

Sedgh, G., Hussain, R., Bankole, A., & Singh, S. (2007). *Women with an unmet need for contraception in developing countries and their reasons for not using a method.* Occasional Report No. 37.

Sharma, R. K., Goyal, A. K., & Bhat, R. A. (2014). Anti-fertility activity of plants extracts on female reproduction. *International Journal of Pharma and BioSciences,* **3**(3), 493-514.

Shinwari, M., & Khan, M. (1998). Indigenous use of medicinal trees and shrubs of Margalla Hills National Park Islamabad. *Pakistan Journal of Forest,* **48**(1-4), 63-90.

Shinwari, Z, K., Rehman, M., Watanabe, T., & Yoshikaw, Y. (2006). *Medicinal and Aromatic Plants of Pakistan (A Pictorial Guide)*. Kohat University of Science and Technology, Kohat, Pakistan 492p.

Shraboni, P., & Singh, R. K. (2015). Addressing unmet need and religious barriers towards the use of family planning method among Muslim women in India. *International Journal of Human Rights in Healthcare*, **8**(1), 22-35.

Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. *Ancient Science of Life*, **15(**25),121-130.

Suhaimi, N. A., Nooraain, H., & Nurdiana, S. (2016). Effects of *Ficus deltoidea* Ethanolic leaves extract on female reproductive organs among Letrozole-induced polycystic ovarian syndrome rats. *Journal of Scientific Research and Development,* **3**(4), 8-14.

Tana River County (n.d).County government of Tana river County. https://www.tanariver.go.ke/. Accessed on 13th May, 2018.

Tsui, A. O., Raegan, M., & Anne, E. B. (2010). Family Planning and the Burden of Unintended pregnancy. *Epidemiology Revision,* **31**(4), 152-174.

Varshney, S., Verma, S., & Arya, R. (2016). A review on scientific validity on medicinal plant used as female contraceptives. *International Journal of Research in Ayurveda and Pharmacy,* **10**(7897), 277-285.

Vijay, K. B., Sharanabasappa, A., & Saraswati, B. P. (2004). Post-coital antiimplantation and pregnancy interruption potency of the seeds of *Crotalaria juncea* Linn. *Ori. Pharm. Experimental Medicine*, **4**(10), 70-76.

World Health Organization (WHO). (2003). *Shaping the future.* Geneva: World Health Organization.

Yahia, E. (2011). *Post-harvest biology and technology of tropical and subtropical fruits*. Oxford: Woodhead Publishing. (pp. 381-418).

Yakubu, M. T., Oladiji, A, T., & Akanji, M. A. (2007)**.** Evaluation of Biochemical indices of male rat reproductive function and testicular histology in Wistar rats following chronic administration of aqueous extract of *Fadogia agrestis* (*Schweinf. Ex Heirn)* stem. *African Journal of Biochemistry Research*, **1(**5), 156-163.

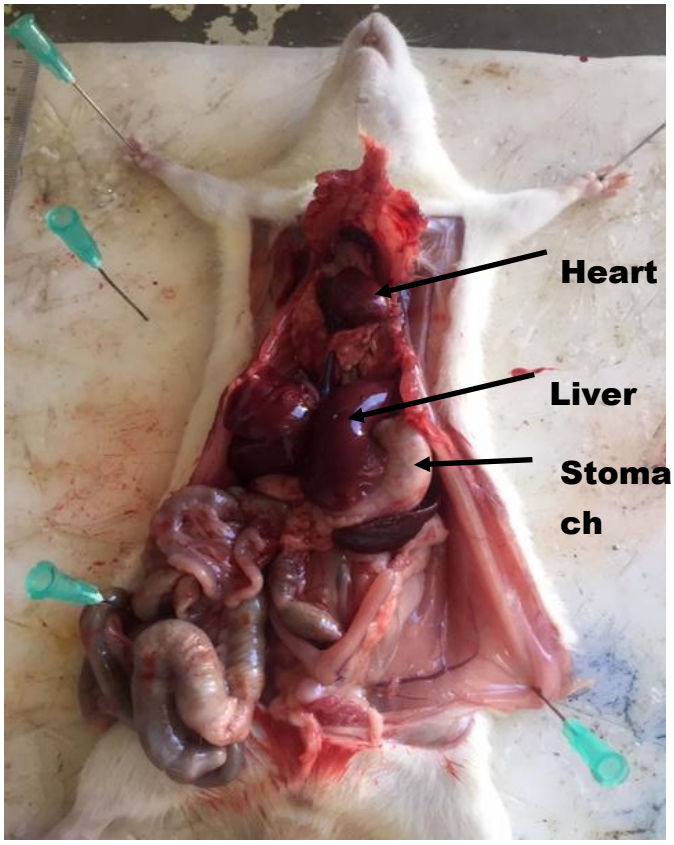
Yakubu, M. T., Olawepo, O. J., & Fasoranti, G. A. (2011). *Ananas comosus*: unripe fruit juice as an abortifacient in pregnant Wistar rats. *European Journal of Contraception and Reproductive Health Care*, **16** (8), 397–402.

APPENDICES

Appendix 1*:*Photograph of prepared *Cissus rotundifolia* aqueous extract ready for lavaging

Appendix 2: Photographs of author administering *Cissus rotundifolia* aqueous extract to the Wistar rats

Appendix 3: A photograph of rat during pathological examination of the vital organs in acute oral studies.



Appendix 4: Biosafety, Animal use and Ethical permit

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenva.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

Ms. Anita Mary Mziray
Egerton University
Dept of Biological Sciences

REF: FVM BAUEC/2019/191

02/02/2019

Dear Ms. Mziray,

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Evaluation of Antifertility properties of Arabian wax leaf *Cissus rotundifolia (Fotssk.) Vahl.* extracts in female winstar rats.

By Ms. Anita Mziray (SM21/11732/2016).

We refer to your revised MSc. proposal submitted to our committee for review and your application letter dated 08/12/2018.

We have reviewed your proposal, particularly section 3.3 that involves use of laboratory animals for acute oral toxicity, estrus cyclicity, fertility and implantation index.

We are satisfied that the proposed treatment and care of the animals meets acceptable standards for animal welfare. Furthermore, the numbers proposed are reasonable.

We have also noted that a registered veterinary surgeon (KVB 1373) will supervise the animal experiments and humane end points.

We hereby give approval for you to proceed with the experiments as outlined in the submitted proposal.

Yours sincerely

Kaluwa

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee
Faculty of Veterinary Medicine.

Appendix 5: NACOSTI Research permit

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
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Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/73129/29027**

Date: **2nd May 2019**

Anita Mary Mziray
Egerton University
P.O. Box 536-20115
NJORO.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Evaluation of the anti-fertility properties of Cissus rotundifolia (forssk.) vahl. extract in female Wistar rats."*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi County** for the period ending **30th April, 2020.**

You are advised to report to **the County Commissioner and the County Director of Education, Nairobi County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County

The County Director of Education
Nairobi County

THIS IS TO CERTIFY THAT:
MS. ANITA MARY MZIRAY
of EGERTON UNIVERSITY, 0-80302 TAVETA,has been permitted to conduct research in *Nairobi County*

on the topic: *EVALUATION OF THE ANTI-FERTILITY PROPERTIES OF CISSUS ROTUNDIFOLIA (FORSSK.) VAHL. EXTRACT IN FEMALE WISTAR RATS.*

for the period ending:
30th April,2020

Permit No : NACOSTI/P/19/73129/29027
Date Of Issue : 2nd May,2019
Fee Recieved :Ksh 1000

.....
Applicant's Signature

.....
Director General
National Commission for Science, Technology & Innovation

Appendix 6: Publication

ORIGINAL ARTICLE
Discovery Phytomedicine 2020, Volume 7, Number 2: 58-64

Anti-fertility properties of *Cissus rotundifolia* (Forssk.) Vahl. Extract using female Wistar rats

Anita Mary Mziray,[1*] Charles Irungu Maina, Catherine Kaluwa Kaingu[2]

ABSTRACT

A third of the worldwide disease burden among women in their reproductive age (15-45 years) is associated with sexual and reproductive complications. The developing world experiences an unmet contraceptive demand that affects nearly 200 million women. The high rates of unintended pregnancies in sub-Saharan Africa increase the prevalence of unsafe abortions in nations. The use of conventional steroids and non-steroid based contraceptives though effective is also linked to increasing side effects. The roots decoction of *Cissus rotundifolia* is used by women in Tana River County, Kenya as a fertility regulator. The study evaluated the phytochemical compounds present in the *Cissus rotundifolia* aqueous extract. It also evaluated the effect of the plant extract on oestrus cycle and other selected female reproductive parameters; mating success, fertility index, gestation length, and litter size using female Wistar rats. The phytochemical screening established the presence of alkaloids, tannins, saponins, phenols, and glycosides in the root aqueous extract of *Cissus rotundifolia*. The plant extract caused a dose-dependent significance increase in proestrus and metestrus phases and a significant reduction in estrus and diestrus phases ($P<0.05$). There was no significant difference in mating success. It caused a dose-dependent reduction in fertility index compared to the control. Gestation length was significantly increased and litter size significantly reduced ($P<0.05$). This probably is the reason for the traditional use of the plant as a fertility regulator. However, further work on reversibility, reproductive hormonal profile and ovarian histomorphology should be undertaken to improve the novel contraceptive pool.

Keywords: *Cissus rotundifolia*, oestrus cycle, phytochemical compound, mating success, fertility index, litter size, gestation length

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INTRODUCTION

Reproductive health is an important feature of socio-economic development. A global recent increase in contraceptive use has coincided with an increment of 25 million unintended pregnancies (Sedgh and Hussain, 2014). This might probably be due to inappropriate use, consistency challenges and possible method failures in fertility regulation. Fifty percent of youth aged below 25 years in Africa have inadequate access to reproductive health-care services (Lule et al., 2007). In Kenya, unmet contraceptive need is linked to a myriad of factors such as women's educational levels, location, autonomy, and income status among others (Campbell and Graham, 2006; Sedgh et al., 2007). The use of conventional steroids and non-steroid based contraceptives though effective is also linked to increasing side effects. It is therefore imperative to pursue alternative novel contraceptive options that are affordable, accessible, reversible and efficacious.

Cissus rotundifolia (Forssk.) Vahl. (Family *Vitaceae*) is commonly in Tana River county, Kenya, to regulate fertility (Kaingu et al., 2013). However, the role of *Cissus rotundifolia* as a potential anti-fertility agent is not clear and the literature is scanty. This study aimed to elucidate the anti-fertility properties of the plant extract on oestrus cyclicity, mating success, fertility index, gestation length and litter size in female Wistar rats.

MATERIALS AND METHODS

Plant collection and extract preparation

The root barks of the plant were collected from Garsen, Itsowe and Ngao sub-divisions of Tana River County, Kenya in February 2019. The roots were then transported to the Department of Veterinary Anatomy and Physiology, University of Nairobi. The roots were thoroughly washed using tap water to remove the soil. They were cut into small pieces using a knife, dried under shade for two weeks. The dried roots were then ground into fine powder using a Cunningham grinder (Artsan Manufacturing, Massachusetts, USA). Three hundred grams of the root powder were put in 3 liters of boiling distilled water for one hour. The mixture was thereafter, allowed to cool, filtered and lyophilised for 48 hours until the extract was completely dry. The plant aqueous extract yield was 205.45 grams. The extract was then kept in labelled sterile vials and stored in the refrigerator (-20°C) until use.

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