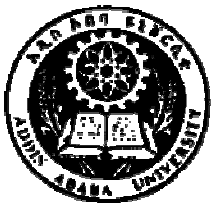


ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

THE EFFECTS OF *RICINUS COMMUNIS* AND *JATROPHA CURCAS* SEED AQUEOUS EXTRACTS ON THE HISTOLOGY OF UTERUS AND OVARY IN MICE

BY:

AMENU TOLERA

JUNE 2004

THE EFFECTS OF *RICINUS COMMUNIS* AND *JATROPHA CURCAS* SEED AQUEOUS EXTRACTS ON THE HISTOLOGY OF UTERUS AND OVARY IN MICE

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

OF

ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT

OF THE

REQUIREMENTS OF MASTERS OF SCIENCE DEGREE IN ANATOMY

BY: AMENU TOLERA

Declaration

Investigator: **Amenu Tolera**

Signature _____

Date of Submission _____

"This thesis is my original work, has not been presented as a thesis work for a degree in this or any other University and that all sources of material used for the thesis have been duly acknowledged".

This thesis has been submitted for examination with my approval as university advisor.

Name _____

Signature _____

ACKNOWLEDGMENTS

I am indebted to Addis Ababa University for sponsoring me to pursue the graduate program and for partially funding my project. Part of the funding comes from a major project of my secured from the Ministry of Health of Ethiopia. I greatly appreciate the offer made to me by my principal advisor.

It gives me a great pleasure to thank my advisors, Dr. Legesse Zerihun, Dr. Mekbeb Afework and Prof. Eyasu Makonnen, all from Faculty of Medicine, Addis Ababa University and Dr. Asfaw Debella, EHNRI, Department of drug research, for their keen interest, unreserved guidance and constructive advise during my research work.

My sincere thanks goes to all staff members of the Department of Anatomy, technicians of Pathology, Pharmacology, Drug Research Department, and EHNRI for their critical comments, assistance in laboratories, and supplying us experimental animals.

My special thanks go to Ato Alehign Kassie, audio-visual center, for his assistance in making the digital photographs. Ato Abdissa Dessa, Endalk Gebre, Eyob Debebe, Mesert Adugna, who were giving me friendly advice regularly during the research.

I also thank my dad and mom as well as my other relatives for their encouragement during the study.

Last but not least, my sincere thanks go to those whose names are not mentioned but contributed a lot for the fulfillment of this study directly or indirectly.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

List of Figures:

List of Tables:

LIST OF ABREVATIONS

AF	Atretic Follicles
a.m	Anti Meridian
ANOVA	One Way Analysis of Variance
B.C	Before the Birth of Christ
C	Cortex of Ovary
CL	Corpus Luteum
CO	Cumulus Oophoroos
cAMP	Cyclic Adenosine Monophosphate
d	Length of long line calibrate
DNA	Deoxy Ribonucleic Acid
DW	Distilled water
EB	Estradiol benzoate
EG	Endometrial Glands
EH	Epithelial Lining cell Height
EHNRI	Ethiopian Health and Nutrition Research Institute
EP	Epithelium
ET	Endometrial Thickness
FA	Follicular Antrum
FSH	Follicle Stimulating Hormone
G	Glands
g	Gram
gm/kg	Gram per kilogram
g/ kg bw	Gram per kilogram of body weight

HP G	Hyperplasia of glands
HP EP	Hyperplasia of Epithelium
JC	*Jatropha curcas*
kg	Kilogram
L	Lumen
LH	Lutinizing Hormone
LD_{50}	Lethal Dose for 50 percent of the Population
M	Medulla of ovary
mg	Milligram
MT	Myometrial Thickness
O	Oocyte
0 c	Degree Celsius
P	Perimetrium
P_p	The number of test points
P_T	The number of total test points
QAL	Queens Anne's Lace
RC	*Ricinus communis*
S	Endometrial Stroma
SEM	Standard Error of the Mean
Vv	Volume density
ZG	Zona Granulosa

ABSTRACT

In developing countries, the problem of population explosion is still a major issue. To combat this issue, many approaches were tried, but the contraception method through explorations of medicinal plants and their mechanism of action on reproductive tissues was found to be more plausible. To this effect, a histological study on the effects of *Ricinus communis* and *Jatropha curcas* seed aqueous extract on the reproductive tissues (uterus and ovaries) was done in this study.

The fresh air dried seeds of *R.communis* and *J.curcas* were employed in the study. The *J. curcas* and *R.communis* seed aqueous extracts were daily administered by gavage to the experimental groups in a doses of therapeutic (1.5 g/kg and 0.02 g/kg), sub-toxic (3 g/kg and 0.04 g/kg), and toxic (6 g/kg and 0.08 g/kg) body weight, respectively, to virgin Swiss Albino Mice for 7 days. The standard groups were treated subcutaneously with a standard drug, Estradiol Benzoate (0.1mg/kg body weight per day) for the same period. The body weight of the mice was recorded daily and they were all sacrificed on the 8^{th} day under Diethyl Ether anesthesia. The uterus and ovaries were dissected out, cleaned of surrounding tissues and weighed on a semi-microbalance sensitive to 0.00 mg and effect of the extracts on the weights of the genital organ weight was recorded. The tissues were fixed in Boun`s Fixative, and histological tissue processing for light microscopy was performed. Histomorphometric analysis of the epithelial cell height, endometrial thickness, myometrial thickness of the uterus, and the number of ovarian corpus luteum and atretic follicles were examined. For histopathological diagnosis, stereological studies of the uterine endometrial gland volume densities were also carried out.

No statistically significant ($P < 0.05$) difference in body weight among treatment groups was observed. The genital organs weights of the experimental groups were decreased significantly ($P < 0.05$) in a dose dependent manner in contrast to those who received the standard drug as compared to the control mice.

Histological observation of the uterus revealed normal structure in the control mice. A dose dependent decrease in epithelial cell height, endometrial thickness, and myometrial thickness ($P < 0.01$) in both extracts was seen showing the weak estrogenic and strong anti-estrogenic effects of the extracts. Complex endometrial hyperplasia was observed at toxic dose of *R.communis* seed aqueous extract but not with *J. curcas*.

In the ovaries of experimental groups, histological investigation showed a dose dependent increase in the number of atretic follicles but decrease in the number of corpus luteum ($P < 0.05$) and an opposite observation in the standard groups as compared to the control mice.

The volume densities of the endometrial gland (epithelium and lumen) were decreased but that of the stroma was increased ($P < 0.05$) significantly at the toxic dose of *R.communis*.

From this study, it can be concluded that the effects of these anti-fertility agents on the uterus and ovaries were through the negative feed back mechanism by causing hormonal disturbance in estrogen and progesterone ratio at hypothalamo-hypophyseal-ovarian- uterine axis and /or by direct action on the uterus and ovaries via inhibition prostaglandin synthesis or desensitization of follicular or steroid hormone receptors.

Further studies need to be perused on the histochemistry and determination of glycogen content, at all doses of the seed aqueous extracts, of both medicinal plants on the reproductive tissues. It is also worth investigating the same parameters of these anti-fertility agents with organic solvents extract of the seeds.

Keywords: *Ricinus communis, Jatropha curcas*, uterus, ovaries, anti-fertility agents, Swiss Albino Mice, complex endometrial hyperplasia.

1. INRODUCTION

1.1. Historical Perspective on the Study of Histology

Vaslius (1514-1564), who often called the father of Anatomy, refuted past misconceptions that Anatomy is confined to isolated observation and description of structures. He described it as a dynamic science that had a long, exciting heritage and currently providing the foundation for medical, biomedical, developmental, cytogenic and biomechanical researches. The importance of anatomy today is in its functional and clinical approach. That is, the invention of microscopes added an entirely new dimension to anatomy and eventually led to explanations of basic body functions. In 1672, a Dutch Anatomist De Graaf described the ovaries of female reproductive system as a site of complex processes such as oocyte maturation, capacitation of spermatozoa, fertilization and embryonic development.

It has been considered that the uterus in addition to hormonal control is influenced by luminal glycoconjugate distribution of the uterine epithelium (1). Lectin profile studies can also provide us further information on the events occurring in the uterus during pregnancy. Investigations at ultra structural level are required to resolve further intra and extra cellular changes (2). Thus, Histology is an essential part of Anatomy and Physiology as it imparts an understanding of the structure and function of genital organs (uterus and ovaries) at microscopic level by employing different types of light and electron microscopes.

1.2. Significance of the Histological Study

The fact that tissues and organs can be recognized histologically implies precise control of many factors such as the size, shape, spacing and orientation of the constituent cells, glands, follicles are known. The patterns produced by these control mechanisms and the pathways by which regulation occurs are well documented (3). Besides, many aspects of histological structure of uterus and ovaries can be rapidly and precisely altered by exposure to hormonal treatment (estrogen and progesterone) and in the presence of intra-uterine contraceptive device as evidenced by ovariectomized rats (4). So one can describe the changes that will happen at cellular level by the application of anti-fertility agents on the uterus and ovaries.

1.3. Structure and Function of the Uterus

1.3.1 Structure of the Uterus

The uterus is a muscular pear-shaped organ of reproduction in the females that lies in the pelvic cavity between the bladder and rectum. It has three regions that include the fundus, a body and cervix. Its size varies from species to species but regardless of species (5), the wall of the uterus is histologically composed of three main layers. Namely, the perimetrium, myometrium and endometrium (Fig. 1.3.1).

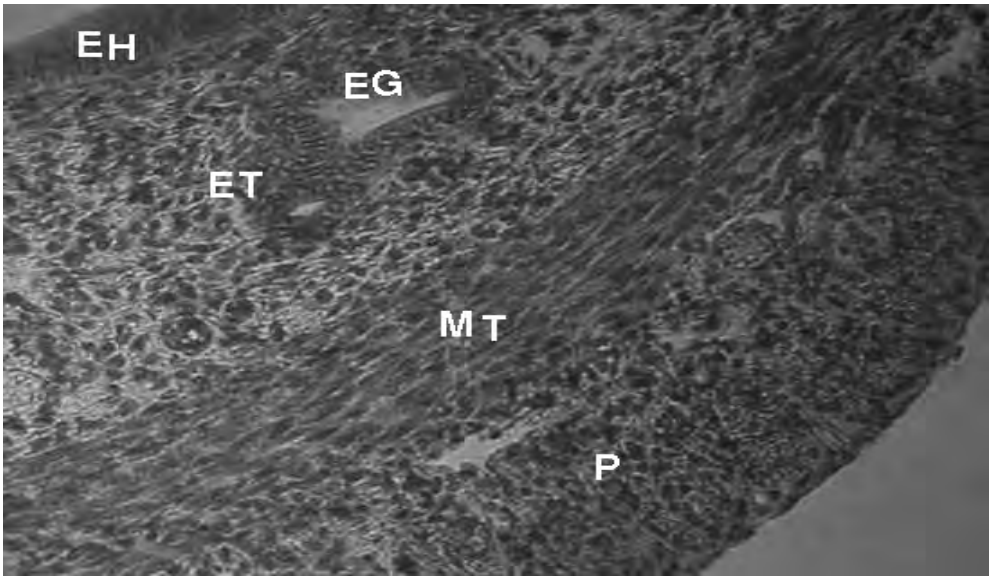


Figure.1.3.1: Histological structure of mice uterus illustrating the perimetrium (P), myometrial thickness (MT), and epithelial cells height (EH), glands (EG) and thickness (ET) of the endometrium. Haematoxylin and Eosin stained, 365 X. (Taken from our study).

The perimetrium is an outer or peritoneal layer also known as tunica advetitia, consisting of a typical loose connective tissue and a large number of lymphatic vessels.

The myometrium is an intermediate or middle layer also called tunica muscularis forming bulk of the uterine wall. It consists of bundles of smooth muscle fibers separated by a thin strand of connective tissue that contains fibroblasts, collagenous and reticular fibers, mast cells and macrophages (4, 5). The muscle forms three layers that are not clearly delineated because of the intermingling of fibers from one layer to another. Generally, internal, middle and outer layers are distinguished. The internal layer is thin and consists of longitudinal and circular smooth muscle fibers. The middle layer is the thickest and shows no regularity in the arrangement of the muscle fibers, which runs longitudinally, obliquely, circularly and transversely. This layer also contains

many large blood vessels and has been called the stratum vascularae. The outer layer consists mainly of longitudinal smooth muscle fibers. The myometrium undergoes contractions after administration of uterine muscle stimulants such as prostaglandin's, oxytocin, and estrogens, which are compounds tested as postcoital contraceptive ("morning after pill") or contra gestational (" one- a-month pill") agents (6).

The endometrium is an inner or mucosal epithelial lining consisting of two sub layers tunica mucosa and tunica submucosa or endometrial stroma or lamina propria. The tunica mucosa of the endometrium is usually made up of simple columnar cells with round or ovoid nuclei and non ciliated secretary cells except in saw and ruminants where it may be stratified or pseudostratified columnar (4, 5). It dips into the stroma to form numerous uterine glands which are simple or branched tubular, secreting mucus, glycogen, protein and lipids. The tunica submucosa of the endometrium is made up of an extracellular matrix full of neutrophils, lymphocytes and many fibroblasts.

The endometrium undergoes a sequence of morphogenic and functional cyclic changes that makes it hospitable for the early embryo if fertilization occurred mainly during menstrual cycles in humans and oestrus cycle in other animals. These changes are controlled by hormones secreted by the ovary (6). Four stages are recognized, the proliferative, secretary, premenstrual and menstrual phases. These phases are, respectively, characterized by a rapid regeneration and repair of the endometrium, lengthening and coiling of uterine glands, intermittent constriction of the spiral arteries, and shedding of the functional layer resulting in menstrual discharge. The proliferative phase, occurring concurrently with follicular maturation is influenced by ovarian estrogen secretion while the secretary phase coinciding with functional activity of the corpus

luteum is primarily influenced by progesterone secretion. The menstrual phase commenced as hormone production by ovary, however, declines with degeneration of the corpus luteum.

1.3.2. Function of the Uterus

Functionally, the endometrium of the uterus is divided into two main zones: 1) the stratum functionalis, which is built up and sloughed off mainly during menstruations, and 2) the stratum basalis, the epithelial and glandular elements that remain to supply replicative cells to regenerate the functionalis of the next cycle. It is specialized for containing, protecting, and nourishing of the nidating embryo from implantation to parturition. Physiological changes in the uterus correlate with functional activity of the ovary (4, 6).

1.4. Structure and Function of the Ovary

1.4.1. Structure of the Ovary

The ovary (female gonad) is an almond-shaped endocrine gland located in the ovarian fossa on each side of the uterus. Its surface is covered with germinal epithelium. Underlying it is a dense connective tissue sheath, tunica albuginea ovarii. In most species, sections of ovary show an inner medulla, consisting of many elastic fibers, smooth muscle cells and neuromuscular bundles (7). And an outer cortex, composed of ovarian follicles (with developing oocytes and associated follicular or granulosa cells), which are at different stages of development (immature to mature): primordial, primary, secondary and mature or Graffian follicles. These follicles are formed by oogenesis which has three stages namely, pre-ovulation, ovulation and post-ovulation. The cortex also consist of interstitial gland cells which are thought to produce estrogen and stromal elements composed of characteristic spindle-shaped fibroblasts which respond in different ways to

hormonal stimuli than fibroblasts of other organs (7, 8). Furthermore, in the cortex is a hormone producing organ, the corpus luteum (Fig. 1.4.1).

A B

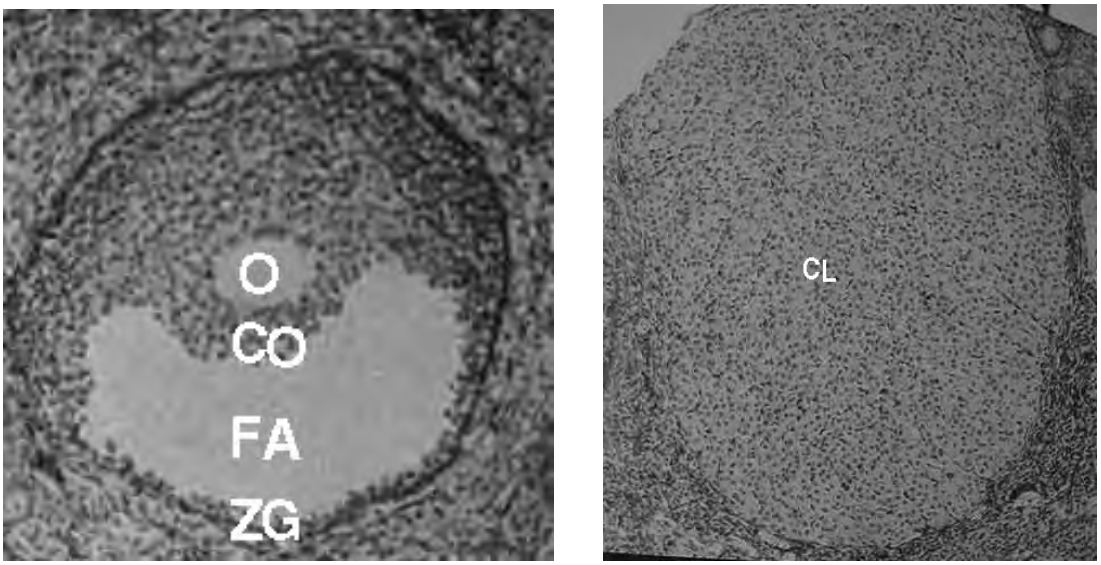


Figure 1.4.1. Histological structure of mice ovary showing an oocyte (O) with cumulus oophoroos (CO), Follicular antrum (FA), Zona granulose (ZG) (A), and corpus luteum (CL) (B) Haematoxylin and Eosin stained. Magnification for both=50x). (Taken from our study).

The corpus luteum or yellow body is surrounded by capsule of the luteal gland formed by theca externa. The parenchyma of the luteal gland consists mainly of two cell types .The granulosa lutein cells are large, polygonal cells that have a large, rounded nucleus and a pale-staining cytoplasm filled with lipid droplets. They secrete steroid hormone progesterone, which promotes the exocrine secretion of uterine glands in the endometrium. The theca lutein cells are smaller in size than the granulosa cells, and have a dark staining cytoplasm and a round nucleus. They are responsible for the secretion of estrogens. Among these glandular cells are numerous capillaries and a small amount of connective tissue that contain some fibroblasts (7, 8).

1.4.2. Function of the Ovary

The function of the ovary is the storage and development of oocytes before their release (gametogenesis), and the production of steroid hormones like progesterone, and paracrine luteotropins locally such as estrogens and insulin-like growth factors which regulated through the ovarian cycle (hormonogenesis).

In the ovarian cycle, maturation of ovarian follicles, their endocrine functions and phenomenon of ovulation are regulated by follicle stimulating hormone (FSH) and lutienizing hormone (LH) which are produced by adenohypopysis. FSH is responsible for maturation of follicles and stimulates the aromatase enzyme complex within granulosa cells to produce estrogens (4, 8). Initial development of follicles is self-regulated and doesn't require FSH, but the hormone is essential for their maturation. Together with FSH, LH induces ripening of the mature follicle and ovulation. Alone, LH coverts the mature follicle to corpus luteum and induces it to secrete estrogen and progesterone. This cyclic nature of follicle formation and ovulation is the reciprocal interaction between pituitary gonadotropins and ovarian hormones.

As production of estrogen by the granulosa cells increase, release of FSH from the adenohypopysis is inhibited, and the level of FSH falls below threshold for maturation of new follicles. However, the rising level of estrogen stimulates the release of LH from the adenohypopysis, resulting in ovulation, formation of corpus luteum, and secretion of progesterone and estrogen by the luteal gland. Increasing level of progesterone now inhibits release of LH from the pituitary, and as the level of LH declines, the corpus luteum is no longer maintained. With decline of corpus luteum, estrogen level wanes, and the anterior pituitary is no longer inhibited from secretion of FSH and a new cycle of folliculogenesis is initiated (8). But

according to Prakash (9), most anti-fertility agents of synthetic or natural origin exert their action by altering the level of estrogen to progesterone ratio, which consequently change the normal reproductive physiology of the animal. Progesterone is essential to maintain pregnancy, by preventing effective uterine contraction, stimulating cAMP production, inhibiting estrogen and oxytocin receptors synthesis. It also promotes the storage of prostaglandin precursors in the decidua and fetal membrane to stimulate the uterus. This was confirmed by the fact that withdrawal or reduced level of progesterone can lead to termination of pregnancy (10). Progesterone is essential for pregnancy to occur; progesterone's function is to prepare the uterine endometrium to receive the fertilized ovum. If the endometrium isn't ready, the fertilized ovum will find implantation very difficult. If it can't be implanted then the opportunity is missed, and it begins to breakdown and is no longer viable and menstruation arrives as usual. So better understanding of the female reproductive cycles will give us a clue on the anti-fertility mechanism of the anti-fertility agents.

1.5. Anti-fertility Agents, Historical Perspectives

Women have used the seeds from *Daucus carota* commonly known as wild carrot or queen anne's lace, for centuries as a contraceptive, the earliest written reference dates back to the late 5^{th} or 4^{th} century B.C. appearing in a work written by Hippocrates. John Riddle (11) writes in *Eve's Herbs*, that Queen Anne's Lace (QAL) seed is one of the more potent anti-fertility agents available, and a common plant in many regions of the world. The seed, harvested in the fall, is a strong contraceptive if taken orally immediately after coitus. Research on experimental animals has shown that extracts of the seed disrupted the implantation process, or if a fertilized ovum has implanted for only a short period, it will cause it to be released. There has been some research done on wild carrot seeds mostly in other countries, the results of those experiments have been

encouraging (12). The Chinese view QAL as a promising post-coital agent, recent evidence suggests that terpenoids in the seed block crucial progesterone synthesis in pregnant animals. Some herbalists associate the contraceptive effect of wild carrot with its capacity to make the uterus so slippery that the fertilized ovum is unable to be implanted. This indicates that, scientific confirmation is helping us to further validate our ancestral knowledge.

In 1926, the presence of estrogenic substances in plants was demonstrated by inducing oestrus in animals. The first isolation of an estrogen from plants was reported in 1933. This initial work was not followed up until the 1950's when people began to recognize that animal infertility could result from consuming estrogen rich plant material. Some people used to employ estrogen rich plant material to fatten livestock. Farnsworth et-al,. listed a number of anti-fertility plants (13). Hughes (14) indicated that over 300 plants from 16 different families contain more than 20 estrogenic substances with four chemically distinct classes (estradiol and estrone, isoflavonoids, coumestans, and resorcylic acid lactones). Anti-fertility agents are substances which interfere with pregnancy or prevent the implantation of the embryo. They are needed for fertility regulation and can be obtained from the folk medicine. *Ricinus communis* and *Jatropha curcas* are among the claimed anti-fertility plants which have been screened positive (15).

1.5.1. Ethnomedical Use of *Ricinus communis*

Ricinus communis (euphorbiaceae) is a tree- like herb 5(-10) m high, with trunk 15 cm thick, stem hollow and its seed contains oil. It is commonly called caster bean (in Amharic, Gulo, in Afan Oromo, Qobbo), and widely distributed in Tropical Africa (15). It is most frequently prescribed traditional drug for treatment of external afflictions. Its oral lethal dose for 50 percent of the population (LD_{50}) was found to be 0.24 g/kg in mice (15, 16, 17).

Figure 1.5.1: *Ricinus communis* with its stem, leaves and seed before ripening. (Courtesy of EHNRI).

1.5.2. Ethnomedical Use of *Jatropha curcas*

Jatropha curcas (euphorbiaceae), is a shrub or a small tree up to 4.5(-8) m high, its seed providing as a purgative, is commonly known as phyic nut (in Amharic, Yeferenji Gulo, in Afan Oromo, Qobbo Addii). It is distributed throughout the tropics including West, East and Southern Africa. It is used traditionally as contraceptive. Its oral LD_{50} was calculated to be 5.25 g/kg in mice (15, 17) (Fig.1.5.2).

Figure 1.5.2 *Jatropha curcas*, with its stem, leaves and seeds before ripening. (Courtesy of EHNRI).

1.5.3 .The Need for Anti-fertility Agents

The world population is increasing each year at an alarming rate (3 % for Ethiopian case) (18) and family planning has received increased attention world wide because of limited natural resources and high maternal mortality rate. To combat the problem of population explosion, there is a need to look for a way of controlling the population growth. Global search on anti-fertility agents is one method to tackle this problem. Although many hormonal drugs are available for this purpose, they are not easily accessible to all people and are not free from side effects. In addition, they may not be available to remote areas at all, where population growth is most significant. Hence the search for a suitable product from traditionally used indigenous medicinal plants were done by many researchers and a number of plants had been screened and found to be safe and effective anti-fertility agents (17, 19). Such plants include: the leaf extracts of *Arthemissia afra (*20*)*, *Leonnotis ocymifolia* (20), the seed extracts of *Ricinus communis* (15) *Jatropha curcas* (15), and the root extracts of *Moringa oleifera* (21).

Furthermore, traditionally used medicinal plants which were shown to possess anti-fertility property are: the fruits of *Cassia fistula (*22), the seed extracts of *Cucurbita moschata*, the stems of *Alphia galanga*, rhizomes of *zingber Cassumunar* and the roots of *Andrographics paniculata* (23). According to Farnsworth (24) a mixture of dried *Betel* extracts, *Piper longum*, oil of *Polianthes* t*uberosa* and dried *Embelia ribes* were given orally daily for 20 days starting from the second day of menstruation and resulted in anti-fertility effect for 4 months in female rats. Though, the parts of the plant which possess anti-fertility activity are variable most of them act by similar mechanisms such as, inhibiting fertilization, increasing uterine contraction and prolonging oestrus cycle (15, 25, 26).

Nevertheless, reversibility in anti- fertility effect after a certain period of time were seen in many of the drugs, which makes it problematic in using these drugs continuously due to lack of complete understanding their mechanisms of action at cellular levels. Besides the fertility or sterility of the females and the anti-fertility or abortiefacient activities of these plants is dictated mainly by the effect they bring on the female genital organs at microscopic level (27). However; there is no study done in Ethiopia in this regard.

1.5.4. Mechanisms of Actions of Anti-fertility Agents

Previous studies showed that plants which possess anti-fertility property act on genital organs by any one or a combination of the following ways. The anti-fertility activity of some anti-fertility agents is due to their estrogenic, anti-estrogenic, progestational and anti-progestational activity. These cause, expulsion of ova from the uterine tube, disrupt the luteotrophic activity of the blastocyst, disturb the functional equilibrium of the hormonal balance or create a non receptive site in the uterus by changing the uterine milieu (28).

Others may act by, rendering the endometrium or stratum functionalis unresponsive to implantation of the fertilized ovum, inhibition of prostaglandin synthesis, preventing the formation of corpus luteum and increasing the number of atretic follicles in the ovary (29). They also mediate by blocking the action of progesterone and its receptors, disturbing the synchronized development of the ovum and endometrium, bringing direct effect or changing histological features of the uterus and ovaries (30). Therefore it could be said that anti-fertility agents act on genital organs for fertility control with or without affecting hormonal balances and /or bringing histoarcitectural changes directly on uterus and ovary (31, 32).

1.5.5. Anti-fertility agents and their effect on body and genital organ weights

According to Singh Shiv Pal (33), the application of *Sebana sebasina* seed aqueous extracts to experimental group rats didn't show any change in body weight at any dose. The genital organ weight, however; was reduced significantly ($P < 0.05$) after treatment with 250 and 400 mg/kg doses for 30 days. Furthermore, from studies done by Makonnen et-al,. (15) by administration of *Ricinus communis* and *Jatropha curcas* , reduction in the weight of genital organs in guinea pigs was reported. Besides absolute volume of endometrium in the uteri of hypothyroid rats was reduced by 45 % ($p<0.05$), and that of myometrium was decreased by 33 % ($P <0.05$). Wider range of reproductive disorders such as irregular menstruation and Frank (permanent) infertility was found in women with hypothyroidism, indicating that thyroid hormones also play important role in conception and maintenance of pregnancy (29). According to Munishi and Rao (34) the reduction in size of uterus after parturition or ovariectomy support that placental estrogens or fetal ovarian steroids may have some effect on development of female genital organs.

1.5. 6. Anti-fertility agents for fertility regulation

Extensive pharmacological, toxicological and clinical profile studies in animals and human volunteers have been conducted to establish the efficacy and safety of anti-fertility agents (*Saheli*, *Ricinus communis*, *Jatropha curcas*, *Arthemisia afra*, *Neem*, *Moringa oleifera* etc.) and suggested that it is possible to develop a new method of fertility regulation based on the prevention or disruption of implantation based on them (35). And most anti-fertility agents also have anti-implantation effect and this will only take place in a favorable uterine milieu. According to Kaushi and Upadhyay (36) the mode of anti-fertility action of intrauterine *Neem* treatment is not because of uterine unresponsiveness to ovarian hormones but is due to impairment of embryo development, confirming that some anti-fertility agents act at the pre-implantation stage.

1.5.7. Effects of Anti-Fertility Agents on the Histology of the Uterus

According to Singh Shiv Pal (33), the uterine histology of rats presented normal structure. The endometrium was provided with large epithelial cells having basal and central nuclei. The uterine glands were numerous, irregular, and tortuous. The uterine lumen was highly distended and the loose stroma with normal vascularity. The dose 100 mg/kg/day for 30 days didn't alter the endometrial height and uterine lumen. The uterine glands were irregular and tortuous. The stroma and vascularity appeared normal. The dose 250 mg/kg/day reduced endometrial height.

Both ponderal and histological changes in the uteri were reported by Prakash (9), by administering the extracts of *Embelia ribes* Burm.seeds in albino female rats. Munshi and Rao (34) have reported the effects on endometrial glands, musculature and uterine lumen in female rats after the application of an indigenous plant preparation. Similar changes were observed by Prakash and Mathur (35) by administrating *Artobotrys odoratissmus* leaf extract.

Dixit (37) reported uterine dysfunction by daily administration of *Malva viscus conzattii* flower extract for 25 days. Bhardwaj and Mathur (38), and Bhardwaj et-al,. (39) presented the results of anti-fertility studies in *Cassia fistula* Linn. Fruit extracts on oestrus cycle, uterus and implantation. The higher the dose showed encouraging activity which is in agreement with findings of Agrawl et-al,. (40) for the fruits of *Juniperus communis* which resulted in 100 % anti-implantation at a dose of 500 mg/kg in female albino rats.

Those studies clearly confirm that the histology of the uterus shows structural changes in a dose dependent manner to anti-fertility agents. Nevertheless, the need for safer alternative to progesterone-estrogen combination pills has been felt since sixties. Clear understanding of the

role of estrogen and progesterone balance in the development of fertilized ovum and priming of uterus for implantation served as a bases for developing an anti-fertility agent that would prevent pregnancy by interfering with implantation and with out disrupting the hypothalamo-hypophyseal-ovarian-uterine axis. Researchers have been designing and synthesizing non-steroidal estrogen antagonists that would act by disturbing the balance between estrogen and progesterone at the uterine level with out interfering with their synthesis of blood vessels (41). *Saheli* presented a major break through in non-steroidal oral contraception that combines dosage convenience with excellent use compliance for fertility regulation in developing countries (41).

1.5.8. Effects of anti-fertility agents on the histology of ovary

According to Sing Shiv Pal (33), Cellular organization of ovaries of rats presented normal structure as evidenced by the presence of all types of follicles, few atretic follicles with normal vascularity in the compact stroma. The germinal epithelium was intact. Administration of *Sebania sebasina* seed aqueous extract in a dose of 100 mg/kg daily for 30 days does not produced any deleterious effect on ovarian tissues; where as, the dose 250 mg/kg daily dose for the same days severely affected the ovarian structure. A large number of developing as well as mature follicles underwent artesia some developing follicles showed lyses of ova. The stroma was compact with poor vascularity. The dose 400 mg/kg daily appeared to decrease endometrial height and effective to cause degenerative changes in the ovary. The administration of this dose for 30 days caused several damage to cellular organization. Even though, large anartesia follicles under want artesia and nuclear degeneration, the stroma become fibrotic with poor vascularity. The germinal epithelium was atrophied and devoid of primordial follicles.

Dexit (37) reported follicular degeneration by daily administration for 25 days of *Malva viscus conzatii* flower extract but *Saheli* exhibited no effect on histology of ovaries at oral dose of 1.25 mg/kg for 7 days (42). But Reed et-al,. (43) indicated that although factors which cause rupture of ovarian follicle and expulsion of the oocyte during ovulation are not yet fully understood, at approach to ovulation in mammals, there is rapid increase in size of the preovulatory follicles as a result of increase in granulosa cells and a marked increase in the vascularity of the theca interna and of the ovary in general. It is probable that both LH and estrogens contribute to increase in vascularity of the ovulatory follicle. In cyclic guinea pigs, injection of either LH or FSH increased vascular supply to theca interna while FSH also increases the size of follicular cells (44).

From pre clinical toxicology study profile, *Saheli,* was found to have an oral LD 50 of 400 mg/kg in rats. It was observed that even 1600 mg/kg produced only 20 % mortality in animal by the oral route and in sub-acute toxicity studies in adult rhesus moneys caused no histopathological changes. At oral doses of 6.25, 12.5 and 2.5 mg/kg/day for 21 days in young adult male and female albino rats. There was no evidence of toxicity in males. The female rats, however; showed decrease in uterine endometrial glands, follicular cysts at different state of artesia and devoid of corpora lutea where seen in the ovaries with the same dose. These observations confirm that, the histology of the ovaries change in a dose and time dependent manner to anti-fertility agents and the human contraceptive dose did not affect the hypothalamo-pitutary-ovarian axis nor reveal any histopathological changes microscopically.

Researchers were emphasizing on the medicinal plants to combat the problem of population explosion. They have screened many of these anti-fertility agents for fertility regulation .However; to be effective, the effect of these anti-fertility agents on the reproductive tissues needs to be undertaken. To this end, this study investigates the effect of *R.communis* and J.curcas seed extracts on the histology of uterus and ovary.

2. OBJECTIVES OF THE STUDY

2.1. General Objective

To investigate the effects of *Ricinus communis* and *Jatropha curcas* seed aqueous extracts on the histology of uterus and ovary in mice.

2.2. Specific Objectives

- To assess any histopathological changes on the uterus and ovaries that may be caused by application of the seed aqueous extracts.
- To get a hint for their possible mechanism of action in female mice.
- To compare and contrast the effects of therapeutic, sub-toxic and toxic doses of these anti-fertility agents on these reproductive tissues.

3. MATERIALS AND METHODS

3.1. Plant Materials Preparation

3.1.1. Plant Collection

The fresh air dried seeds of *J.curcas* were collected from Shewarrobit, 220 kilometers North of Addis Ababa, Ethiopia, in the month of October, 2002 and authenticated by a Botanist from Ethiopian Health and Research Institute (EHNRI), Department of Drug Research. Mounted voucher specimens of parts of the plants were prepared by the investigator and his advisor, and have been deposited at the Herbarium of Medicinal Plants of EHNRI. The ethnomedical information about the plant was obtained from publications of Makonnen et-al,. (15) as well as from oral interviews with traditional healers of the local people. The seeds of *R. communis* were supplied directly by EHNRI.

3.1.2. Plant Extraction

After grabbing (removal of extraneous matter such as dirt and adulterants), the seeds were further dried at room temperature to avoid chemical changes, facilitate fixing of the constituents and grinding. The dried specimens were grounded in to powder and the fine particles of plant materials were obtained. The powdered plant materials were macerated with water for 24 hours. The maceration process embraces soaking of the plant material in cold solvents (water), filtering with Whatman filter paper number one, concentrating the extract and was commonly used to avoid decomposition of thermo liable components of the extract. The filtrate was lyophilized (freeze dried) with a Lyophilizer to prevent loss of active components of the plant and weighed by a sensitive balance. The resulting dry weight of the extract was then reconstituted to the desired concentrations for administration at the therapeutic, sub-toxic and toxic doses of the extract to the mice.

3.2. Animals Preparation

3.2.1. Type of Animal

Pregnant female Swiss albino mice (aging three month, weighing 40-50 g) were purchased from EHNRI, Addis Ababa, Ethiopia and brought to animal breeding house of Faculty of Medicine. They were housed in a temperature-controlled room (25 ± 0.5 0c) maintained on 12/12 hour light /dark cycle. They were feed on commercial pellet diet and drinking water *ad libitum* through out the study.

3.2.2. Animal Breeding and Acclimatization

The mice were checked at 9:00 a.m. each day to determine if they have given birth. After two weeks of birth, the female litters were isolated and placed in separate cages. When they were matured, the virgin forty-eight Swiss female Albino Mice (aging 8-10 weeks, weighing 25-32 g, showing 1-7 days regular oestrous cycle) were randomly assigned either to 6 controls, 36 experimentals and 6 standard groups from the breeding stock.

Eight groups of virgin mature female Swiss Albino Mice were used in this study. Before starting of the experiment, all the animals were acclimatized and maintained under laboratory conditions with free access of food and tap water according to the method described by Anokbonggo (45). Body weight was recorded throughout the experiment starting from the 1st day of administration.

3.3. Administration of the Extracts

The seeds powder as an aqueous solution was administered orally to the experimental groups by gavage. The control groups (I) received the vehicle (distilled water) every day for 7 days. The experimental groups (II-VII) received therapeutic (1.5 g/kg and 0.02 g/kg), sub-toxic (3 g/kg and 0.04 g/kg) and toxic (6 g/kg and 0.08 g/kg) body weight doses of *J. curcas* and *R. communis* were administered to each mice respectively, by the same route for the same period. While the standard groups (VIII), were given standard drug (Estrogen pills, Estradiol Benzoate (EB)) at the therapeutic dose for the same period subcutaneously. On the 8^{th} day, all of the mice were weighed to investigate the effect of the extract on their body weight after administration of the extracts.

3.4. Determination of Estrogenic and Anti-estrogenic Activity of the Extracts.

For this study the experimental groups were treated with therapeutic, sub toxic and toxic doses of the test extracts while the standard groups were treated with EB in olive oil (0.1 mg/kg body weight) daily. This group was used as positive control. The control group which was used as negative control received the vehicle. On the 8^{th} day of the experiment, all animals were sacrificed under diethyl ether anesthesia. Both tissues were dissected out, cleared of the surrounding tissues, placed on filter paper and weighed quickly on a semi-micro balance sensitive to 0.001 mg. The uteri and ovarian ratios were calculated by dividing the uterine and ovary weight in milligrams to body weight in grams as described by Vogel (46).

3.5. Effects of the Extracts on Body and Genital Organs Weight of Mice

Abdominal incision was done to expose the uterus and ovaries of the mice by using surgical blades, scissors and /or forceps. The uteri and ovaries were freed from surrounding tissues, blotted on filter paper and weighed quickly on a semi-micro balance sensitive (Precisa 125A, Switzerland) to 0.001 mg in the Core Laboratory and recorded according to the method described in Sing Shiv Pal (33), to observe the effect of the extract on ovaries and uteri as compared to body weight. The body weight gain was calculated on the basis of the weight taken on the 1st day soon after oral administration considered as the initial weight and the weight taken on the 8th day before anesthesia and dissection was considered as the final weight. The genital organs weights were taken soon after dissection. Data were expressed per 100 g body weight to ensure normalization for statistical analysis. The whole of the ovaries and the middle one centimeter long part of the uterine horn (where implantation commonly is taking place) were cut in longitudinal and transverse pieces and placed immediately in fixative for further tissue processing.

3.6 Effects of the Extracts on Uterine and Ovarian Histomorphology

3.6.1. Histological Processing for Light Microscopy

Both tissues were fixed overnight in Bouin`s fixative consisting of picric acid (75%), distilled water (25%) and glacial acetic acid (5%). They were dehydrated through graded series of ethanol (70-100 %) for six hours, cleared in xyelene for six hours, transferred to dissolved paraffin wax solution and allowed to stay in an oven at 60 0c overnight. Early morning, the tissues were embedded in Histology Laboratory in a paraffin wax and blocks were prepared for sectioning. But before sectioning, the tissue blocks must be frozen in deep freeze refrigerator (- 4 0c). Serial sections (every 10th) at 6 µm were taken using Leitz ultramicrotome with disposable microtome

knife in Pathology Laboratory. One in 10 of these sections was chosen by a systematic random sampling procedure as described by Bedi et-al,. (47), placed on albumin coated glass slides, and placed in oven overnight so as to dissolve the wax. Then the specimens were cooled at room temperature for an hour and stained in the routine Ehrlich`s Haematoxylin and Eosin. The staining procedure successively includes clearing in xyelene for eight minutes, rinsing in decreasing concentration of alcohol (100-70 %), washing in tap water, staining the cytoplasm by putting in Heamatoxylin for ten minutes, washing in tap water, rinsing in borax for bluing, washing in tap water, staining the nucleus by putting in Eosin for two minutes, washing in tap water, rinsing in increasing concentration of alcohol (70-100 %) and putting in xyelene until mounting. Sections were mounted on glass slides by using depex and cover slips.

3.6.2. Morphometric Analysis of the Uterus and Ovary

After death, the uteri were fixed in overnight in Boun`s fixative and were processed for routine paraffin embedding with Heamatoxylin and Eosin staining. The sections were studied in Histology laboratory, Faculty of Medicine using a light microscope (Leitz Dialux 20) fitted with Camera, wild MPS 51 Heerbrugg, Switzerland and eye piece graticule with subdivisions. The endometrial epithelial cell height, the endometrial stromal thickness, and myometrial thickness were measured by divisions of the graticule and then were converted to an equivalent standard micrometer at 25X magnification objective lens according to the method described by Nephew et-al,. (48). Besides the presence or absence of any histopathological changes were examined for all the treated groups at each dose.

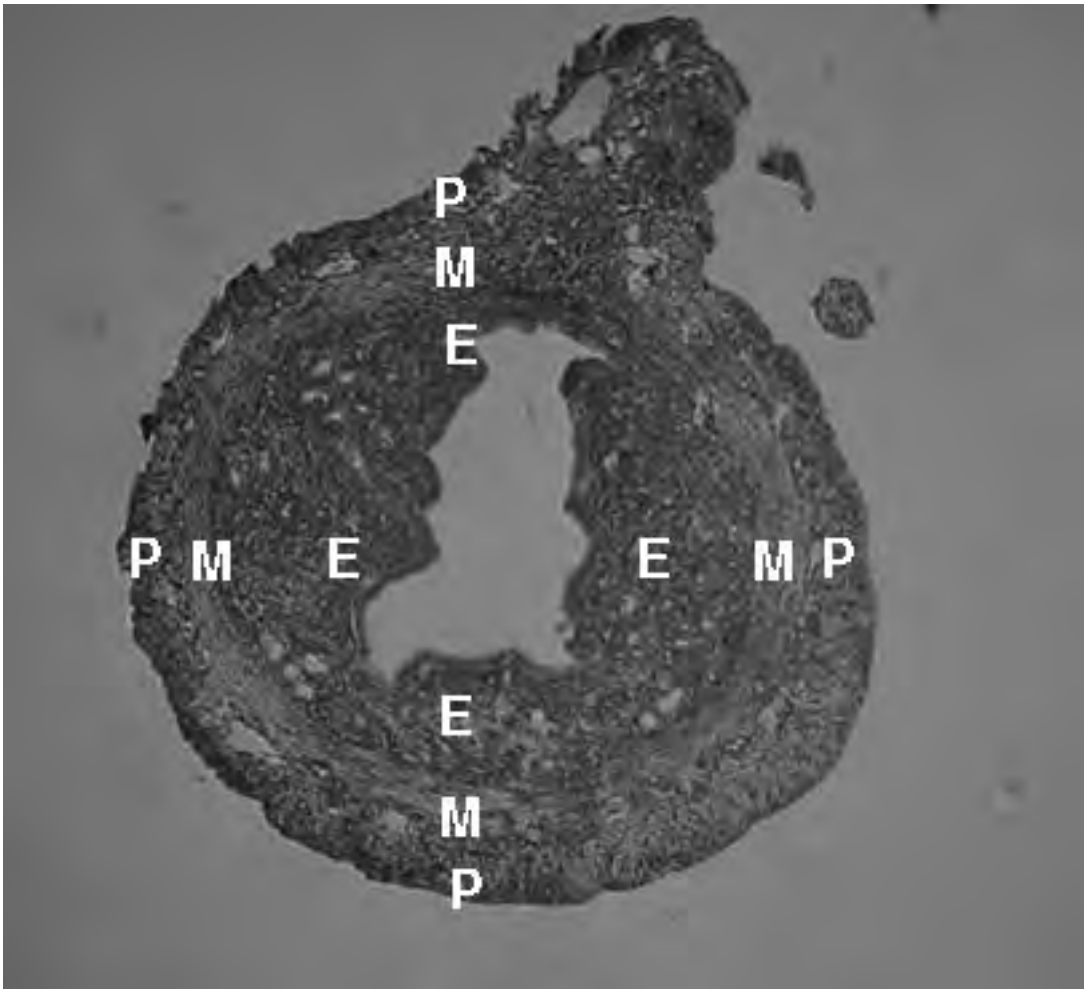


Figure 3.6.2: The Histomorphometry used in measuring epithelial cells height, endometrial thickness and myometrial thickness demonstrated on uterus of mice. Note: perimetrium (P), myometrium (M), endometrium (E) and the uterus of mice is almost circular histologically in transverse section. Stain is Haematoxylin and Eosin, 235X. (Taken from our study).

For the ovaries too, the same procedure was followed and the number of, atretic and corpus luteum as well as presence or absence of histophatological changes were observed by the same microscope at the 4X magnification objective lens for all the groups at each dose as described by Prakash et-al,.(49). Follicles were classified into two groups as proposed by Osman (50). That is,

a follicle is considered to be atretic follicles when ever two or more pyknotic granulosa cells would be found in a single section, follicular cells are disorganized, oocyte shows signs of degeneration, degeneration of zona pellucida, and healthy follicle otherwise.

3.7. Test-System M_{42} Stereological Studies of the Uterus

The scope of the stereology is to determine three-dimensional quantitative parameters of morphological structures from bi-dimensional counts. For that, stereology uses geometry and probabilistic statistics, and is determined from counts of test-points and test-intersections applying some previously defined mathematical formulas. The evaluation of endometrial glands and stroma of uterus was done on six microscopic fields of each randomly selected 6 μm thick of Hematoxylin and Eosin stained sections. The test study used was named Test-system $M_{42,}$ a system of lines (straight or curve lines) and points that has 42 test-points, the test-line measures 21d (where d is length of short line calibrate of the test-system) and the test-area measures $36.36d^2$ as used by Weibel (51). This was superimposed on a morphologic image for the stereological count (52). Using this method stereological parameters of the tissue was achieved. The endometrial glands volume densities were calculated separately. Vv of the glands (lumen, epithelium) and stroma were determined by point counting. To avoid overestimation, all structures falling on forbidden lines (dotted lines) were not counted. That is, Vv of (epithelium, stroma and lumen) of the endometrial gland is found by a formula:

$$Vv = \frac{P_p}{P_T} \%$$

Where: P_p = is the number of test points in the structure.

P_T = is the number of total test points.

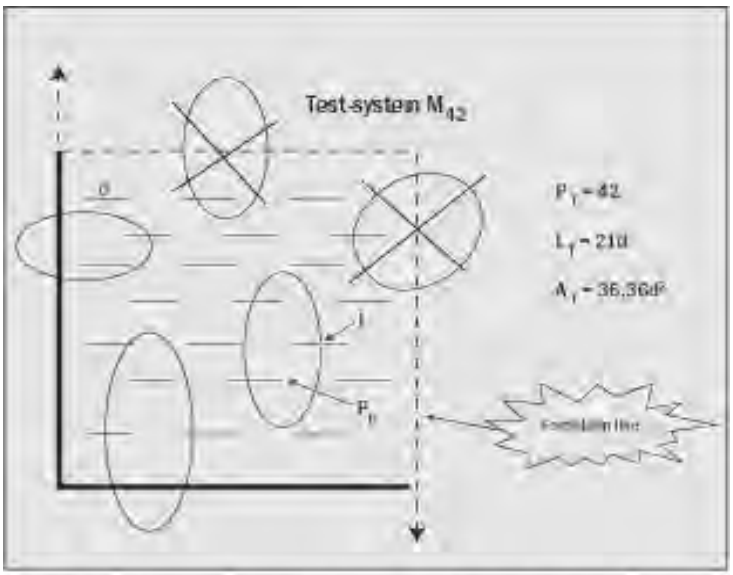


Figure 1 – *Test-system M42. All structures falling on the 'forbidden line' (dotted lines) are not counted to avoid overestimation. The short line length 'd' calibrates this test-system and its extremes are considered test-points (P_p), all short lines are the test-line (L_T), and the test-area (A_T) is the area inside the frame.*

Figure 3.7. The Test-System M_{42} used for Stereological Study of the Uterus

(Source**:** J. Bras.pathol. med. lab.Vol. 39.1 Riode Janerio 2003. From internet).

In summary, mounted slides were examined and sections were photographed on slide films. The films were processed, printed and the effects of the extracts on histology of the tissues were observed and statistically analyzed.

3.8. Statistical Analysis

The results were analyzed statistically by using one way analysis of variance (ANOVA) together with student t-test to test the level of significance between control, experimental and standard groups of the datum. All data were expressed as mean ± standard error of the mean (SEM). A probability of less than 0.05 with 95 % confidence interval ($P < 0.05$), and less than 0.01 with 99 % confidence interval ($P < 0.01$) were assumed to denote a significant and very significant difference respectively. The number of mice used for each group was six (n=6).

4. RESULTS

4.1. Effects of *Ricinus Communis* Seed Aqueous Extract on the Body and Genital Organs Weight of Mice

Table 1 shows the initial and final body weight in grams, and uterine and ovarian weight ratio at different treatment doses of *R.communis* and EB as compared to the control mice. Treatment with therapeutic, sub-toxic, and toxic doses of the seed aqueous extract showed no statistically significant difference in body weight in all of the treated groups. However; the weight of both uterus and ovary was significantly decreased in mice treated with sub-toxic and toxic doses of the extract but significantly increased in the standard groups (EB treated) as compared to the control mice.

Table 1: The effects of *Ricinus communis* seed aqueous extract for one week on the body and genital organs weight of mice.

Treatment (mg/kg bw)	Body Weight (gm)		Uterine/Body Weight Ratio	Ovarian /Body Weight Ratio
	Initial	Final		
control (DW)	28.99 ± 0.54	28.99 ± 0.42	4.92 ± 0.01	0.903 ± 0.006
Standard (0.00001 EB)	28.44 ± 0.94	32.09 ± 0.67	6.57 ± 0.03**	1.263 ± 0.003**
Therapeutic (0.00002 RC)	27.17 ± 0.10	27.13 ± 0.21	4.59 ± 0.05	0.894 ± 0.014
Sub-toxic (0.00004 RC)	28.32 ± 0.09	27.56 ± 0.29	3.90 ± 0.01**	0.798 ± 0.001**
Toxic (0.00008 RC)	26.52 ± 0.38	25.03 ± 0.50	3.47 ± 0.03**	0.780 ± 0.010**

** = Statistically Significant (P< 0.05) compared to control.

4.2. Effects of *Jatropha Curcas* Seed Aqueous Extract on the Body and Genital Organs Weight of Mice

Table 2 shows the initial and final body weight in grams, and uterine and ovarian weight ratio. Treatment with therapeutic, sub-toxic, and toxic doses of the extract showed no statistically significant difference in body weight among all the *J.curcas* treated groups. Nevertheless; the weight of genital organs was significantly decreased in those mice treated with toxic doses of the extract. A statistically significant increase in genital organ weight was observed in standard groups as compared to the control mice. Besides the decrease in genital organ weight was greater in *R.communis* than *J.curcas* although it was not statistically significant (See Table, 1 and 2).

Table 2: The effects of *Jatropha curcas* seed aqueous extract for one week on genital organs and body weight of mice.

Treatment (mg/kg bw)	Body Weight (gm)		Uterine /Body Weight Ratio	Ovarian /Body Weight Ratio
	Initial	Final		
Control (DW)	28.99 ± 0.54	28.99 ± 0.42	4.92 ± 0.01	0.903 ± 0.006
Standard (0.00001 EB)	28.44 ± 0.94	32.09 ± 0.67	6.57 ± 0.03 **	1.263 ± 0.003**
Therapeutic (0.0015 JC)	28.52 ± 0.19	25.80 ± 0.94	4.14 ± 0.02	0.835 ± 0.002
Sub-toxic (0.003 JC)	31.34 ± 0.29	26.77 ± 0.36	4.07 ± 0.01	0.882 ± 0.001
Toxic (0.006 JC)	27.97± 0.81	25.55 ± 0.98	3.94 ± 0.01**	0.704 ± 0.001**

** = Statistically Significant ($P < 0.05$) compared to control.

4.3. Estrogenic and Anti-estrogenic Activity of *Ricinus Communis* in Mice

The Bar graph shown in Figure 1 depicts uterine weight ratio versus treatment doses of aqueous seed extract of *R.communis* and EB in mice. Administration of EB showed a statistically significant increase in uterine wet weight but administration of *R.communis* provoked a highly dose dependent decrease in uterine wet weight as compared to control mice.

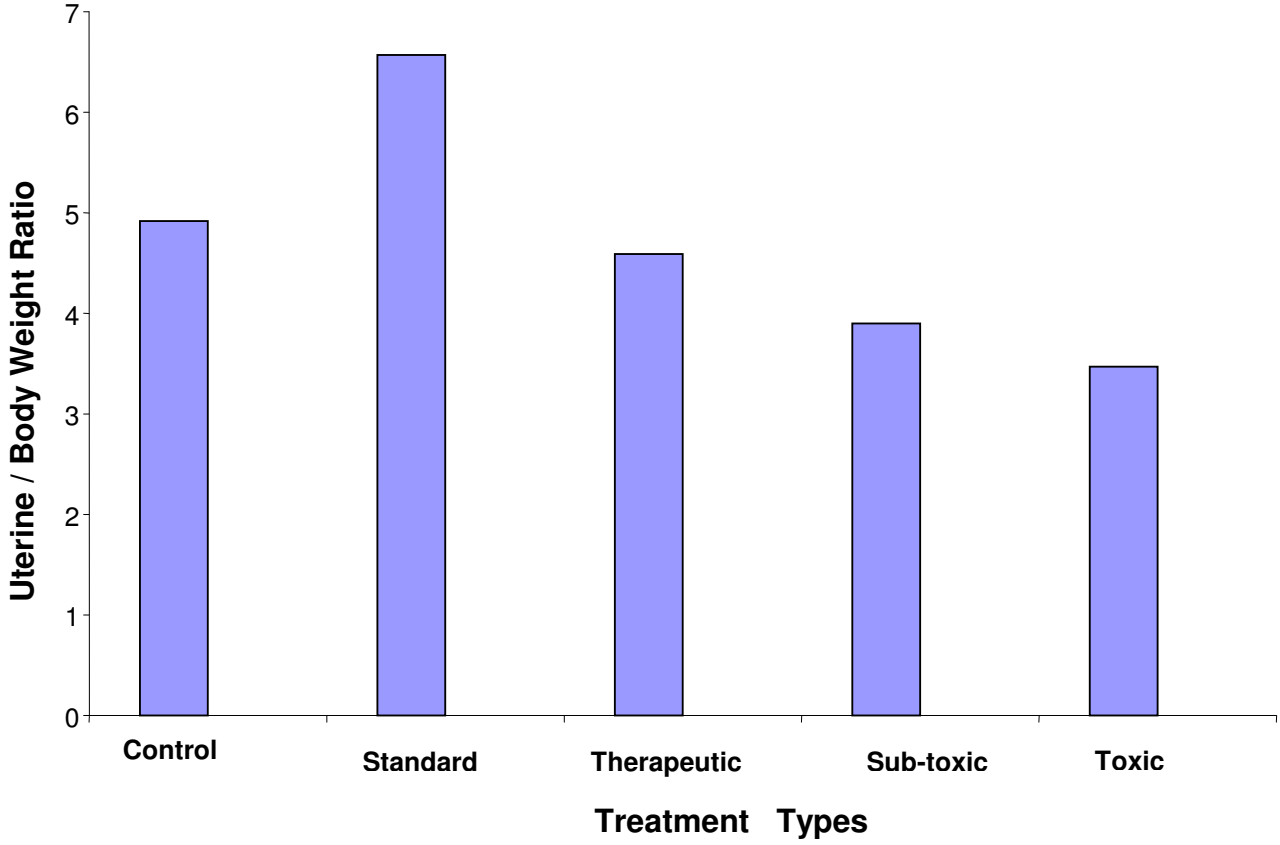


Figure 1: The estrogenic and anti-estrogenic effect of *Ricinus communis* seed aqueous extract in mice treated with the standard drug, therapeutic, sub-toxic, toxic doses as compared to the control mice. Note: the dose dependent decrease in uterine wet weight ratio.

4.4. Estrogenic and Anti-estrogenic Activity of *Jatropha curcas* in Mice

The Bar graph shown in figure 2 demonstrates uterine weight ratio versus treatment doses of aqueous seed extracts of *J.curcas* and EB in mice. Administration of EB resulted in a statistically significant increase in uterine wet weight but administration of *J.curcas* showed a dose dependent decrease in uterine wet weight as compared to the control mice. Moreover the decrease in weight was greater for *R.communis* than *J.curcas* treated groups.

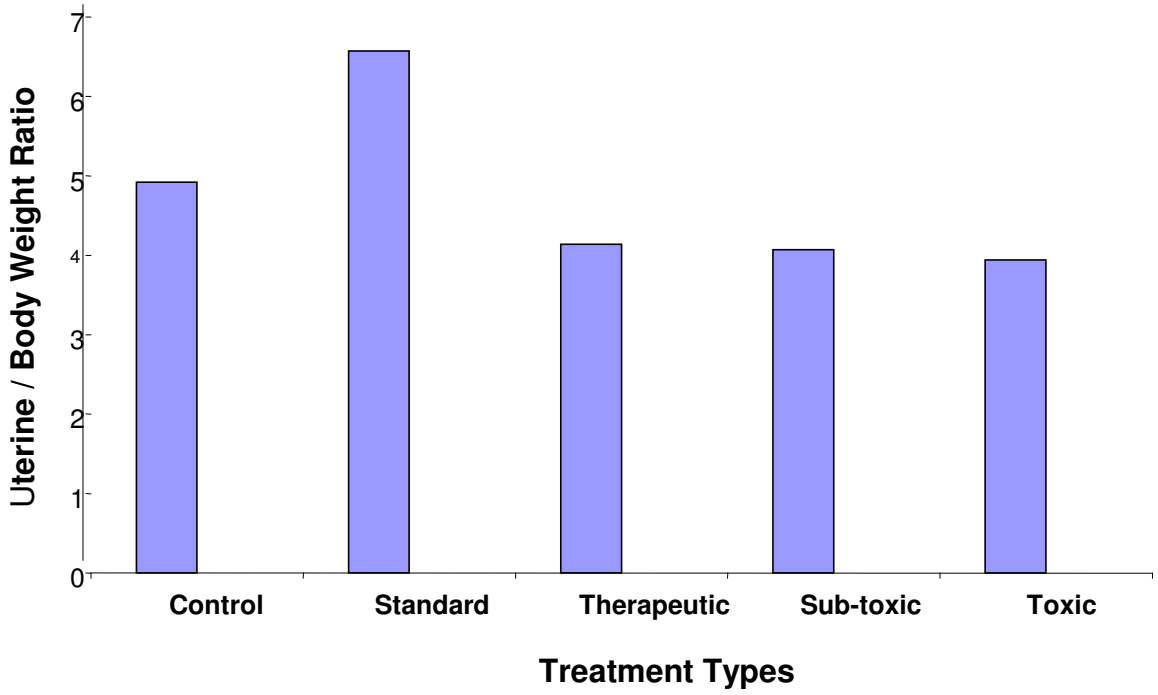


Figure 2: The estrogenic and anti-estrogenic effect of *Jatropha curcas* seed aqueous extract in mice treated with the standard drug, therapeutic, sub-toxic, toxic doses as compared to the control mice. Note: the increase in uterine wet weight in those treated with the standard drug.

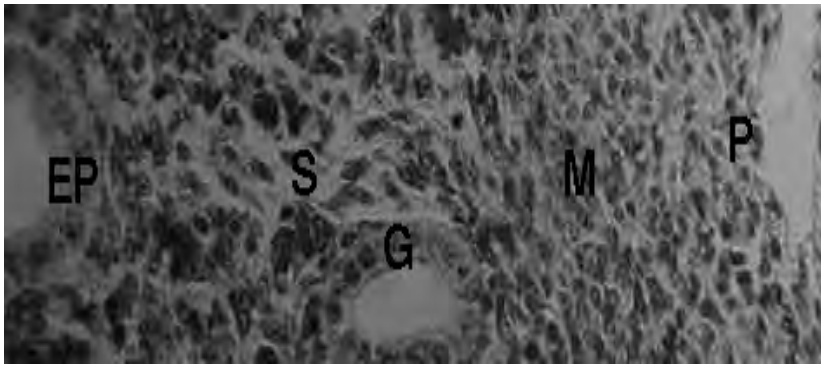
4.5. Effect of *Ricinus Communis* Seed Aqueous Extract on Uterine Histomorphometry

Table 3 and Figure 3(A-E) summarizes the morphometric measurements and histopathological effect of the extract on histology of the uterus. Histological observation of Heamatoxylin and Eosin stained sections of the control mice presented a normal structure (Fig.3A). That is, the endometrium was lined by simple columnar cells having central nuclei, the stroma with normal vascularity, the uterine glands which are simple tubular and numerous, and the lumen was wider to the treated groups. Treatment with therapeutic dose of *R.communis* seed aqueous extract did not alter uterine histomorphometry and histoarcithecture significantly. However; treatment with sub-toxic and toxic doses of the extract brought reduction in uterine epithelial cell height, endometrial thickness, and myometrial thickness significantly (Fig. 3(C, D, and E) respectively). Treatment with EB showed a significant increase in the above parameters as compared to control mice (Fig.3B). Furthermore, at toxic dose of the extract, histology revealed the presence of histopathological effect of the extract on the uterus with complex endometrial hyperplasia.
(Fig, 3E and 3E*)

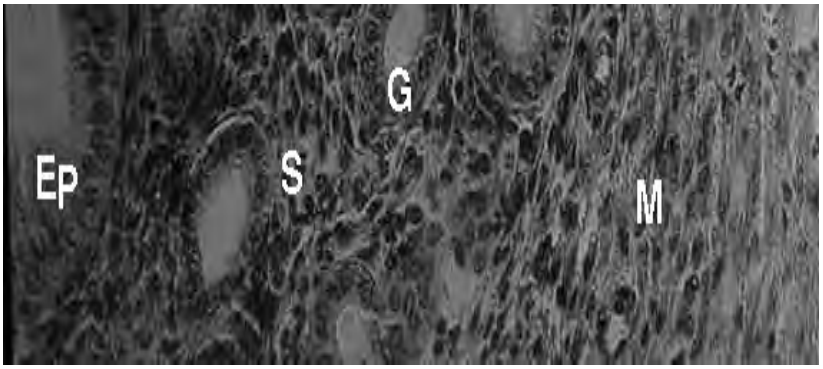
Table 3: The effect of *Ricinus communis* Seed Aqueous Extracts on Uterine Histomorphometry

Treatment (mg/kg bw)	Height of Endometrial Epithelium (µm)	Thickness of Endometrium (µm)	Thickness of Myometrium (µm)	Histopathological change (Yes or No)
Control (DW)	30.8 ± 2.72	201.6 ± 3.89	71.6 ± 3.58	No
Standard (0.00001 EB)	34.0 ± 2.53 *	294.6 ± 4.52*	181.0 ± 5.26*	No
Therapeutic (0.00002 RC)	19.7 ± 1.65	200.6 ± 6.18	53.6 ± 3.20	No
Sub-toxic (0.00004 RC)	18.3 ± 0.80*	190.2 ± 5.35*	48.3 ± 4.60*	No
Toxic (0.00008 RC)	12.0 ± 0.73*	182..6 ± 6.05*	41.0 ± 6.09*	Yes, Endometrial Hyperplasia

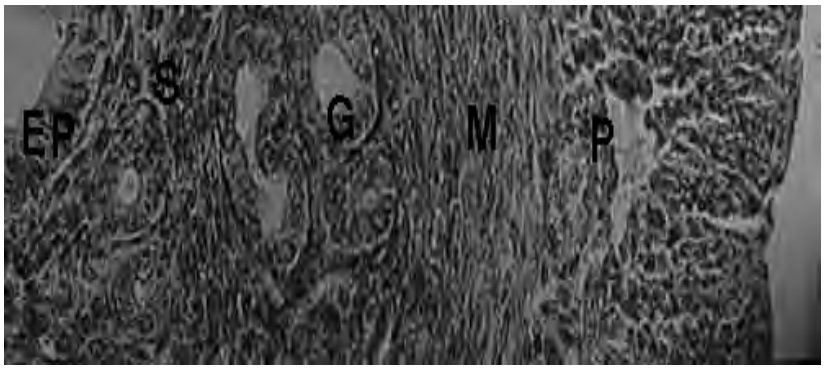
* = Statistically Significant (P< 0.01) compared to control.



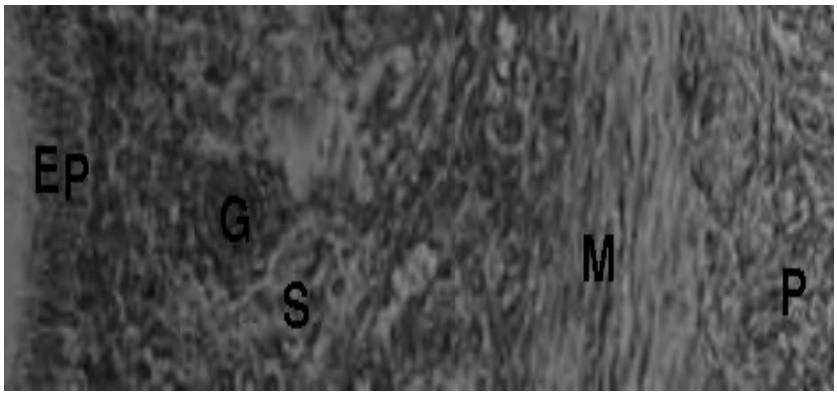
A



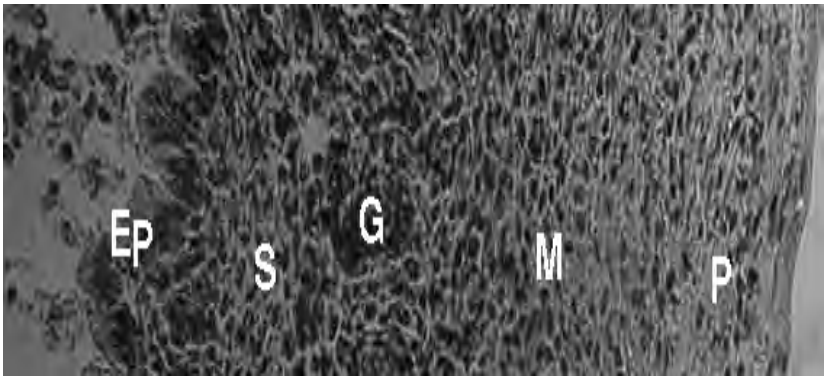
B



C

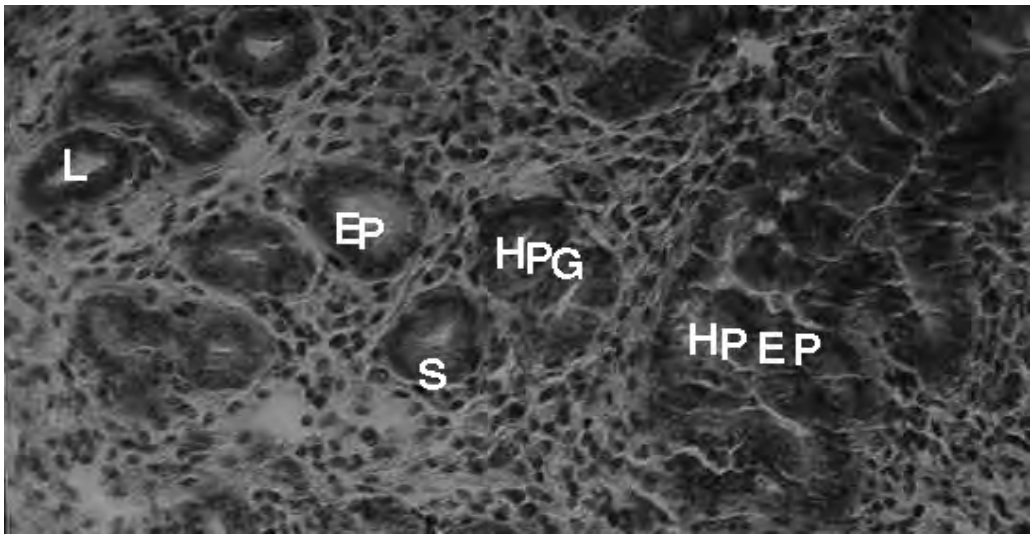


D



E

Figure 3(A-E) Haematoxylin and Eosin stained sections of mice uterus illustrating epithelial lining cells height (E), endometrial stromal thickness (S), and myometrial thickness (M), perimetrium (P), and gland (G). In the control mice (A), normal histological structure was observed. In those treated with a standard drug for 7 days, note: the increase in epithelial cells height, endometrial stromal and myometrial thickness. In those treated for the same period with therapeutic (C), sub-toxic (D), toxic (E and E*) doses of *Ricinus communis* seed aqueous extract, a dose dependent structural changes (decrease in epithelial cell height, stromal and myometrial thickness) are seen. Note: the histopathology (complex endometrial hyperplasia) of epithelium and glands in uteri treated with toxic dose of *R.communis*. Magnification for all =365X.



E*

Figure 3E*: Note the complex hyperplasia of the endometrial gland (HP G), and hyperplasia of the epithelium (HP EP). Lumen of endometrial gland (L), endometrial epithelium (EP), and stroma (S).

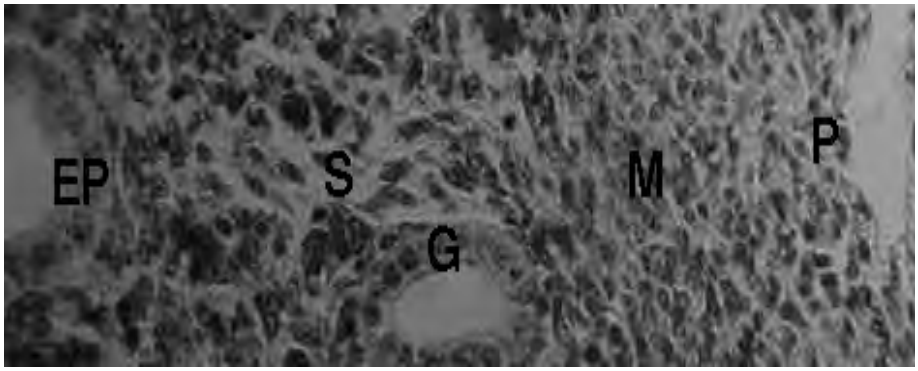
4.6. Effects of *Jatropha curcas* Seed Aqueous Extracts on Uterine Histomorphometry

Table 4 and Figure 4 (A-E) shows morphometric analysis of the uterus in *J.curcas* and EB treated mice as compared to the control mice. Almost similar findings were obtained as those of the *R.communis* treated mice (Fig. 4 (A, B, C, D, and E) respectively. But, at all doses of the extract there is no statistically significant histopathological effect on the genital organ of the mice observed. Nevertheless; decrease in epithelial cell height, endometrial and myometrial thickness was much more in *R.communis* treated mice than *J.curcas* treated mice although it failed to attain statistical significance (See Table, 3 and 4).

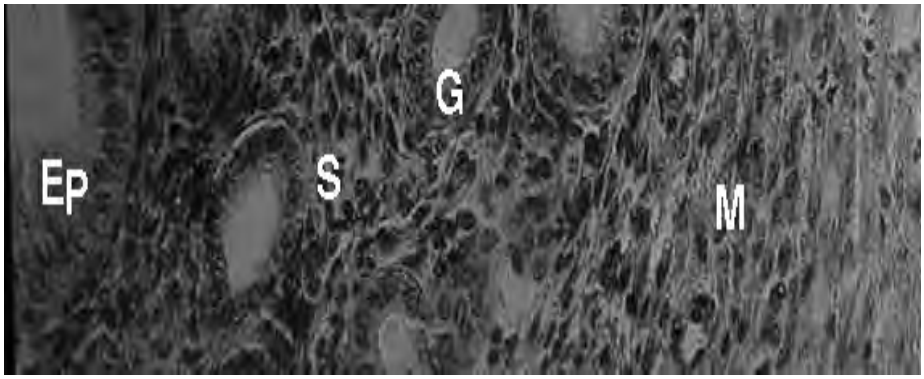
Table 4: The effect of *Jatropha curcas* Seed Aqueous Extracts on Uterine Histomorphometry

Treatment (mg/kg bw)	Height of Endometrial epithelium (µm)	Thickness of Endometrium (µm)	Thickness of Myometrium (µm)	Histopathological Change (Yes or No)
Control (DW)	30.8 ± 2.72	201.6 ± 3.89	71.6 ± 3.58	No
Standard (0.00001 JC)	34.0 ± 2.53*	294.6 ± 4.52*	181.0 ± 5.26*	No
Therapeutic (0.0015 JC)	24.8 ± 0.80	201.3 ± 4.51	58.6 ± 2 .21	No
Sub-toxic (0.003 JC)	15.6 ± 1.72	203.0 ± 2.34	48.3 ± 1.42	No
Toxic (0.006 JC)	14.8 ± 0.80*	194.6 ± 5.84*	47.00 ± 1.26*	No

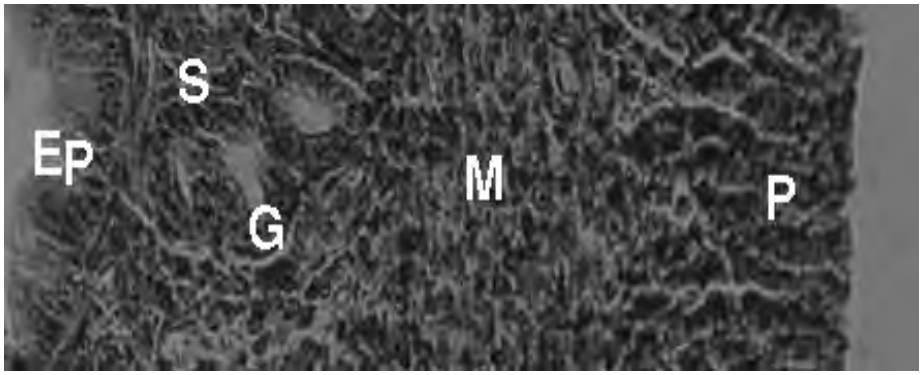
* = Statistically Significant (P< 0.01) compared to control.



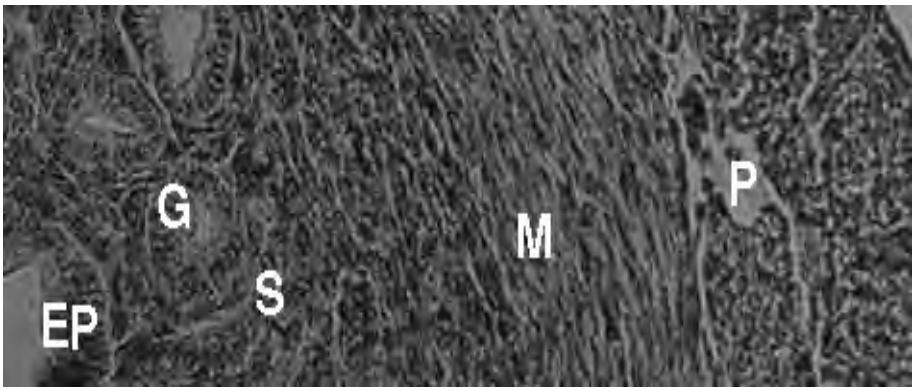
A



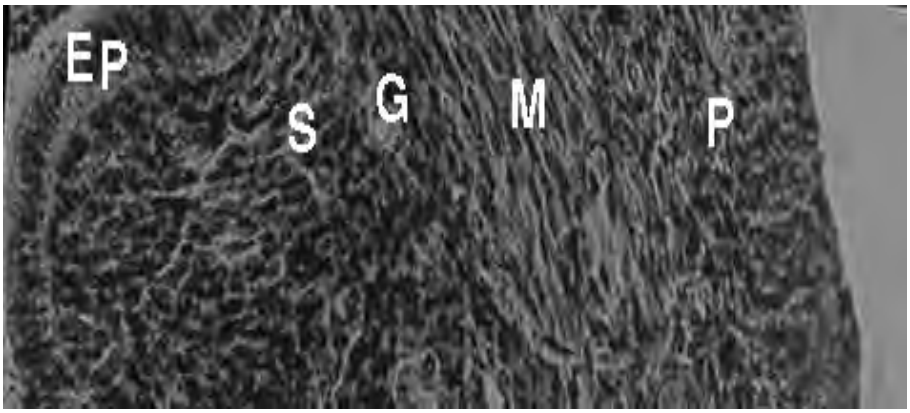
B



C



D



E

Figure 4(A-E) Haematoxylin and Eosin stained sections of mice uterus showing epithelial lining cells height (EP), endometrial stromal thickness (S), myometrial thickness (M), perimetrium (P), and glands (G). In the control (A) mice, histology reveals a normal structure. In those treated with EB (B) for one week, an increase in epithelial lining cells height, stromal and myometrial thickness worth noting. In those treated for the same period with therapeutic (C), sub-toxic (D), toxic (E), note that the epithelial lining cells height, stromal and myometrial thickness were decreased. No histopathological change of the epithelium and glands is observed at any dose. Magnification for all = 365X.

4.7. Effect of *Ricinus Communis* Seed Aqueous Extract on Ovarian Histomorphometry

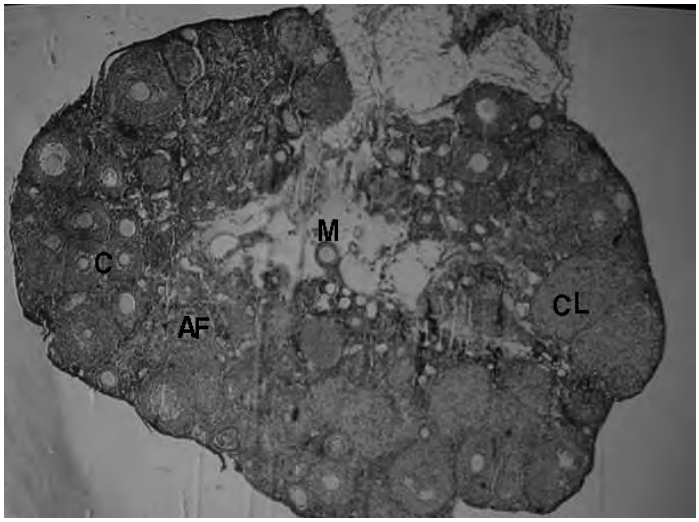
Figure 5 (A-E) and Table 5 presents histological observations of Heamatoxylin and Eosin stained sections. Morphometric analysis of ovaries of control mice showed normal structure (Fig.5A) .Treatment with therapeutic and sub-toxic doses did not provoke a statistically significant change in number of atretic follicles and corpus luteum (Fig. 5(C and D). But treatment with toxic doses of *R.communis* aqueous seed extract caused a statistically significant decrease in number of corpus luteum with a concomitant increase in number of atretic follicles (Fig.5E) and vice versa for EB treated groups (Fig. 5B). There is no statistically significant histopathological change seen histologically at all of the treatments.

Table5: The effect of *Ricinus communis* Seed Aqueous Extract on Ovarian Histomorphometry

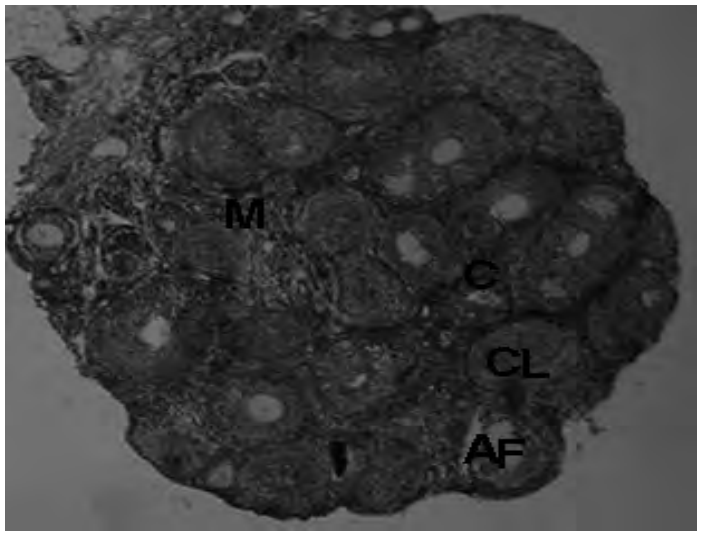
Treatment (mg/kg bw)	Number of Atretic Follicles	Number of Corpus Luteum	Histopathological Change (Yes/ No)
Control (DW)	7.5 ± 0.76	4.8 ± 0.30	No
Standard (0.00001 EB)	3.1 ± 0.47**	7.3 ± 0.66**	No
Therapeutic (0.00002 RC)	6.6 ± 0.66	4.0 ± 0.36	No
Sub-toxic (0.00004 RC)	7.6 ± 0.7	3.8 ± 0.60	No
Toxic (0.00008 RC)	8.6 ± 1.05 **	3.0 ± 0.57 **	No

* *= Statistically Significant (P< 0.05) compared to control.

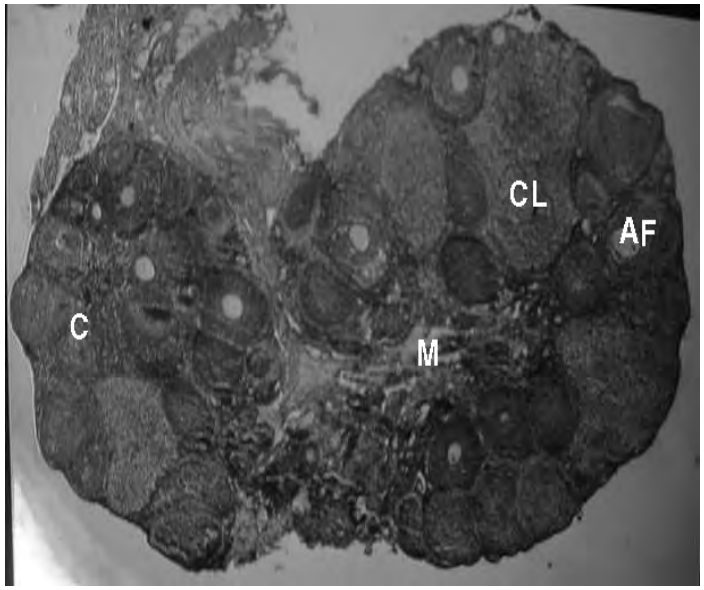
A



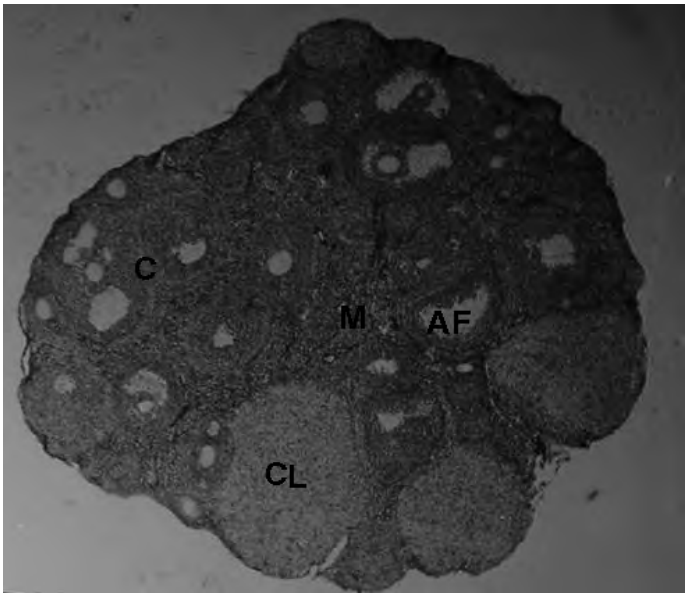
B



C



D



E

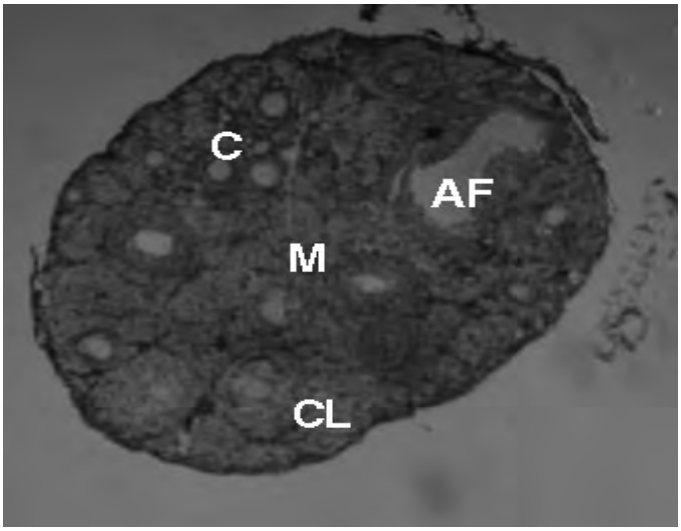


Figure 5 (A-E) Haematoxylin and Eosin stained sections of mice ovary demonstrating cortex (C), medulla (M), atretic follicles (AF), corpus luteum (CL).Obtained from controls (a) with normal structure. Treated for 7 with EB (B), therapeutic (C), sub-toxic (D), toxic (E) doses of *Ricinus communis* seed aqueous extract .Note: the increase in number of corpus luteum, but decrease in number of atretic follicles in the standard groups, in contrast to the treated groups and the absence of histopathological change at any dose. Magnification for all =50X.

4.8. Effects of *Jatropha Curcas* Seed Aqueous Extract on Ovarian Histomorphometry

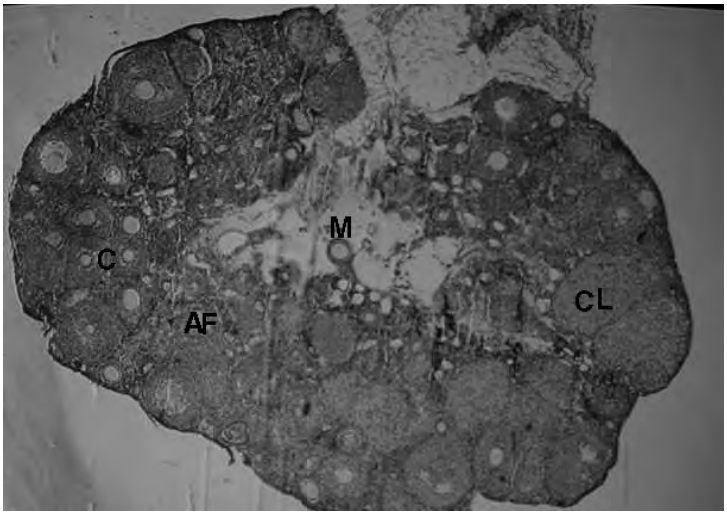
Table 6 and Fig 6 (A-E) depict microscopic study of the ovary of mice. Result showed a dose dependent increase in the number of atretic follicles and decrease in number of corpus luteum in *J.curcas* treated mice (Fig 6(C, D, and E) respectively, as opposed to EB treated once significantly (Fig 5B).

Table 6: The effects of *Jatropha curcas* Seed Aqueous Extract on Ovarian Histomorphometry.

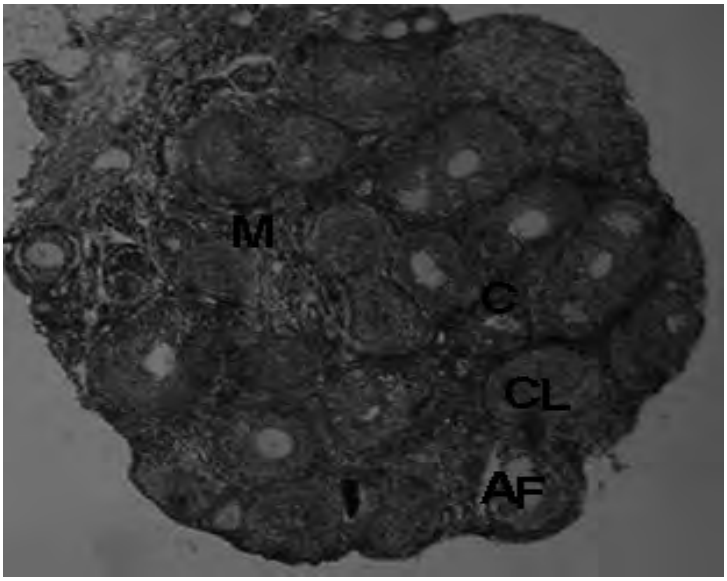
Treatment (mg/kg bw)	Number of Atretic Follicles	Number of Corpus Luteum	Histopathological Change (Yes / No)
Control (DW)	7.5 ± 0.76	4.8 ± 0.30	No
Standard (0.00001 JC)	3.1 ± 0.47**	7.3 ± 0.66**	No
Therapeutic (0.0015 JC)	7.0 ± 0.87	4.2 ± 0.47	No
Sub-toxic (0.003 JC)	7.2 ± 0.47	3.8 ± 0.54	No
Toxic (0.006 JC)	8.0 ± 1.06**	3.3 ± 0.61 **	No

* * = Statistically Significant (P< 0.05) compared to control.

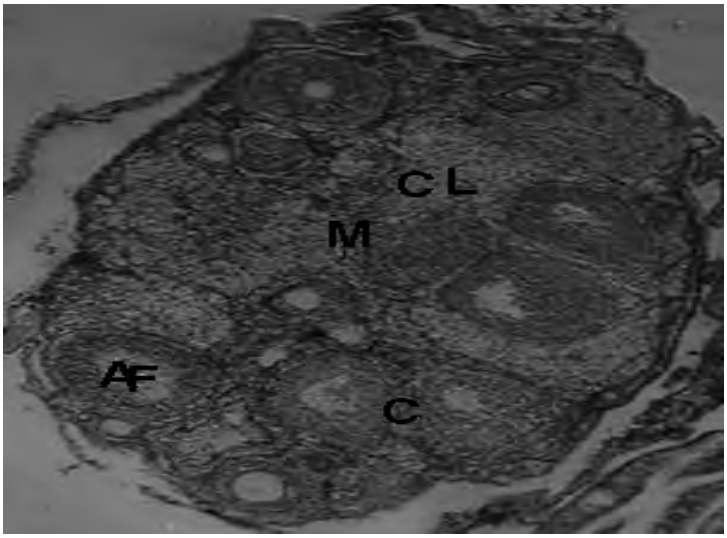
A



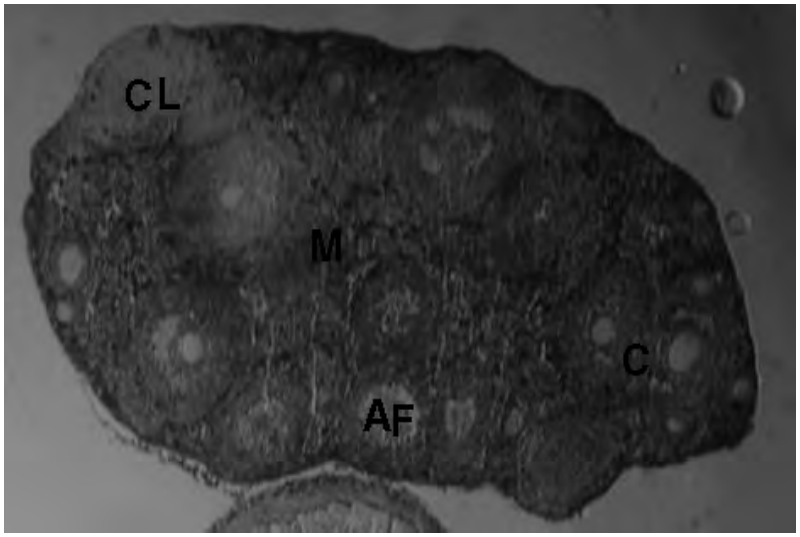
B

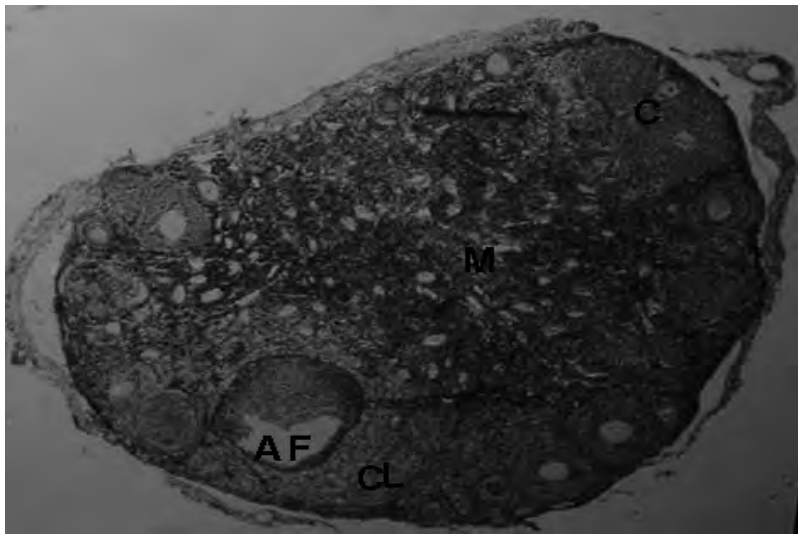


C



D





E

Fig 6 (A-B): Haematoxylin and Eosin stained sections of mice ovary illustrating the cortex (C), medulla (M), atretic follicles (AF), corpus luteum (CL). In the control mice (A) cellular organization of ovary appears normal structure. In those treated for 7 days with EB (B), the number of atretic follicles are decreased but the number of corpus luteum are increased. In those treated for the same period with therapeutic (C), sub-toxic (D), toxic (E), of *Jatropha curcas* seed aqueous extract, a dose dependent increase in number of atretic follicles but decrease in number of corpus luteum was observed. No histopathology seen. Magnification for all=50X.

4.9. The Volume Densities of Endometrial Glands of the Uterus Treated With Seed Aqueous Extract of *Ricinus communis* in Mice

Table 7 and figure 7 demonstrates the volume density (Vv) of endometrial glands (epithelium, lumen & stroma) calculated by point counting using test-system M_{42} of the mice. Results showed statistically significant differences in the stereological parameters only for those treated with toxic dose of *R.communis*. That is, Vv epithelium, Vv lumen were decreased for the toxic dose treated groups than others and vice versa for Vv stroma of the same group.

Table 7: The Volume densities of endometrial glands treated with *Ricinus communis*

Treatment (mg/kg bw)	Endometrial Gland		Endometrial Gland
	Vv Epithelium	Vv Lumen	Vv stroma
Control (DW)	26.9 ± 0.7	13.8 ± 0.7	59.3 ± 0.3
Standard (0.00001 RC)	26.6 ± 0.9	11.5 ± 0.9	61.9 ± 0.1
Therapeutic (0.00002 RC)	27.3 ± 0.8	13.0 ± 0.8	59.7 ± 0.4
Sub-toxic (0.00004 RC)	27.3 ± 0.8	11.5 ± 0.7	61.2 ± 0.5
Toxic (0.00008 RC)	22.6 ± 0.5**	9.1 ± 0.7**	68.3 ± 0.2**

* * = Statistically Significant ($P< 0.05$) compared to control.

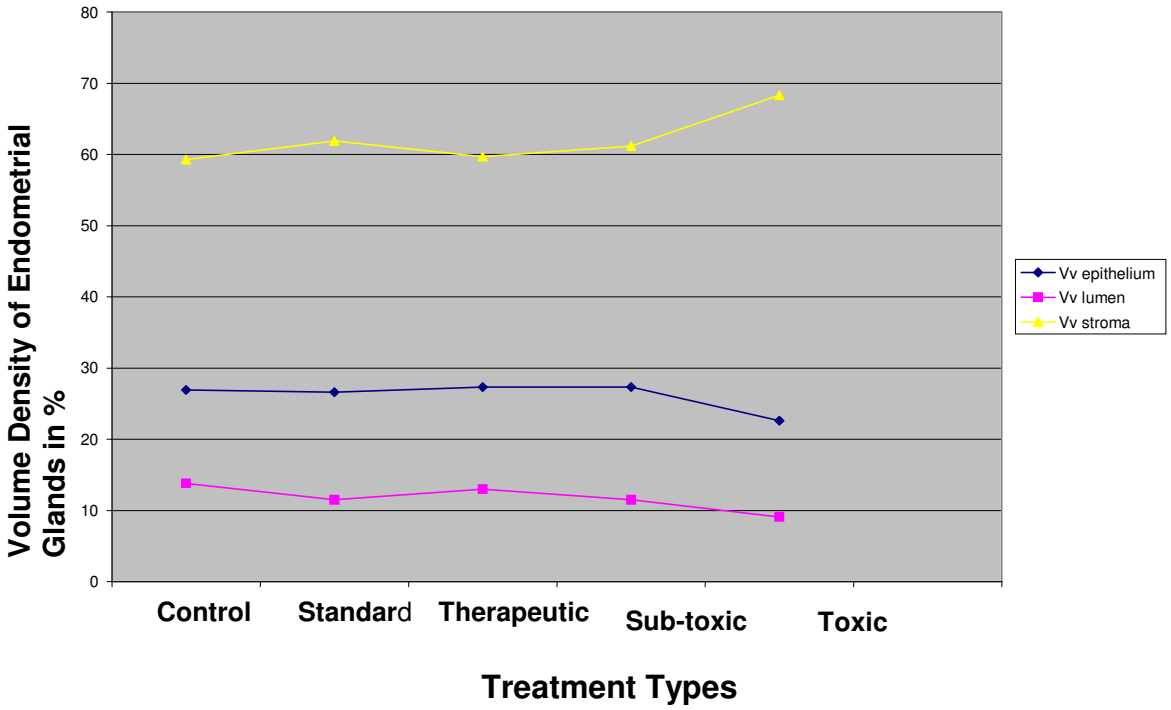


Figure 7: Line graph showing the volume densities of endometrial glands of the uterus treated with seed aqueous extract of *Ricinus communis* .Note the decrease in volume densities of the epithelium and glands at toxic dose.

4.10. The Volume Densities of Endometrial Glands of the Uterus Treated with Seed Aqueous Extract of *Jatropha curcas* in Mice

The differences in Vv epithelium, Vv lumen and Vv stroma of the endometrial glands for the *J.curcas* treated groups showed change in the stereological parameters studied in a dose dependent manner as compared to control, although it failed to attain statistical significance (See Fig , 8). Compared to *R.communis*, the Vv was smaller for *J.curcas.*

Table 8: The Volume densities of endometrial glands treated with *Jatropha curcas*

Treatment (mg/kg bw)	Endometrial Gland		Endometrial Gland
	Vv Epithelium	Vv Lumen	Vv stroma
Control (DW)	26.9 ± 0.7	13.8 ± 0.7	59.3 ± 0.3
Standard (0.00001 EB)	26.6 ± 0.9	11.5 ± 0.9	61.9 ± 0.1
Therapeutic (0.0015 JC)	26.9 ± 0.8	12.6 ± 0.7	60.5 ± 0.5
Sub-toxic (0.003 JC)	28.5 ± 0.6	11.4 ± 0.7	60.1 ± 0.4
Toxic (0.006 JC)	26.1 ± 0.8	11.0 ± 0.7	62.9 ± 0.5

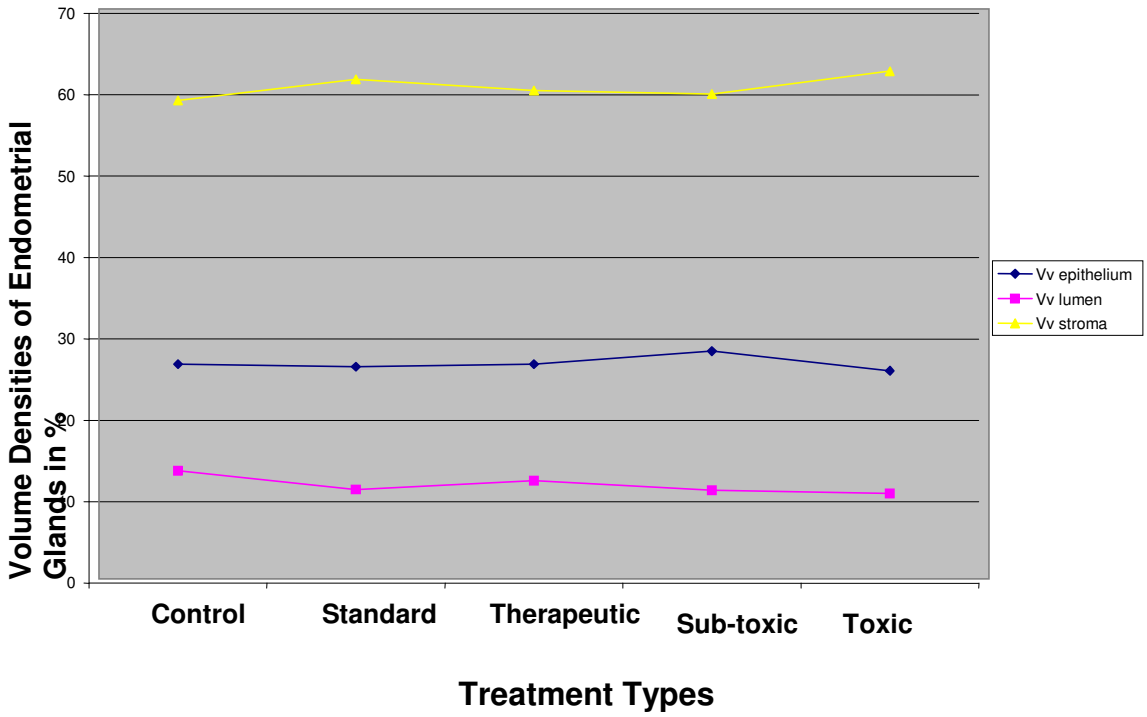


Figure 8: Line graph showing volume densities of endometrial glands of uterus treated with seed aqueous extract of *Jatropha curcas.*

5. DISCUSSIONS

Numerical control of the human population has been a major medical and social concern of our time. With population explosion exceeding the natural resource of a country, for instance in developing countries, it is very difficult to meet the demand for food of their population. In modern era, steroid hormones (estrogens and progesterone) have an edge on protein hormones as far as cellular accessibility is concerned because of their lipid solubility, steroids can transverse the plasma membrane and enter cells by simple diffusion. Once inside, steroid hormones bind to specific receptors to alter the genetic programming apparatus of their target and modify cellular function (53). Recent comparative histological and physiological analysis indicate that steroid receptors belong to a conserved family composed of three major domains; an amino terminus, a DNA-binding motif, and a carboxyl terminus steroid-binding region (54). The contraceptive potential for ovarian steroid hormones was recognized early in this century by physiologists who observed that ovulation was inhibited in the presence of the corpus luteum (55, 56). In 1950, Pincus and Chang (57) had reported an anti-ovulatory effect of oral progestin in the rabbit. Since its introduction in 1960, pill has liberated millions of women from adversities of excessive child bearing. Although steroidal hormones are crucial determinants in the regulation of normal reproductive process of the females, success of steroidal contraception in women faced some constraints. Because, the same hormones can agument begnin (localized) tumor and malignant diseases manifested by steroid sensitive organs (uterus and ovaries). Moreover, endocrine management of tumors of reproductive system involves two approaches surgical removal of gonads or hormone therapy. The pharmacological options have entailed attempts to production of pituitary gonadotrophins or interfering directly with steroid hormone action. Drawbacks of these therapies include undesirable systemic side-reactions, low efficacy, and repetitive or costly treatment regimens. These are partly due to lack of clear knowledge of the mechanism of action

of naturally or synthetically derived steroid hormones at their pharmacological doses histologically on the reproductive tissues.

This study clearly shows the fact that an endocrine system displays an elegant system of checks and balances in the form of feed back loops to facilitate the normal functioning of all body systems. In the present study, no reduction in the body weight of the mice was observed after 7 days of administration of both extracts except symptoms of diarrhea and loss of stamina were noticed at higher doses of both extracts and this was in agreement with findings of Singh Shiv Pal (33). A significant reduction in genital organ weight was noticed at sub-toxic dose of *R.communis* and toxic doses of both extracts. This may be is due to toxicity of the extracts at higher doses resulting in uterine dysfunction and follicular degeneration. Prakash and Mathur, and Dexit (35, 37) have also obtained similar results with administration of Artobotrys *odoratissimus* leaf extracts to female rats. The difference in reduction of genital organ weight between the two extracts is may be due to their difference in toxicity of the extracts in mice.

A significant increase in diestrus with concomitant decrease in oestrus phase in mice was reported by Makonnen et-al,. (15) by administration of the extracts in mice. The dose dependent decrease in wet weight ratio of the uterus indicates the weak estrogenic and strong anti-estrogenic effects of the extracts in mice. The increase in wet weight ratio of the standard groups was due to the estrogenic nature of the standard drug (58). The decrease in wet weight of ovarian ratio in treatment groups as compared to the control mice shows the anti-ovulatory effect via suppression of FSH. This explains the possible mechanism of action of these extracts is to be through the cyclic hormonal changes. That is, the extracts may act on the genital organs by causing disturbance in estrogen-progesterone ratio which is essential for implantation. This is caused by

reduced synthesis of steroids in the ovary. Hence, anti-estrogens if administered to mice can prevent the process of ovum implantation. This was in agreement with findings on *Rumex steudelli* root extract in rats (59), and on *sesban* seed extracts in mice (33). The resulting difference in anti-estrogenic effect of the extracts is may be due to species difference between the plants or difference in their active components.

The significant reduction in uterine histomorphometry at the toxic and sub-toxic dose of the extracts is may be due to anti-estrogenic, uterotropic effects of these anti-fertility agents in mice. Since estrogenic compounds increase overall uterine size and weight (60). Anti-estrogens induce uterine muscle contractions and increase sensitivity of myometrium to prostaglandin's which are involved in ovulation and luteal regression (61). This histological evidence supports the possible mechanism of action of these anti-fertility agents through inhibition of prostaglandin synthesis which disrupt the special utero-ovarian vascular connection. This connection allows the uterus of some species to communicate directly with the ovaries.

The highly deviated, very significant increase in uterine epithelial cell height, endometrial thickness, and myometrial thickness in standard groups might be because of estrogenic activity of EB, and/or interference of endogenous estrogens, since ovariectomy was not done for obvious reasons. This result may have implications for risk assessment of these anti-fertility agents prior to their administration for fertility control in women.

Furthermore, the presence of histopathological change at toxic dose of *R.communis* treated mice can be explained by the effect of the extract on hypothalamo-hypopyseal-ovarian-uterine axis. Similar findings were reported by Prakash (9) by administration of *Embelia ribes* Brum.seeds in

female albino rats. The absence of histopathological effect in *J.curcas* treated animals implies the more risk free contraceptive potential of the extract as compared to *R.communis* treated groups, and is may be due to less toxicity of *J.curcas* as compared to *R.communis*, which contain the most toxic substance to animals' Rincin (62). This further corroborates the fact that the effect of some anti-fertility agents on uterine milieu is through disturbance of hormonal balance, which is important for normal implantation of the embryo.

Plowchalk et al,.(63) have reported that the quantitative assessment of follicle number is an indicator of the normal function as well as toxic responses in the ovary, as follicles are the physiological units of the ovary. The most important controllers of the development of the follicles and corpus luteum are FSH and LH produced by basophilic cells in the pars distalis in adenohypopysis, the gonadotropes and steroid estradiol produced by granusolsa cells (64). Although all follicles are apparently exposed to the same fluctuations in these hormones, not all are equally responsive, some ovulate and others become atretic, indicating the presence of intragonadal regulatory factors which modulate the effect of these major hormones like cholesterol (65). In this study the increase in number of atretic follicles and decrease in the number of corpus luteum at toxic doses of the extracts is attributed to the damage caused by the higher doses of the extracts on these cells through action at the level of hypothalamo-pituitary-ovarian-uterine axis. Prakash et-al,.(49) have reported that, oral administration of Carbofuran to normal virgin Swiss albino mice for 30 days destroyed the endocrine homeostasis, by suppressing gonadotropic releasing hormones (FSH releasing and LH releasing factors) release, which in turn may act directly on the synthesis and secretion of gonadotropins by gonadotropes or indirectly by altering the cells resposiveness to gonadothropic releasing hormones or gonadal steroids which result in the change in the levels of FSH and LH by negative feed back

mechanisms. This also indicates the premise that these anti-fertility agents affect gonadotropin secretion via central nervous system mechanism by causing imbalance in estrogen-progesterone ratio which in fact is not due to estrogenic activity for the extracts as it would be the case for the increase in the number of corpus luteum decrease in the number of ateric follicles in the EB treated groups. The absence of histopathological change on the ovary with both extracts does not imply absence of the effect of the extracts on the ovary instead; it could be due to insensitizing follicular or estrogen and progesterone receptors which directly affect the follicullogenesis.

Stereology can be used as a complementary method in histopathological diagnosis (66). Because the difference in Vv of epithelium, lumen and stroma was found to be best discriminant factor between normal and hyperplasic endometrium as they show high correlation with endometrial pathologies (67). In the present study, the Vv stroma was increased in the toxic doses of *R. communis* treated groups than others. This difference could be explained by the increase in the number of the endometrial glands as well as hyperplasia of the epithelium, reflecting pseudostratified epithelium histoarchitecturally. But the Vv of epithelium and lumen presented an opposite tendency to both. Because in the Vv of endometrium, the densities that are related to the gland (Vv epithelium plus Vv lumen) and the stroma are complementary. This result substantiates the presence of histopathological effect at the toxic doses of the *R.communis* extract on the uterus which was found to be complex endometrial hyperplasia. This will in turn confirm the presence of disturbed hormonal balance caused by administration of the extracts in mice.

6. CONCLUSIONS

From this study the following conclusions can be drawn:

1. *Ricinus communis* and *Jatropha curcas* possess weak estrogenic and strong anti-estrogenic activities in Swiss female albino mice.
2. The effects of *Ricinus communis* and *Jatropha curcas* seed aqueous extracts on genital organs are:
    - 2.1 reductions in weight of the uterus and ovaries in Swiss albino mice.
    - 2.2 decrease in epithelial cell height, thickness of endometrium and myometrium at sub-toxic and toxic doses of *Ricinus communis* and toxic dose of *Jatropha curcas*.
    - 2.2 decrease in the number of corpus luteum and increase number of atretic follicles in a dose dependent manner in the ovary.
    - 2.3 complex endometrial hyperplasia at the toxic dose of *Ricinus communis*.
3. The possible mechanisms of action of these anti-fertility plants are:
    - 3.1 through negative feed back mechanism by causing disturbance in estrogen-progesterone ratio.
    - 3.2 by direct action on the uterus and ovaries via inhibition of prostaglandin synthesis.
    - 3.3 by desensitizing follicular or steroid hormone receptors.
    - 3.4 by combinations of the above factors.

7. RECOMMENDATIONS

1. As the present study concentrates only on the two anti-fertility plants, further studies should be pursued on others that are claimed to posses anti-fertility activities.
2. Before *Ricinus communis* and *Jatropha curcas* are used as a contraceptive, further investigations on the histochemistry and estimation of glycogen content at each doses of the extracts are necessary. It is also worth investigating the same parameters with organic solvents of the seeds in these tissues.
3. Attention should be given by the governmental, nongovernmental organizations and research institutions to combat the problem of population explosion in Ethiopia through explorations of indigenous medicinal plants, which are not yet exploited may be because of misunderstanding or lack of clear idea about their effect on the female reproductive system.
4. We also recommend that, the farmers to conserve well these folk medicinal plants for the future generations.

8. REFERENCES

(1) Wutc L.S, Jih M, Liu T, Wan Y. (1993). Differential distribution of glycocongugates in human reproductive tract. Fertility and Sterility, 59: 60-64.

(2) Ingrid and Baudeks (1997). Lectin binding patterns of porcine oviduct mucosa and endometrium during oestrus cycle. J. of Anatomy. Cambridge University Press, 190 (2): 299-305.

(3) Gunin AG (2001): Estrogen changes mitotic orientation in the uterine epithelia. European J. of obstetrics & Gynecology & reproductive biology, 97: 82-86.

(4) Gunin AG, Mashin IN, Zakharou DA (2001): Proliferation, mitosis orientation & morphogenetic changes in the uterus of mice following chronic treatment with both estrogen and glucocorticoid hormones. J. of endocrinology, 16(9): 23-31.

(5) L. Carlos J. Jose and Roberto K (1995). Basic Histology. An international 8th edition. Appleton and Lange: 423-436.

(6) Pakarinen, P.I., Lahteenmaki, P., Lehtonen, E. and Reima, I. (1998).The ultra structure of human endometrium is altered by administration of intrauterine levonorgestrel. *Hum. Reprod.,* 13 : 1846–1853.

(7) Lesson Lesson Paparo (1985).Text Book of histology.5th edition W.W Saunders Company: 462-489.

(8) Pedersen T, Peters H (1968). A proposal for classification of follicles in the mouse ovary. J.of Reprod Fertil., 17: 555–562.

(9) Prakash A.O (1979a). Effects of *Embelia ribes* Brum. extracts on the uterus of rat. A histological study. probe, 18 (3):178-184.

(10) Prakash A.O (1979 b). Uterine ponderancy in rats under the effect of *Embelia ribes*

Brum.extracts .probe, 18 (14):267-270.

(11) Riddle, John M. (1997). Eve`s herbs a history of contraception and abortion in the West. Cambridge, Harvard University Press: 32-44.

(12) Bennet, Robin Rose. Summer (1994).Wild Carrot Seeds for Herbal Contraception *Northeast Herbal Association Newsletter*, 6:32-34

(13) Farnsworth, N.R., Bingel, A.S., Cordell, G.A., Crane, F.A., Fong, H.H.S., (1975). Potential Value of Plants as Sources of New Anti-fertility Agents II', *Journal of Pharmaceutical Sciences*, 64: 717.

(14) Hughes, C.L. (1988). Phytochemical mimicry of reproductive hormones and modulation of herbivore fertility by phytoestrogens, *Environmental Health Perspectives*, 78: 21-52.

(15) Makonnen E, Zerihun L, Assefa G, and Rostom A.H (1999). Anti-fertility activity of *Ricinus communis* seed in female Guinea pigs. East African Medical Journal ,76: 335-337.

(16) Sue E, Tadesse M, and Inga H (1995). Flora of Ethiopia and Eritrea 2(2).Canellaceae to Euphorbiaceae, Uppsala Sweden: 230-241.

(17) Rostum A.H, Makonnen E, Zerihun L, and Assefa G (1998). Toxicity study of Jatropha curcas and Ricinus communis seed extracts in experimental animals. Egyptian Journal of Medical Laboratory Sciences,7(2): 93-101.

(18) United nations fund for population activities (1991).The state of world population. New York: 23-53.

(19) Singh M.M., Kamboj, V.P., Kar, A.B. (1973).Effect of some non-steroidal anti-fertility agents on histology and biochemistry of the uterus and uterine fluid during delayed implantation in rats: Contraception, 8: 235-253.

(20) Mekonnen Y, Tolera A (2000). Anti-fertility and abortificient properties of *Arthemessia afra* and *Leonotis ocymifolia*. Unpublished: 1-25.

(21) Shukla S, Mathur R, Prakash A.O (1988)..Anti-fertility profile of the aqueous extracts of *Moringa oleifera* roots.Jouranal of Ethnopharm 22: 51-62.

(22) Bharadwaj and Mathur R (1979).Anti-fertility screening of fruits *Cassia fistula* Linn. in female albino rats .Comp. Physical Ecol., 4: 277-279.

(23) Duke J.A (1985). Hand Book of Medicinal Herbs. CRC Press Inc. Bocartaton Florida: 253-254.

(24) Fransworth N (1992).The Medicinal Plants. Prachon co.Ltd.Thailand:211-252.

(25) Chaudhury R.R (1965). Plants with possible anti-fertility activity. Special report Series, 55(1).O.M.R New Delhi: 10-21.

(26) Bolt HM (1981) Metabolism of estrogens: Natural and synthetic. In: International Encyclopedia of Pharmacology & Therapeutics, Section, 106: 19-39.

(27) Nityanand, S., Chandrawati, Singh, L. Srivatsava, J.S., Kamboj, V.P (1990). Clinical evaluation of *Centchroman*: A new oral contraceptive. In: hormone antagonists for fertility regulation. ISSRF: Bombay: 223-230.

(28) Nigam,P.K,,Kamboj, V.P., Chandra,H (1985). Effect of long term centehremaon treatment on some estrogen sensitive biochemical constituents in the genital organs of female rhesus monkeys (Macaca mulatta) Contraception, 32: 483-490.

(29) Bygdeman M, Swahn ML (1985): Progesterone receptor blockage .Effect on uterine contractility and early pregnancy. Contraception, 32 (1): 45-51.

(30) Geoge. B, Alan .S, Barbara .Y, James .L (1996): Wheater`s basic histophatology 3rd edition: 194-212.

(31) Fathalla M.F (1993) .Contraception and women health. British medical bulletin 49: 245-251.

(32) Singh, M.M., Wadhwa, V,, Kamboj, V.P (1982). Effect of Centohromanon ovarian Responsiveness to gonadtrophins in immature female rats. J. Exp Biol , 20 : 448-451

(33) Singh Shiv Pal (1990). Fertility control of female through Sesbania sesban seeds. The Journal of Research and Education in Indian Medicine, 9: 27-32.

(34) Munishi, S.R and Rao,S.S (1972).Anti-fertility activity of an indigenous plant preparation ROC-101.effect on reproduction. Indian J. Med. Res., 60:1 054-1060

(35) Prakash A.O, and Mathur, R (1977b). Anti-fertility investigations on the green leaves of *Artobotrys odoratissimus* Linn. in female albino rats.Probe,16 (2):115-118.

(36) Kaushic C and Upadhyay, S (1995): Mode of long term anti-fertility effect of intrauterine neem treatment .contraception, 51(3): 203- 207

(37) Dexit, V.P (1977).Effects of chronically administered on *Malva viscus* conzatti flower extract on the female genital tract of Gerbil, Merionis Hurrianae, Jerdon Indian J. Exp.biol., Biol., 15(8):1650-1652.

(38) Bharadwaj and Mathur R (1979). Anti-fertility screening of fruits *Cassia fistula* Linn. in female albino rats .Comp. Physical Ecol. 4: 277-279.

(39) Bharadwaj.B, Goshi, C.R, Bose A.N, Moza B.K and Basu B.P (1986). Anti-fertility activity of some medicinal plants Indian J.Exp.Biol., 6 : 252-253.

(40) Argawal .O, Bhardwaj, S and Mathur, R (1980). Anti-fertility effect of *Juniperus communis*. Planta Medica : 98-101.

(41) Singh MM, kamboji Vp (1980): Estrogenic and anti-estrogenic activities of two non-

steroidal anti-fertility agents. Endokrinologie J. 76(1): 38-44.

(42) Chak, T.M., Dua, PR., Kar, K. Srimal, R.C.Dhawan, B.N (1977): Acute toxicity and pharmacology of Centohroman. Indian J Exp Biol .15: 1159-1161

(43) Reed, AH, Rakoff AE, Kramer S (1965): A histologic study of the effects of low dosage irradiation of rabbit ovaries. Fertil Steril 16: 370–381.

(44) Chakrabotti, B Chaudhury, A and Chaudhury R.R (1968). Anti-fertility effect of green leaves of *Artobotrys odoratissimus*.J. Indian Med. Asso. 51 (5): 227-229.

(45) Ankobonggo W.W (1987).Pilot study of the anti-fertility effects of the extracts from the roots *Abrus canseus* and *Eriosema psoralioiedes* in Guinea pigs. Sc.Tech.Pub.Series 235: 138-147.

(46) Vogel, h.G. (1997).0varian hormones. Pharmacological Assay. 2nd edition. Springer, Berlin, Hideelberg; 637-645.

(47) Bedi KS, Campbell LF, Mayhew TM (1992): A fractionator study of the effects of under nutrition during early life on the rat Purkinje cell numbers (with a caveat on the use of nucleoli as a counting units) .J. of anatomy, 181:1999-208.

(48) Nephew Kenneth P, Elizabeth Osbrone, Ronald A.lubet, Clinton J.Grubbs and sohaib A.khan (2000): Effects of oral administration ofTamoxifen, Toremifew,Dehydro Epiandrosterone, and Vorozole on uterine histomorphology in the rat. Experimental Biology and medicine, 223: 288-294.

(49) Prakash N., Balligar & Basappa B, Katwal (2002): Reproductive toxicity of Carbofuran to the female mice. Effects on estrus cycle & follicles. Industrial Health.40: 345-352.

(50) Osman P (1985): Rate and course of atrasia during follicular development in the adult cycle rat. J Reprod Fert, 73: 261–270.

(51) Weibel, E.R. (1979): Stereological methods. *In*: *Practical methods for biological morphometry*. London: Academic Press: 1-27.

(52) Artacho-Pérula, E.M.(1993): Histomorphometry of normal and abnormal endometrial samples. *Int. J. Gynecol. Pathol*., *12*: 173-179.

(53) Ray, S. Grover, P.K. Kamboj, V.P. Setty, B.S., Kar, A.B. An and, N. Anti-fertility agents 12.(1976): Structure- activity relationship of 3,4-dephenyl chromense and-chromans. J .of Med Chem , 19: 276-279.

(54) Bygdeman M, Swahn ML (1985). Progestrone receptor blockage. Effect on uterine contractility and early pregnancy, 32(1): 45-51.

(55) Hirshfield AN (1990).Development of follicles in the mammalian ovary. Int.Rev.cytol, 124 : 43-100.

(56) Dorrington JH, Chuma AV, Bendel JJ (1988).Transforming growth factor and follicle stimulating hormone promote rat granulose cell proliferation. Endocr.123: 353-359.

(57) Pinus and Chang (1997): Differential follicle counts a screen for chemically induced ovarian toxicity in mice. Results from continuous breeding Bioassays.Fund Appl Toxicol. , 39: 1–10.

(58) Ulmann a. (1987) .The antiprogestin, recent advances in fertility regulation J. of Steroid Biochem, 27:1009-1012.

(59) Endalk Gebre (2003). Efficacy and safety evaluation of traditionally used anti-fertilty plant: *Rumex steudellii*. Unpublished: 31-40.

(60) Nwafor, P.A, Okwuasaba, F.K and Onoruvwe, O.O (1998).Contraceptive and non-estrogenic effects of methanolic extract of *Asparagus pubescens* root in experimental animals. J. of Ethnopharmacology, 62(2): 117-122.

(61) Prapas,P.A (1987).Inhibition of egg development and implantion in rats after post-coital administration of the progesterone antagonist Ru-486.Journal of reproduction and fertility. 80(2): 487-491.

(62) Knight, B. (1979). Ricin, a potent homicidal poison. Br. Med. J. 278: 350-351.

(63) Plowchalk DR, Smith BJ, Mattison DR (1993). Assesment of toxicity to the ovary using follicle quantification and morphometrics. In: Methods in toxicity: Female Reproductive toxicity, editions by Heindel JJ, Chapin RE, Vol.3: 57-68.

(64) Evans ACO, Kumar CM, Wandji SA, Fortune JE (1997) .Changes in androgen Secretion and lutinizing hormone pulse amplitude are associated with recruitment and Growth of ovarian follicles during luteal phase of the bovine estrus cycle. Biol. Reprod. 57:394-401.

(65) Goldman JM, Cooper RL, Laws SC (1990). Chlordimeform-induced alterations in endocrine regulation within the male rat reproductive system. Toxicol Appl. Pharmacol. 22: 467-472.

(66) Baak, J.P *(1977)*: Quantitative morphology: methods and materials I. Stereology and morphometry. *Europ. J. Obstet. Gynec. Reprod. Biol.*, *7(1)*: 43-52.

(67) Elyzabeth Avud-portari, Nadia D.Gomes, Carlos A, mandarim de-lacerda (2003). Simple hyperplasia vs proloferative endometrium, stereological study .J .bras.pathol.med lab., 39 (1): 25-39.