EVALUATION AND COMPARISON OF *TRIGONELLA FOENUM GRAECAUM* (FENUGREEK) AND CONTRACEPTIVE PILLS ANTIFERTILITY EFFECT IN FEMALE RATS

BY

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A dissertation submitted in fulfilment of the requirement for the degree of Master of Medical Sciences

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SEPTEMBER 2015

ABSTRACT

Antifertility agents are one of the most common medication used in this modern era. With advances in medical sciences, many natural compounds are also used. One of these herbal plants is *Trigonella foenum-graecum* (fenugreek). The purpose of this study was to evaluate the anti-fertility effects of fenugreek seeds aqueous (FSA) extract on female rats by determining serum reproductive hormone concentrations and observing their uterus tissue histology, as well as determining the thickness of endometrium uterus, the uterine weight and the implantation rate to compare the effectiveness of fenugreek with marketed combined oral contraceptive pills (COCPs). Twenty four 8-week-old Sprague Dawley rats divided into four groups (A, B, C and D). Animals in Group A (positive control group) were not given any treatment and therefore the rats were pregnant. Groups B (negative control group) were not given any treatment and therefore the rats were not pregnant. Group C (treatment group) were administered orally with 0.05 mg/kg body weight of COCPs. Group C (treatment group) were administered orally with 750 mg/kg body weight of FSA extract for 15 days. Daily vaginal smear cytology was examined. Blood samples were taken by retro orbital technique on day 16 for evaluation of the reproductive hormones assay. Uteruses of the animals were removed for histological study. Administration of 0.05 of COCPs led to decrease the serum FSH concentration to 71.56 ng/ml while 750 mg/kg FSA extract led to decrease it to 25.62 ng/ml which shows that FSA extract effective three times more than COCPs. The endometrium thickness of the uterus in the FSA extract treated group was normal while in the COCPs increased. The uterine weight in the FSA extract treated group was normal while the COCPs treated group was insignificantly high. The uterus tissues in the FSA extract treated group was normal while in the COCPs treated group, there were histopathological changes in the uterine tissues; the glands distribution decrease which a remarkable sign of atrophic endometrium and there were abnormal deciduiod cells (decidual like cells).

ملخص البحث

على الرغم من الاستعمالات المعاصره لطرق منع الحمل، لا زال بعض النسوة والأجيال متعاقبة يعتمدن بعض النباتات كمضادات للخصوبة. من هذه النباتات المستعمله هي (الحلبة). تهدف هذه الدراسه الى تقييم تأثير المستخلص المائي لحبوب الحلبة على خصوبة اناث الجرذان وذلك بقياس مستوى هرمون التكاثر في مصل الدم , فحص التركيب النسيجي للرحم وتحديد سماكة الطبقة الداخلية للرحم ووزن الرحم وعدد الأجنة ومقارنة فعالية الحلبة بمانع حمل من المتواجد في الأسواق. شملت هذه الدراسة ٢٤من الجرذان (بعمر ٨ أسابيع). قسمت هذه الجرذان الى اربعه مجوعات (أ, ب, ج, د). المجموعة أ (المجموعه الضابطه الموجبة) لم تعطى هذه المجموعة أي معالجة وكانت حوامل. المجموعة ب أ (المجموعه الضابطه السالبة) لم تعطى هذه المجموعة أي معالجة ولم تكن حوامل. المجموعة ج أعطيت مانع حمل من المتواجد في الأسواق ٠.٠٥ ملغ/كغم والمجموعة د أعطيت من المستخلص المائي لحبوب حلبة ٧٥٠ ملغ/كغم بواسطه الفم لمده ١٥ يوم. درست المسحه المهلبيه يوميا ثم اخذت عينات من الدم في اليوم السادس العشر لفحص هرمون التكاثر المحرض لنمو الحويصلات وتم رفع المبيض للدراسه النسيجية ثم اوقفت المعامله لمدة يوم ١٥ وبعدها قتلت الحيوانات لدراسه الهرمونات والتركيب النسيجي للرحم. أدت المعامله ب ٧٥٠ ملغ/كغم من مستخلص الحلبه الى إنخفاض الهرمون المحرض لنمو ثلاث مرات أكثر من مانع الحمل المتوافر بالأسواق. تم أخذ رحم الجرذان. إزداد وزن الرحم في المجموعة التي أعطيت مانع حمل من الأسواق والمجموعة التي أعطيت حلبة كان وزن الرحم طبيعيا. سماكة الطبقة الداخلية للرحم في المجموعة التي أعطيت مانع حمل من الأسواق إزدادت والمجموعة التي أعطيت حلبة كانت سماكة الطبقة الداخلية للرحم طبيعية. كان هنالك تغيرات في أنسجة الرحم للمجموعة التي أعطيت مانع حمل من الأسواق فقد قل عدد الغدد وهذه علامة على ضمور بطانة الرحم وكان هنالك خلايا غير طبيعية في بطانة الرحم.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Medical Sciences.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Medical Sciences.

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Azmi Md Nor
Dean, Kulliyyah of Medicine

DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Ayah Rebhi Mohammad Hilles

Signature……. Date ….....

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

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Affirmed by Ayah Rebhi Mohammad Hilles

……..……..…… ……..
Signature Date

ACKNOWLEDGEMENTS

The real of infinite and grateful praises, thanks to **ALLAH** for all this gift thus this text would not have been possible without the help of **ALLAH** under whose blessing and guidance I have completed this dissertation as a requirement for my Master of Medical Sciences (MMDSc) and may His peace and blessings be upon our beloved Prophet Mohammad. Almighty **ALLAH** was the most merciful in providing me the opportunity to have worked with great people who have contributed in various ways to my research and thesis that deserve special mention.

First and foremost, special thanks goes to my parents, my Father Rebhi Mohammad Hilles, my Mother Amaal Saqer Hilles, who have been my backbone, source of motivation and courage, to keep me moving forward, they brought me up to reach this level. The preparation of this research was a challenging time in my life. I would not have succeeded in anything without my parents who made everything possible. I dedicate this research to them, without their support, this research would not have seen the light. I would like to extended heartfelt gratitude to my siblings; Basil, Nazik, Saeed, Masood and Yara, who have gave me effective incentive and encouragement which kept me motivated, sane and on schedule to complete this research. Without their support and pep talks, this research and thesis would not have been possible. My deepest gratitude to all my other family members especially Aunty Wafaa, my all colleagues and friends especially my best friends Ghadeer, Menna and Sayyada for all their support and encouragement during my master journey.

Secondly, it was not possible for this work to be real, in this form, without the experience and confident guidance of my supervisor Assistant Prof. Dr. Ahmed Kaid Naji Allow, who has shown faith in me and my abilities as a student from day one. Without his assistance, this research would not have been possible, my highest appreciation goes to him. My heartfelt gratitude also goes to my co-supervisor Prof. Dr. Anil Kumar Saxena, who has given me support and encouragement. Without his promptness in reading and commenting, this research would not have come to fruition.

My greatest appreciation flows to all of the Postgraduate Office, helpful lecturers and staff at Department of Basic Medical Sciences (Kulliyyah of Medicine) who contributed their experiences to help me through this project particularly to all the technical stuff who have helped me with their skillful knowledge, the challenging in the lab work became much easier.

I would like to express my deepest gratitude to Syed Mahmood Al-Jafri, who has helped me throughout my research with preparing the extract, using the instruments and guiding in so many other things. I could not have imagined having a better advisor and mentor for the research part I have done in Kulliyyah of Pharmacy. His continuous support, immense knowledge, motivation and guidance gave a real value to my research. Words cannot express how grateful I am to him and I will be grateful forever. I have been blessed from ALLAH to have him on my way.

Last but not least, I wish this piece of work will be beneficial to the Ummah and will encourage other fruitful work in the future.

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LIST OF ABBREVIATIONS

FSA	Fenugreek Seeds Aqueous
FSH	Follicle Stimulating Hormone
gm	Gram
kg	Kilogram
GnRH	Gonadotropin-Releasing Hormone
H&E	Hematoxylin and Eosin
IIUM	International Islamic University Malaysia
KOP	Kulliyyah of Pharmacy
LD_{50}	Lethal Dose 50
LH	Luteinizing Hormone
mg/kg	Milligram per kilogram
COCPs	Combined Oral Contraceptive Pills
SD	Standard Deviation
SDF	Soluble Dietary Fiber
HTGL	Hepatic Triglyceride Lipase
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
VLDL	Very Low Density Lipoprotein
NWHRCI	National Women's Health Resource Center Inc.
CRP	C - reactive protein
SD	Standard Deviation

LIST OF SYMBOLS

%	Percentage
ºC	Degree Celsius
+	Plus
=	Equal to
<	Less than
>	More than
±	Plus minus

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Every year in the developing countries, one out of eight women aged 15 to 49 gets pregnant. Two-fifths of these pregnancies which is 75 million in total are unintended. Unintended pregnancies occur among women who had wanted to delay having a child or who did not want to become pregnant at all. It carries a lot of risks to a woman's health, and in turn, it also endangers the well-being of her family and her children. Moreover, it also may hinder a woman's ability to continue the education, contribute in formal labor, and participate fully in her community. Yet, in 2008, 26% (215 million) of the 818 million women in developing countries who wanted to avoid pregnancy, become pregnant either because they were not using any contraceptive method or they were using the traditional ones. These women accounted for 82% of all unintended pregnancies (Figure 1.1). The remaining unintended pregnancies occurred among the 603 million women who were using a modern contraceptive and conceived because they had difficulty using their method correctly and consistently or because of method failure. (Singh S et al., 2009).The number of unintended pregnancies in developing countries can be decreased by 71%, from 75 million to 22 million annually if all women who want to avoid unintended pregnancy, use modern contraceptives, (Cates W et al., 2010).

The impact on women, their families and their countries would be great: There would be 22 million fewer unplanned births and 25 million fewer induced abortions, which in turn would result in 15 million fewer unsafe abortions, 90,000 fewer

maternal deaths and 390,000 fewer children who would lose their mothers. (Cleland J et al., 2006).

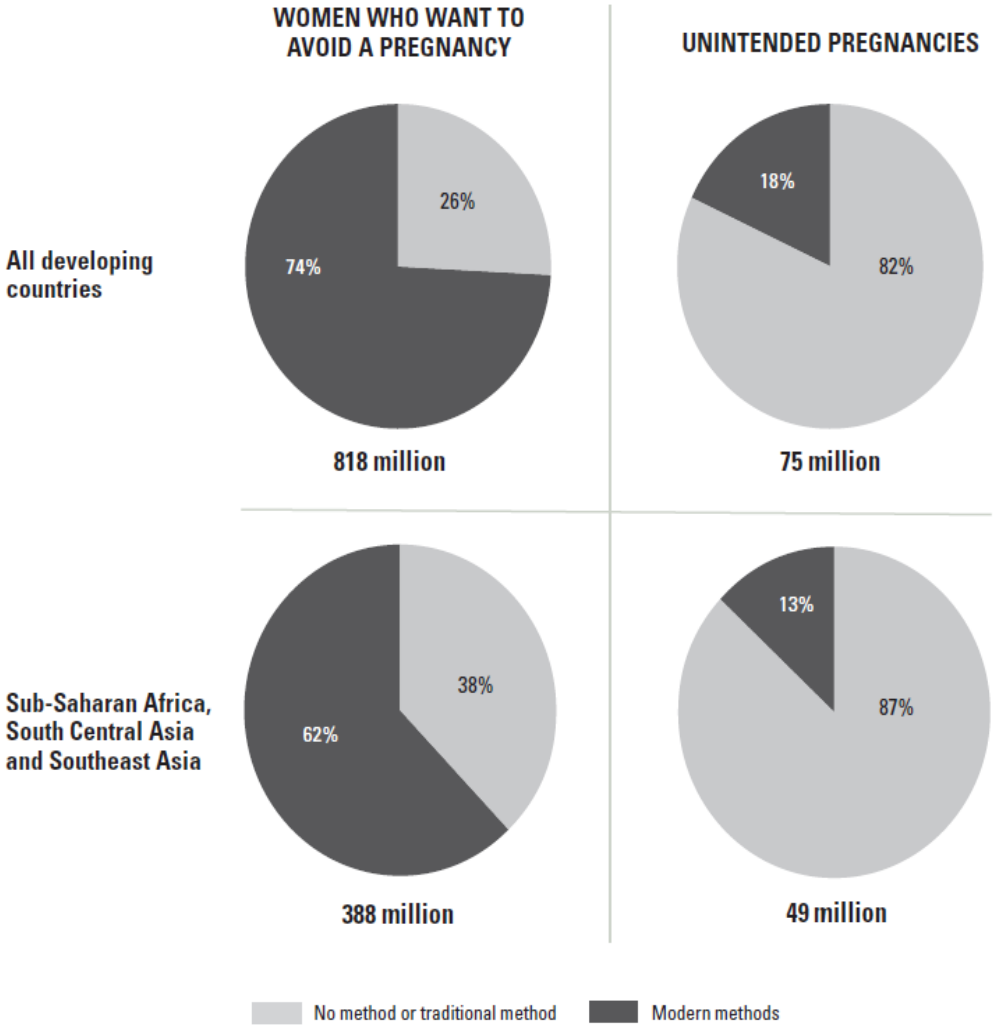

Figure 1.1 Women who want to avoid pregnancy but do not use a modern method account for a disproportionate majority of unintended pregnancies.

For decades, calls for more acceptable family planning options for women and men have been hindered by the inadequate attention and resources afforded to the contraceptive research and development field, as well as by a shift in the focus of remaining efforts from discovery to adaptation. (Harper MJK, 2005).

Most of today's modern methods including hormonal methods, oral contraceptive pills, injectable contraceptives, intrauterine devices (IUDs), non-hospital methods for vasectomy and tubal ligation depend on mechanisms of action discovered before 1960 and on forms of delivery developed during the contraceptive revolution of the 1960s and early 1970s. (Darroch JE, 2007).

Contraceptive methods that have emerged over the last 20 years or so—such as contraceptive implants, patches, female condoms, copper and hormonal IUDs, vaginal rings and newer vaginal barrier methods—have taken longer than predicted to arrive on the market, in large part because contraceptive development has garnered less attention and funding in the public and private sectors than it did during the period of greatest contraceptive innovation and what make the attention more less is the adverse effects of standard contraceptive pills which create a vital need to produce new contraceptive technologies which might be more satisfactory than existing methods for many women, and they might alleviate some of the difficulties related to access and use (Burkman, 2011).

Several factors make this a good time to reinvigorate the field of contraceptive research and development. These include an increasing understanding among international agencies and policymakers of the economic, social and personal costs of unintended pregnancy, and growing commitment to achieving universal access to family planning; (Cohen SA, 2011) the expansion of consumer markets in developing countries, which represent potentially profitable new markets for contraceptive products ;(Sharma S, 2005) a rising demand for contraceptives, because the number of reproductive age women is increasing in developing countries, and because the desire for smaller families and levels of premarital sexual activity are growing in some regions ;(Ross J, 2009) and advances in scientific knowledge and tools, which pave

the way for the development of innovative contraceptive methods. (Harper MJK, 2005) and (Nass SL, 2004).

Women and their partners face all manner of barriers to modern method use, from access issues and misinformation to inequities in social and sexual relationships, and not all of these can be overcome by developing new forms of birth control. Yet, there is an ongoing, vital need to produce new contraceptive methods. What is needed is a broad array of contraceptive methods that require less complex distribution system, safer and less discomforting than current methods; that combine high acceptability with high continuity of use; and that are suited to the diverse requirements imposed by the variety of conditions throughout the world. (Greep RO, 1976).

1.2 PROBLEM STATEMENT

Recently, oral contraceptive pills (OCPs) is the most common contraceptive method used worldwide (Shufelt, 2009). However, using OCPs has a lot of adverse effects such as venous thrombosis, myocardial infarction and worsening migraine (Sabatini, 2011). Therefore, some women still rely on traditional herbs for family planning based on its common use for many year. These herbs are more affordable, easily available, and believed to cause fewer side effects. One of these herbs is *Trigonella foenum-graecum* (fenugreek), which is a famous an annual plant, cultivated worldwide as a semiarid crop. It has potential antifertility effect (Kassem, 2006) with minimal side effects and lower cost of production. The aim of ongoing research to focus on the potent anti-fertility activity of fenugreek by comparing the effectiveness between fenugreek seeds aqueous extract with stantard combined oral contraceptive pills (COCPs) to determine if fenugreek seeds can be as effective as COCPs.

1.3 GENERAL OBJECTIVE

Evaluation of the potential antifertility activity of fenugreek seeds (*Trigonella Foenum Graecum*) aqueous extract (FSA extract) compared with stantard combined oral contraceptive pills (COCPs) in female rats.

1.4 SPECIFIC OBJECTIVES

- To use the dose of 750 mg/kg of *Trigonella Foenum Graecum* aqueous extract which is below the safety limit dose to determine the optimal effective dose of possible anti-fertility effects.
- To investigate the effect of *Trigonella Foenum Graecum* aqueous extract compared with stantard combined oral contraceptive pills (COCPs) on the serum concentration of reproductive hormone; Follicle Stimulating Hormone (FSH).
- To study the differences of the uterine weight in the normal control group, FSA extract treated group and COCPs treated group.
- To observe the effect of *Trigonella Foenum Graecum* aqueous extract compared with stantard combined oral contraceptive pills (COCPs) on the implantation rate.
- To evaluate the endometrium uterine thickness in the normal control group, FSA extract treated group and COCPs treated group.
- To calculate the endometrium gland duct density in the normal control group, FSA extract treated group and COCPs treated group.

- To study the effect of *Trigonella Foenum Graecum* aqueous extract compared with stantard combined oral contraceptive pills (COCPs) on the uterus by histology of uterus tissues.

1.5 HYPOTHESIS

- *Trigonella Foenum Graecum* aqueous extract has significant anti-fertility effect on the female rats.
- *Trigonella Foenum Graecum* aqueous extract has significant anti-fertility effect on the serum concentration of reproductive hormones; Follicle Stimulating Hormone (FSH).
- *Trigonella Foenum Graecum* aqueous extract has significant anti-fertility effect on the implantation rate.
- *Trigonella Foenum Graecum* aqueous extract causes histopathological changes on the uterus tissues.

CHAPTER TWO

LITERATURE REVIEW

2.1 LITERATURE REVIEW ABOUT FENUGREEK

2.1.1 Taxonomy of fenugreek

Fenugreek (*Trigonella foenum-graecum*) is annual leguminous crop (Figure 2.1), which is native to the Indian subcontinent and the Eastern Mediterranean region (Petropoulos, 2002). It is currently widely cultivated in central Europe, central Asia, northern Africa, North America and some parts of Australia. India is considered as a leader fenugreek producer in the world, it claim to produce 70-80 % of the world's exported fenugreek, (Fotopoulos, 2002). Fenugreek suited in mild to cool growing regions with low to moderate rainfall. The plant is adapted to rain fed growing conditions and is suitable for growth within the semi-arid regions of western Canada especially those genotypes of Mediterranean origin due to similarity in day length (Acharya *et al*., 2008). Fenugreek, perhaps, is best known for presence of the distinctive, pungent aromatic compounds in the seed (Max, 1992) that impart flavor, color and aroma to foods, making it a highly desirable supplement for use in culinary applications.

As a spice, it constitutes one of the many ingredients that make up curry powders (Srinivasan, 2006). In countries like India, fenugreek leaves are consumed as leafy vegetables in the diet (Sharma, 1986b), while in Egypt and Ethiopia, the plant is used as a supplement in maize and wheat flour for bread-making (Al-Habori and Raman, 2002). In Persia and Yemen, fenugreek represents a key ingredient in the preparation of daily meals among the general population (Al-Habori and Raman,