

Effect of Trigonella Foenum-Graecum Foenugreek oil extract on some parameters of sperms and in vitro fertilization in mice

Khulood W.AL-Samarrae^{*}, Hazim Ismaeel.AL-Ahmed^{*}
and Ikbal AL-Kateeb^{**}

^{*}Biotechnology Research Center / AL-Nahrain
University. ^{**} Pharmacy College / Baghdad University

تأثير المستخلص الزيتي لبذور نبات الحلبة على بعض معايير النطف والاختصاص خارج الجسم في الفئران

خلود وهيب السامرائي^{*} ، حازم اسماعيل الاحمد^{*} ، د. اقبال الخطيب^{**}

^{*} مركز بحوث التقنيات الاحيائية / جامعة النهرين ، ^{**} كلية الصيدلة / جامعة بغداد

الخلاصة :

الهدف من هذا البحث ، دراسة تأثير المستخلص الزيتي لنبات الحلبة على وزن الجسم ووزن الخصى وبعض معايير النطف في ذكور الفئران ، وعلى الاختصاص الخارجى ومراحل تطور الجنين في الاناث . استخدم للدراسة ١٥ ذكر وانثى بالغة باعمار (٨-٩ اسابيع) من الفئران قسمت الى ثلاثة مجاميع ، كل مجموعة تضم ٥ فئران ، عوملت مجموعتان بتركيزي ٢ و ٤ ملغم من المستخلص الزيتي لنبات الحلبة /كغم من وزن الجسم عن طريق الحقن داخل الخلب البريتوني . وعوملت المجموعة الثالثة بالمحلول الملحي الفسيولوجي كمجموعة سيطرة . قتلت الحيوانات (الذكور والاناث) بعد ٣٥ يوم من المعاملة عن طريق فصل الرقبة . عزلت النطف من ذيل البربخ وتم اجراء الفحوصات التالية ، حركة النطف ، نسبة النطف الحية والميتة وتشوهات النطف . وجمع المصل لاجراء تحليل هرمون الشحمون الخصوي كذلك تم عزل البويضات من قناة البيض لاناث الفئران بعد سحقها في ٥٠٠ مايكرو لتر من الوسط الزرعى النسيجي المحور ١٩٩ وجمع المصل لاجراء تحليل الهرمون المحفز لنمو الجريبات والهرمون اللوتيني . حضنت النطف والبويضات الناضجة في الوسط الزرعى النسيجي المحور ١٩٩ لغرض التلقيح . وتمت متابعة تطورات الجنين بعد ٢٤ ساعة من التلقيح . اظهرت النتائج وجود زيادة معنوية في وزن الجسم والخصى لمجموعتي الحيوانات المعاملة بالمستخلص وزيادة معنوية في حركة النطف وانخفاض في نسبة النطف

الميتة والتشوهات في مجموعتي الحيوانات المعاملة بالحلبة . وظهرت النتائج زيادة معنوية في هرمون الشحمون الخصوي والهرمون المحفز للجريبات والهرمون اللوتيني في مجموعتي الحيوانات المعاملة بالحلبة . اما دراسة الاخصاب الخارجي فقد اظهرت زيادة معنوية في نسبة الانتاج للبويضات ، والاخصاب ومراحل تطور الاجنة (٢-٤ خلية) .

Abstract:

This project, aims to study the effect of *trigonella* seeds oil extract on body weight , testes weight and some parameter of reproductive system in male mice , and on *In vitro* fertilization (IVF) , cleavage stages of embryos in females. Fifteen adult (8-9 weeks) males and females mice were divided into three groups , each group containing 5 mice , first and second groups were treated with (2 and 4 mg/kg body weight) of *Trigonella* oil extract (intraperitoneally) while the third group treated with normal saline as control group . After 35 days of treatment with *Trigonella* the animals (male and female) were sacrificed by dislocation of cervical vertebrae. Isolated sperms from tail of epididymis were used for the following tests. Sperm motility, dead / live sperm, sperm abnormalities and serum ws collected to assay testosterone. Isolation of ova from oviduct after micing in 500µl of TCM-199 media serum blood to assay FSH and LH . Sperm and matured ova were incubated in TCM-199 media for insemination. The results showed increase in body weight and testes weight in groups treated with *trigonella*. significant increase in sperm activity and decrease in percentage of dead sperm and abnormalities in groups treated with *trigonella* oil. The male testosterone and female FSH, LH increased significantly in groups treated with *trigonella*. IVF study showed significant increase in percentage of maturation, fertilization and cleavage (2-4 cells).

Key words: *Trigonella* , oil , sperms , *in vitro* fertilization.

Introduction

The name of the plant fenugreek comes from *Foenum-graecum*, meaning Greek Hay, the plant being used to scent inferior hay. The name of the genus, *Trigonella*, is derived from the old Greek name, denoting three-angled, and the form of its corolla. The seeds of Fenugreek have been used medicinally all through the ages and were held in high repute among the Egyptians, Greeks and Romans for medicinal and culinary purposes [1]. Fenugreek is an erect annual herb, growing about 2

feet high, seeds are brownish, about 1/8 inch long, oblong, rhomboidal, with a deep furrow dividing them into two unequal lobes. They are contained, ten to twenty together, in long, narrow, sickle-like pods [2].

Leaves and seeds of the plants have been used for centuries to prepare extracts and powders for medicinal use. In ancient Rome, fenugreek was thought to be used to aid labor and delivery. It was used as incense and in the embalming of mummies in ancient Egypt. In traditional Chinese medicine, fenugreek seeds were used to treat weakness and oedema of the legs[3]. In India, fenugreek is used as a lactation stimulant. Many other medicinal uses of the plant (e.g., the treatment of baldness and indigestion) have been reported. The preliminary results of human and animal studies suggest that oral fenugreek seeds have hypoglycemic and antihyperlipidemic properties [3]. Fenugreek has very important roles in improving fertility due to the presence of diosgenin that considered precursor for synthesis of sex hormone [4 , 5, 6]. *In vitro* fertilization (IVF) has been frequently used for treatment of sterility in humans', this project, aimed to study the effects of *trigonella* oil extract on some reproductive parameters and on *In vitro* fertilization IVF in mice.

Materials and Methods:

Preparing of *trigonella* seeds oil extract

Dry seeds of *Trigonella* were purchased from local markets and identified by the Iraqi national herbarium, the seeds were cleaned, dried and finally powdered . The *Trigonella* oil was extracted by using petroleum ether , dosage of *trigonella* oil used was 2 and 4 gm/kg B.W[7].

Laboratory animals and experimental design

Adult male mice (30-36 gm) were purchased from Biotechnology Research Center and maintained on a 14:10-hour light dark cycle in the animal house control and treated mice were provided with feed and water ad libitum; there were no differences in feed intake. Males were randomly divided into 3 groups, each composed of 5 mice. First and second groups were treated with (2,4 gm/kg B.W) respectively of *trigonella* oil intraperitoneally while administered for 35 days and the third group was given normal saline as a control group. The animals in each group were sacrificed by dislocation of cervical vertebrae. Sperms were obtained from the two tails of epididymides by mincing in 500 µl

TCM-199, and maintained at 37°C in 5% CO₂ incubator. Sperms maintained prior treatment to capacitation. (sperms motility , percentage of dead/live sperms and abnormalities of sperm were recorded).

As well as , in females the ovaries and oviduct were removed and placed in a sterile disposable petridish containing 1ml TCM-199 medium, then the oviducts were isolated, ova were obtained from the two oviduct by mincing in 500 µl of TCM-199 media, and maintained at 37°C in 5% CO₂ incubator.

Reproductive hormones assay

Serum hormones (Testosterone , FSH, LH) concentrations were evaluated with a Bio merieux Italia S.P. a vidia campigliano, 58 50015-point A EMA (F₁) Italia miniVIDAS. following the manufacturer's recommendations.

Microscopic examination

Sperms parameters were assessed according to WHO Laboratory manual [8] for Motility , percentage of dead/live and abnormalities sperms. Ova were examined to isolate matured ova by obtained first polar body.

***In vitro* fertilization (IVF):-**

Sperm and matured ova were co-incubated in TCM-media-199 having (20mg/ml B.S.A. and heparin) for insemination. Ova were observed for cleavage after 24h.of inseminate under phase contrast microscope [9].

Statistical Evaluation

Data were analyzed by one-way analysis of variance (ANOVA- test).

Data are presented as means ± SE. The level of significance was $P < .05$. [10].

Results and Discussion

Body and testes weights were increased in mice treated with *Trigonella* oil extract as compared with control group (table 1). The results indicated that treatment with *Trigonella* oil extract may caused increase the appetite to feed , which lead to increase body weight [11,12].

Table (1): Effect of *Trigonella* oil extract on body weight and testes weight

Treatment groups	Final Body Weight gm(μ +SE)	Testes weight mg/100gm(μ +SE)
Control	33.98+1.91	0.20+0.006
Trigonella (2gm/kg B.W)	39.81+1.06	0.36+0.098
Trigonella (4gm/kg B.W)	38.44+0.89	0.34+1.020

n=(5) numbers of animals in each group

Several studies suggested that *Trigonella* extract can affect the metabolism of animals treated with it due to the principle materials necessary in maintaining metabolism, such as amino acids and protein which are found in *Trigonella* [13,14,15,16].

Significant differences in mean testes weight in mice treated with *Trigonella* extract was observed when compared with control mice. This may be due to water accumulation in testes tissue, coumarine found in *Trigonella* is one of the compounds considered to be responsible [17]. Table (1).

The results obtained pointed significant increase in percentage of sperm activity and significant decrease in percentage of dead sperm and abnormalities of sperm (77.71+1.99, 80.6+1.05) (23.21+2.02, 21.11+2.06) (25.55+0.98, 23.87+2.91) respectively in treated mice as compared with the control mice (65.87+2.05, 35.6+1.43, 33.3+3.2) respectively (Table 2 and Fig1).

Table (2): Effect of *Trigonella* oil extract on sperm motility, dead sperm and abnormalities of sperm %

Treatment groups	Sperm motility % (μ +SE)	Dead sperm %(μ +SE)	Abnormalities sperm(μ +SE)
Control	A 65.87+2.05	A 35.6+1.43	A 33.34+3.2
Trigonella 2gm/kg B.W	B 77.71+1.99	B 23.21+2.02	B 25.55+0.98
Trigonella 4gm/kg B.W	B 80.6+1.05	B 21.11+2.06	B 23.87+2.91

Differences A, B, C are significant (P<0.05) to compared rows

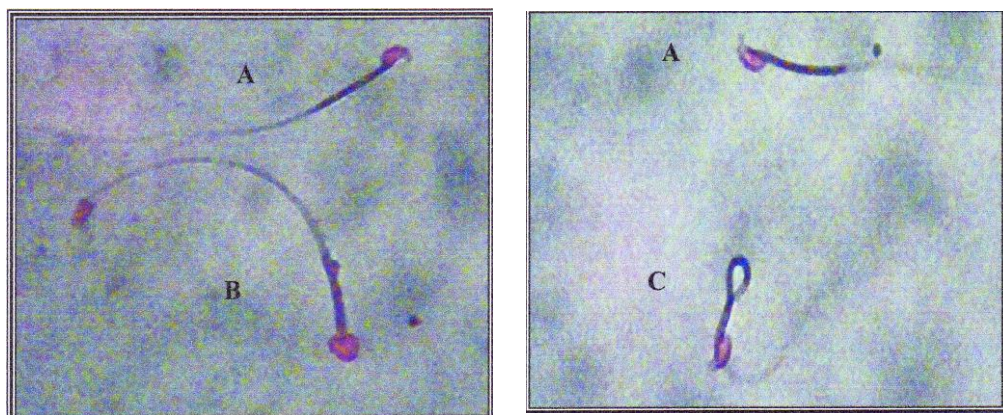


Figure (1): Sperm morphology , showing : normal sperm (A), sperm hummer head (B), sperm curve tail (C) (40X)

Clement , [18] demonstrated that spermatogenesis is regulated by the accurately coordinated expression of many genes ,disruption of this process can be brought about mutations of many genes leading to abnormalities.

The increase observed in mitotic index MI of mouse testis may be returned to the *Trigonella* extract which may have mitogenic effects through the presence of active compounds acts as or stimulate the division process without addition any mitogenic agents [19].These plants extracts may improve animal fertility and it can cross testis barriers.

Decreasing in the abnormalities of sperms (head and tail)especially after 35days of treatment with *trigonella* extracts occur when the sperms are in the spermatogonia stage and before mitotic division which represent a source of sperms , and this will be in agreement with AL-Rubia , [20] and AL-Rubia , [21] who clarified that *Trigonella* extract dos not contain any mutagenic agents , As we know, these mutagenic agent are responsible of abnormalities in sperms head and tail [22] compounds found in *Trigonella* extract may play a role in antioxidant protection against sperm damage [23]. This test represent a more sensitive test for detection the mutagenic compounds. On the other hands *trigonella* extracts has protective effectiveness in germ stem cells (spermatogonia) which act as a source of all sperms [24].

Significant increase in levels of hormones (male testosterone and female FSH , LH) in treated mice also was noticed as compared with control mice .(table 3).

Table (3): Effect of *Trigonella* extract on reproduction hormones (male testosterone , female FSH and LH) after 35 days treatment in mice

Treatment groups	Male testosterone ng/ml (μ +SE)	Female FSH IU/ml(μ +SE)	Female LH IU/ml(μ +SE)
Control	A 0.97+0.29	A 0.34+0.87	A 0.28+0.06
Trigonella 2gm/kg B.W	B 1.88+1.03	B 0.51+0.92	B 0.43+0.95
Trigonella 4gm/kg B.W	C 2.02+1.21	C 0.82+1.02	C 0.94+0.93

Differences A, B, C are significant ($P<0.05$) to compared rows

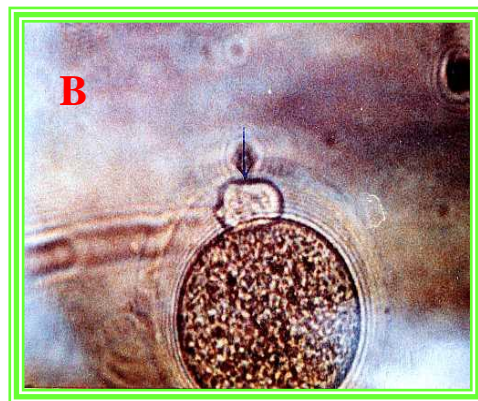
This increase may be due to diosgenin found in *trigonella* extract which have important role in synthesis sex hormones which have very important role in reproduction [25,26].

The male reproductive function is under hormonal control ,spermatogenic process is under control of follicular stimulating hormone (FSH) and testosterone [27],while the formation of type A spermatogonia and conversion of primary spermatocyte into secondary spermatocyte (Meiosis I) are testosterone dependante and the final step of maturation of spermatids is dependant on FSH [28].

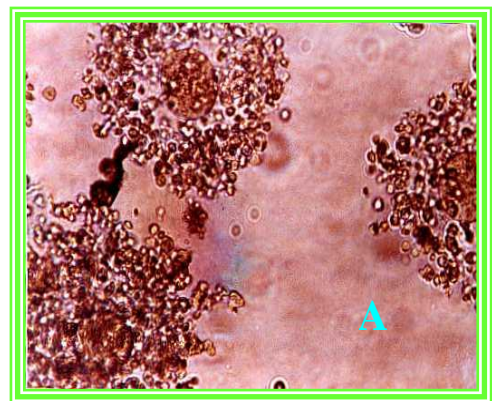
Table 4 shows significant increase in mean number of mature ova , fertilized ova and ova cleavage (2_4 cells).Fig.2

Table (4): Effect of *Trigonella* extract on *in vitro* fertilization (% incubated ova , maturated ova , fertilized ova cleavage ova (2-4 cells) after 35 days treatment in mice

Treatment groups	% of incubated ova	% of maturated ova	% of fertilized ova	% ova cleavage (2-4 cells)	% ova cleavage (4 cells)
Control	38	57.84	54.54	58.31	42.84
Trigonella 2gm/kg B.W	29	72.41	61.91	69.2	55.54
Trigonella 4gm/kg B.W	50	76.00	63.14	70.81	58.8



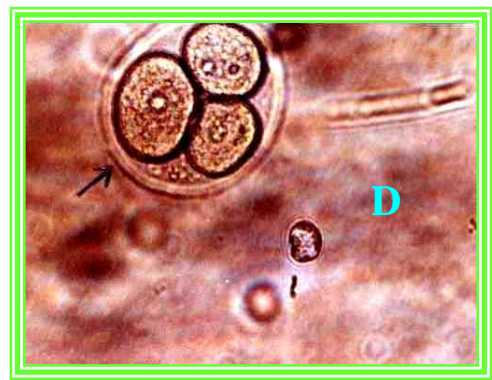
Mature ova



Immature ova



fertilized ova (2 cell)



Embryo (4 cells)

Figure (2):Ova stages (Immature ova (A), Mature ova (B), fertilized ova (2 cell)(C) and Embryo (4 cells)(D)

The seed of Fenugreek contains alkaloids, Colin, bitter material, fatty acid, minerals, protein and vitamin C, which are excellent remedies for dysiorea and weakness resulting from emaciation. It improves the appetite, it is a general health-restorer, it has a fattening effect, increases the number of red blood cells and restores the strength of a physically strained body. It is rich in phosphorus, organic iron and carbohydrates [29, 30].

Whole fenugreek seeds contain 48% total fibre, which includes 20% gum and 28% neutral detergent fibre (NDF) and about 4% of saponins [31]. Further, fenugreek seeds are reported to contain high levels of flavonoids (>100 mg/100 g) [32]. It could be possible that saponins and flavonoids present in fenugreek seeds enhance the

antioxidant potential and thus are responsible for the reduction in lipid peroxidation in tissues[32].

As we know herbs active compounds including flavonoids , minerals and vit.C exhibit strong healing effects on reproductive organs by increasing vitality and circulation and supporting the hormonal system by nourishing the endocrine gland, steroid compound found trigonella can help in regulating estrogen / progesterone ratio.[29]

References

- 1- Derrida, M. (2004). What is Fenugreek. www.Wholebodyhealth.com
- 2- Al-Khalisi, M.H.H. (2000). The Effect of Fenugreek seeds on mammary gland. A histological and Histochemical Approach. Ph.D Thesis . Baghdad University.
- 3- Gupta, A.; Gupta, R. and Lal, B. (2001). Effect of *Trigonella foenum graecum* (Fenugreek) seeds on glycemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study. J. association of physicians of India. 49 :1057- 1061. (Abstract).
- 4- Taylor, W.G. Zulyniak, H.J.; Richard, K.W.; Acharya, S.N.; Bittman, S. and Elder, J.L. (2002). Variation in Diosgenin levels among 10 Accessions of fenugreek seeds in western Canada. J. Agric. and Food. Chemis. 50 (21): 5994-5997.
- 5- McWharter, L.S. and Pharm, D. (2001). Biological complementary therapies: A focus on Botanical products in Diabetes. Diabetes Spectrum. 14: 199 -208.
- 6- Hiroshi , S. , Keigo , Y. , Toshihiko , W. , Masao , and Jiro , A . (1996). Rederivation of mice by means of *in vitro* fertilization and embryo transfer . Exp . Anim . 45(1) , 33-38 .
- 7- Anuradha , C. and Ravikumar, P. (2001) . Restoration on tissue antioxidants by Fenugreek seeds (*Trigonella foenum graecum*) in alloxan-diabetic rats . Indian J physiol pharmacol ; 45(4): 408 _420.
- 8- WHO Laboratory manual for the examination of human semen and sperm – cervical mucus interaction. (1992). Third Edition. Cambridge University press.
- 9- AL- Ahmed H.I. (2002). Uses of hormonal regimes for oestrus synchronization , superovulation , ova and embryos collection and *in vitro* fertilization in iraqi local does. A thesis submitted to the council of the college of veterinary medicine university of Baghdad .
- 10- Al-Mohammed, N.T.; Al-Rawi, K.M.; Younis, M.A. and Al-Morani, W.K. (1986). Principle of Statistics. J. Al-Mosul University.

- 11-** Petit,PR.; Sauvaire,YD.; Hillaire-Buvs,MD.; Lecort,DM.; Baissac,YG. Panson, GR. And Ribes, GR. (1995). Steroid saponins from fenugreek seeds: Extraction, purification. (10):674-80. (Abstract).
- 12-** Hooda,S.and Jood,S. (2004).Nutritional evaluation of wheat-Fenugreek blends for product making. Plant. Foods. Hum. Nutr. 59(4) : 149- 54. (Abstract).
- 13-** Awadalla, MZ.; El-Gedaily, AM.; El-Shamy, AE. And El-Aziz,KA (1980).Studies on Egyptian foods,part2:The effect of protein on blood constituents of rats. Z.Ernahrungswis. 19 (4) :. 248-50. (Abstract).
- 14-** Makai, P.S.;Makai, S.and Kismanyoky,A. (2004).Comparative test of fenugreek *Trigonella foenum-graecum* L./varieties.J.Cent.Euro.Agric. 5(4): 259-62.
- 15-** Zubay, G.; Parson, W. & Vance, D.(1995). Principles of Biochemistry. 50-66 : Brown publishers. England.
- 16-** Landau, BR.(1980).Essential Human Anatomy and Physiology.2nd ed . Scott, forseman and company London. England. : 685-693.
- 17-** Raju,J.;Patlolla,J.M.; Swamy,M.V. and Rao,C.V.(2004).Diosgenin, a steroid saponin of *Trigonella foenum-graecum*. (Fenugreek),inhibits azoxymethane-induced aberrant crypt foci formation in F344 rats and induces apoptosis in HT-29 human colon cancer cells.Cancer.Epidemiol. Biomarkers. Prev. 13(8):1392-8 .(Abstract).
- 18-** Clermont , Y. (1970).Dynamics of human spermatogenesis in the human testis Rosenberg , E.; and Panlsen , C.A.(eds) . New York , London : Plenum Press. 47-1.
- 19-** Hagelstrom ,A.H.; Gorla , N.B.; and Larripa ,I.B. (1995) . Chromosomal damage in workers occupationally exposed to chronic low level ionizing radiation . Toxicol . Lett., 76:113-7.
- 20-** AL-Rubaie, A.H.M. (1999). The effect of garlic extract on the inhibiting of mutagenic action of zinc phosphide and gamma ray. In albino mice .Ph.D. Thesis,College of Science, University of Babylon.
- 21-** AL-Rubaie,F.A.A.(2000). A study of the mutagenic and anti mutagenic ability of some Iraqi medicinal plants in albino mice.M.Sc. Thesis ,College of Education, (IBn-ALhatham), University of Baghdad .
- 22-** Wyrobek, A.J.; and Bruce , W.R. (1975). Chemical induction of sperm abnormalities in mice. Proc. Natl. Acad. Sci. U.S.A., 72:4425-4429.
- 23-** Sierens , J.; Hartley , J.A. ; Camphell , M.J.; Leathem , A.J.; and Woodside , J.V. (2002) . In vitro isoflavone supplementation reduces hydrogen peroxide induced DNA damage in sperm. Teratogenesis Carcinogenesis and Mutagenesis, 22: 227-34.

- 24- Tate ,A. ; and Natarajan ,A. (1976). A correlation on the genetic damage induced by chemical mutagen in bone marrow and spermatogonia . *Mutat . Res .*, 37:267-278.
- 25- Wesley , G . ; Holly , J . , Ken , W. ; Surya , N. ; Shabtai , B. and James , L. (2002) . Variation in diosgenin levels among 10 accessions of fenugreek seeds produced in western Canada . *Journal of agricultural and food chemistry* . volume 50 , number 21 , pages 5994_5997 .
- 26- Seeley , R.R.; Stepheus , T.D.; and Tate , P. (1996).Essentials of anatomy and physiology .2nd ed . Medical economics company , U.S.A, :110.
- 27- Ganong, W.F. (1991). Review of medical physiology .Connecticut: Appleton and lauge , 401:94-95.
- 28- Fournier, P. (1972): Trigonelle. Les quatre flores de la France. N.05.
- 29- Paris, N. – Sauvaire, Y. – Baccou, I.C. (1975): ProcM6 d' extraction de v6getaux pour la production de sapog6nines steroidique et de sousproduits utilisable industriellement. Brevet fi'ancais No.75.
- 30- Searle AJ, Wilson R. (1980) Gultathione peroxidase: effect of superoxide, hydroxyl and bromine free radicals on enzyme activity. *Int J. Rad. Biol.* 37: 213-217.
- 31- Kukreja CR, Okabe E, Schrier MG, Hess LM.(1988). Oxygen radical-mediated lipid peroxidation and inhibition of Ca ATPase activity of cardiac sarcoplasmic reticulum. *Arch Biochem Biophys.* 261: 447-457.

Recived (11/5/2008)
Accepted (3/9 /2008)