SCREENING FOR ANTIFERTILITY POTENTIAL AND SAFETY EVALUATION OF FIVE LOCALLY USED MEDICINAL PLANTS IN ALBINO MICE (*Mus musculus*)

by

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DECLARATION

I hereby declare that this submission is my own work towards the M.Phil and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

This study was undertaken to evaluate the antifertility potential and toxicity of the crude ethanol extracts of *Anthocleista nobilis* (ARE), *Macaranga heterophylla* (MLE), *Palisota hirsuta* (PRE), *Trichillia monadelpha* (TBE) and *Waltheria indica* (WLE). Four hundred and twenty (420) albino mice were used for the study. Acute toxicity test was carried out using the Acute Toxic Class method (OECD, 2001). The LD_{50} of each plant extract was determined to be above 5000 mg/kg. In a preliminary fertility test, treatment of mice with ARE, MLE, PRE, TBE and WLE did not significantly alter their reproductive indices. Nevertheless, mice treated with ARE showed complete foetal resorption. Administration of TBE caused foetal resorption and abortion in treated mice. Hence ARE and TBE were selected for further testing. Repeated exposure of mice to ARE and TBE did not produce treatment related changes or toxicity. *Anthocleista nobilis* had no significant effect on the oestrous cycle and other reproductive parameters of treated mice. *Trichilia monadelpha* significantly altered phases of the oestrous cycle and exhibited antiimplatation and abortifacient effect. *In vitro* studies revealed that TBE possesses antispasmodic effect. It significantly inhibited the contractile response elicited by acetylcholine. The effect of *Trichilia monadelpha* on the uterus may be mediated by muscarinic receptors. The crude ethanol extracts of ARE, MLE, PRE and WLE did not show antifertility effects in mice at the doses tested.

DEDICATION

To my family for their unflinching support to get me this far.

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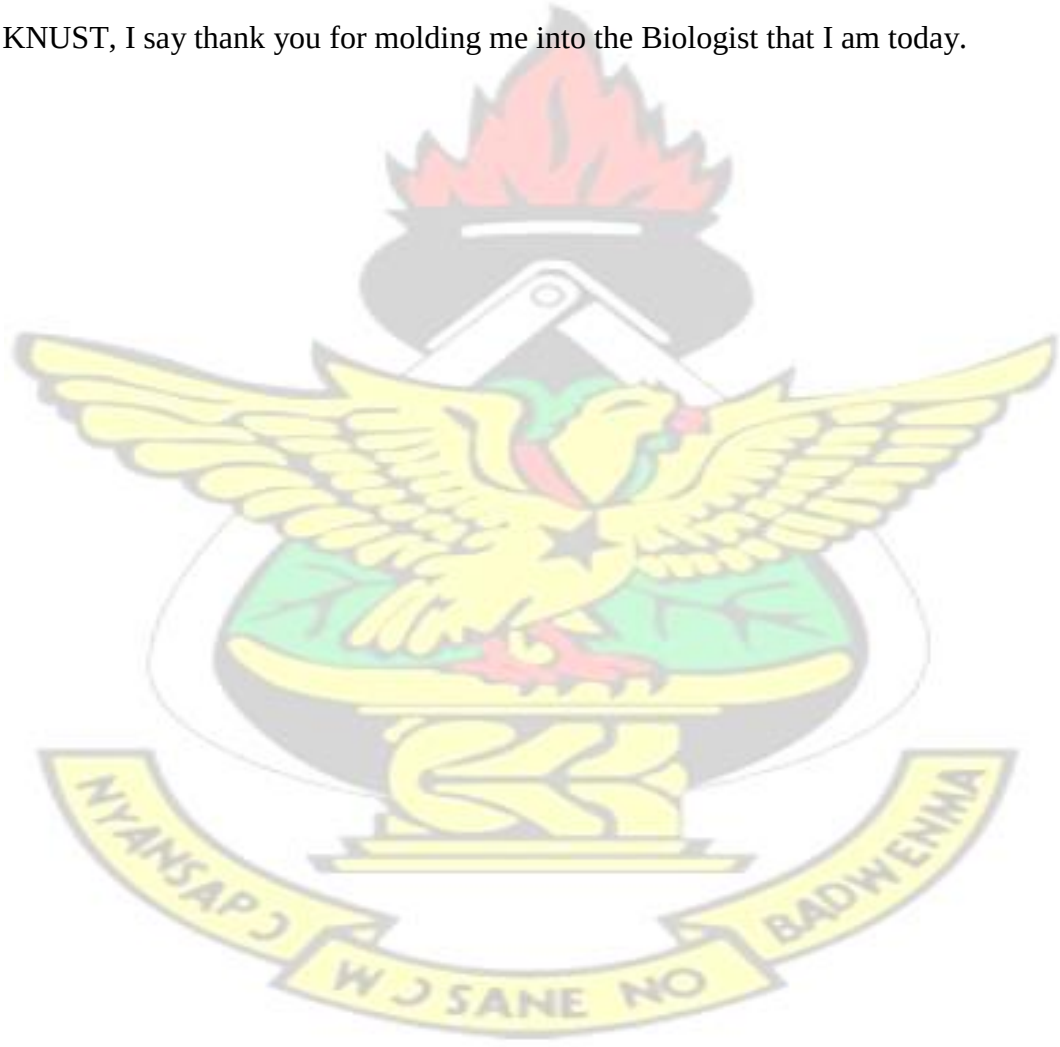

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CHAPTER ONE

1.1 INTRODUCTION

Plants have long been a source of nutrition and therapy to man (Solomons, 2000; Schippmann, 2002; Denny and Buttriss, 2007; Salim *et al*., 2008; Rao *et al*., 2011). Good health and reduced risk of developing chronic diseases have long been associated with the consumption of plant-derived foods. The presence of vitamins, antioxidants and bioactive compounds in plant foods have been linked to systemic functions such as reproduction and immunity. The potential of managing disease using nutritional therapy has generated numerous epidemiological studies (Solomons, 2000; Denny and Buttriss, 2007). Globally, about 85,000 plant species have been recognized as medicinally useful (Bhattarai *et al*., 2009).

From ancient times to the present day, plants continue to play an indispensable role in the prevention and treatment of diseases (Hostettmann *et al*., 2000; Calixto, 2005). Health practices of various cultures worldwide identify with the use of medicinal plants as curative and preventive medicines. The history of phytotherapy can be traced as far back as 60,000 years ago (Fabricant and Farnsworth, 2001; Gossel-Williams *et al*., 2006). The knowledge of medicinal plant use formed the basis for traditional systems of medicine which were the only source of health care before the advent of orthodox medicines (Gurib-Fakim, 2011). In developing countries, majority of the population in rural communities rely heavily on medicinal plants for their everyday health care needs. For the people in these communities, traditional medicine is the only form of health care available which is affordable (WHO, 2002).

The practice of traditional medicine is usually fused with the religious and cultural practices of the indigenous people (Gossel-Williams *et al*., 2006; Salim *et al*., 2008). In most areas, the practice of traditional medicine is associated with rituals, superstition and witchcraft (WHO, 2002; Andemariam, 2010). Often, the healer uses plants based on similarities between the plant and the ailments being treated.

This practice known as the doctrine of signatures was used to select plants for treating diseases. Followers of the doctrine believed that the appearance of a plant may give clues to its medicinal properties. Hence plants with red colour were used to treat blood-related diseases and those with yellow colour were used to treat jaundice. These unconventional methods of healing associated with the practice of traditional medicine have always raised concerns about its purported benefits (Gurib-Fakim, 2006; Salim *et al*., 2008). However, empirical findings from traditional societies worldwide have provided several effective medicines for the treatment of diseases (Iwu *et al*., 1999).

The isolation of digoxin from foxglove and morphine from poppies opened a new chapter for the discovery of natural molecules from medicinal plants used as medicines. This marked the beginning of the discovery of evidence-based drug from plants (Balunas and Kinghorn, 2005; Rishton, 2008; Salim *et al*., 2008). The discovery of natural molecules led to the development of a defined and dose controlled medicine which formed the bases for conventional medicine and modern pharmaceuticals (Rishton, 2008). It was at this point that the effectiveness of natural products was attributed to their active constituents. Following these discoveries,

secondary metabolites from plants were used widely as medicines in their original forms as well as modified forms (Iwu *et al*., 1999; Fabricant and Farnsworth, 2001; Salim *et al*., 2008).

Fabricant and Farnsworth (2001) indicated that globally there were 122 compounds from 94 species of plants which were used as single entity medicinal agents. Most of these drugs were obtained as a result of examining the plants based on their traditional use. Over the past few years, a considerable number of drugs used in modern medicines have come from plant sources. Notable examples of plant-derived drugs include the good old antimalarial-quinine, extracted from the bark of Cinchona species which is receiving a strong revival as a drug in use to face resistance to the malaria parasite. Diosgenin obtained from wild yams in Mexico and Central America is used for the synthesis of all anovulatory contraceptive agents and various steroid hormones. Salicylic acid, a precursor for aspirin was originally derived from white willow bark (Salim *et al*., 2008; Gurib-Fakim, 2011).

Currently, many people still use traditional medicine to satisfy their health care needs. The World Health Organization (WHO) defines traditional medicine as including diverse health practices, approaches, knowledge and beliefs incorporating plants, animals and mineral-based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness (WHO, 2002). WHO officially recognized traditional medicine and its practitioners in health care delivery as important

resources in achieving health for all by the year 2000 in their 1978 Alma-Ata Declaration on primary health care (Addae-Mensah, 2005).

Since then, a number of resolutions and policies have been adopted in WHO member countries to integrate traditional medicine into national health care delivery systems (Ameh *et al*., 2010).

During the last decade, the use of traditional medicine has expanded globally and demand for plant derived drugs is increasing. WHO estimates that about 80% of the population in Africa depend on traditional medicines for their primary health care needs. The broad use of traditional medicines in developing countries is attributed to accessibility and affordability (WHO, 2002; Adeneye, 2014; Akhigbe, 2014; Gadaga and Tagwireyi, 2014). In developed countries, the use of traditional medicine as an alternative form of therapy is on the rise on account of the increasing cost of personal health maintenance as well as failure of modern medicine to cure chronic diseases like AIDS and the side effects associated with the use of synthetic drugs (Hoareau and DaSilva, 1999; Rates, 2001; Haramati and Lumpkin, 2004; Ameh *et al*., 2010; Heinrich, 2010).

Tremendous opportunities exist in the exploitation of potential plants as medicines in developed and developing countries. However this would require rigorous scientific validation and appropriate regulatory procedures in terms of safety assessment, efficacy evaluation and standardization of medicinal plant products (Matthews *et al*., 1999; WHO, 2002).

1.2 Problem statement

Fertility regulation appears fundamental to the promotion and sustenance of economic growth and development. Modern contraceptives have played a central role in population control and fertility transitions observed worldwide. Despite the success of modern contraceptive in fertility regulation, several countries still have low levels of contraceptive usage. Reasons for underuse of effective methods of contraception include; lack of access to contraceptive methods of choice, fear of side effects and a user's dissatisfaction with a particular method. In spite of this under usage in some parts of the world, there is a growing body of evidence that suggests that contraceptive services are often unable to meet the growing demand of couples for fertility regulation in other parts. This short fall has created a gap between desired and achieved contraception (WHO, 2004; Lule *et al*., 2007; IPPF, 2008; Smith *et al*., 2009; DFID, 2011).

Many women in developing countries do not have access to the contraceptive methods of their choices. Inability of contraceptive services to meet the demand of couples for fertility regulation results in increased number of unplanned pregnancies, some of which are terminated by induced abortion (WHO, 2007). It has been estimated that almost 40% of pregnancies worldwide are unplanned, the result of non-use of contraception, ineffective contraceptive use or method failure. Analysis of data from developed countries shows that an increase in the use of effective contraceptive methods will reduce the incidence of abortion. However, nowhere in the world has abortion levels reached zero even in areas of high levels of contraceptive prevalence and use (WHO, 2004; IPPF, 2008; Smith *et al*., 2009;

DFID, 2011).

Method failure or ineffective use of contraceptives results in about 27 million unintended pregnancies out of which 6 million occur even though the method has been used correctly and consistently (Smith *et al*., 2009). The exigency at this hour is not only to expand and improve family planning services but to broaden the choices to meet the individual's need to space or limit births. In the African sub-region, particularly for those in rural areas where current methods of contraception are inaccessible or unaccepted by the people, safe, tested and efficacious herbal contraception might be an alternative (Ahmed *et al*., 2011; Kaur *et al*., 2011). It is worth noting that if women had only the number of pregnancies they wanted and at the intervals they wanted, maternal mortality would drop by one-third. Providing women with contraception of their choices would avert over a million needless deaths of women due to complications related to pregnancy and childbirth (WHO, 2007; IPPF, 2008; Smith *et al*., 2009).

1.3 Justification

Medicinal plants have been used for centuries in traditional medicine based on the empirical findings of several thousands of years (Gurib-Fakim, 2006). Available evidence suggests that, about 75% of the Ghanaian population rely exclusively on traditional health practitioners and medicinal plants for their primary health care needs (Abel and Busia, 2005). Traditional medicine and its practitioners are recognized by WHO as important resources to increase health care delivery worldwide especially in developing countries. However, the apparent lack of scientific evidence on the quality, efficacy and safety of medicinal plants has been a major setback in the incorporation of traditional medicine into national health care

systems (Farnsworth and Soejarto, 1991; Matthews *et al*., 1999; WHO, 2002; Adeneye, 2014; Gadaga and Tagwireyi, 2014; Teke and Kuete, 2014). It is therefore necessary to contribute to research into the efficacy and toxicity of medicinal plants to provide scientific justification for their use and safe guard the health of users.

1.4 Main and specific objectives

1.4.1 Main objective

The objective of the study was to screen and assess the toxicity and reproductive effect of the crude ethanol extracts of five plants, i) *Anthocleista nobilis* ii) *Palisota hirsuta* iii) *Macaranga heterophylla* iv) *Trichillia monadelpha* and v) *Waltheria indica* in albino mice.

1.4.2 Specific objectives

The study seeks to:

- Determine the acute toxicity effect of the extracts in mice
- Assess the preliminary fertility effects of the extracts
- Assess the effect of the extracts on the oestrous cycle of female albino mice
- Determine the effect of the extracts on the relative organ to body weight ratio, histoachitecture of the liver and serum biochemical markers of treated mice
- Evaluate the general effect of the extracts on fertility and embryonic development
- Assess the effect of the extracts on the isolated uterus

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Female reproductive system

The female reproductive system consists of internal organs and genitalia. The internal organs consist of the ovaries, fallopian tubes, uterus and vagina. The opening of the vagina is bordered by the clitoris, labia minora and labia majora. The ovaries produce ova and the sex hormones oestrogen, progesterone, relaxin and inhibin. An egg is released from the ovary into the fallopian tube during ovulation. The egg is transported slowly by the fallopian tubes through muscular contraction and ciliary action into the uterus (Vander *et al*., 2001; Saladin, 2004; Marieb *et al.*, 2015; Martini *et al.*, 2015).

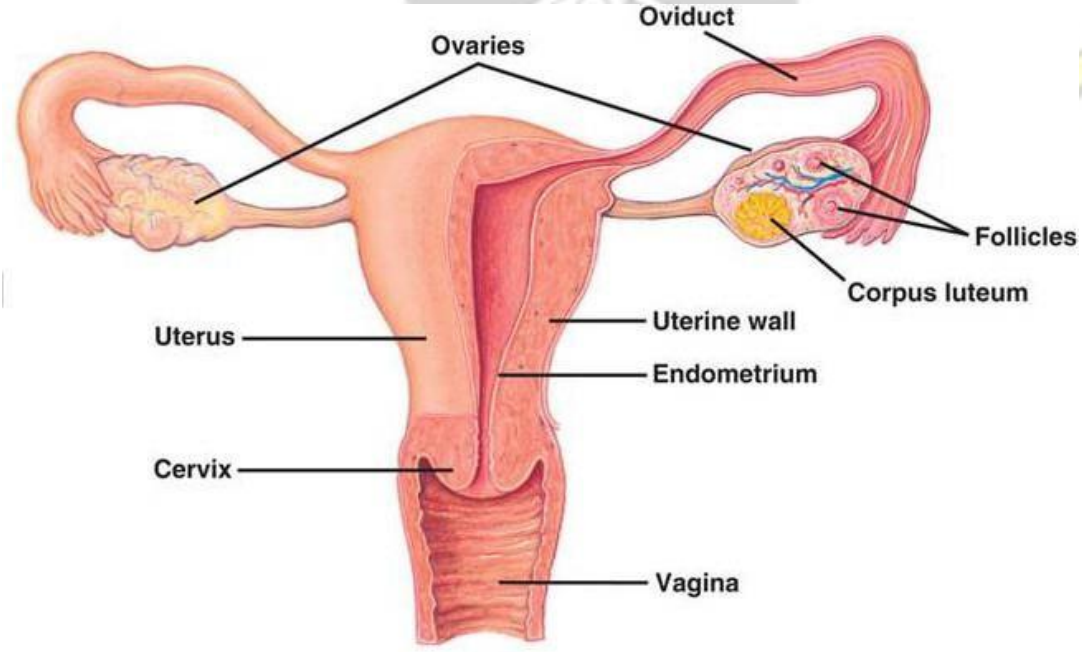

Figure 1: Diagrammatic representation of the female reproductive system **(**University of California, 2015).

The egg is viable for approximately 24 hours after ovulation therefore fertilization occurs when it is still in the fallopian tube, because a fertilized egg takes roughly 7

days to be implanted. On its journey into the uterus, the zygote undergoes proliferation to form a morula which later develops into a blastocyst before it embeds itself into the walls of the uterus (Saladin, 2004; Marieb *et al.*, 2015; Martini *et al.,* 2015).

2.2 Hormonal regulation of the female reproductive cycle

A normal female reproductive physiology requires a well-coordinated action of the hypothalamus, the pituitary, the ovaries and the endometrium (Tamm *et al*., 2012; Marieb *et al.,* 2015; Martini *et al.,* 2015). Hormones from the hypothalamus and pituitary gland regulate events in the ovaries which in turn controls the changes that occur in the uterus. Disturbances in this hormonal equilibrium would affect ovulation, ova transport and successful implantation of a fertilized ovum (Totora and Grabowski, 2003; Widmaier *et al*., 2004; Marieb *et al.*, 2015; Martini *et al.,* 2015). Thus compounds that interfere in the function of the hypothalamic pituitary axis may have negative impact on fertility.

2.2.1 Gonadotropin Releasing Hormone (GnRH)

GnRH produced by hypothalamus stimulates the synthesis and release of follicle stimulating hormone and luteinizing hormone by the anterior pituitary (Totora and Grabowski, 2003; Marieb *et al.*, 2015; Martini *et al.*, 2015).

2.2.2 Follicle Stimulating Hormone (FSH)

Follicle stimulating hormone is released by the anterior pituitary under the influence of GnRH from the hypothalamus. It stimulates the growth and

maturation of follicles in the ovary. It also stimulates granulosa cells to multiply and produce more oestrogen (Widmaier *et al*., 2004).

2.2.3 Luteinizing Hormone (LH)

Luteinizing hormone is released by the anterior pituitary under the influence of GnRH from the hypothalamus. It stimulates the multiplication of theca cells and their subsequent production of androgens. The androgens diffuse into the granulosa cells where they are converted into oestrogen by the enzyme aromatase (Widmaier *et al*., 2004). Increasing levels of oestrogen produced by the developing follicle promotes the release of GnRH which leads to the mid-cycle LH surge. The elevated levels of LH induce ovulation and the transformation of the remaining granulosa cells of the Graafian follicle into the corpus luteum. It sustains the corpus luteum for about 14 days before it eventually degenerates into the corpus albicans in the absence of a zygote (Totora and Grabowski, 2003;
Widmaier *et al*., 2004; Marieb *et al.*, 2015; Martini *et al.*, 2015).

2.2.4 Oestrogen

The term oestrogen denotes a group of steroid hormones with related effects on the female reproductive system (Vander *et al*., 1985). These are beta (β) estradiol, estrone and estriol (Totora and Grabowski, 2003; Lebrun *et al*., 2013). The principal premenopausal oestrogen produced by the ovary and placenta is beta (β) estradiol (Simpson *et al*., 1999; Guncu *et al*., 2005; Findlay *et al*., 2010).

Oestrogen is synthesized from cholesterol under the influence of follicle stimulating hormone (FSH). The action of oestrogen is regulated by the rate of production and

the presence of receptors on target cells. Oestrogen is known to produce a negative feedback effect on the hypothalamic-pituitary axis.

Moderate levels of oestrogen inhibits the release of gonadotropin releasing hormone (GnRH) by the hypothalamus and the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by the anterior pituitary gland. This effect is exploited as the underlying mechanism of action of contraceptive pills (Totora and Grabowski, 2003; Findlay *et al*., 2010; Marieb *et al.,* 2015; Martini *et al.,* 2015). High level of plasma oestrogen induces the LH surge required for ovulation. Oestrogen is crucial for other reproductive functions including development and maintenance of secondary sexual characteristics, maintenance of pregnancy and the growth of breast in pregnant women. It is also implicated in fluid and electrolyte balance as well as fat metabolism (Totora and Grabowski, 2003; Marieb *et al.,* 2015; Martini *et al.,* 2015). Oestrogen is known for its protective cardiovascular effect by decreasing total cholesterol and low-density lipoprotein (LDL) levels and increasing high-density lipoproteins (HDL) (Lebrun *et al*., 2013).

2.2.5 Progesterone

Progesterone is produced by corpus luteum after ovulation and the placenta during pregnancy. It acts synergistically with oestrogen to build and maintain the endometrium for implantation of the fertilized ovum as well as preparation of the breast for lactation after child birth. High levels of progesterone in the presence of oestrogen have a negative feedback effect on GnRH and LH thus inhibiting follicle maturation and ovulation (Widmaier *et al*., 2004). After fertilization, progesterone induces decidual formation in the uterine endometrium to nourish the embryo

during development. It is known to inhibit uterine contractility thus preventing premature expulsion of the foetus (Totora and Grabowski, 2003;
Marieb *et al.*, 2015; Martini *et al.*, 2015).

2.2.6 Relaxin

Relaxin is produced by the corpus luteum after ovulation. It inhibits contractions of uterine smooth muscle in the uterus which is believed to promote fertilization. Relaxin relaxes the pubis symphysis and dilates the uterine cervix to ease delivery (Totora and Grabowski, 2003; Marieb *et al.*, 2015; Martini *et al.*, 2015).

2.2.7 Inhibin

Inhibin is a peptide hormone produced by the granulosa cells and the corpus luteum (Widmaier *et al*., 2004). It has a negative feedback effect on the release of FSH as well as LH to a lesser extent (Totora and Grabowski, 2003; Marieb *et al.*, 2015; Martini *et al.*, 2015).

2.3 Physiology of the female reproductive system

The female reproductive cycle is characterized by a series of events that recur monthly and concurrently in the ovaries and uterus in the absence of pregnancy. These rhythmic changes result in the release of a mature egg from an ovary and the preparedness of the uterus for the implantation of a fertilized ovum. The duration of the cycle averages 28 days. However, it varies from 20 to 45 days in normal cycling females.

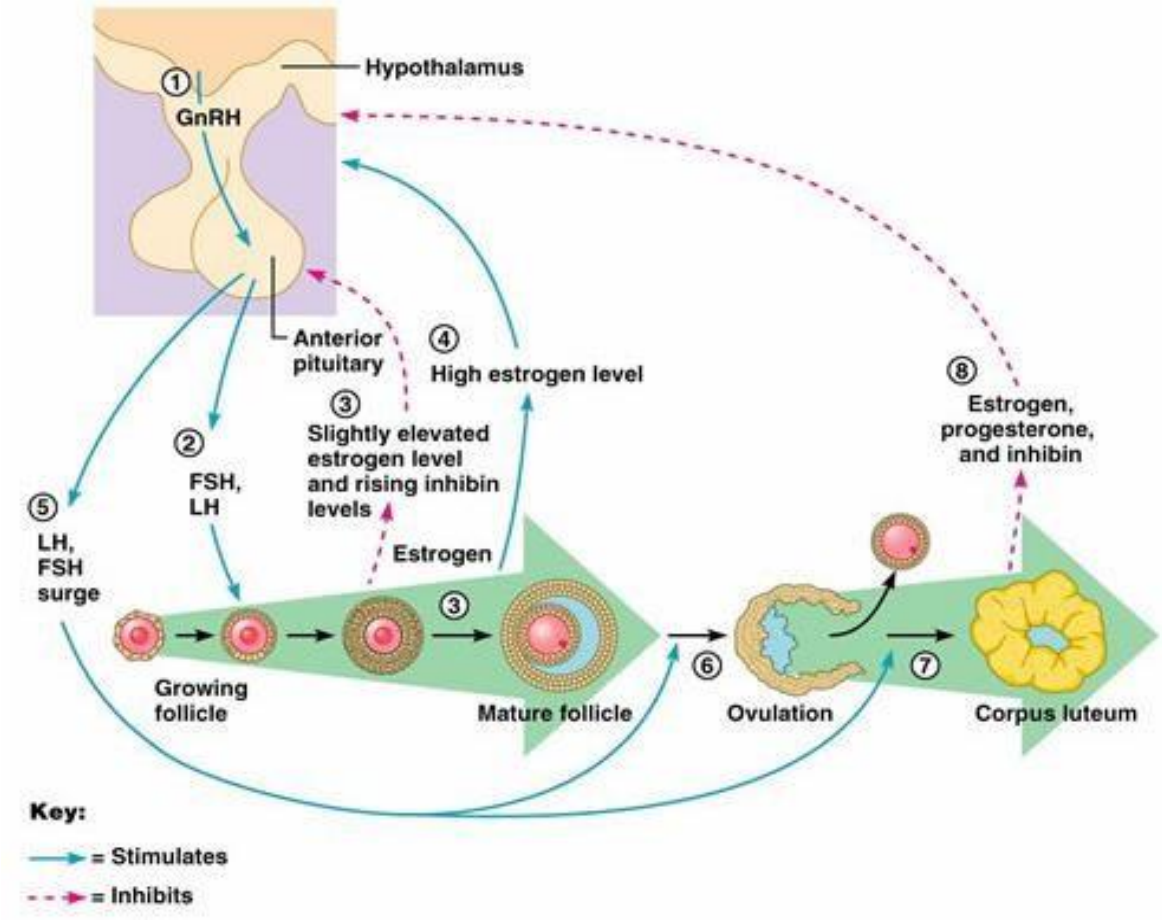

Figure 2: Diagrammatic representation of the hormonal control of ovarian cycle by the hypothalamic-pituitary axis (Cummings, 2006).

2.3.1 Ovulation

The formation of a female gamete in the ovary is termed oogenesis. This process begins at puberty and continues through the reproductive period of females till menopause. The ovary consists of eggs at various levels of development known as follicles. At birth, the ovary of a female child contains about one million primordial follicles. Each primordial follicle consists of an egg surrounded by a single layer of granulosa cells. Further development of the follicles takes place once puberty is attained under the influence of the pituitary hormones, FSH and LH. The hormones stimulate the growth of the egg and proliferation of the surrounding granulosa cells.

Also a second layer of cells called theca cells grow from the ovarian connective tissue cells to surround the egg (Widmaier *et al*., 2004).

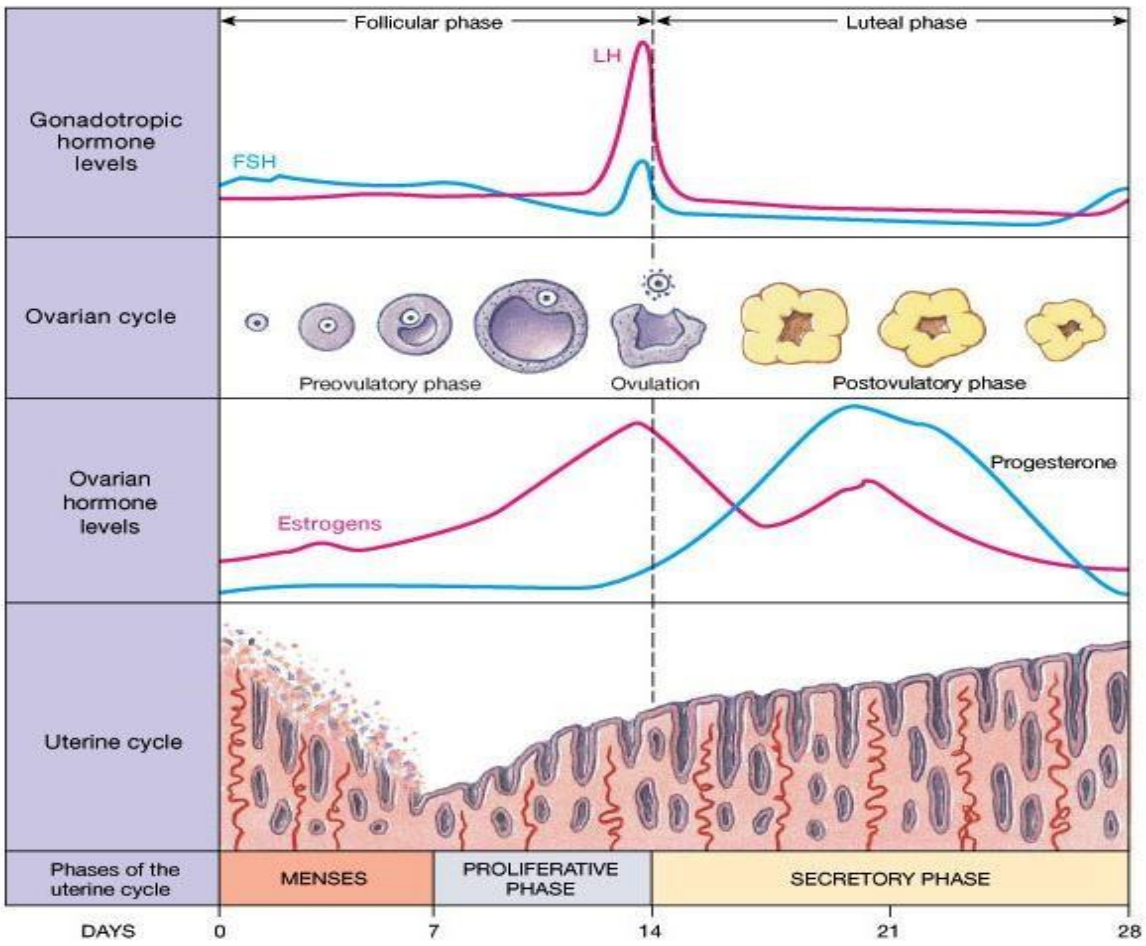

Figure 3: Diagrammatic representation of plasma hormonal levels and corresponding ovarian and uterine changes during the menstrual cycle (Marieb *et al.*, 2015).

The granulosa cells secrete a thick layer of material, the zona pellucida around the egg. They also secrete a follicular fluid that builds up into a cavity called antrum. A number of the ovarian follicles develop to this stage however, only one of the large antral follicles continues to develop while the rest undergo degeneration through a process called atresia. As the antrum enlarges, it pushes the egg surrounded by several layers of granulosa cells to the edge of the follicle. The

granulosa cells surrounding the egg forms a small hill that projects into the antrum and is termed cumulus oophorous (Saladin, 2004; Widmaier *et al*., 2004).

Just before ovulation, the egg (primary oocyte) completes the first meiotic division to become a secondary oocyte. The cumulus oophorous breaks from the rest of the follicles and the egg floats freely in the antral fluid. The matured follicle becomes so large that it bulges out on the surface of the ovary. Enzymes and prostaglandins secreted by the granulosa cells break down the follicularovarian membranes (Widmaier *et al*., 2004). Ultimately, the Graafian follicle ruptures releasing its antral fluid and the egg surrounded by the corona radiate (Saladin, 2004). The remaining granulosa cells of the follicle transform into the corpus luteum. It is sustained for about 14 days before it eventually degenerates into the corpus albicans in the absence of a zygote (Totora and Grabowski, 2003;

Widmaier *et al*., 2004; Marieb *et al.*, 2015; Martini *et al.*, 2015).

2.3.2 Uterine changes in the menstrual cycle

The changes that occur in the ovary resulting in ovulation correspond to changes in the uterus in anticipation for pregnancy. These changes influenced by the ovarian hormones oestrogen and progesterone consist of a build up of the endometrium followed by its breakdown and vaginal discharge in the absence of pregnancy. This cycle that last about 28 days is divided into the menstrual phase, proliferative phase, secretory phase and premenstrual phase. The first day of menstrual discharge marks the beginning of a new sexual cycle (Saladin, 2004;

Marieb *et al.*, 2015; Martini *et al.*, 2015).

The menstrual phase is the period in which blood, serous fluid and endometrial tissue are discharged from the vagina. This phase lasts about 5 days. The menstrual phase is followed by the proliferative phase around day five. The proliferative phase is mediated by oestrogen and it involves the rebuilding of the endometrial tissue lost at the last menstruation. The high level of oestrogen produced by the ripening follicles stimulates regrowth of blood vessels as well as mitosis in the stratum basalis to produce a new stratum functionalis. It also stimulates the production of progesterone receptors in the endometrium. During this phase, the cervix rises and becomes softer. Cervical mucus becomes transparent, slippery and stretchy (Totora and Grabowski, 2003; Saladin, 2004;
Marieb *et al.*, 2015; Martini *et al.*, 2015).

After ovulation, the secretory phase commences around day 15 under the influence of oestrogen and progesterone secreted by the corpus luteum. The uterine endometrium differentiates into a secretory tissue. The endometrium thickens and it becomes edematous and highly vascularized. Its glands become coiled and filled with glycogen. The cervical mucus becomes thicker, viscous and opaque inhibiting sperm mobility. The mucus forms a plug that blocks the entry of sperms and bacteria from the vagina. These uterine events prepare the endometrium for implantation. The progesterone inhibits contractions of uterine smooth muscle induced by locally produced prostaglandins. This ensures that the zygote successfully implants without being swept away (Widmaier *et al*., 2004).

In the absence of pregnancy, the corpus luteum degenerates depriving the developed endometrium of its hormonal support. Uterine blood vessel constricts and the endometrium undergoes degenerative changes due to low oxygen and nutrient

supply. The smooth muscles of the uterus begin to undergo uterine contractions. This is mediated by prostaglandins from the endometrium in response to decreasing levels of oestrogen and progesterone. The endometrium begins to slough off concluding the cycle. The amount of blood loss is about 50 to
150 ml (Widmaier *et al*., 2004).

2.3.3 Fertilization

Fertilization is the union of the male and female gametes. This process usually occurs in the ampulla of the fallopian tube. Out of the about half a billion sperms that are released into the vagina of the female during ejaculation, only a few hundreds succeed in reaching the uterine tubes. It takes minutes for sperms to reach the fallopian tubes after ejaculation into the vagina however, the sperms remain in the tubes for several hours before fertilization can take place. This period enables capacitation of sperms before fertilization. Transport of the sperm through the cervical mucus in the uterus into the uterine tubes is aided by contraction of the uterus and uterine tube stimulated by the hormone oxytocin released from the posterior pituitary gland of the female and also by the prostaglandins within the seminal fluid of the male (Totora and Grabowski, 2003;
Marieb *et al.*, 2015; Martini *et al.*, 2015).

For a sperm to reach the egg nucleus, it must first penetrate the corona radiate, a multiple layer of granulosa cells that surround the egg and bind with the zona pellucida. Binding of a sperm to the receptors on the zona pellucida triggers the release of enzymes from the acrosome that digests a pathway in the zona pellucida as the sperm advances. Once the sperm enters the cytoplasm of the egg, the secondary oocyte completes the second meiotic division

which gives rise to a matured ovum and a second polar body. The nuclear material of the ovum is reorganized in the female pronucleus. In the egg's cytoplasm, the nucleus of the sperm swells as it forms the male pronucleus and the rest of the sperm breaks down. The two pronuclei fuse and their chromosomes pair to form a zygote with 23 pairs of chromosomes (Totora and Grabowski, 2003; Marieb *et al.*, 2015; Martini *et al.*, 2015).

2.3.4 Implantation

The fertilized egg takes about 3 to 4 days to reach the uterus. During this period, it undergoes a series of mitotic divisions without growth to form a solid mass of tiny cells known as a morula. After reaching the uterus, the morula absorbs fluids from the uterine cavity as it transforms into a blastocyst. The blastocyst consists of an inner mass of cells, a fluid filled cavity and an outer layer of cells called trophoblast. The inner mass of cells develops into the embryo and the trophoblast develops in to the placenta and umbilical cord. On about the 5^{th} day of fertilization, the blastocyst sheds the zona pellucida through a process known as hatching. It absorbs more fluid and nutrients from the uterine cavity causing it to enlarge before implantation into the uterine wall. Implantation normally occurs about 6 days after ovulation. This process requires an active interaction between the blastocyst and the endometrium (Totora and Grabowski, 2003; Marieb *et al.*, 2015; Martini *et al.*, 2015).

Prior to implantation, the endometrium of the uterus undergoes structural changes under the influence of progesterone secreted by the corpus luteum. The blastocyst loosely attaches itself to the endometrium with inner mass of cells oriented towards the endometrium. Implantation induces proliferation of the trophoblast making it several layers thick. The endometrium also undergoes changes called the decidual

response at the point of contact with the blastocyst. The trophoblast secretes proteolytic enzymes that digest the endometrial lining enabling the blastocyst to bury itself in the endometrium. Digested uterine glands release nutrients needed to support the early stages of embryonic development (Vander *et al*., 2001; Totora and Grabowski, 2003; Marieb *et al.,* 2015; Martini *et al.,* 2015). The trophoblast also secretes human chorionic gonadotropin (hCG) which maintains the production of progesterone and oestrogen by corpus luteum. The inner mass of cell separates from the trophoblast and differentiates into two layers; the primitive endoderm and ectoderm on the 8th day after fertilization

(Totora and Grabowski, 2003; Marieb *et al.,* 2015; Martini *et al.,* 2015).

2.3.5 Organogenesis

In humans the gestation period is 36 weeks of which the embryonic period is from week 1 to 8 and the fetal period is from week 9 to 36. Formation of body organs and systems occurs from week 4 to 8 during the embryonic period. During the fetal period, the formed organs undergo differentiation and maturation (Totora and Grabowski, 2003; Marieb *et al.,* 2015; Martini *et al.,* 2015).

2.4 Oestrous cycle of rodents

All female mammals experience fertility cycles – the oestrous cycle during sexual maturity. In humans, the oestrous cycle is called the menstrual cycle because of the associated uterine bleeding. Aside primates, most other mammals do not exhibit uterine bleeding during the cycle (Stoddard, 2014). Humans and a few primates are the only mammals which use coitus for recreation and procreation, all others use it

for procreation (Meston and Buss, 2007; Balcombe, 2009). It is during a particular phase of the oestrous cycle that the females are most receptive to a mate and said to be ‘on heat’.

Some mammals like antelopes and deers come on heat once a year hence they are termed monoestrous. Dioestrous mammals like dogs come on heat twice a year. Polyoestrous mammals like the mouse (our test animal) exhibit a 4-6 days oestrous cycle throughout the year. Oestrous cyclicity is controlled by the ovarian hormones oestrogen and progesterone (Malashetty and Patil, 2007; Caligioni, 2009). The levels of these ovarian hormones are in turn influenced by the gonadotropins FSH and LH, hence, oestrous cyclicity is a reasonable index of good functioning of the hypothalamic-pituitary-gonadal axis (Caligioni, 2009;
Abu and Uchendu, 2011).

The cycle stops during pregnancy, pseudo pregnancy and lactation except for one oestrous lasting for about 12-20 hours postpartum (Hill, 2014). The oestrous cycle of mice is divided into four clear phases (proestrous, oestrous, metaoestrous and dioestrous) which can readily be charted by examining vaginal smears. The three types of cells that can be found in such vaginal smears are i) cornified epithelial cell ii) nucleated cells iii) leukocytes; and the relative abundance of these cells determines the phase of the oestrous cycle under consideration. Although it is labour intensive, the evaluation of vaginal smear cytology is the most accurate method used to determine the various phases of the rodent oestrous cycle
(Marcondes *et al*., 2002; Goldman *et al*., 2007; Caligioni, 2009; Byers *et al*., 2012).

2.4.1 Proestrous

Proestrous is the first phase of the oestrous cycle and it is characterized by the growth and maturation of follicles in the ovary and development of the endometrium. During this phase the vagina is gaping and the tissues are pink, moist and swollen. Numerous longitudinal striations could be seen on the dorsal and ventral lips of the vagina (Byers *et al*., 2012; Hill, 2014). This stage shows mostly nucleated cells with some cornified epithelial cells (Byers *et al*., 2012).

2.4.2 Oestrous

Oestrous signifies the period of ovulation also called heat period. Female animals are receptive to sexual activity during the heat period (Hill, 2014). The uterus is distended because of the activity of uterine glands. Vaginal tissues are oedematous, lighter pink and less moist. Striations observed in proestrous become deepened. The oestrous phase is characterized by the presence of cornified epithelial cells (Byers *et al*., 2012). The cornification of vaginal epithelial cells is mainly due to high levels of oestrogen secreted by the granulosa cell of matured follicles. It is known that exogenous administration of oestrogen consistently stimulates the proliferation of the vaginal epithelium in adult spayed animals

(Malashetty and Patil, 2007).

2.4.3 Metaoestrous

Metaoestrous occurs shortly after oestrous. The ovary at this stage contains corpora lutea secreting progesterone. Vaginal tissues are pale, dry and less oedematous.

The cell types of metaoestrous smears include cornified epithelial cells and leukocytes (Byers *et al*., 2012; Hill, 2014).

2.4.4 Dioestrous

The corpora lutea begin to regress during the dioestrous phase of the oestrous cycle. Vaginal tissues are similar to that observed in metaoestrous (Byers *et al*., 2012; Hill, 2014). The vaginal opening is small and closed with no tissue swelling. This is the longest stage of the oestrous cycle in mice lasting about 2 days. Vaginal smear during the dioestrous phase shows predominantly leukocytes
(Byers *et al*., 2012).

2.5 Human population growth and family planning

Population growth is inevitable in the face of medical advancement and improved global health care systems. However, fast population growth in times when more than 2.6 billion people lack basic needs is an issue of worldwide concern. The first billion human population was recorded in 1800 from the beginning of human history. According to the US population bureau, it took 130 years for the second billion to be added and in only 30 years afterwards, the world population reached
3 billion. Each additional billion followed more rapidly. In 1999, the world population rose to 6 billion from 5 billion in 12 years, the shortest interval between any of the previous billions (Population reference bureau, 2010).

The world's population would continue to grow, however, for the first time in history, world population growth rate is projected to slow down. This development is attributed to the decline in population of low fertility countries like Europe,

northern America and Japan (UN, 2011). Nonetheless, the populations of high fertility countries, like Ghana, which are also developing countries, are expected to continue growing substantially. More than 85 percent of the population growth of the past 100 years had taken place in developing countries; of the projected population growth in 2050, 97 percent is expected to be in developing countries. Over the next few years, any growth in the developed countries will likely be limited to North America, Australia and New Zealand, and much of that growth will arise from immigration from developing countries (Population Reference Bureau, 2010; UN, 2014). The world population explosion has intensified the search and development of better contraceptive options for men and women worldwide (Farnsworth *et al*., 1975).

For millennia, men and women have made use of different methods to regulate fertility and plan families. Traditional methods that date back centuries provided inspiration for the development of some of today's most reliable contraceptives including the birth control pill and the copper-laden Intrauterine-devices (IUD) (James and Kepron, 2002; PPFA, 2006; Mann, 2010). Modern contraceptives, provided under the banner of family planning, have played a key role in reducing the rate of the world's population growth. One of the main objectives of the family planning program has been to encourage couples to use modern contraceptives to space their children and thereby invariably reduce their family size. Countries across the globe adopted family planning into population policies to slow down the rate of population growth (Badasu, 2001).

The widespread adoption of family planning represents one of the most dramatic changes of the 20th century. The still growing use of contraception in the 21st century around the world has given couples the ability to choose the timing of pregnancy, the number and spacing of their children. It allows women to pursue education and careers so childbirth is delayed until when ready. That in itself limits the number of children the woman has and thereby contributes to the reduction of the rate of the world's population growth. Family planning is one of the most effective means of population control that has tremendous lifesaving benefits (Smith *et al*., 2009). The era of modern contraception began in the 1960s, when both the birth control pill and intrauterine contraceptives became commercially available. The widespread use of these effective and convenient contraceptive methods resulted in significant changes in births and fertility rates worldwide (PPFA, 2006; Mann, 2010).

Fertility levels have declined steadily over the last three decades but the pace of decline varies among regions. Countries that have achieved a high level of contraceptive use have reached a lower fertility level (Lule *et al*., 2007). Studies of the American population revealed drastic demographical changes since the introduction of modern contraceptives. Between 1800 and 1900, the family size in the Unites States declined from 7.0 to 3.5 children, and by 1933, the average family size had shrunk to 2.3 children (CDC, 2000). Despite the success of modern contraceptives in population control, contraceptive use is low and the need for contraception is high in the world's poorest and populous places (WHO, 2004, Smith *et al*., 2009).

All over the world, millions of women want to decide for themselves when, and how many children to have (IPPF, 2008). Compared to women elsewhere in the world, women in sub-Saharan African countries have larger families and make much less use of family planning. Several factors have contributed to the sustenance of a high level of fertility in this region. These factors include; i) early marriages ii) high levels of infant and child mortality and iii) the high social value placed on child bearing. Children are a priced possession in African societies signifying status and wealth. In some areas, it is a way of insuring oneself from poverty since more children meant more helping hands. Child spacing has inadvertently and reasonably been carried out by employing long period of breastfeeding and traditional postpartum abstinence periods to promote infant survival rates (Cadwell and Cadwell, 2002).

Today, only few people could afford the pride of having a large family amidst the rising cost of living and increasing expenses of child education. Education of the girl child and the economic empowerment of women have also negatively impacted the desire to have and maintain large family resulting in increased attempts to limit and manage family sizes. Recent research on data from world surveys reveal conclusively that fertility has started to decline in many African countries despite variations in speed and timing (Cadwell and Cadwell, 2002).

The key explanatory variable for the decline revolves around the increasing levels of urbanization and the concomitant contraceptive prevalence associated with it. Ghana was among the few countries that adopted family planning methods to control the fast rate of population growth in the late 1960's (Badasu, 2001). Contraceptive use in Ghana increased steadily between 1988 and 1998. The

increase in contraceptive use mirrored a similar decline in fertility during the same period. The total fertility rate in Ghana declined by an average of 2 births from 6.4 to 4.4 per woman. Despite the significant reduction in fertility, contraceptive prevalence has not increased appreciably. There is a gap between the actual and the desired family size, resulting in unintended pregnancies (GDHS, 2008).

The Ghana health demographic shows that out of the 98% of married women who have knowledge about at least one modern method of contraception, only 17% were using a method at the time of the survey. While the percentages of women who have never used any method of contraception have declined over time, there has been a significant increase in the number of sporadic users i.e. women who have used contraception in the past but were not doing so currently (Parr, 2003). Demographic surveys conducted over the years show a weak link between contraceptive use and the significant reduction in total fertility.

According to Badasu (2001), modern contraception has not contributed significantly to Ghana's fertility transition that begun decades ago. Thus the experience of low and unstable levels of contraceptive use and significant reduction in fertility is considered something of a mystery. A number of studies have attributed to the decline in fertility partly to induced abortion (Badasu, 2001; The ACQUIRE Project, 2005). Anarfi (2003), suggested that the role of herbs in fertility decline needs to be investigated, given the high prevalence of their use among Ghanaian women, even though the potency of some as contraceptives have not been scientifically established.

The gap between desired and achieved levels of contraception keeps increasing. Analysis of fertility preferences and contraceptive practices from various demographic surveys indicates that there are many women who either do not want any more children or want to delay the next birth but are not using contraception or need more effective contraceptives. The reasons given for non-use of modern contraception included; i) fear of side effects, ii) lack of access, iii) cost and iv) inconvenience of use (Badasu, 2001; Williamson *et al*., 2009). For the desired levels of contraception to be realized, a lot needs to be done to remove the barriers to modern contraceptives as well as increase the options of contraception available for women. Well documented, local readily available and safe herbal contraceptives might offer alternatives for women who have problems with or lack access to modern contraceptives options particularly women living in the rural areas in developing nations (Ahmed *et al*., 2011; Kaur *et al*., 2011).

2.6 Review of medicinal plants with antifertility activity

Several plants have been in folkloric use throughout history as fertility regulators. A number of these plants have been reported in scientific work to affect fertility in rodents. Sheeja *et al*. (2009), reported that acetone and ethanol extract of *Plumbago rosea* leaves produced anti-ovulatory effects in rats. They showed that the extracts possess oestrogenic activity, as judged by increased uterine weight and vagina opening in immature rats. The extracts however, antagonized the oestrogenic effect of ethinyl estradiol when co-administered to female rats. Montaserti *et al*. (2007) showed that the alcoholic extract of *Physalis alkekengi* significantly decreased the number of implantation sites and weight of neonates in treated rats. Reports by Sani and Sule (2007) indicates that subcutaneous injection of methanol extracts of

Ricinus communis seeds induced 100% antifertility effects in mice at a dose of 200 mg/kg.

According to Mishra *et al*. (2011) the fresh juice of *Raphanus sativus* reduces the number of implantation sites, number of neonates delivered, the number of corpora lutea and ovarian weights significantly in treated rats. They also reported that the juice disrupted the oestrous cycle and altered the levels of cholesterol and protein. Rao and Alice (2001) reported that the alcohol extract of *Phyllantus amarus* inhibits implantation and disrupts the oestrous cycle with a prolongation of the dioestrous phase. However, it does not significantly change body weight, organ weights, blood and serum parameters except for the levels of 3β and 17βhydroxy steroid dehydrogenase.

2.7 Selected plants under study

2.7.1 Anthocleista nobilis

2.7.1.1 Botanical description

Botanical name: *Anthocleista nobilis* G. Don

Family: Loganiaceae

Local name: Awudifuakete (Twi)

Anthocleista nobilis is a medium sized tree which grows to a height of 30m. The trunk is unbuttressed and branchless for up to 15 m with a girth of 14 to 90 cm. The bark of the tree is smooth and pale grey; inner bark is cream yellow and granular. Leaves are simple, opposite and crowded at the end of branchlets. In young plants the apex of the leaf is rounded with wavy margin. Inflorescence is terminal in stiff branched cymes of white flowers. The fruit is a green ellipsoid berry, 3-4cm by 2-

2.5 cm and thick walled. Seeds are obliquely ovoid and dark brown (Burkill, 1985; Iwu, 1993; Mosango, 2007).

Figure 4: Photograph of aerial parts of *Anthocleista nobilis* trunk with terminal leaves (X 0.04)
(Useful Tropical Plants, 2014).

2.7.1.2 Ecological and geographical distribution

The plant is common in moist places, in swamps and on river banks. It occurs on the west coast of Africa from Eastern Senegal to Nigeria and the Central African Republic (Iwu, 1993; Mosango, 2007)

2.7.1.3 Traditional usage

The plant is used throughout its distribution range as a strong purgative and diuretic. A root decoction is usually taken to treat constipation, to regulate menstrual cycle, as abortifacient and a birth aid. A decoction of the leaves is taken to treat abdominal pains of uterine origin, epilepsy, convulsions, small pox, chicken pox, measles and spasms (Burkill, 1985; Mosango, 2007; Iwu, 2014). A bark decoction is used to

treat gonorrhea, dysmenorrheal, leprosy, oedema, dropsy and gout. It is also taken as a laxative, vermifuge, febrifuge, and as an antidote to venomous bites and stings. In Liberia, a bark infusion is given to dogs with diarrhea. A decoction of the roots with lemon is taken to treat hepatitis. A poultice of young twigs and stem bark is applied on abscesses and ulcerous wounds. A mixture of grounded leaves with soil from the fire place and water is believed to promote closure of the fontanelle in babies (Burkill, 1985; Mosango, 2007;

Schmelzer *et al*., 2010).

2.7.1.4 Previous studies on *Anthocleista nobilis*

Oral administration of the ethanol extract of *A. nobilis* root bark showed a pronounced reduction of pentobarbitone-induced sleep in carbon tetrachloride poisoned mice; an effect comparable to that of silibinin. It also reduced the toxic effects of carbon tetrachloride (CCL_4) by inhibiting the elevation of serum GOT and GPT. The LD_{50} in mice at 24 hours was 200mg/kg when administered by the intraperitoneal route. In an in vitro assay, it induced a concentration dependent relaxation in isolated guinea pig ileum (Madubunyi and Asuzu, 1996). Report by Annan and Dickson (2005) indicates that the plant possesses potent anti-oxidant activity. The ethanol root extract is also reported to improve immunity in fowls challenged with the Newcastle disease virus (Ayodele, 2011).

2.7.1.5 Phytochemistry of plant

The plant is reported to contain alkaloids, glycosides, saponins and steroids (Burkill, 1985).

2.7.2 Macaranga heterophylla

2.7.2.1 Botanical description

Botanical name: *Macaranga heterophylla* (Müll.-Arg.) Müll.-Arg.

Synonyms: *Macaranga guinguelobata* Beille., *Mappa heterophylla* Müll.-Arg. and *Tanarius heterophylla* (Müll.-Arg.) Kuntze.

Family: Euphorbiaceae

Local names: Opam (Akan-Asanti) and Opamnua (Twi)

Figure 5: Photograph of *Macaranga heterophylla* leaves (X 0.2)

Macaranga heterophylla grows as a shrub or small tree up to 10m tall. The stem and twigs are covered with woody spines. Leaves are simple, hairy and alternate with ovate stipules of 4cm long. The blade of the leaf has about 3 to 7 lobes with toothed margins. Flowers are unisexual and occur in auxiliary panicles lacking petals and disks. The fruit, a rounded 2 lobed drupe is coloured pink to red. Each fruit contains 2 nearly globose black seeds. Wood from the tree is light and soft exuding a yellow translucent gum when cut (Burkill, 1985; Schmelzer, 2007).

2.7.2.2 Ecological and geographical distribution

Macaranga heterophylla is a perennial plant abundant in secondary forest wet localities and riverine forests. It occurs from Eastern Senegal to Cameroon

(Schmelzer, 2007).

2.7.2.3 Traditional usage

The plant is generally used for medicinal purposes in West Africa. Various parts of the plant are indicated as strong laxatives, enemagogue and abortifacient. Preparations made from the plant are used to facilitate delivery, hasten the expulsion of retained placenta after delivery. It is a remedy for impotence, female sterility and venereal diseases. In Senegal, root decoction is taken for amenorrhoea. It is used to treat asthma, tuberculosis, snake bites, scorpion stings, cough and gonorhoea (Burkill, 1985; Schmelzer, 2007).

2.7.3 Palisota hirsuta

2.7.3.1 Botanical description

Botanical name: *Palisota hirsuta* (Thunb.) K. Schum.

Synonyms: *P. Thyrsiflora* Benth.

Family: Commelinaceae

Local names: Mpetima, Akwabe, Sommenini (Twi); Sombanyi (Fante); Sumbe, Klugbogbo (Ewe); Ofonugba (Guan); Nakutsokpo (Krobo).

Figure 6: Photograph of aerial parts of *Palisota hirsuta* showing leaves and inflorescence (X 0.18)

Palisota hirsuta is a robust herb of about 2-4 min height. The stem is rigid and fleshy with a layer of soft brown hairs at the base. It is spanned with swollen nodes which are about 30 cm apart. The nodes are covered by short ragged sheaths of leaves in whorls. The leaves are mostly at the top of the stem arranged in rosettes. They are obovate to oblanceolate, about 15 to 40 cm long and 4 to 11.5 cm wide. They are acute at the apex and narrow to the base terminating in flat, densely hairy petioles that are about 3 cm long. The margins and midribs have soft brown hairs. The undersurface of the leaf blade is dark green and hairy.

The flowers are white to purplish bearing glossy and black fruits. The inflorescence is a loose and spreading panicle about 10 to 30 cm long that has many slender lateral branches (Akobundu and Aggakwa, 1998).

2.7.3.2 Ecological and Geographical distribution

Palisota hirsuta is a resilient perennial herb found in forest regrowth and farmlands. It is widely distributed in the tropics (Akobundu and Aggakwa, 1998).

2.7.3.3 Traditional usage

Various parts of the plant are used for several ailments. In Ghana and Nigeria, the roots are added to soup taken by women in pregnancy. A preparation of the plant is taken internally for difficult childbirth and female sterility in Ivory Coast. The leaves in groundnut soup are taken by suckling mothers to 'cleanse' their milk (Burkill, 1985; Neuwinger, 1996). The leaves and roots boiled in water are used in the preparations of enemas to provoke bleeding and induce abortion in Ghana (Bleek and Asante - Darko, 1986). The Tiv people of Nigeria use the leaves and roots in aid of conception. A decoction of the whole plant serves as a remedy for urethral discharge. Boiled roots with lime is said to cure gonorrhea if taken immediately within three days. The plant is known to ease stomach ache and the roots when put in warm water are used as enemas for constipation. The dried powdered leaves in water are also used as anti-dysentery enemas (Burkill, 1985).

With a good reputation for pains and wounds, the Igbo people of Obampa prepare an ointment from the plants for gunshot wounds and swellings (Burkill, 1985; Odugbemi and Akinsulire, 2008). Leaf infusion is taken for piles and given to babies to heal the navel. In Gabon, stem shavings are also used to promote healing of wound particularly of the umbilicus. The sap from roasted leaves is instilled in the ear for earache (Burkill, 1985). Heated leaves are applied over the lumbar region for kidney pains; the sap is applied as a dressing for furuncles, arthritic pain,

fractures, yaws and guinea worm sores. A draught of plant stem infusion is also taken for coughs, chest pains and bronchitis (Burkill, 1985). In Cameroon, a decoction of the plant is used to treat conjunctivitis, gastralgia and boils (Jiofack *et al.*, 2009).

2.7.3.4 Previous studies on the activity of *Palisota hirsuta*

Ethanolic leaf extract of *Palisota hirsuta* exhibited anti-arthritic (Wood *et al.*, 2009a) and anti- nociceptive effects in rats (Wood *et al.*, 2009b). The antinociceptive effect was attributed to the stimulation of peripheral opiod receptors through the activation of the nitric oxide- cyclic GMP-ATP-Sensitive K channels. The anti-inflammatory and anti-pyretic effects of the ethanolic root extracts of the plants have also been documented (Boakye-Gyasi *et al.*, 2008). A study conducted by Benson *et al.*, (2008) showed that total flavonoids isolated from the leaves of *Palisota hirsuta* possess aphrodisiac property in rats. Leaf extract of the plant possesses antibacterial activity against Gram positive organisms with no activity against Gram negative organisms. The root extract however, showed no activity against both groups of organisms (Falodun *et al.*, 2009). Methanolic extract of the plant also exhibited antiviral activity against herpes simplex virus, the sindbis virus and the poliovirus (Anani *et al.*, 2000).

2.7.3.5 Phytochemistry of plant

A preliminary phytochemical analysis of the powdered leaves of *Palisota hirsuta* showed that it contains tannins, reducing sugar, flavonoids, steroids and terpenoids with traces of alkaloids (Boakye-Gyasi, 2009). A report on the antinutritive

component in the leaves indicates that it contains 1.97 % tannins, 17.40 mg g^{-1}phytin and 1.72 mg g^{-1} hydrogen cyanide. It also contains 93.60% dry matter, 15.34% crude protein, 10.80 % ash, 10.90% crude fiber, 2.10% ether extract, 54.46% nitrogen free extract, 48.75% acid detergent fiber, 49.40% neutral detergent fiber and 0.66% hemi-cellulose (Okoli *et al*., 2003).

2.7.4 Trichilia monadelpha

2.7.4.1 Botanical description

Botanical name: *Trichilia monadelpha* (Thonn.) JJ de Wilde

Synonyms: *Trichilia heudelotii* Planch. Ex Oliv. (1868)

Family: Meliaceae

Local names: Otanduro (Twi), Tanaduro (Fante), Tenuba (Nzema)

Figure 7: Photograph of a branch of *Trichilia monadelpha* with leaves (X 0.15)

Trichilia monadelpha is an evergreen medium sized tree that grows up to 20 m high without buttress. It has a short, straight and cylindrical trunk with a girth of about 1.7m. The bark surface is pale grey to dark brown, more or less smooth with very fine longitudinal fissures. The slash of the bark is pink, darkening to orange

brown. Leaves alternate and are pinnately compound consisting of 4 to 6 pairs of leaflets without stipules and petioles of about 4 to 13cm long. Leaflets are opposite, ovate to obovate, 4-26cm by 1.5 -9cm, cuneate to obtuse at the base. Flowers are unisexual, scented, greenish yellow to greenish white in colour. The fruits are green and globose with bright red seeds (Burkill, 1985; Amponsah *et al*., 2002; Lemmens, 2008).

2.7.4.2 Ecological and geographical distribution

Trichilia monadelpha is a plant in evergreen and semi-decidous secondary forests. Mostly along rivers and other wet localities. It occurs from Eastern Guinea Bissau to Central Africa Republic, Democratic Republic of Congo and South to Northern Angola. It is also found in lowlands, evergreen rain forests in Ghana and many parts of West Africa (Lemmens, 2008).

2.7.4.3 Traditional usage

The bark of the plant is of high value in traditional medicine. Preparations from the bark of the plant are used to treat pulmonary troubles, oedema, arthritis, rheumatism, yaws, cough, skin infections, skin ulcers, dyspepsia and gouts (Burkill, 1985; Amponsah *et al*., 2002; Lemmens, 2008; Odugbemi and Akinsulire, 2008). Bark decoctions are used as an aphrodisiac, abortifacient and ecbolic. The plant is used as a remedy for diarrhoea, dysentery, malnutrition and to cure venereal diseases (Burkill, 1985; Lemmens, 2008).

2.7.4.4 Previous work done on *Trichilia monadelpha*

Owusu (2009) reported that the aqueous, alcoholic and petroleum ether extracts of the stem bark of *T. monadelpha* possess anti-inflammatory as well as analgesic effects with no evidence of toxicity after two weeks of oral administration in rats.

A significant decrease in testosterone level was observed in rats treated with 400mg/kg of the stem bark extract of *T. monadelpha.* However, no adverse effect was reported on testicular function (Oyelowo *et al*., 2011).

2.7.4.5 Chemical composition of the plant

The bark of the plant is reported to contain tannins, alkaloids, saponins, flavonoids, steroids, terpenoids and glycosides (Burkill, 1985; Owusu, 2009).

2.7.5 Waltheria indica

2.7.5.1 Botanical description Botanical name: *Waltheria indica* Linn.

Synonyms: *Waltheria Americana* L.

Family: Sterculiaceae

Local names: Sawai (Twi)

Waltheria indica is a short-lived shrub sometimes reaching 2 m in height and 2 cm in stem diameter with a weak taproot, but with robust lateral roots and abundant fine roots. The young stems and leaves are covered with grey, velvety hairs. The alternate leaves are narrowly ovate or oblong with a rounded to heartshaped base, irregularly serrate edges, and a rounded to acute tip. The stalks are 0.5-3.3 cm long and the blades are 2-12 cm long and 1-7 cm broad. Inflorescences are usually dense clusters in leaf axils that contain fragrant, yellow flowers. The plant is commonly

known as sleepy morning, velvet leaf, marsh-mallow, monkey bush, boater bush, leather coat and buff coat (Burkill, 1985).

Figure 8: Photograph of aerial parts of *Waltheria indica* showing leaves and flowers (X 0.08)
(Wikimedia Commons, 2012)

2.7.5.2 Ecological distribution

Waltheria indica is common in open places, in savannah grasslands and on abandoned farmlands. It grows throughout the tropics and warmer regions. It is also common in the new world of the Americas (Burkill, 1985).

2.7.5.3 Traditional usage

The plant is used in the treatment of skin diseases, malaria, typhoid fever, sickle cell anemia and epilepsy (Oliver-Beyer, 1986; Bala *et al*., 2011). It is used to treat infected wounds and convulsion in children (Newmark, 2002). Aerial parts of the plant are used to treat cough and emollient (Vardhana, 2008). In Ghana it is used as an abortifacient and to regulate the menstrual cycle (Farnsworth *et al*., 1975; Burkill, 1985; Abbiw, 1990; Leonard, 2006; Khare, 2007; Kumar *et al*., 2012). It is also used as a purgative and the root is prescribed for internal haemorrhages (Khare, 2007).

2.7.5.4 Previous work done on plant

A study was conducted to evaluate the inhibitory activity of polar and non-polar extracts from *Waltheria indica* against *Trypanosoma brucei brucei* and the results indicated that the ethanol extract was the most active against the organism (Bala *et al*., 2011). The ethyl acetate fraction of the aqueous ethanol extract of the plant blocked leptazole-induced convulsion, potentiated amylobarbitone sleeping time and decreased exploratory activity, indicating anticonvulsant and sedative actions (Hamidu *et al*., 2008).

Three flavonoids isolated from *W. indica* exhibited strong anti-inflammatory effect associated with a dose dependent reduction in the production of inflammatory mediator nitric oxide (NO), tumor necrosis factor alpha (TNF-α) and interleukin 12 (IL-12) (Rao *et al*., 2005). Extracts from *W. indica* showed potent antibacterial activity against strains of s*treptococcus* causing *pneumonia* including strains resistant to penicillin (Kone *et al*., 2006). According to Zailani *et al*. (2010), various parts of the plant possess antibacterial activity against *Escherichia coli, Salmonella typhi* and *Pseudomonas aerogenosa*. The leaf extract of *Waltheria indica* had the highest antibacterial effect compared to the extracts of the stem and root.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1. Collection of plant samples

Roots of *Anthocleista nobilis* were collected from Aburi in the Eastern region of Ghana. The stem bark of *Trichillia monadelpha* (Fig. 9), the leaves of *Macaranga heterophylla* (Fig. 10) and the roots of *Palisota hirsuta* (Fig. 11) were collected from Bobiri reservation forest in the Ashanti region of Ghana. The plant samples were authenticated by a plant taxonomist at the Forestry commission, Kumasi, Ghana. The leaves of *Waltheria indica* were collected from the Legon botanical garden, Accra after having been similarly authenticated.

Figure 9: Photograph of stem bark of *T. monadelpha* (X 0.2)

Figure 10: Photograph of air dried leaf sample of *M. heterophylla* (X 0.2)

Figure 11: Photograph of stem and roots of *P. hirsuta* (X 0.14)

Voucher specimens of the plant samples were deposited at the herbal medicine herbarium at KNUST with the following herbarium numbers:

i) *Anthocleista nobilis*: KNUST/HM1/2013/L004 ii)

Macaranga heterophylla: KNUST/HM1/2013/L001 iii)

Palisota hirsuta: KNUST/HM1/2013/L002 iv)

Trichillia monadelpha: KNUST/HM1/2013/L003

v) *Waltheria indica*: KNUST/HM1/2014/L095

3.2 Preparation of plant extracts

The five plant materials were air dried for 14 days and grounded into powder using the hammer mill. The powdered materials were extracted in 70% ethanol using the Soxhlet apparatus. The extracts obtained were concentrated with the rotary evaporator and the residue dried to a constant weight in an electric oven at 50^{o}C. The percentage yield was as follows:

i) *Anthocleista nobilis* root extract (ARE): 13.7% w/w ii)

Macaranga heterophylla leaf extract (MLE): 3.89% w/w iii)

Palisota hirsuta root extract (PRE): 5.94% w/w iv) *Trichillia monadelpha* stem bark extract (TBE): 8.49% w/w

v) *Waltheria indica* leaf extract (WLE): 10% w/w

All extracts were kept in a refrigerator during the experiment. Various doses of the extracts were prepared by dissolving weighed amounts in distilled water. Prepared samples were orally administered using a stainless steel gavage and no animal received more than 1ml/kg body weight of the test organisms.

3.3 Test animals

Four hundred and twenty (420) albino mice of the ICR strain between 2 and 3 months old (20g to 35g) were purchased from Centre for Scientific Research into Plant Medicine (CSIRPM) Mampong-Akwapim, Ghana and housed and acclimatized for 14 days before any experimentats began at the animal facility of the Department of Theoretical and Applied Biology, KNUST. They were kept in stainless steel cages of dimensions 34 x 47 x 18 cm with soft wood shavings as bedding, and fed with commercially formulated pellet diet from GAFCO, Tema, Ghana. Water was provided *ad libitum*. The mice were handled humanely throughout the experimental period. Prior to mating, all females were isolated during the 14 days acclimation period to rule out existing pregnancy.

3.4 Preparation of vaginal lavage

Vaginal lavage was obtained according to the method of Byers *et al*. (2012). In this, sterile pipettes fitted with sterile pipette tips were used to collect the vaginal samples. The animals were restrained by the nape and tail in a supine position and

the pipette with about 0.2ml of normal saline was gently introduced into the vagina. The saline solution was used to wash the vagina twice and the pipette was withdrawn. The collected sample was placed on a clean slide and observed under the microscope. The stages of the oestrous cycle were determined based on the presence or absence of cornified cells, nucleated epithelial cells and leukocytes. Samples with predominantly cornified cells were classified as oestrous. Those with a numerous nucleated cells were classified as proestrous whilst smears with equal proportion of leukocytes and nucleated cells were classified as metaoestrous. A sample with a large number of leukocytes was characterized as dioestrous.

3.5 Acute toxicity study

Acute toxicity study was carried out according to the method described by the Organization of Economic Co-operation and Development test guideline 423, Acute Toxic Class method (OECD, 2001). The test substance was administered orally to three groups of three female animals using defined doses of 300mg/kg, 2000mg/kg and 5000mg/kg. Absence of compound related mortality at a given dose was taken as an indication of an LD_{50} above that dose.

One hundred and twelve female mice were randomly divided into six groups. Group I (n=7) served as the control group and received only distilled water by gastric intubation throughout the treatment period using stainless steel gavage. Animals in groups II to VI received extracts of *Anthocleista nobilis* (II), *Macaranga heterophylla* (III), *Palisota hirsuta* (IV), *Trichillia monadelpha* (V) and *Waltheria indica* (VI) respectively. Groups II to VI were each subdivided into three with seven animals each as follows: IIA, IIB, IIC, ……. to VIA, VIB and VIC. The animals

in subdivisions A, B and C received a single dose of 300 mg/kg, 2000 mg/kg and 5000 mg/kg of the various extracts respectively. All animals were observed closely for mortality and or treatment related abnormalities over a period of 24 hours to underscore possible toxicity. The animals were observed for a further 14 days to observe any delayed signs of toxicity.

3.6 Preliminary fertility test

This preliminary test was carried out to assess the effect of the test plants on pregnancy, fertility index and embryonic development in order to select promising plants for further testing. In this study, a limit dose of 1000mg/kg was used according to accepted pharmacological standards described by the ICH Harmonised Tripartite Guidelines S5(R2) (ICH, 2005).

3.6.1 Effect of post coital treatment on pregnancy

This study was carried out by the method described by Gebrie *et al*. (2005) with some modification. According to the protocol, two groups of animals (n = 5) labelled as control and test group were orally given water and the test substance respectively for a period of 10 days after mating. Both groups were sacrificed on day 11 to determine the number of implantation sites in the horns of the uteri. The test animals in this study were sacrificed on day 15 of gestation according to the ICH Harminised Tripartite Guidelines S5(R2) (ICH, 2005) instead of day 11 to differentiate between implantation and resorption sites.

Fifty five virgin female mice were randomly divided into six groups. Group I (n=5) served as the control group and received only distilled water throughout the treatment period by gastric intubation. Animals in groups II to VI received plant extracts as ordered above for the toxicity test of section 3.5. Groups II to VI were each subdivided into two with five animals each as follows: IIA, IIB, ……. to VIA and VIB. All cohort A animals received 500 mg/kg of the extracts in the order they have been assigned; whereas all the cohort B animals were given 1000 mg/kg of their respective extracts.

Animals in all groups were left overnight with males of proven breeding ability (in the ratio of 2 females to 1 male) and females were examined for the presence of copulatory plugs in the morning. The presence of copulatory plug in the vagina was used as an indicator of successful mating (Fig. 12). The day copulatory plug was seen was taken as day 1 of pregnancy. The animals were treated from day 1 to day 10 of pregnancy. During this period, the mice were examined daily for signs of abortions and morbidity. On gestation day 15, all the test animals were sacrificed by cervical dislocation and laparotomized to determine the number of implantation sites in the horns of the uterus. The number of resorption sites, live and dead foetuses, if any, were also counted and recorded.

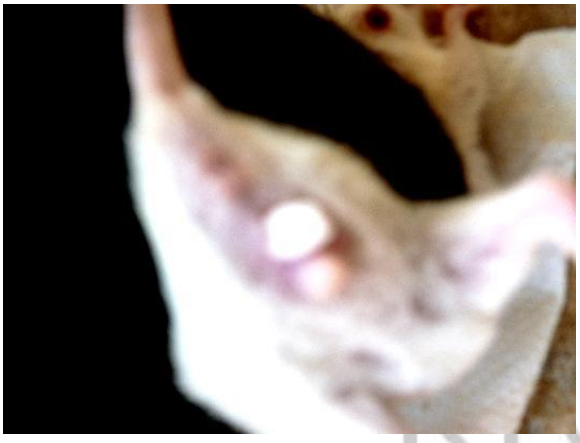

Figure 12: Photograph of female mouse with copulatory plug (X 1.6)

3.6.2 Effect of treatment on embryonic development and measurement of fertility index

This study was carried out according to the ICH Harmonised Tripartite Guidelines S5(R2) (ICH, 2005). Following the protocol, control and test groups were dosed for 14 days before mating and 10 days after mating. Both groups were sacrificed on day 15 to determine the number of implantation and resorption sites in the horns of the uteri.

Fifty five female mice were randomly divided and labelled into the six groups. Group I (n=5) served as the control group and received only distilled water by gastric intubation throughout the treatment period. The remaining five of six groups were given doses of the extracts used in the toxicity test (reproduced here as a reminder) *Anthocleista nobilis* (II), *Macaranga heterophylla* (III), *Palisota hirsuta* (IV), *Trichillia monadelpha* (V) and *Waltheria indica* (VI) respectively.

Groups II to VI were each subdivided into two with five animals each as follows: IIA, IIB, ……. to VIA and VIB. The subdivisions A and B received doses of 500 mg/kg and 1000 mg/kg of the five extracts respectively.

Animals in all 10 sub-groups were pretreated for 14 days with their respective extracts at the prescribed doses before cohabitation with untreated males. Females were examined for the presence of copulatory plugs which was used as an indicator of successful mating. The day copulatory plug was seen was taken as the first day of pregnancy. Treatment continued after successful mating for a further 10 days. The mice were examined daily especially for signs of abortions and or any other signs of morbidity. On gestation day 15, all the animals were sacrificed by cervical dislocation and laparotomized to determine the number of implantation sites in the horns of the uterus. The number of resorption sites, live and dead foetuses, if any, were also counted and recorded.

Fertility index was calculated as the number of animals pregnant divided by the number of animals mated multiplied by 100.

Fertility index: $\frac{\text{Number of animals pregnant}}{\text{Number of animals mated}} \times 100$

3.7 Effect of the most promising treatments, ARE and TBE on oestrous cycle of female mice

Nulliparous female mice with regular cycles were used for this study. Vaginal lavage of the mice were taken daily and examined under the microscope for 15 days and only females with regular oestrous cycles were selected for the study.

Forty nine female mice with regular cycles were randomly divided into three groups of I, II and III. Groups I (n=7) served as the control group and received only distilled water by gastric intubation throughout the treatment period. Animals in groups II and III received extracts of the roots of *Anthocleista nobilis* (ARE) and the bark of *Trichillia monadelpha* (TBE) respectively. Groups II and III were each put into

three sub-groups (n = 7) as follows: IIA, IIB, IIC, IIIA, IIIB and IIIC. The subdivisions A, B and C received doses of 750 mg/kg, 1500 mg/kg and 3000 mg/kg of the extracts respectively.

All the animals were treated for 21 days and the oestrous cycle was monitored daily and recorded throughout the study period. Mice were sacrificed on day 22 by cervical dislocation. Blood samples were collected through venous puncture into tubes without anticoagulants for biochemical analysis. Liver of test animals were harvested, weighed and preserved in 10% formal saline for histological examination.

3.7.1 Organ to body weight ratio determination

Selected organs, liver, kidney, ovary and uteri were excised, trimmed of excess fat and weighed. The relative organ to body weight ratio was calculated as follows:

$$\frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

3.7.2 Serum analysis

Animals were decapitated using a surgical blade. Blood samples were collected into sterile sample tube without anticoagulants. The samples were centrifuged and the serum was analyzed using Flexor junior automated analyzer.

3.7.3 Histopathological studies

Liver isolated from test animals were preserved in 10% formal saline solution. Portions of the organ were embedded in paraffin and sectioned on a Reichart microtome according to standard micro techniques. Sections were stained with hematoxylin and eosin. Photomicrographs were made and examined for

histopathological changes. The changes observed were graded on a scale of normal, moderate, and severe limit of injury.

3.8 Anti-implatation effect of ARE and TBE

The effect of the extracts on implantation was carried out according to the method described by Gebrie *et al*. (2005) with some modification as stated in section 3.6.1. Forty nine female mice were randomly divided into three groups. Groups I (n=7) served as the control group and received only distilled water. The remaining 42 animals were split into two groups of 21 each as II and III received extracts of *Anthocleista nobilis* and *Trichillia monadelpha* respectively. Each sub-groups of 21 were further divided into three of seven animals as IIA, IIB and IIC for ARE and IIIA, IIIB and IIIC for TBE. The animals in A, B and C received doses of 750 mg/kg, 1500 mg/kg and 3000 mg/kg of their respective extracts.

The animals in all groups were similarly male-paired and mated and were examined for the presence of copulatory plugs in the morning. This inspection was done until all 49 females were successfully mated. The day copulatory plug was seen was taken as day 1 of pregnancy.The animals were treated from day 1 to day 10 of pregnancy. During this period, the mice were examined daily for signs of abortions and morbidity. On gestation day 15, all the test animals were sacrificed by cervical dislocation and laparotomized to determine the number of implantation sites in the horns of the uterus. The number of resorption sites, live and dead foetuses, if any, were also counted and recorded.

3.9 Effect of ARE and TBE on fertility index and embryonic development

This study was carried out according to the ICH Harmonised Tripartite Guidelines S5(R2) (ICH, 2005). Another forty nine female mice were similarly divided into groups as of 3.8 and offered same doses of ARE and TBE. However, except for the animals the control group which were offered distilled water, the treatment groups were pretreated for 21 days with their respective extracts before being male-paired and subsequently examined for copulatory plugs. Treatment continued after successful mating for a further 10 days. The mice were examined daily for signs of abortions and morbidity. On gestation day 15, all the animals were sacrificed by cervical dislocation and laparotomized to determine the number of implantation sites in the horns of the uterus. The number of resorption sites, live and dead foetuses, if any, were also counted and recorded. Fertility index was calculated as the number of animals pregnant divided by the number of animals mated multiplied by 100.

Fertility index: $\frac{\text{Number of animals pregnant}}{\text{Number of animals mated}} \times 100$

3.10 Effect of TBE on smooth muscle preparation

This *in vitro* test was carried out to evaluate the uterotonic or tocolytic effect of TBE, a factor which is known to contribute to the reproductive effects of some medicinal plants (Naseri *et al*., 2008; Gruber and O'Brien, 2011).

3.10.1 Isolation and mounting of rat uterus

This study was carried out according to the method of Boye (2010). Nulliparous virgin rats were injected with estradiol benzoate 24 hours before the day of the experiment. Each rat was killed by a gentle blow to the head. The abdomen was

opened and the two horns of the uterus were identified and dissected out. The uteri horns were separated and freed from surrounding fat and each was cut opened longitudinally so that the preparation was a sheet of muscle instead of a narrow tube. A piece of this sheet, about 2-3 cm was mounted in 25ml organ baths containing De Jalon´s solution.

A thread was attached to one end of the isolated strip of uterus and was tied to the aerator tube in the organ bath containing De Jalon's physiological solution. Another thread was attached to the other end of the isolated uterus and fixed to a lever system fitted with a frontal writing point moving on a white kymograph paper wound around a cylinder of 30 cm diameter revolving at a rate of 4 mm per minute. A load of 0.5 g (tension of 5 mN) was applied on the tissue to minimize spontaneous activity if any.

The bath was thermostatically regulated and maintained at 32^0C. The tissue was aerated with ordinary air using Corning - Eel 850 air compressor (Evans Electroselenium Ltd, Halstead Essex England). The isolated strip of uterus was allowed to stay in the De Jalon's physiological solution for at least one hour before starting the test making sure there were no spontaneous uterine contractions.

3.10.2 Administration of TBE on isolated uterine muscle

The effects of the extract (1mg/ml-50mg/ml) and Acetylcholine (5µg/ml80µg/ml) were investigated on the isolated rat uterus. A concentration - response curve was constructed to determine the concentrations that induced half-maximal contraction of the uterine tissue, EC50. Different concentrations of the plant extracts were added to the solution bathing the tissue in the bath. The effect of the extract on the

isolated uterus was monitored within a 30 second intervals (0 second: kymograph was started; 30 seconds: drug was administered; 60 seconds: kymograph was stopped and tissue washed; 180 seconds: kymograph was started again).

Various concentrations of the extracts were added until the steady, largest amplitudes, defined as the maximal contractions were obtained. The uterine contraction agonist, acetylcholine was used as a control. This was added to the tissue in the same manner as the extracts and a dose response curve was constructed and the EC50 was determined.

3.10.3 Effect of TBE on acetylcholine (Ach) induced contractions

The tissue was pretreated with TBE (1 mg/ml and 3 mg/ml) for 5 minutes after which the uterus was contracted with acetylcholine (5µg/ml-80µg/ml) till the maximal response was obtained. The effect of the acetylcholine on the isolated uterus in the presence of the extract was recorded and a concentration- response curves was constructed. The uterine muscle contractions inhibitor atropine (2 x 10^{-8} mg/ml) was used as a control.

3.11 Data Analysis

The results of the study were analyzed using Graphpad Prism version 5.01. Results expressed as mean ± Standard Error of Mean (SEM) were analyzed by one way ANOVA followed by Newman keuls post hoc test to compare columns.

Data on preliminary fertility test and oestrous cycle were analyzed using two way ANOVA followed by Bonferroni *post hoc* test. A value of $p < 0.05$ was used as the criterion for statistical significance.

3.12 Justifiction for choice of ARE and TBE

Statistical analysis of the preliminary fertility test and the occurance with rare events such as complete foetal loss and abortion (ICH, 2005) were used to select promising plants for further studies.

CHAPTER FOUR

4.0 RESULTS

4.1 Acute toxicity study

Throughout the period of the acute toxicity study, none of the treated animals showed any signs of toxicity to the five plant extracts of

i) *Anthocleista nobilis* root extract (ARE) ii) *Macaranga heterophylla* leaf extract (MLE) iii) *Palisota hirsuta* root extract (PRE) iv) *Trichillia monadelphia* stem bark extract (TBE)

v) *Waltheria indica* leaf extract (WLE)

There were no observable behavioural, motor or neurological changes in animals treated with the plant extracts. No death was recorded during the acute toxicity studies therefore the LD_{50} of ARE, MLE, PRE, TBE and WLE exceeds 5000mg/kg based on categorization established by Hodge and Sterner scale, an indication that the extracts are practically non-toxic (Teke and Kuete, 2014). No latent toxicity was observed in the treated animals after keeping them for extra 14 days. Therefore a limit dose of 1000 mg/kg was used for the preliminary fertility test (ICH, 2005).

4.2 Preliminary fertility test

Preliminary fertility test results show that treatment of animals for 10 days after mating with ARE did not affect implantation (Table 1). The number of implants was not significantly different compared to controls ($p > 0.05$). Treatment with ARE caused early foetal death at all doses as judged by the presence of resorption sites. Foetal loss was not observed in control animals (Fig. 13). None of the animals showed signs of mortality or vaginal bleeding during treatment. The number of live foetuses was lower in animals treated with a dose of 1000 mg/kg
(Table 1) although not significant compared to controls ($p > 0.05$).

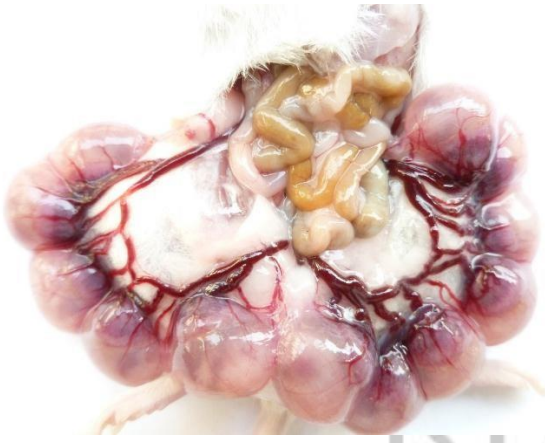

Figure 13: Photograph of uterine horns of a mouse showing implants (X 0.8)

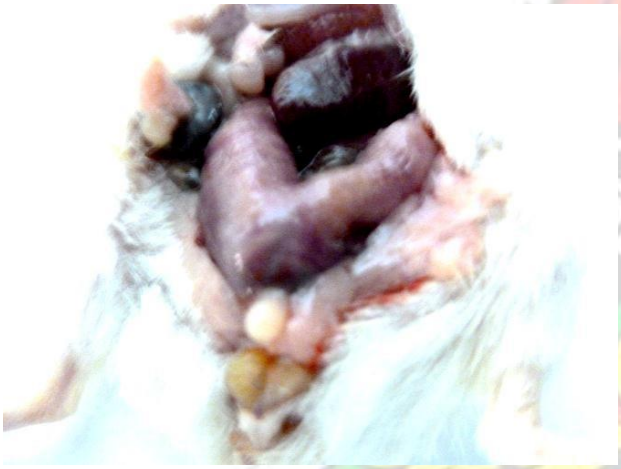

Figure 14: Photograph of uterine horns of mouse treated with ARE (1000 mg/kg) showing complete foetal resorption (X 1)

Treatment of mice with ARE for 14 days before mating and 10 days after mating did not affect fertility index but resulted in decreased number of implants and live foetuses at all doses (Table 2). One out of the five animals (20%) treated with a dose of 1000 mg/kg showed complete resorption of foetuses. It appeared resorption occurred late during development as judged by the size of implants (Fig. 14). Post implantation loss was however, not significantly different ($p > 0.05$) compared to controls (Table 2).

Mice treated with MLE after mating had reproductive indices comparable to controls at all doses (Table 1).The number of implants was not significant ($p > 0.05$) at all doses compared to controls (Table 1). No foetal loss was observed in treated animals. Treatment of mice before and mating with MLE did not affect fertility index (Table 2). Although the number of implant and live foetuses was lower in mice treated with a dose of 1000 mg/kg, this was not significantly different ($p > 0.05$) compared to controls (Table 2). Foetal death was not observed in treated animals at all dose levels.

Mice treated with PRE after mating had reproductive indices comparable to controls at all doses (Table 1). The number of implants and live foetuses were not significantly different ($p > 0.05$) compared to controls (Table 1). Fertility index of mice treated with PRE before and after mating was not affected at all doses (Table 2). The number of implants and live foetuses were not significantly different ($p > 0.05$) compared to controls (Table 2). Foetal death was not observed in treated animals and none of the animals showed signs of mortality or vaginal bleeding during treatment.

Treatment of animals for 10 days after mating with TBE did not affect implantation (Table 1). The number of implants and live foetuses was not significantly different compared to controls ($p > 0.05$). Foetal death was observed in treated animals at all doses (Table 1). None of the animals showed signs of mortality or vaginal bleeding during treatment. Animals treated with TBE at a dose of 1000 mg/kg before and after mating had reduced fertility index (60%) (Table 2). One out of the five treated animals (20%) had no foetuses but ammonium sulphide staining showed early foetal death and tissue resorption (Fig. 15). Furthermore, another animal (20%)

from the same group exhibited signs of abortion by the expulsion of uterine content (Fig. 16). Examination of the uterus showed highly thickened uterine wall (Fig. 17).

Foetal death was also observed in animals treated with TBE at a dose of 500 mg/kg (Table 2). Although the number implant and live foetuses were reduced in mice treated with TBE before and after mating at all doses (Table 2), it was not statistically significant compared to controls ($p > 0.05$). Post implantation loss was also, not significantly different ($p > 0.05$) compared to controls (Table 2).

Figure 15: Photograph of uterine horns stained with ammonium sulphide showing sites of early embryonic resorption (X 1.1)

Figure 16: Photograph of mouse treated with TBE (1000mg/kg) showing signs of abortion (X 1.4)

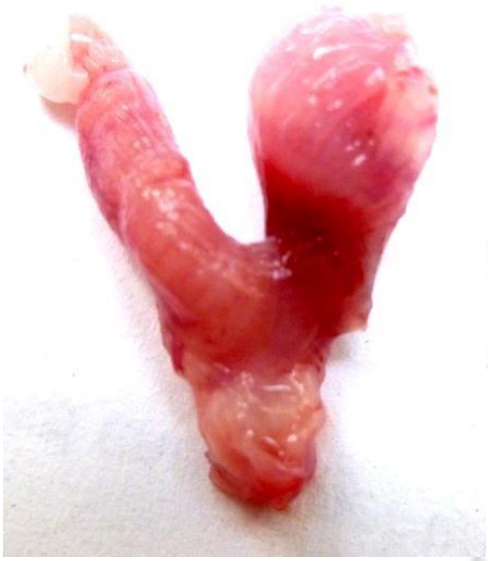

Figure 17: Photograph of aborted mouse uterus showing thick walls without implants (X 1.7)

Mice treated with WLE after mating had reproductive indices comparable to controls (Table 1). The number of implants and live foetuses were not significantly different ($p > 0.05$) compared to controls (Table 1). Fertility index of mice treated with WLE before and after mating was not affected at all doses (Table 2). The number of implants and live foetuses were not significantly different ($p > 0.05$) compared to controls (Table 2). Foetal death was not observed in treated animals at all doses and none of the animals showed signs of mortality or vaginal bleeding during treatment.

Based on the results of the preliminary fertility test, ARE and TBE were selected for further tests. The effective dose in the preliminary test (1000 mg/kg) was increased three folds and used as the high dose in the subsequent studies. The high dose, 3000 mg/kg was reduced sequentially by a factor of half ($\frac{1}{2}$) to obtain a median dose of 1500 mg/kg and a low dose of 750 mg/kg.

Table 1: Effect of 10 days post coital treatment on implantation and foetal viability

Group/Dose of treatments (mg/kg)	Mean number of implants	Mean number of live implants	Post implantation loss (%)
I (Control)	9.60 ± 1.208	9.60 ± 1.208	0.00
IIA (ARE 500)	9.60 ± 0.927	9.40 ± 0.814	1.67
IIB (ARE 1000)	8.20 ± 1.393	7.80 ± 1.463	6.82
IIIA (MLE 500)	10.80 ± 0.583	10.80 ± 0.583	0.00
IIIB (MLE 1000)	11.00 ± 0.316	11.00 ± 0.316	0.00
IVA (PRE 500)	11.20 ± 0.374	11.2 ± 0.374	0.00

IVB (PRE 1000)	10.80 ± 0.374	10.80 ± 0.374	0.00
VA (TBE 500)	9.80 ± 0.734	9.60 ± 0.678	1.82
VB (TBE 1000)	9.80 ± 1.356	9.20 ± 1.020	4.68
VIA (WLE 500)	11.00 ± 0.447	11.00 ± 0.447	0.00
VIB (WLE 1000)	10.80 ± 1.068	10.80 ± 1.068	0.00

Results are expressed as mean ± SEM (n = 5). Post implantation index = (number of implantationnumber of life foetuses) / number of implantation x 100.

Table 2: Effect of 14 days pretreatment of female mice prior to mating with continued treatment after mating on reproductive indices

Group/Dose of treatments (mg/kg)	Fertility index (%)	Mean number of implants	Mean number of live implants	Post implantation loss (%)
I (Control)	100	9.60 ± 1.208	9.60 ± 1.208	0.00
IIA (ARE 500)	100	8.40 ± 2.015	7.20 ± 1.985	10.91
IIB (ARE 1000)	100	7.80 ± 1.068	5.80 ± 1.685	21.82
IIIA (MLE 500)	100	10.60 ± 0.748	10.60 ± 0.748	0.00
IIIB (MLE 1000)	100	8.60 ± 1.435	8.40 ± 1.327	1.67
IVA (PRE 500)	100	9.00 ± 1.414	9.00 ± 1.414	0.00

IVB (PRE 1000)	100	10.00 ± 1.871	10.00 ± 1.871	0.00
VA (TBE 500)	100	9.00 ± 0.894	7.60 ± 0.8718	15.00
VB (TBE 1000)	60	6.40 ± 2.638	6.00 ± 2.449	3.33
VIA (WLE 500)	100	10.40 ± 0.510	10.40 ± 0.510	0.00
VIB (WLE 1000)	100	9.80 ± 1.200	9.20 ± 1.241	6.82

Results are expressed as mean ± SEM (n = 5). Post implantation index = (number of implantation number of life foetuses) / number of implantation x 100.

4.3 Effect of 21 days of treatment on the oestrous cycle of female mice Treatment of mice with ARE at doses of 750 mg/kg, 1500 mg/kg and 3000 mg/kg for 21 days did not alter the various phases of the oestrous cycle (Fig. 18). Cycle length of treated female mice was also not significantly different ($p > 0.05$) compared to controls at all doses (Table 3). Mice treated with TBE at doses of 1500mg/kg and 3000mg/kg showed a significant prolongation of the dioestrous phase compared to controls ($p < 0.05$); proestrous and metaeostrous phases also reduced significantly ($p < 0.05$) in mice treated with 3000mg/kg of TBE (Fig. 19).

Cycle length was insignificantly prolonged ($p > 0.05$) compared to controls (Table 3).

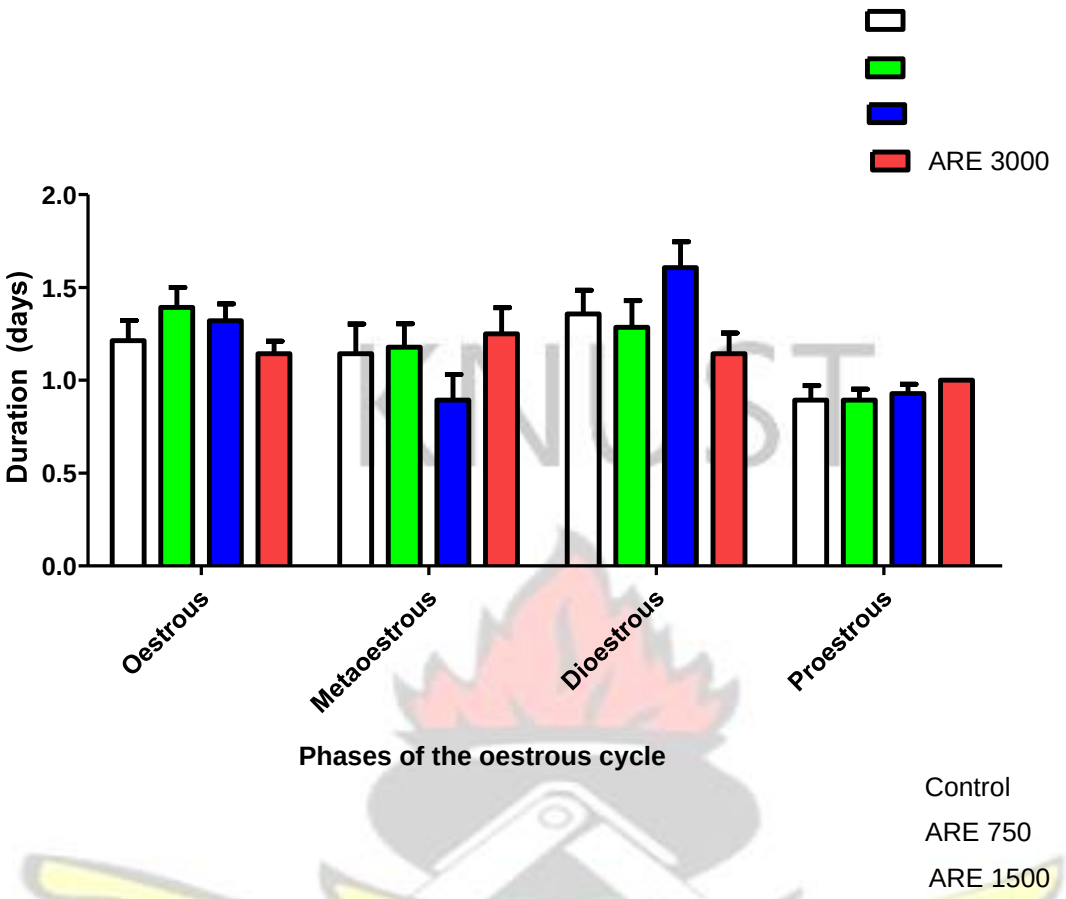

Figure 18: Effect of 21 days of treatment with ARE on the oestrous cycle of female mice.
Results are expressed as mean ± SEM (n = 7).

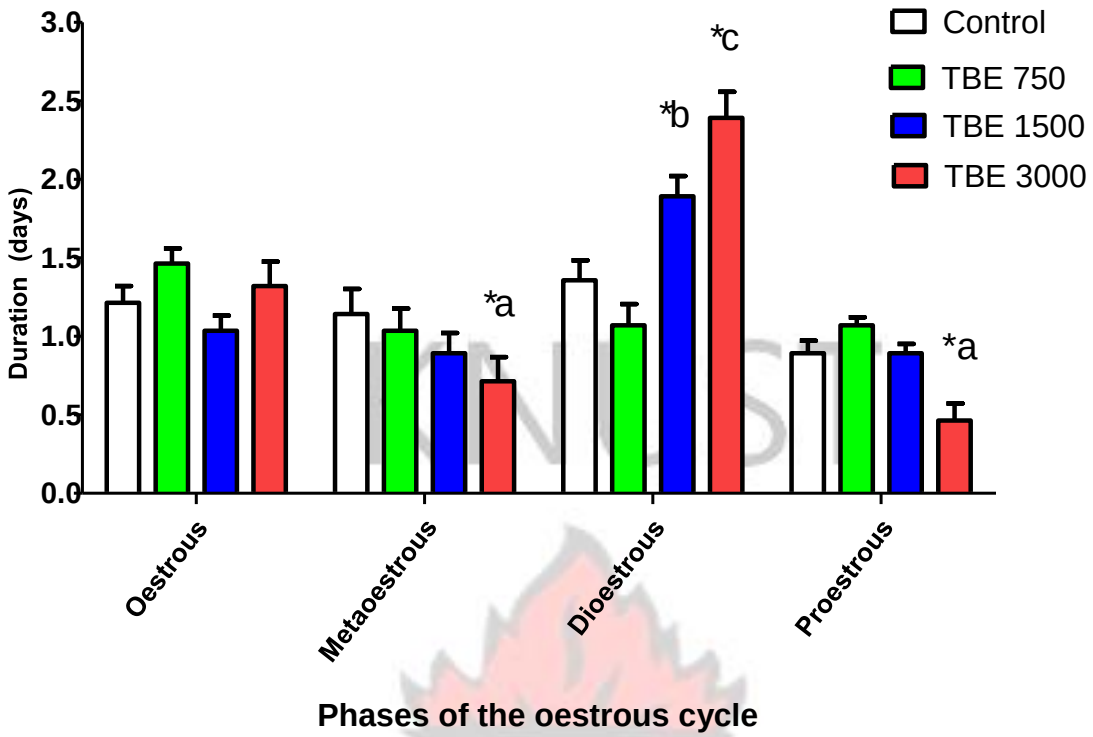

Figure 19: Effect of 21 days of treatment with TBE on the oestrous cycle of female mice. Results are expressed as mean ± SEM (n = 7). Statistically significant differences*$^{a}P \leq 0.05$, *$^{b}P \leq 0.01$, *$^{c}P \leq 0.001$ compared to control by two way ANOVA followed by Bonferroni *post hoc* test.

Table 3: Effect of 21 days of treatment on the duration of the oestrous cycle of female mice

Group/Dose of treatments (mg/kg)	Duration of cycle (days)
I (Control)	4.607 ± 0.15
IIA (ARE 750)	4.75 ± 0.13
IIB (ARE 1500)	4.75 ± 0.13
IIC (ARE 3000)	4.536 ± 0.12
IIIA (TBE 750)	4.643 ± 0.12
IIIB (TBE 1500)	4.714 ± 0.12
IIIB (TBE 3000)	4.929 ± 0.14

Results are expressed as mean ± SEM (n = 7).

4.4 Toxicological profile of ARE and TBE

4.4.1 Effect of 21 days of treatments on the body weight of mice

Treatment of mice with ARE for 21 days did not result in significant ($p > 0.05$) body weight changes compared to control (Fig. 20). Animals treated with ARE at all doses increased in body at the end of the study. Increased feed intake was observed in animals treated with ARE at all doses. Significant body weight changes were not recorded ($p > 0.05$) in mice treated with TBE at all doses compared to controls (Fig. 20). Mice treated with the low (750 mg/kg) and median doses (1500 mg/kg) gained weight at the end of the study while weight loss was observed in animals treated with the high dose (3000 mg/kg) of TBE. It was also observed that feed intake in mice treated with TBE at a dose of 3000 mg/kg was reduced.

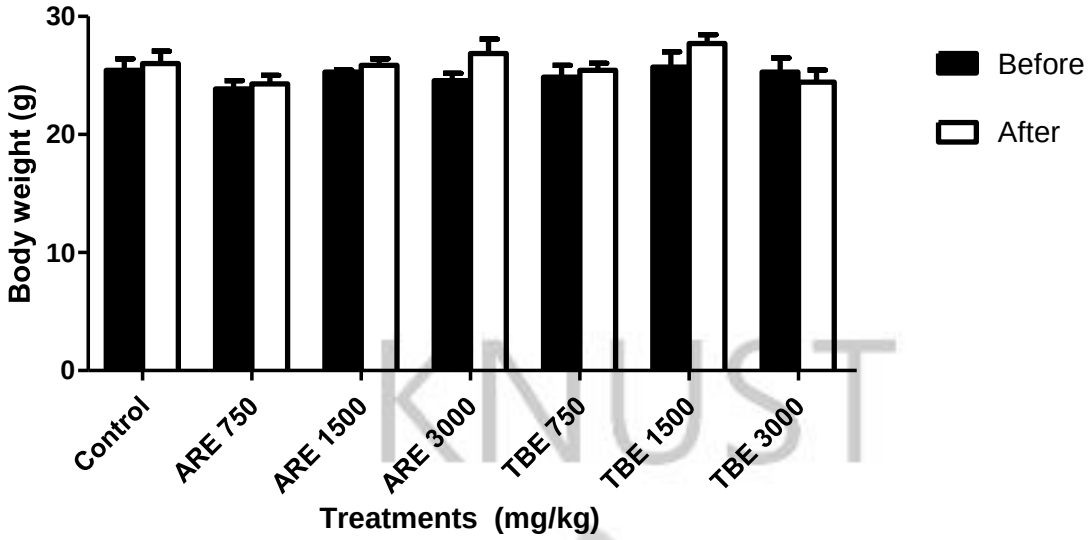

Figure 20: Effect of 21 days of treatments on body weight of female mice.

4.4.2 Effect of 21 days of treatments on relative organ weight of mice

Relative liver weights of animals treated with ARE at dose of 1500 mg/kg and 3000 mg/kg were reduced but not significantly different ($p > 0.05$) compared to controls (Fig. 21). Relative kidney weights were comparable to controls ($p > 0.05$) (Fig. 22). A dose dependent reduction of relative ovarian weight (Fig. 23) and a dose dependent increase in relative uterine weights (Fig. 24) were recorded at all doses but these changes were insignificant ($p > 0.05$) compared to controls. Relative weights of liver, kidney and ovaries of mice treated with TBE at all doses were not significantly different compared to controls ($p > 0.05$) (Fig. 25-27). Although relative uterine weight showed a dose dependent reduction, this was not statistically significant ($p > 0.05$) compared to controls (Fig. 28).

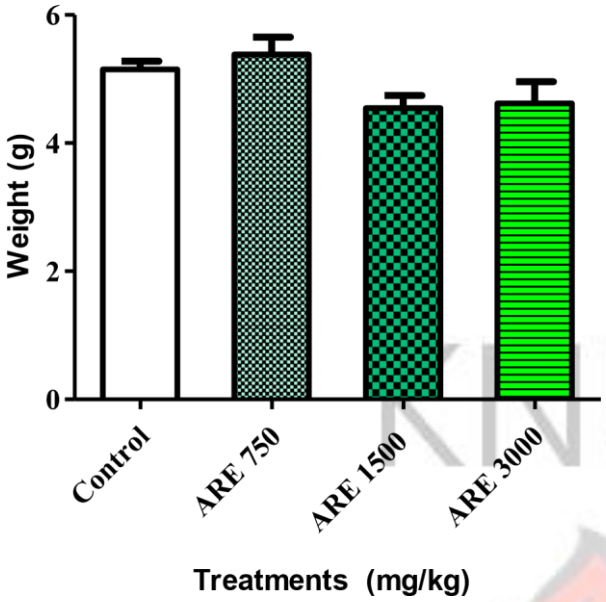

Figure 21: Effect of 21 days of treatment with ARE on relative liver weight

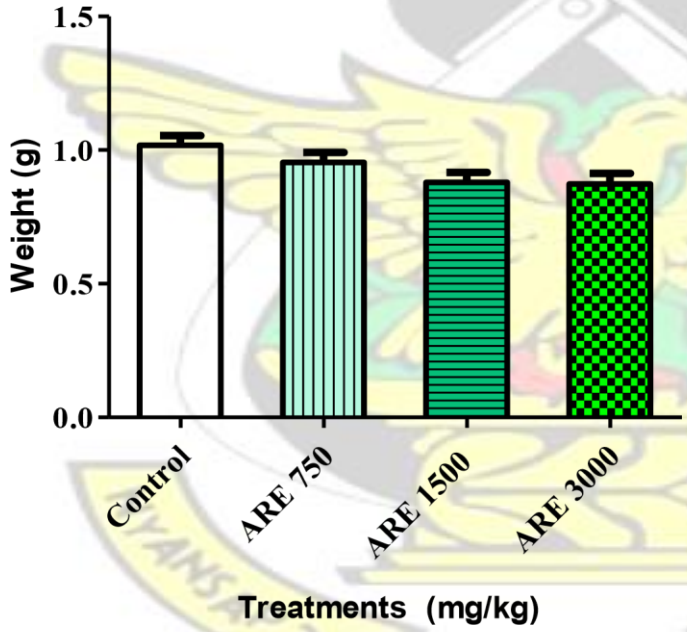

Figure 22: Effect of 21 days of treatment with ARE on relative kidney weight

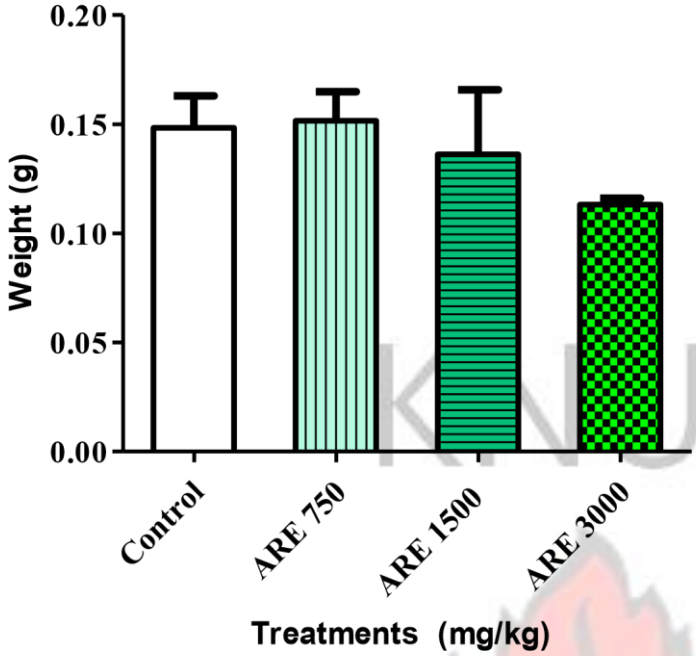

Figure 23: Effect of 21 days of treatment with ARE on relative ovarian weight

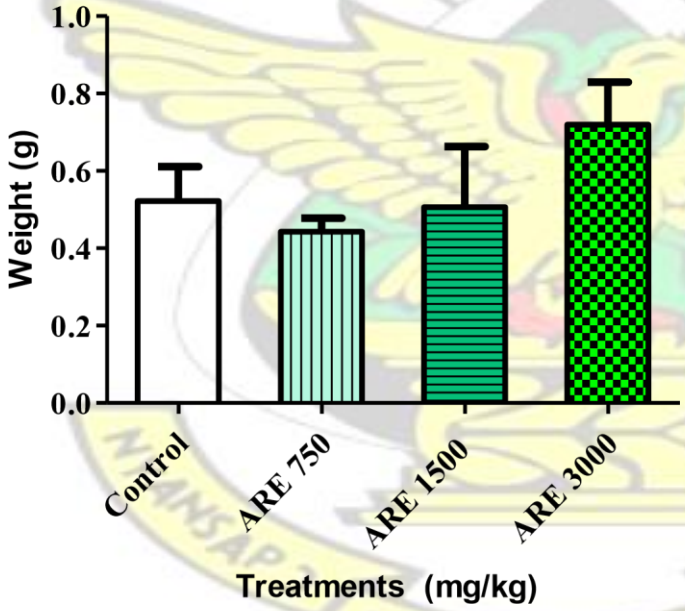

Figure 24: Effect of 21 days of treatment with ARE on relative uterine weight

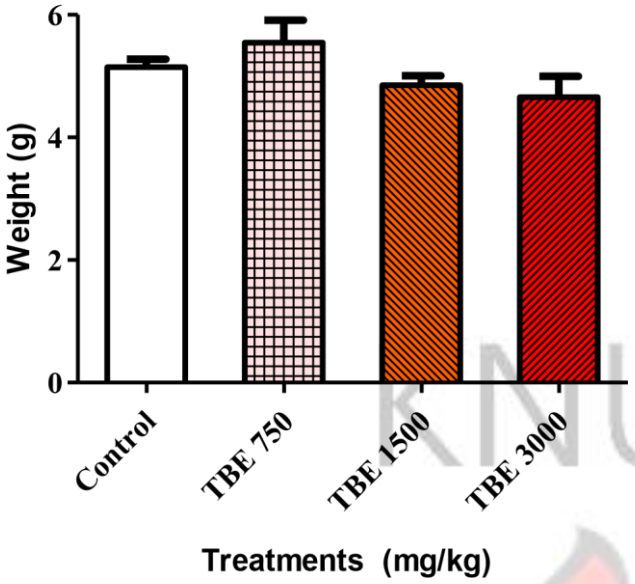

Figure 25: Effect of 21 days of treatment with TBE on relative liver weight

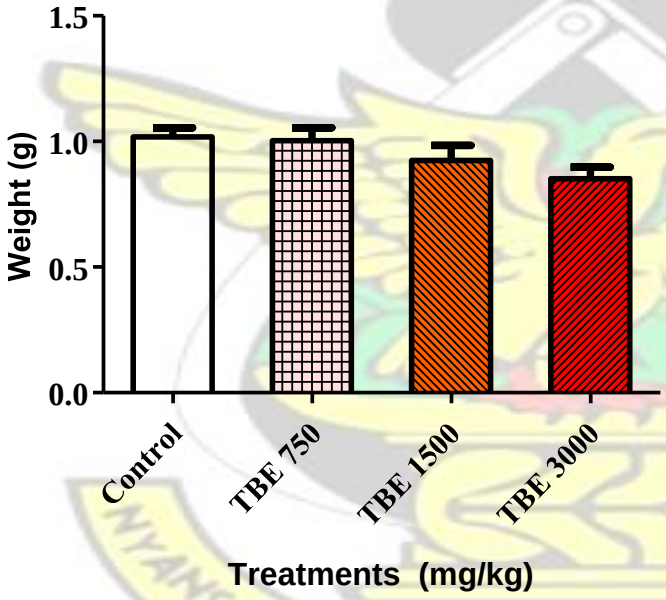

Figure 26: Effect of 21 days of treatment with TBE on relative kidney weight

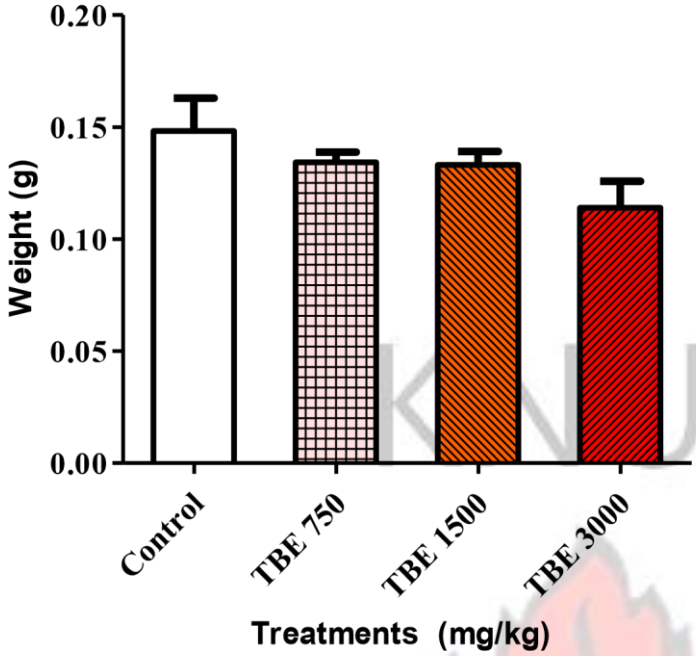

Figure 27: Effect of 21 days of treatment with TBE on relative ovarian weight

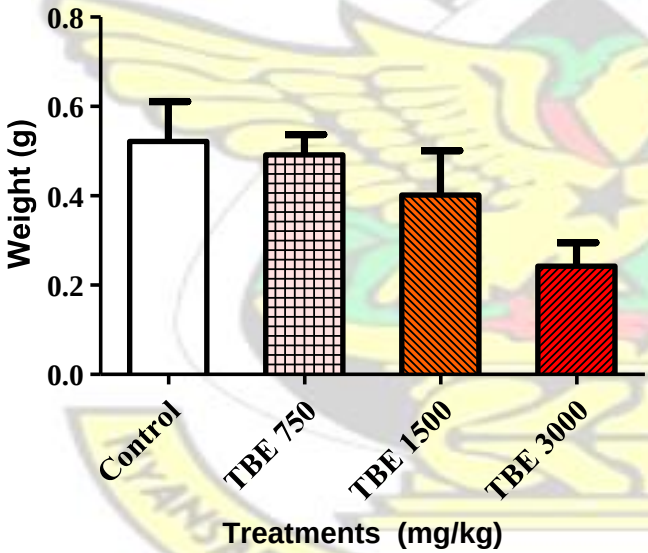

Figure 28: Effect of 21 days of treatment with TBE on relative uterine weight

4.4.3 Effect of 21 days of treatments on serum biochemical parameters As shown in Table 4, administration of ARE for 21 days did not have any significant effect (*p*

> *0.05*) on biochemical parameters of treated animals compared to controls. There was a reduction of creatinine levels at all doses although not significantly different ($p > 0.05$) compared to controls (Table 4). Levels of cholesterols, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and alkaline phosphatase (ALP) were not significantly different
($p > 0.05$) compared to controls (Table 4).

Treatment with TBE resulted in lower levels of creatinine at all doses. However this was not statistically significant ($p > 0.05$) compared to controls (Table 4).
Mice treated with 1500 mg/kg and 3000 mg/kg of TBE had high levels of ALP. There was however no change in cholesterols, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) levels in treated animals at all doses (Table 4).

Table 4: Effect of 21 days of treatment with three different doses of the two plants (ARE and TBE) on serum biochemical markers.

Group/Dose of treatments (mg/kg)	Serum biochemical markers					
	Cholesterol	Creatinine	ALP	Triglycerides	HDL	LDL
I (Control)	2.97 ± 0.26	83.47 ± 10.92	94.29 ± 5.34	1.32 ± 0.10	1.94 ± 0.31	0.59 ± 0.12
IIA (ARE 750)	3.05 ± 0.30	58.76 ± 4.31	96.29 ±10.51	1.32 ± 0.10	2.01 ± 0.16	0.50 ± 0.08
IIB (ARE 1500)	2.42 ± 0.23	64.81 ± 7.45	96.71 ± 20.81	1.21 ± 0.05	1.62 ± 0.27	0.47 ± 0.10
IIC (ARE 3000)	2.67 ± 0.26	48.97 ± 6.72	77.57 ± 7.16	1.08 ± 0.16	1.77 ± 0.16	0.41 ± 0.13
IIIA (TBE 750)	2.94 ± 0.11	66.86±13.40	91.86 ± 6.86	1.40 ± 0.20	1.80 ± 0.09	0.63 ± 0.06
IIIB (TBE 1500)	2.98 ± 0.34	61.50±12.38	118.70 ± 18.18	1.35 ± 0.24	1.66 ± 0.26	0.71 ± 0.15
IIIB (TBE 3000)	2.46 ± 0.24	64.50 ± 17.89	103.70±16.63	1.17 ± 0.18	1.94 ± 0.31	0.44 ± 0.10

Results are expressed as mean ± SEM (n = 7).

4.4.4 Histopathological examination of the liver of mice treated with ARE for 21 days

The biochemical observations reported in the study were supplemented by the histopathological examination of liver sections. No test related changes were observed in the light micrographs of selected organs of control and treated animals. Morphological structure of liver from control and treated animals showed clear hepatic lobule and sinusoid without necrosis or denaturing (Fig. 29).

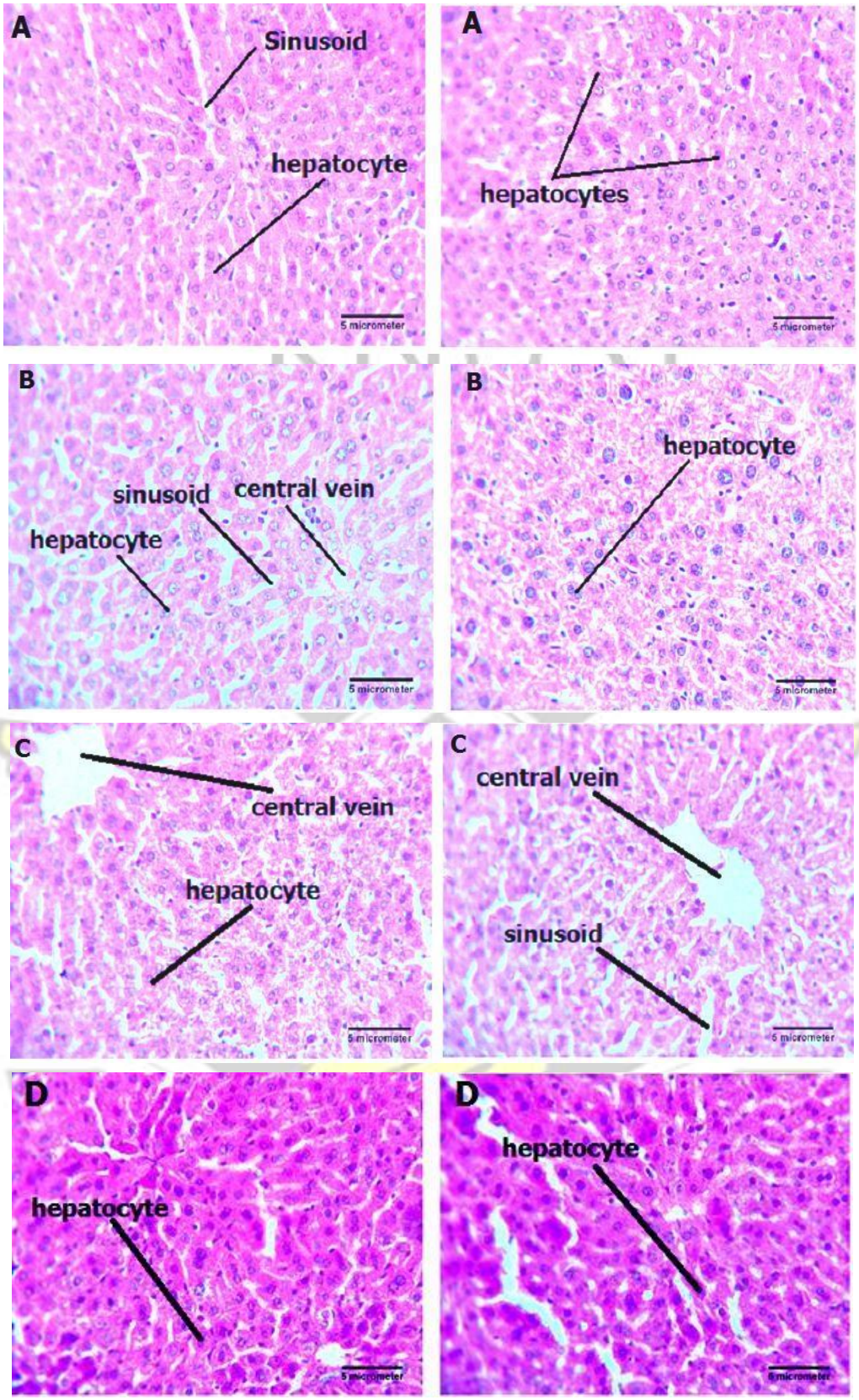

Figure 29: Light micrographs of transverse section of liver of control and treated animals (Haematoxylin and eosin staining, Bar represents 5µm). (A) Control, (B-D) mice treated with 750mg/kg 1500mg/kg and 3000mg/kg of ARE respectively.

4.4.5 Histopathological examination of the liver of mice treated with TBE for

21 days

No test related changes were observed in liver of mice treated with 750mg/kg, 1500mg/kg and 3000mg/kg of TBE. The morphology of hepatic lobules and hepatocytes of the livers from treated and control animals were normal without necrosis or fibrosis. No infiltration of inflammatory cells was observed in the portal area (Fig. 30).

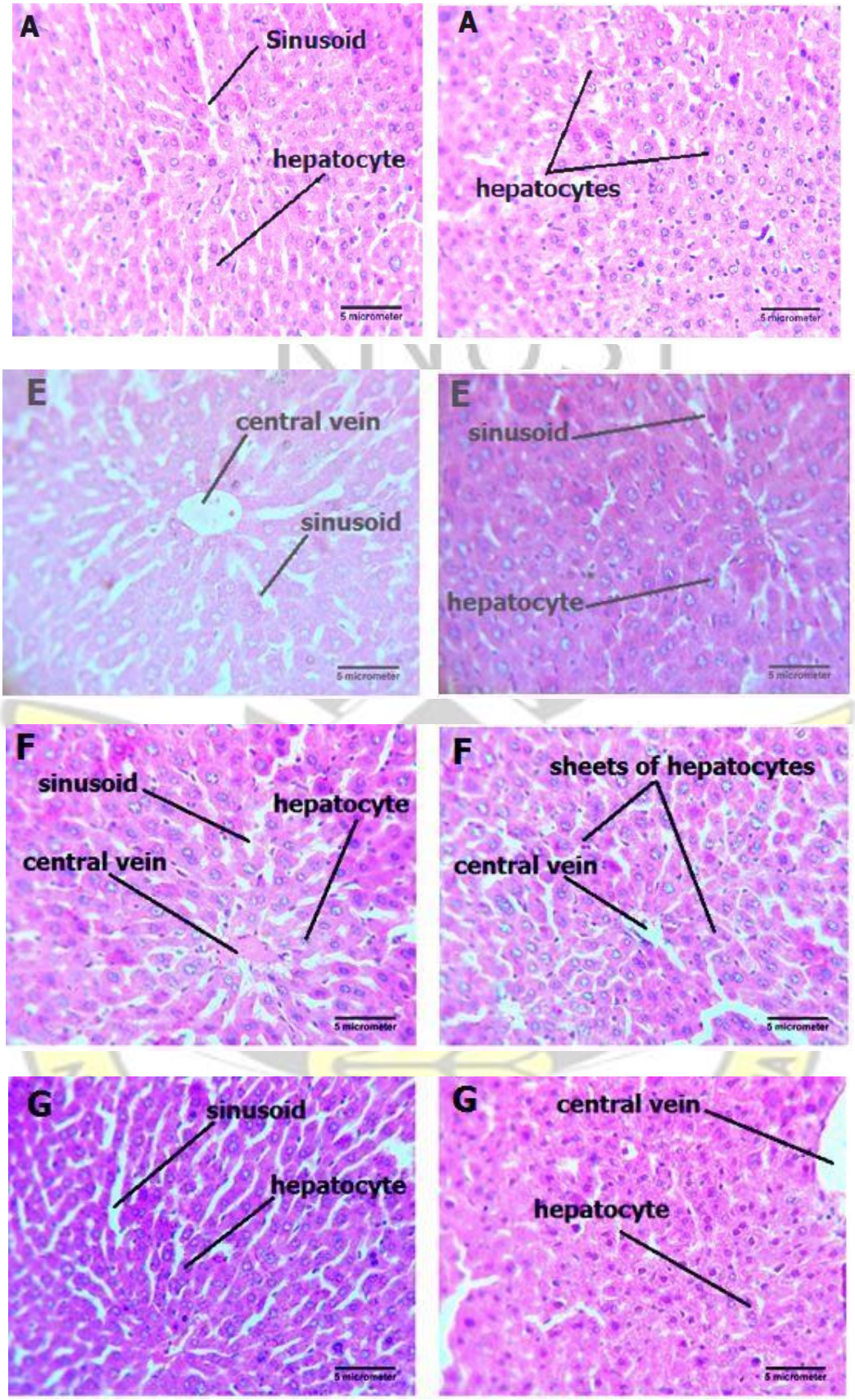

Figure 30: Light micrographs of transverse section of liver of control and treated animals (Haematoxylin and eosin staining, Bar represents 5µm). (A) Control, (E-G) mice treated with 750mg/kg, 1500mg/kg and 3000mg/kg of TBE respectively.

4.5 Effect of post coital treatment on implantation

Treatment of female mice with ARE at doses of 750mg/kg, 1500mg/kg and 3000mg/kg after mating for 10 days did not affect implantation (Table 5). The number of implants and live foetuses at all doses were not significantly different ($p > 0.05$) compared to controls (Table 5). Post implantation loss at all doses was also not statistically significant compared to controls ($p > 0.05$).

Animals treated with TBE showed a dose dependent reduction in the number of implants however this was not statistically significant ($p > 0.05$) at all doses compared to controls (Table 5). The mean number of live foetuses was significantly lower ($p < 0.05$) in mice treated with doses of 1500mg/kg and 3000mg/kg. Post implantation loss was significantly high ($p < 0.05$) in animals treated with 1500 mg/kg of TBE compared to controls (Table 5).

Table 5: Effect of post coital treatment with three different doses of the two plants (ARE and TBE) on implantation and foetal viability

Group/Dose of treatments (mg/kg)	Mean number of implants	Mean number of live implants	Post implantation loss (%)
I (Control)	10. 57 ± 0.37	10. 57 ± 0.37	0.00
IIA (ARE 750)	10.29 ± 0.18	10.14 ± 0.26	1.43
IIB (ARE 1500)	10.14 ± 0.26	10.00 ± 0.22	1.20
IIC (ARE 3000)	9.71 ± 0.61	9.43 ± 0.69	3.34
IIIA (TBE 750)	10.43 ± 0.20	10.29 ± 0.29	1.43
IIIB (TBE 1500)	8.14 ± 1.35	5.43 ± 1.51*[c]	37.81*[b]
IIIC (TBE 3000)	7.86 ± 0.51	7.71 ± 0.42*[a]	1.43

Results are expressed as mean ± SEM (n = 7). Statistically significant differences *[a]$P \leq 0.05$, *[b]$P \leq 0.01$, *[c]$P \leq 0.001$ compared to control by one way ANOVA followed by Newman Keuls *post hoc* test.

4.6 Effect of ARE and TBE on fertility index and embryonic development

Maternal body weight of ARE treated mice at all doses was not significantly different ($p > 0.05$) compared to controls (Fig. 31). Treatment of mice with ARE for 21 days before mating and 10 days after mating did not affect fertility index at all doses (Table 6). The number of implants and live foetuses were reduced in a dose dependent manner, however, this effect was not statistically significant ($p > 0.05$) compared to controls (Table 6). Post implantation loss was observed only in animals treated with 1500 mg/kg and 3000 mg/kg (Table 6). Antifertility effect was 14% at a dose of 3000 mg/kg (Table 7).

Treatment of test animals with TBE caused significant reduction ($p < 0.05$) in maternal body weight at doses of 1500 mg/kg and 3000 mg/kg (Fig. 31). Fertility indices of animals treated with TBE at doses of 1500 mg/kg and 3000 mg/kg were reduced to 42.85% and 28.57% respectively (Table 6). The number of implants and live foetuses were significantly reduced ($p < 0.05$) in animals treated with doses of 1500 mg/kg and 3000 mg/kg (Table 6). Vaginal bleeding was observed in three out of seven animal (42%) treated with a dose of 1500 mg/kg (Table 7). Antifertility activity was 57% and 71% for doses of 1500 mg/kg and 3000 mg/kg respectively (Table 7).

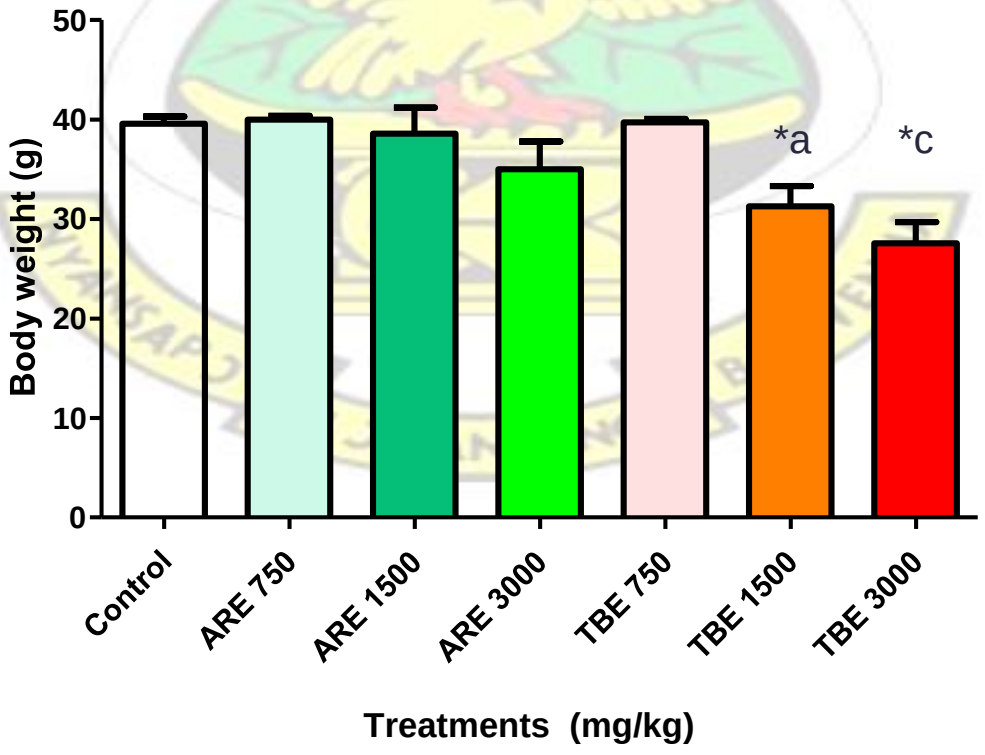

Figure 31: Maternal body weight at sacrifice of mice treated 21days before mating, and 10 days after mating. Statistically significant differences $*^{a}P \leq 0.05$, $*^{b}P \leq 0.01$, $*^{c}P \leq 0.001$ compared to control by one way ANOVA followed by Newman Keuls *post hoc* test.

Table 6: Effect of three different doses of the two plants (ARE and TBE) on fertility index and foetal viability when given before and after mating.

Group/Dose of treatments (mg/kg)	Fertility index (%)	Mean number of implants	Mean number of live implants	Post implantation loss (%)
I (Control)	100	10. 57 ± 0.37	10. 57 ± 0.37	0.00
IIA (ARE 750)	100	10.29 ± 0.18	10.29 ± 0.18	0.00
IIB (ARE 1500)	100	7.43 ± 1.74	7.29 ± 1.69	1.19
IIC (ARE 3000)	100	6.43 ± 1.76	5.71 ± 1.61	8.23
IIIA (TBE 750)	100	9.86 ± 0.40	9.71 ± 0.42	1.43

IIIB (TBE 1500)	42.85	4.57 ± 2.16*[a]	3.86 ± 1.86 *[b]	6.49
IIIC (TBE 3000)	28.57	2.43 ± 1.67*[c]	2.29 ± 1.54 *[c]	1.30

Values are expressed as mean ± SEM (n = 7). Statistically significant differences *[a]$P \leq 0.05$, *[b]$P \leq 0.01$, *[c]$P \leq 0.001$ compared to control by one way ANOVA followed by Newman Keuls *post hoc* test.

Table 7: Effect of three different doses of the two plants (ARE and TBE) on some reproductive parameters of treated mice

Parameters	**Group/Dose of treatments (mg/kg)**						
	I (Control)	IIA (ARE 750)	IIB (ARE 1500)	IIC (ARE 3000)	IIIA (TBE 750)	IIIB (TBE 1500)	IIIC (TBE 3000)
No. of mice pregnant/ No. of mice treated	7/7	7/7	7/7	6/7	7/7	3/7	2/7
No. of mice with vaginal bleeding	-	-	-	-	-	3	-
No. of implants	74	72	59	54	69	32	17
% Antifertility	0	0	0	14	0	57	71

% antifertility = (number of animals shoeing no implants / total number of animals) x 100

4.7 Effect of TBE on the isolated rat uterus

TBE (1 mg/ml - 50 mg/ml) did not elicit any contractile effect on the isolated myometrial strips but rather a relaxation of spontaneous contraction of the isolated uterus was observed (Fig. 32). A uterine agonist, acetylcholine ($5 \times 10^{-3} – 80 \times 10^{3}$ mg/ml) evoked uterine contractions with an EC_{50} of 0.436 µg/ml. Incubation of the uterine tissue with the extract significantly antagonized ($p < 0.05$) the contractile effects of acetylcholine (Fig. 33). At a concentration of 1mg/ml and 3mg/ml, TBE inhibited the contractile response of acetylcholine (Ach) by 62% and 77% ($p < 0.05$) respectively. Atropine (2×10^{-8} mg/ml) also inhibited the contractile response of acetylcholine by 89% ($p < 0.05$) (Fig. 33).

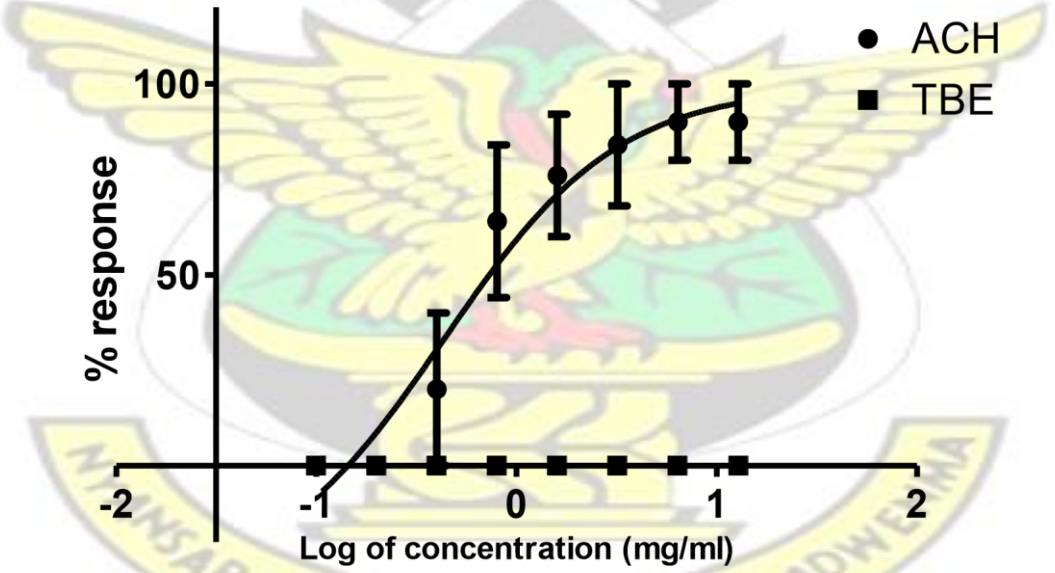

Figure 32: Effect of TBE (1 mg/ml - 50 mg/ml) and acetylcholine ($5 \times 10^{-3} – 80 \times 10^{-3}$ mg/ml) on the isolated rat uterus.

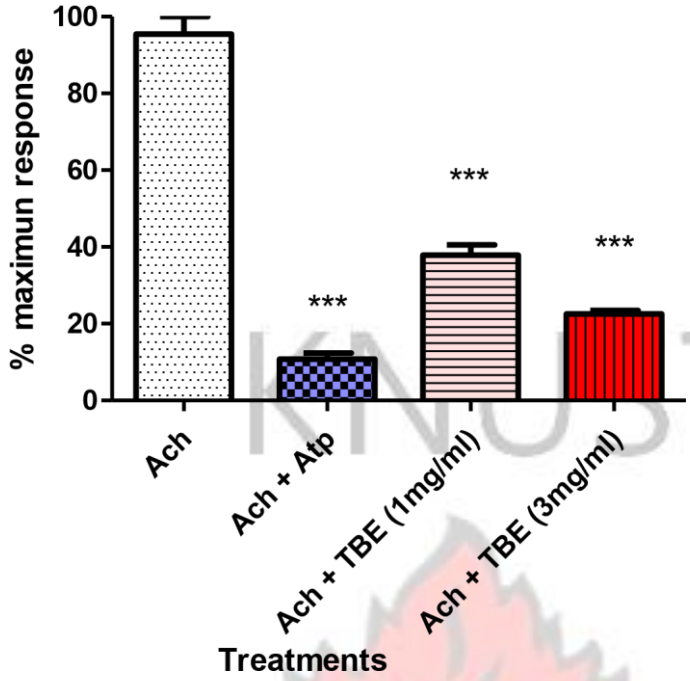

Figure 33: Effect of TBE and atropine on the contractile response of acetylcholine on the isolated rat uterus.

CHAPTER FIVE

DISCUSSION

Safety tests are required for new medicines, which in spite of their potential efficacy, might affect the health of humans. Acute toxicity study is the first step for the toxicological assessment of an unknown substance. The aim of the test was to determine the range between the dose that causes no observed effect and the dose that is life threatening (OECD, 2001; Tamokou and Kuete, 2014). Herbal remedies have been used for several thousands of years for their therapeutic effects. Herbalists state long years of practice as a proof of safety of their preparations. However, the growing demand and use for herbal medicines worldwide have raised concerns about their purported safety (WHO, 2002;
Fennell *et al*., 2004; Tamokou and Kuete, 2014).

Reports on *Anthocleista nobilis* (ARE) indicate that the plant strengthens the immune system (Oyedele, 2011), possesses antioxidant activity (Annan and Dickson, 2005) as well as hepato-protective effect (Madubunyi and Asuzu, 1996). Despite the wide spread use of the plant few scientific studies have been undertaken to ascertain its safety. The results of the acute toxicity study showed that the oral administration of ARE did not produce any signs of toxicity or death in the treated mice; suggesting an LD_{50} above 5000 mg/kg (OECD, 2001).
According to the Hodge and Sterner scale, ARE is practically non-toxic (Teke and Kuete, 2014).

This outcome is contrary to the work of Madubunyi and Asuzu (1996) which reported an LD_{50} of 200 mg/kg in mice using the same ethanol root bark extract but

given through the intraperitoneal route (*i.p.*). Mahama (1983) also reported the same LD_{50} of 200 mg/kg when the aqueous root bark extract of *A. nobilis* was similarly given i.p. in rats though. The disparity shown in this work may be due to the oral route of administration.

It is suggested here that perhaps the enzymes in the alimentary canal and the liver may help reduce the toxicity; a proposition worth investigating in future work. The intraperitoneal method of dosing is thought to occasionally provide information on both local as well as systemic toxicity (Teke and Kuete, 2014). Work done by Anyanwu *et al*. (2013) on *A. vogelii*, a close relative of *A. nobilis* reported an LD_{50} higher than 5000mg/kg. This is supported by the work of Nguessom *et al*. (2013) which showed that the LD_{50} of *A. vogelii* is higher than 2000 mg/kg in mice. This perhaps supports the suggestion made here that *A. nobilis* has low toxicity following oral administration.

Oral administration of *Palisota hirsuta* root extract (PRE) was well tolerated with no mortality up to the maximum dose of 5000mg/kg. This indicates that the LD_{50} of PRE is above 5000 mg/kg hence it is practically non-toxic (OECD, 2001; Teke and Kuete, 2014). This result is corroborated by the work of Boakye-Gyasi (2009) who reported that the LD_{50} of ethanol extract of *P. hirsuta* leaf is above 5000 mg/kg.

The family Euphorbiaceae provides food and various medicinal properties used in ethnobotany. Some are however, known to be toxic. An example is ricin, a wellknown poisonous compound contained in *Ricinus communis* that elicits violent purgative action in man. The leaves of *Euphorbia kamerunica* are also reported to

be toxic in rats (Uduak and Kola, 2010). *Macaranga heterophylla* leaf extract (MLE) did not cause mortality or induce signs of toxicity in treated animals at all doses, hence the LD_{50} of MLE is above 5000 mg/kg. A close relative, *M. gigantean* was similarly found to be well tolerated in mice with an LD_{50} above 5000 mg/kg (Amin, 2010). According to the Hodge and Sterner scale, substances administered orally that show an LD_{50} above 5000 mg/kg are practically non-toxic (Teke and Kuete, 2014).

T. monadelpha is of high value in traditional medicine. It is reported to possess potent anti-nociceptive and anti-inflammatory activity (Owusu, 2009). It is also used in a poly-herbal decoction for the treatment of arthritis (Donkor *et al*., 2014). The results of the acute toxicity study showed that oral administration of *T. monadelpha* back extract (TBE) did not cause death of treated animals at all dose levels. The LD_{50} of TBE is therefore greater than 5000mg/kg. This result is supported by the work of Owusu (2009) which reported that the LD_{50} of aqueous stem bark extract of *T. monadelpha* exceeds 5000 mg/kg in rats.

The acute toxicity study of *W. indica* leaf extract (WLE) confirmed with previous studies (Osman *et al*., 2013; Atif *et al*., 2014; Basiru and Olayemi, 2014). The results in this study showed that the LD_{50} of WLE is above 5000mg/kg. Contrary to this result, Hamidu *et al*. (2008) reported that the LD_{50} of the ethanol extract of the aerial plant part of *W. indica* is 875 mg/kg. A possible explanation for this difference may lie in the plant parts used since the concentration of secondary metabolites is not the same throughout a plant. The result indicates that WLE is practically non-toxic (Teke and Kuete, 2014).

Before undertaking the experiments in this study, no information describing the effect of parts of the selected five plants had been found in literature search on pregnancy and foetal viability. A preliminary fertility study was therefore used to screen the five plants to select promising plants for further tests. Post coital treatement was used to assess the effect of the plant extracts on conception and implantation. Treatment given before mating, through mating and implatantion was expected to discover effects on oestrous cycle, tubal transport, conception and implantation (ICH, 2005).

Post coital administration of ARE for 10 days with doses of 500 mg/kg and 1000 mg/kg had no significant effect ($p > 0.05$) on implantation compared to controls (Table 1). This suggests absence of anti-implantation activity when given after mating; though a dose dependent reduction in implantation in the treated groups was seen (Table 1). Furthermore, some prenatal mortality (Fig. 14), maybe an indication of adverse effect on pregnancy was observed in treated animals at all doses though none was statistically significant ($p > 0.05$) compared to controls (Table 1). Perhaps higher doses of the extract may induce pronounced effect on reproductive processes as reported in literature by Burkill (1985), Mosango (2007) and Iwu (2014).

The fertility index of mice pretreated for 14 days followed by continued treatment after mating with ARE was not affected at all doses (Table 2). This could suggest that ARE has no direct influence on ovulation or conception in mice. The number of implants in treated animals was comparable to controls ($p > 0.05$) but the number

of live foetuses was reduced at all doses though not statistically different from controls (Table 2). It is known that chemical insults before and after the implantation process can result in implantation losses (Yakubu and Bukoye, 2009; Srikant *et al*., 2013). The incidence of foetal resorption suggests interruption of pregnancy after implantation (Yakubu and Bukoye, 2009).

One of the five animals (20%) treated with a dose of 1000 mg/kg showed complete resorption of foetuses (Fig. 14). Such adverse effect on foetal viability may be due to maternal toxicity, direct foetal toxicity or delayed tubal transport (Farnsworth *et al*., 1975; Kimmel and Buelke-Sam, 2001). The result suggests that perhaps ARE may be foetotoxic or abortifacient. This is an indication of the antifertility potential of ARE.

Post implantation loss was increased in mice that were pretreated (Table 2) compared to those treated only after mating (Table 1), though it was not significant compared to controls ($p > 0.05$). This, however, may suggest a negative impact on preimplantation events. Perhaps prolonged pretreatment may increase the adverse effect of ARE on pregnancy. The loss of foetuses may be due to foetotoxicity or a disturbance in the hormonal milieu of the uterus (Lim *et al*., 2002; Song *et al*., 2007). The uterus in the refractory phase can be toxic to the blastocyst hence delayed transport of the blastocyst to the implantation site could also result in foetal loss (Farnsworth *et al*., 1975; Yoshinaga, 1988; Paria *et al*., 1993). *A. nobilis* is reported to induce a dose dependent relaxation of the guinea pig ileum (Madubunyi and Asuzu, 1996). It is possible that ARE delayed transport of the blastocyst by relaxation of the oviduct.

A. nobilis is reported to be used as an abortifacient and to regulate menstruation (Burkill, 1985; Mosango, 2007; Iwu, 2014). It is used in a preparation of long term contraceptive. It is also employed in the treatment of abdominal pains of uterine origin (Mosango, 2007; Iwu, 2014). Although, *A. nobilis* has reputed folkloric use as an antifertility plant, no anti-implantation or abortifacient activity was observed in treated animals at all dose levels except for the adverse trend on foetal development which was dose dependent (Table 2). The results of the preliminary fertility test showed that perhaps ARE has no anti-implantation activity, however, it may have detrimental effect on foetal development. It is possible that the doses tested here according to accepted pharmacological standards after toxicicity tests were not high enough to produce reported results.

Hence ARE was selected for further testing with higher doses.

Various parts of *M. heterophylla* are used as purgative. A root decoction is taken to treated amenorrhoea and as an abortifacient (Schmelzer, 2007). Treatment of mice with *M. heterophylla* leaf extract (MLE) after mating resulted in an insignificant increase ($p > 0.05$) in the number of implants compared to controls (Table 1). This may suggests that MLE has fertility enhancing property when given post coitum. It is also possible that the leaf does not contain compounds that negatively impact conception. The absence of dead foetuses or resorption sites in treated animals at all doses (Table 1) suggests that MLE has no adverse effect on pregnancy when given post coitum (Kimmel and Buelke-Sam, 2001).

Pretreatment of mice with MLE followed by continued treatment after mating did not alter fertility indices of treated mice (Table 2). This suggests that MLE has no anti-ovulatory activity or contraceptive effect in mice. Although the number of implant and live foetuses was lower in mice treated with a dose of 1000 mg/kg, this was not significantly different ($p > 0.05$) compared to controls (Table 2). Modification of the normal hormonal pattern or uterine environment necessary for embryonic development results in lower number of implant (Kimmel and BuelkeSam, 2001). Hence failure of MLE to significantly alter the fertility and implantation indices of treated mice suggests that perhaps MLE may have no influence on reproductive processes in mice.

Prenatal death was observed in mice treated with a dose of 1000 mg/kg but, it was of no statistical significant ($p > 0.05$) compared to controls (Table 2). The percentage of post implantation loss indicates disturbances in embryonic development (Brugiolo *et al*., 2010). The absence of significant foetal loss in mice treated with MLE could suggest that the extract has no effect on implantation and foetal development in mice. It is not always the case that medicinal plants with folkloric reputation result in anti-implantation or abortifacient effect. Work done by De Freitas *et al*. (2004) showed that aerial parts of *Ruta graveolens,* a reputed abortifacient did not cause pre-implantation loss, resorptions or fetal death.

Mice treated with *Palisota hirsuta* root extract (PRE) after mating had reproductive indices comparable to controls at all doses (Table 1). Foetal loss was not observed in treated animals at all doses (Table 1). This suggests that PRE has no adverse effect on pregnancy when given after mating. Fertility index of mice treated with

PRE before and after mating was not affected at all dose levels (Table 2). This suggests that PRE may have no effect on the oestrous cycle and implantation. Prenatal death which indicates disturbances in embryonic development (Brugiolo *et al*., 2010) was not observed in any of the treated mice at all doses (Table 2). This suggests that perhaps PRE is well tolerated in pregnant mice and has no antifertility activity.

P. hirsuta is used in folkloric medicine for mitigating difficult childbirth and female sterility (Burkill, 1985; Neuwinger, 1996). It is also used in the preparations of enemas to provoke bleeding and induce abortion in Ghana (Bleek and Asante - Darko, 1986). However, no antifertility activity was observed in treated mice at all doses tested. Similar finding was reported by Montanari and Bevilacqua, (2002). They showed that the leaves of *Maytenus ilicifolia* which is used locally for fertility control similarly had no effect on implantation or organogenesis when administered orally at a dose of 1000 mg/kg/day to mice.

In this study, folkloric use of plant parts as antifertility agents may produce similar "no effect" on implantation or organogenesis and thus supporting the assertion that with the exception of a comparatively small number of patently toxic plants, complementary and neutralizing substances as found in whole plants generally serve to mitigate and/or eliminate any toxicological effects of the identified active ingredients (Obomsawin, 2011). It may thus be safe to say here that when plants are touted with efficacious uses one must stay on the side of caution for such claims may just be the extract in unregulated high doses that can poison the whole organism (Ciganda and Laborde, 2003).

Post coital treatment of mice with *T. monadelpha* bark extract (TBE) at doses of 500 mg/kg and 1000 mg/kg did not significantly affect ($p > 0.05$) implantation compared to controls (Table 1). This may suggest TBE has no anti-implantation activity when given after mating. A dose dependent increase in foetal loss was observed in treated animals, though not statistically significant ($p > 0.05$) compared to controls. This suggests detrimental effect of TBE on foetal development. The adverse effect caused by the crude extract may be due to maternal toxicity or foetotoxicity or both (Kimmel and Buelke-Sam, 2001; Yakubu and Bukoye, 2009; Srikant *et al*., 2013). The fertility index of mice treated before and after mating with TBE at a dose of 1000 mg/kg was reduced to 60% (Table 2). This may suggest a possible anovulatory or contraceptive property of TBE.

One out of the five treated animals (20%) had no foetuses but showed implantation sites after ammonium sulphide staining (Fig. 15) indicating early foetal loss and tissue resorption (ICH, 2005; Tyl, 2013). Perhaps TBE contains compounds that are embryotoxic and or abortifacient. The loss of foetuses may be caused by maternal toxicity, direct chemical insults on developing embryo or an alteration of hormonal function (Kimmel and Buelke-Sam, 2001; Yakubu and

Bukoye, 2009; Srikant *et al*., 2013). An inbalance in the sequential action of oestrogen and progesterone may be responsible for the loss of foetuses in treated mice (Wood *et al*., 2007; Young and Lesssey, 2010; Cooke *et al*., 2015). Embryonic death may also be attributed to the presence of oestrous cycle disrupting chemicals in the extract. Irregularity of oestrous cycle may cause distortions of endometrial

function which may in turn lead to failure of implantation and pregnancy (Abu and Uchendu, 2011).

Another animal of the same group exhibited signs of abortion by the expulsion of uterine content (Fig. 16). This perhaps supports the folkloric reputation of TBE as an abortifacient. The abortifacient activity of TBE may be produced by compounds like oestrogen or oxytocin that stimulate uterine contractility (Farnsworth *et al*., 1975; Tafesse *et al*., 2005). Examination of the uterus of aborted mouse showed highly thickened uterine wall (Fig. 17) indicating that TBE may contain chemicals that act on the uterine wall to induce abortion. Uterine contractility may also be due to the stimulation of prostaglandins synthesis or the presence of compounds with such effect (Clark and Myatt, 2008). Further studies into the effect of TBE on uterine muscle may provide an insight into the mechanism of action of the extract.

The extract at a lower dose of 500 mg/kg had no significant effect on fertility index of treated mice compared to controls. This may show that the effect of the extract on conception and foetal viability depends on the dose. This result is in agreement with the work of Padmashali *et al*. (2006) which reported that the abortifacient activity of *Balanites Roxburghii* was dose dependent. It is also comparable to the effect of *Plumbago rosea* and *Alchornia chordifolia* (Satter *et al*., 2007; Lembe *et al*., 2014).

A reduction in the number of implants was observed in mice pretreated with TBE though not statistically significant ($p > 0.05$) compared to controls (Table 2). This may suggest that perhaps administration of TBE influenced the oestrous cycle and

implantation. Post implantation loss of foetuses was also observed at all doses though not statistically significant ($p > 0.05$) compared to controls (Table 2). This suggests that TBE may contain compounds that influence events before and after implantation (ICH, 2005). The preliminary fertility results suggest that TBE possesses abortifacient activity and has adverse effect on embryonic development depending on the dose. Based on the results of this preliminary study, TBE was selected for further testing with higher doses.

The preliminary fertility test indicated that *W. indica* leaf extract (WLE) does not exhibit significant anti-implantation activity compared to controls (Table 1). Prenatal death was not observed in treated animals at all doses. This suggests that perhaps WLE has no antifertility effect when given orally after mating. Treatment of mice with WLE before and after mating had no significant effect on fertility and implantation indices compared to controls (Table 2). This may suggest that WLE has no anti-ovulatory property at the doses tested. Post implantation loss was observed in mice treated with a dose of 1000 mg/kg though, not significantly different ($p > 0.05$) compared to controls (Table 2) suggesting that perhaps WLE may not affect embryonic development.

W. indica has a folkloric reputation as an abortifacient and regulator of the menstrual cycle (Farnsworth *et al*., 1975; Burkill, 1985; Abbiw, 1990; Leonard, 2006; Khare, 2007; Kumar *et al*., 2012). Ovulation has been likened to an inflammatory response (Richards *et al*., 2002) therefore plants with strong antiinflammatory effect would affect female reproductive function (Mendonca *et al*., 2000; Richards *et al*., 2002; Wang *et al*., 2007; Clark and Myatt, 2008; Jabbour

et al., 2009; Ricciotti and FitzGerald, 2011). *W. indica* is reported to possess strong anti-inflammatory activity mediated by the inhibition of nitric oxide (NO), tumor necrosis factor alpha (TNF-α) and interleukin 12 (IL-12) (Rao *et al*., 2005). Interestingly, WLE did not show contraceptive, anti-ovulatory or antiimplantation effect at the doses tested. The possible explanation may be that the antifertility agent is not present in the leaf in large amounts. The dose used may also not be high enough to produce reported effect.

The lower number of live foetuses and the loss of feotuses in mice treated with ARE and TBE suggests their antifertility potential. Although the number of implants and live foetuses of mice treated with ARE, TBE, PRE, MLE and WLE were not significantly different ($p > 0.05$) compared to controls, the number of live foetuses in mice pretreated with ARE and TBE was significantly lower ($p < 0.05$) compared to mice pretreated with MLE and WLE. Based on observations made and statistical analysis of the preliminary fertility results, ARE and TBE were selected for further testing. Since the preliminary results clearly showed that the antifertility effect of ARE and TBE was dose dependent, higher doses were used for the subsequent tests. The highest dose used in the preliminary study (1000 mg/kg) was increased three folds (3000 mg/kg) to evaluate the effect of higher doses on the oestrous cycle, conception and embryonic development.

The oestrous cycle is known to be a reasonable index of good functioning of the hypothalamic-pituitary-gonadal axis (Goldman *et al*., 2007; Caligioni, 2009; Abu and Uchendu, 2011). The pattern of events in the oestrous cycle may provide a useful indicator of the normality of reproductive neuroendocrine and ovarian

function in the non-pregnant female (Kimmel and Buelke-Sam, 2001). The results of this study showed that administration of *A. nobilis* root extract (ARE) to female mice for 21 days did not alter oestrous cyclicity (Fig. 18) or the duration of the various phases of the oestrous cycle at all doses (Fig. 18). This suggests that perhaps ARE does not influence steroidogenesis at the doses tested in mice. The prenatal mortality observed in mice treated with ARE during the preliminary fertility test may be due to embryotoxic effect of the extract.

Treatment of mice with TBE at doses of 1500mg/kg (Fig. 19) and 3000 mg/kg for 21 days significantly prolonged ($p < 0.05$) the dioestrous phase of the oestrous cycle (Fig. 19). The dioestrous phase of the oestrous cycle is characterized by the formation of the corpus luteum which secretes oestrogen and progesterone necessary for a successful pregnancy (Totora and Grabowski, 2003; Groothuis *et al.*, 2007; Marieb *et al.*, 2015; Martini *et al.*,2015). Prolonged dioestrous is an indication of the maintenance of the corpus luteum beyond its 2 days life span and it is associated with high levels of plasma progesterone (Westwood, 2008; Bertolin and Murph, 2014). Aqueous extract of *T. monadelpha* was reported to have significantly reduced serum testosterone level in male mice (Oyelowo *et al*., 2011). It is possible that administration of TBE altered the hormonal pattern necessary for the maintenance of ovarian function hence oestrous cyclicity.

The low dose of 750 mg/kg did not alter oestrous cyclicity of treated mice. This suggests that TBE contains phytochemicals that act on the oestrous cycle depending on the dose. Furthermore, proestrous and metaoestrous phases of the cycle were significantly reduced ($p < 0.05$) in mice treated with 3000 mg/kg of TBE. During

proestrous, follicles develop and start to produce oestrogens that stimulate endometrial growth (Totora and Grabowski, 2003; Groothuis *et al*., 2007; Marieb *et al.,* 2015; Martini *et al.,* 2015). The result suggests that TBE may contain phytochemicals that interfere with the production of oestrogen necessary to stimulate endometrial growth. This finding is supported by the reduction of the relative uterine weight in mice treated with TBE at a dose of 3000mg/kg (Fig. 28), suggesting that TBE may contain anti-estrogenic compounds.

The oestrous phase of the cycle was not affected at all doses, an indication that TBE may not directly influence ovulation (Fig. 19). The reduction in metaoestrous may have accomodated the prolonged diestrous phase (Fig. 19) resulting in the insignificant increase ($p > 0.05$) in cycle length (Table 3).

Oestrous cyclicity is controlled by the hypothalamic-pituitary-gonadal axis. Consequently, loss of cyclicity would indicate an imbalance in ovarian hormones or gonadotropins (Goldman *et al*., 2007; Malashetty and Patil, 2007; Abu and Uchendu, 2011). Reduced fertility has been reported in animals with prolonged diestrous phase (Uchendu *et al*., 2000). A similar finding was reported by Sheeja *et al*. (2009) which showed that *Plumbago rosea* leaves prolonged the dioestrous phase of the oestrous cycle. Oestrous cycle disruption may be one of the ways by which TBE acts on the reproductive processes in mice and impair fertility.

Monitoring of body changes during toxicity studies can be an indicator of overall health of an animal. Studies have shown a positive correlation between toxicity and a reduction in body and organ weights of an animal following exposure to a chemical substance (Adeneye *et al*., 2010; Chang *et al*., 2012). Treatment of

animals with *Anthocleista nobilis* root extract (ARE) for 21 days insignificantly increased ($p > 0.05$) body weight of treated mice at all doses (Fig. 20). This suggests that ARE stimulates appetite but causes bad food assimilation or lipid lowering effect. A similar finding was reported in a related species of ARE, *A. vogelii* is reported to increase feed consumption (Anyanwu *et al*., 2013; Nguessom *et al*., 2013). Body weight changes in TBE treated mice at all doses were not significantly different ($p > 0.05$) from controls (Fig. 20). However, the insignificant weight loss observed in mice administered with 3000 mg/kg may suggest appetite lowering effect of TBE at a high dose.

The relative organ weight is an important index of physiological and pathological status of animals (Akhigbe, 2014). Unfavourable drug related interactions with major organs might cause cellular constriction and inflammation, which are reflected in relative organ weights (Devaki *et al*., 2012). The liver is the largest organs in the mammalian body and its function includes detoxification and metabolism of xenobiotics in the body. The results did not reveal any significant differences ($p > 0.05$) in the relative liver weight of animals treated with ARE (Fig. 21) and TBE (Fig. 25) at all doses compared to controls. This suggests that perhaps the plant extracts do not contain compounds that would interact with the liver (Fennell *et al*., 2004).

Cells of the body continually adapt to minor and major internal stimuli to preserve structural integrity and function. Inability of the cells to cope with physiologic stresses results in subcellular alterations which may be reversible or irreversible. Reversible cell injury is characterized by cellular swelling and the appearance of

large lipid vacuoles in the cytoplasm. If the damaging stimulus persists, the injury becomes irreversible as the cell cannot recover from the loss of key structural component leading to cell death. Hence light microscopic changes used as a criterion for pathological assessment includes necrosis, cellular swelling, fatty change and infiltrationtion of inflammatory cells (Crowley, 2004; Boyer *et al*., 2012; Kumar *et al*., 2012). The photomicrograph of the liver confirmed that ARE (Fig. 29) and TBE (Fig. 30) are non-toxic because there were no structural changes in the liver of treated animals. The absence of observable changes such sinusoidal congestion supports the idea that repeated exposure of mice to ARE and TBE have no hepatotoxic effect.

Biochemical analysis of serum was carried out to determine possible hepatic dysfunction, tissue damage or changes in biliary excretion evoked by repeated exposure to the extract. Alkaline phosphate (ALP), a marker of hepatobiliary injury in rodents and humans (Boone *et al*., 2005) was not significantly different ($p > 0.05$) in mice treated with ARE and TBE at all doses compared to controls (Table 4). This suggests that liver function was preserved in mice treated with the plants extracts. There was no significant change ($p > 0.05$) in serum creatinine level in animals treated with ARE and TBE at all doses compared to controls (Table 4). Serum creatinine is a useful marker of renal function that measures the amount of functional nephrons (Levey *et al*., 2006; Lascano and Poggio, 2010). This is an indication that ARE and TBE at the doses employed do not cause renal impairment. Similar finding was reported by Nguessom *et al*. (2013) using the aqueous stem bark extract of *A. vogelii*.

Cholesterol is a compound synthesized in the liver and necessary in the formation of cell membranes and hormones. It is transported in the blood from cell to cell by lipoproteins. The lipid profile of animals may indicate drug induced liver damage which compromise glucose metabolism and the synthesis of proteins, lipids and coagulation factors (Boone *et al*., 2005). From the results, cholesterol level of animals treated with ARE and TBE at all doses was not significantly different ($p > 0.05$) compared to controls (Table 4). Other serum parameters like triglycerides, low and high density lipoproteins were also not significantly different ($p > 0.05$) compared to control (Table 4). This suggests the non-toxicity of the plant extracts to the liver within the treatment period.

A dose dependent reduction in relative kidney weights was observed in animals treated with ARE (Fig. 22) and TBE (Fig. 26) though not significantly different ($p > 0.05$) compared to controls. The primary organs affected by toxicant induced metabolic reactions are the liver and kidney (Kanote *et al*., 2012). Hence the absence of marked effect on relative liver and kidney weight in treated animals at all doses suggest that ARE and TBE are relatively safe. Renal function test was also performed to assess possible nephrotoxicity which might be associated with repeated exposure of the plant extracts. High creatinine levels are associated with renal dysfunction (Boone *et al*., 2005; Akhigbe, 2014). From this study creatinine levels were reduced in animals treated with ARE and TBE at all doses although not significantly different ($p > 0.05$) compared to controls (Table 4). This suggests the absence of nephrotoxic compounds in the plant extracts. This finding on TBE is corroborated by the work done by Owusu (2009) which showed that *Trichilia monadelpha* was well tolerated in rats without any signs of toxicity after 14 days of

treatment. The absence of significance in body weight, organ weight and biochemical parameters in treated animals at all doses lends support to the safety of ARE and TBE.

In a reproductive study, information on relative weight of ovaries and uteri is valuable since significant increases or decreases indicate reproductive toxicity (Kimmel and Buelke-Sam, 2001). Treatment of animals with the plant extracts for 21 days did not result in significant changes ($p > 0.05$) in relative ovarian weight compared to controls. Although the relative ovarian weight in mice treated with ARE at a dose of 1500 mg/kg and 3000 mg/kg showed a slight reduction, it was not significant ($p > 0.05$) compared to controls. The reduction in ovarian weight may suggest possible anti-oetrogenic effect (Kimmel and Buelke-Sam, 2001). The result suggests that the effect of ARE on reproduction may be dose depend since no effect was observed in mice treated with the low dose (750 mg/kg).

The relative uterine weight of animals treated with ARE at all doses were comparable ($p > 0.05$) to controls (Fig. 24). Although relative uterine weight showed a dose dependent reduction, this was also not significantly different ($p > 0.05$) compared to controls (Fig. 28). The ovarian hormone oestrogen is known to stimulate endometrial growth (Groothuis *et al*., 2007). Therefore a reduction in the weight of the uterus may reflect the effects of compounds that inhibit steroidogenesis and cyclicity and dramatically reduce the weight of the uterus so that it appears atrophic and small (Kimmel and Buelke-Sam, 2001). Perhaps TBE contains compounds that exhibit anti-oestrogenic effect. The absence of significant

differences in body weight, relative organs weights and serum biochemical parameters of treated and control mice support the safety of ARE and TBE.

The effect of ARE and TBE on conception and inplantation was assessed using the newly selected doses of 750 mg/kg and 1500 mg/kg and 3000 mg/kg. Post coital treatment with ARE at all doses did not significantly reduce ($p > 0.05$) the number of implants and the number of life foetuses compared to control (Table 5). This suggests that perhaps the crude extract of ARE may not possess antiimplantation effect when given after mating. Although prenatal death of foetuses was observed in treated animals at all doses, this was not significantly different ($p > 0.05$) compared to controls (Table 5).

Post coital administration of TBE did not significantly reduce ($p > 0.05$) the number of implants in treated compared to controls (Table 6). This may suggests that TBE does not interrupt implantation events when given post coitum. It is possible that a plant extract having abortifacient effect may not possess antiimplantation activity (Chirotaw, 2006). The result agrees with the finding of Shrestha *et al*. (2011) which reported that *Arecha catechu* possesses abortive activity with no associated anti-implantation effect. The result of Savadi *et al*. (2009) also supports the present finding.

Judging by the significant reduction ($p < 0.05$) in the number of live foetuses, it is evident that TBE has adverse effect on foetal development (Table 6). The results suggest that TBE may contains phytochemicals that modify of the uterine

environment which is critical to blastocyst survival (Yoshinaga, 1988; Carson *et al*., 2000; Lim *et al*., 2002; Ma *et al*., 2003). The receptivity for blastocyst implantation is controlled by the synergistic action of progesterone and estrogen (Yoshinaga, 1988; Lydon *et al*., 1995; Ma *et al*., 2003; Al-Asmakh, 2007; Jerry, 2007; Song *et al*., 2007, Young and Lessey, 2010; Sargis, 2014). Therefore phytochemicals that inhibit the action of oestrogen or progesterone would negatively influence reproduction (Oluyemi *et al*., 2007).

Post implantation loss of foetuses in animals treated with a low dose of TBE was not significantly different ($p < 0.05$) compared to controls (Table 6). This suggests that the effect of TBE is dose dependent. A crude extract contains a myriad of compounds that may be acting synergistically depending on the dosage (Fennell *et al*., 2004). A similar result was reported by Kage *et al*. (2009) which stated that the antifertility effect of *Trichosanthes cucumerina* variety *cucumerina* is dose dependent.

Maternal body weight at sacrifice of mice treated before and after mating with ARE was not significantly different ($p > 0.05$) compared to controls (Fig. 31).
This may suggest the lack of toxicity in mice since body weight changes is an index of toxicity (Akhigbe, 2014). Administration of TBE resulted in a significant reduction ($p < 0.05$) of maternal body weight at sacrifice compared to controls (Fig. 31). Weight loss associated with treatment of mated animals may result from loss of appetite or adverse effect on foetal development (Kimmel and Buelke-Sam, 2001).

The reduction in maternal weight (Fig. 31) may not be a consequence of maternal toxicity since unmated mice treated with the same doses did not show significant weight changes ($p > 0.05$) compared to controls (Fig. 20). Changes in oestrogen and progesterone levels might be the underlying cause of this observation. These ovarian hormones are known to influence food intake and uterine receptivity in animals (Carson *et al*., 2000; Toth *et al*., 2001; Ma *et al*., 2003; Augustine *et al*., 2008; Olofsson *et al*., 2009; Faas *et al*., 2010). The result suggests that perhaps TBE may contain phytochemicals with oestrogenic or progestinal activity.

The fertility index of ARE treated animals at all doses was not significantly different ($p > 0.05$) compared to controls (Table 6). This suggest that ARE has no contraceptive effect at the doses tested. This finding may be supported by the inability of ARE to alter the oestrous cycle in treated mice (Fig. 18). Contrary to expected results based on the preliminary fertility test, the number of implants and live foetuses showed a dose dependent reduction but was not significantly different ($p > 0.05$) compared to controls (Table 6). This suggest that perhaps ARE has no anti-implantation activity and an increase in dosage does not increase its effect on implantation. It is possible that massive insult induced by ARE may have caused damage to the blastocyst thereby causing its death. Data found in this research indicate that, despite the fact that *A. nobilis* is reputed locally as an antifertility plant, oral administration of the ethanol root extract up to a dose of 3000 mg/kg does not alter reproductive performance of female mice. It is possible that mice metabolize ARE in ways that the observed effect in humans cannot be reproduced in mice. Inter species differences may have accounted for the absence of antifertility effect in treated mice (Farnsworth *et al*., 1975).

The fertility index of mice treated before and after mating with TBE was reduced in a dose dependent manner (Table 6). Ammonium sulphide staining of the uteri of non-pregnant animals showed the mice had implantation sites but no foetuses indicating early embryonic loss and tissue resorption (ICH, 2005; Tyl, 2013). This result suggests that TBE possess abortifacient activity. Out of the seven animals treated with TBE at a dose of 1500mg/kg, vaginal bleeding was observed in three (42%) (Table 7). Normal endometrial function requires a balance in the sequential action of oestrogen and progesterone (Wood *et al*., 2007; Cooke *et al*., 2015), and a disruption of this balance is a significant factor in many reproductive problems including abnormal bleeding and pregnancy loss (Young and Lesssey, 2010). This observation suggests that TBE contains chemicals that influence hormonal function in treated mice.

The number of implants was reduced in a dose dependent manner in treated animals. However, this observation was significant ($p < 0.05$) only in mice administered with TBE at doses of 1500mg/kg and 3000mg/kg (Table 6). It is possible that TBE produces antifertility effect through the alteration of the oestrous cycle (Abu and Uchendu, 2011). The number of mice showing no implantation (Table 7) in this study might be due to the prolonged dioestrous phase observed (Fig. 19) which reduces the chance of ovulation hence fertilization
(Tafesse *et al*., 2005).

The number of live foetuses was also reduced significantly ($p < 0.05$) in mice treated with doses of 1500 mg/kg and 3000 mg/kg (Table 6). This supports the idea

that TBE may contain chemicals that alter the oestrous cycle or cause an unreceptive uterus as a result of hormonal imbalance (Yoshinaga, 1988; Carson *et al*., 2000). Irregularity of the oestrous cycle may cause distortions in endometrial function which may in turn lead to failure of implantation and pregnancy (Abu and Uchendu, 2011). Some toxic agents may directly interfere in the synthesis and secretion of gonadal hormones or indirectly alter the responsiveness of the pituitary glands to gonadotropin releasing hormone (GnRH) which in turn influence secretion of gonadal hormones (Makori *et al*., 2009; Ambali *et al*.,
2010).

The antifertility effect of TBE at doses of 750 mg/kg, 1500 mg/kg and 3000 mg/kg are 0%, 57% and 71% respectively (Table 7). Antifertility activity of medicinal plants have been attributed to the phytoconstituents present in plant extracts (Yakubu and Bukoye, 2009; Abu and Uchendu, 2011; Sheeja *et al*., 2009; Vivekanandan *et al*., 2014). Alkaloids and flavonoids have been shown to reduce serum concentrations of LH, FSH and estradiol (Al-Imari, 2012). Flavonoids isolated from *Butea monosperma* and *Stachyslav andulifoli* have similarly been reported to possess antifertility activity (Khama and Choudhury, 1968) and abortive effect (Jafarzadeh *et al*., 2012). *T. monadelpha* is reported to contain phytochemicals such as alkaloids, saponins, phytosterol and flavonoids (Owusu,

2009; Anyanwu *et al*., 2013; Ben *et al*., 2013). Perhaps the abortifacient effect of TBE may also be due to the presence of alkaloids and flavonoids.

Compounds having estrogenic activity have also been repoted to impair fertility in many species. Estrogenic chemicals cause infertility by shortening the time of transport of egg, disrupting oestrous cycle and lowering the plasma progesterone levels which finally stops development of the endometrium (Farnsworth *et al*., 1975; Tafesse *et al*., 2005; Sattar *et al*., 2007; Clark and Myatt, 2008). Abortifacient activity can be produced by antifertility agents that promote uterine contractility. Propulsion of the ova may result in its expulsion from the reproductive tract similar to what was observed in mice treated with TBE (Fig. 16). Furthermore, accelerated transport of ova may cause degeneration of the zygotes when introduced too early into the uterus (Farnsworth *et al*., 1975; Yoshinaga, 1988; Paria *et al*., 1993; Tafesse *et al*., 2005). Perhaps the abortifacient activity of TBE may be mediated by uterotonic agents. In light of this background, the effect of TBE on isolated uterine tissue was assessed.

The crude ethanol extract of *T. monadelpha* failed to elicit uterine contractile effect on the isolated uterus (Fig. 32) however, it significantly inhibited the acetylcholine induced contractions on the isolated uterus (Fig. 33). The result suggests that the abortifacient effect of TBE may not be mediated by uterotonic agents. This implies that other mechanisms may be involved in the observed abortifacient effect of TBE (Farnsworth *et al*., 1975). Foetal expulsion during abortion can occur due to accelerated transport of zygotes in the endometrium (Farnsworth *et al*., 1975; Tafesse *et al*., 2005; Sattar *et al*., 2007; Clark and Myatt, 2008). Estrogenic and oxytocic compounds have been reported to stimulate uterine contractility and excitability (Tafesse *et al*., 2005; Naseri *et al*., 2008). The antispasmodic effect of

TBE suggests that perhaps the extract contains phytocompounds that inhibit the action of oestrogen or oxytocin.

Contraction of uterine smooth muscles can be stimulated by a number of agonists including acetylcholine (Matsui *et al*., 2002). Acetylcholine elicits uterine contractions through the stimulation of muscarinic receptors in the uterine tissue. Atropine is a non-specific competitive muscarinic receptor inhibitor that reduces the effect of acetylcholine and relaxes the uterus (Nwafor *et al*., 2002; Bigovic *et al*., 2010; Bose *et al*., 2014). Atropine significantly inhibited ($p < 0.05$) the acetylcholine induced contractions on the isolated uterus (Fig. 33). Similarly, TBE also significantly inhibited ($p < 0.05$) the acetylcholine induced contractions on the isolated uterus (Fig. 33). This indicates that the presence of utero-active compounds in the plant extract that might be involved in the inhibitory effect (Sattar *et al*., 2007). It is possible that inhibitory effect of TBE on acetylcholine induced contractions may be mediated by muscarinic receptors. The antispasmodic effect of TBE may be mediated by other mechanisms that need further experimentation.

With reference to the studies above, it is creditable to say that TBE contains compounds that influence the hormonal milieu of the female reproductive system. Taken together, the result of the study showed that the ethanol extract of *T. monadelpha* possesses abortifacient, embryotoxic and oestrous cycle disrupting effect in mice. The antispasmodic activity of TBE may be mediated by muscarinic receptors. This finding supports the folkloric use of the plants as an abortifacient (Burkill, 1985; Lemmens, 2008). The antifertility effect of TBE observed in this

study merits further investigation. Fractionation of the extract would be a worthwhile effort to isolate the active compounds responsible for the observed activity.

CHAPTER SIX

6.0 SUMMARY OF FINDINGS, CONCLUSION AND

RECOMMENDATIONS

6.1 Summary of findings

- The results of this study have led to the conclusion that oral administration of the crude ethanol extracts of *Anthocleista nobilis, Macaranga heterophylla, Palisota hirsuta, Trichillia monadelpha* and *Waltheria indica* are relatively safe up to doses of 5000 mg/kg.
- The crude ethanol extracts of *A. nobilis, M. heterophylla, P. hirsute* and *W. indica* do not possess antifertility effect at the doses tested in mice.
- The crude extract of *A.* nobilis does not alter the oestrous cycle of female mice and does not exhibit antifertility property in mice.
- The crude ethanol extract of *T. monadelpha* disrupts the oestrous cycle in female mice and exhibits antifertility effect accompanied with abortifacient property.
- Repeated exposure of mice to ARE and TBE for 21 days is well tolerated with no accompanied toxicity since body weight, relative weight of liver and kidney, serum biochemical markers and histoachitecture of liver did not show significant differences between treated and control animals.
- *In vitro* studies showed that *T. monadelpha* possesses antispasmodic activity which may be mediated by muscarinic receptors.

6.2 Conclusion

In all, the results indicate that the crude ethanol extracts of *Anthocleista nobilis, Macaranga heterophylla, Palisota hirsuta, Trichillia monadelpha* and *Waltheria*

indica are relatively non toxic. Out of the five plants tested, only *T. monadelpha* exhibited significant antifertility activity, abortifacient, antispasmodic and oestrous cycle disrupting effect. The antispasmodic effect of *T. monadelpha* may be mediated by muscarinic receptors.

6.3 Recommendations

- As part of the search to establish the biological effects of TBE, efforts should be made to isolate and identify the active compounds responsible for the antifertility effect.
- Futher studies are needed to determine the mechanism of action of TBE.
- The reversibility or otherwise of the antifertility activity of TBE can be investigated in futher studies.
- Higher doses of TBE can be used to assess it toxicity and antifertility activity.
- Other methods of extraction should be used to extract non polar coumpounds which may be present in *Anthocleista nobilis, Macaranga heterophylla, Palisota hirsuta, Trichillia monadelphia* and *Waltheria indica* and exhibit antifertility activity.

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