Wasit Journal for Science & Medicine **2009 2 (1) : (11 - 29)**

EFFECTS OF Origanum Vulgare ON SOME SPERMS PARAMETERS , BIOCHEMICAL AND SOME HORMONES IN ALLOXAN DIABETIC MICE

Kadhum Jhyied Gattia - Wassit University

تأثيرات مستخلص نبات المردقوش على بعض معايير النطف ، الكيماحياتية وبعض قياسات الهرمونات في الفئران المستحث فيها داء السكري باستخدام الالوكزان

كاظم جهيد كاطع - جامعة واسط

الخلاصة

تم دراسة تاثير نبات المردقوش على خصوبة ذكور الفئران البيض باستخدام الفحوصات التالية (قياس مستوى هرمون التيستوسترون ، والهرمون اللوتيني والهرمون المحفز لنمو الجريبات المبيضية ، حيوية النطف و نشاط النطف ، حركة النطف والتشوهات) وقياس تركيز مستوى انزيم ال GOTو GPTوALP وقياس تحليل الدهون ،جميعها درست للفئران الطبيعية (السيطرة) والفئران المستحث فيها داء السكري باستخدام الالوكزان.

حقنت الفئران المستحدث فيها داء السكري باستخدام الالوكزان بمستخلص نبات المردقوش لمدة ٣ اسابيع وبجرعة 18,. غم/كغم من وزن الجسم ، اظهرت االنتائج نقصان في مستوى الكلوكوز للفئران المستحدث فيها داء السكري ، وزيادة في مستوى هرمون التيستوستيرون والهرمون اللوتيني والهرمون المحفز لنمو الجريبات المبيضية ، وقلة في نسبة الحيامن الميتة والتشوهات وزيادة فعالية الحيامن وانخفاض في مستوى انزيم ال GOT , GPT , ALP وتحليل الدهون مقارنة مع حيوانات السيطرة.

ABSTRACT

The influence of Origanum Vulgare on male fertility by using following tests (measurement of testosterone , LH and FSH level , sperm viability , activity , motility and abnormalities) and on GOT, GPT,AL.Ph. and Lipid profile status was studied in normal and alloxan diabetic mice.

Alloxan diabetic mice were injected with Origanum Vulgare extract for 3 weeks at a dosage of 0.18 g/kg body weight. The Alloxan diabetic mice showed significantly decreased in serum glucose level. however testosterone , LH and FSH level were increased and decreased in dead sperms ,

abnormalities and increase motility , decrease in GOT, GPT, AL.Ph. and lipid profile, in alloxan diabetic mice.

INTRODUCTION

Origanum vulgare is a species of Origanum, native to Europe, the Mediterranean region and southern and central Asia. It is a perennial herb, growing to 20-80 cm tall, with opposite leaves 1-4 cm long. The flowers are purple, 3-4 mm long, produced in erect spikes(Faleiro and Leonor,.2005).

Oregano has an antioxidant activity, due to a high content of phenolic acids and flavonoids (Dragland and Steinar , 2003). Additionally, oregano act as antimicrobial against food-borne pathogens such as Listeria monocytogenes, both of these characteristics may be useful in both health and food preservation. In Philippines, oregano (Coleus aromaticus) is not commonly used for cooking but is rather considered as a primarily medicinal plant, useful for relieving children's coughs.

Main constituents include carvacrol, thymol, limonene, pinene, ocimene, and caryophyllene. The leaves and flowering stems are strongly antiseptic, antispasmodic, carminative, cholagogue, diaphoretic, emmenagogue, expectorant, stimulant, stomachic and mildly tonic. Oregano is taken by mouth for the treatment of colds, influenza, mild fevers, indigestion, stomach upsets and painful menstruation. It is strongly sedative and should not be taken in large doses, though mild teas have a soothing effect and aid restful sleep. Used typically, oregano is one of the best antiseptics because of its high thymol content (Faleiro and Leonor,.2005).

MATERIALS AND METHODS

PLANT MATERIAL

Origanum were purchased from the local market.

Extraction of Origanum compound (Alcohol Extract):

(50g) of plant powder was extracted with ethanole (250 ml) by Soxhelt apparatus for 6 hours at 40-60ºC, then the cooled solution was evaporated to dryness by rotary evaporator at 40ºC and kept until used (Al-Jeboory, 1994).

ANIMALS CARE

Fifty adult males of Swiss albino strain mice were obtained from animal house. 50 mice were used throughout this study , the age of the mice

were in the range of 2.5 to 3 months, and the weight in the range 25-30 grams. The animals were housed in small plastic cages, which were cleaned weekly by washing with soap and tap water and sterilized with 70% ethyl alcohol during the period of the study. The room temperature was maintained at (24±2) º C, and the animals were exposed to 14 hours light program. Food of animal was pellet from local market.

INDUCTION OF DIABETES

Diabetes was induced by a single intraperitoneal injection of alloxan monohydrated (5% w/v) in physiological saline at a dose of 150 mg/kg body weight in a volume of 0.1ml. The diabetic state was confirmed 48 hours after alloxan injection by weight loss (Benedict , 1911), and hyperglycaemia (Sasaki and Mastui 1972).There was 75% mortality in animals treated with alloxan. Surviving mice with a fasting blood glucose level higher than 200ml/dl were included in the study. five groups consisting of five animals for each group were maintained as follows:

EXPERIMENTAL GROUPS:

CONTROL GROUP:-

Normal mice injected with 0.1ml of physiological saline .

GROUP A:-

Normal mice injected with alloxan 0.1ml to induce diabetic mice.

GROUP B:-

Diabetic mice treated with 0.1ml of Origanum extract after one week from treated with alloxan.

GROUP C:- 2 week
GROUP D:- 3 week

GENERAL PROCEDURE:-

Blood sugar levels were determined periodically by heart puncture at the end of (1,2and 3 weeks) from treated with alloxan, the mice fasted over night and killed by cervical dislocation.

TREATMENTS OF MALE

The testes were removed and placed in a sterile disposable Petri dish containing 1ml TCM-199 medium, then the epididymes were isolated and spermatozoa were obtained from the two tails of epididymes by mixing in 1ml TCM-199, and maintained at 37°C in 5% CO_2 incubator prior treatments.

Microscopically examination:-

Spermatozoa were assessed according to WHO Laboratory manual for viability, activity, Motility and abnormalities.

Testosterone , FSH , LH assay:

Bio merieux Italia S.P. a vidia campigliano, 58 50015-point A EMA (F_1) Italia miniVIDAS. Was used for the hormonal assay.

In testosterone , FSH , LH tests the assay principle combines an enzyme immuno assay sandwich method with a final fluorescent detection (ELFA).

BIOCHEMICAL MEASUREMENTS:-

1.GOT,GPT.:-

According to Reitman and Frankel (1957), blood was collected from the mice by heart puncture .The serum was separated by centrifugation at 2000 rpm for 10 min. Then, the serum was taken and treated as follows:

Two test tubes were used for each sample, the 1st one contained the blank reagent and 2nd contains the sample. These samples were treated as in the following:-

	GPT	GOT
Reagent 1 Reagent 2	1 ml —	— 1 ml
Incubate for 5 min at 37°C.		
Serum	0.2 ml	0.2 ml
Mix and incubate at 37°C	— 1 hour	— 30 min.
Reagent 3	1 ml	1 ml
Mix. Let stand for 20min at room temp		
NaOH 0.4 N	10 ml	10 ml
Mixed wait 5 min. measure under condition identical to those used for the standard curve.		

Wavelength: 505 nm (490 - 520nm)

Activities of these two enzymes in the serum were estimated from the activity table attached with kit of each enzyme.

3. ALP(alkaline phosphates)

Sample used in this test was the same of serum sample used for GPT& GOT tests. To estimate the activity of the ALP enzymes, procedure of Kind and King (1945) was used: four test tubes for each sample were prepared, the 1st one contain the sample, the 2nd is the blank sample, the 3rd contain the standard sample and the 4th is the blank reagent, as shown below:-

	Serum sample	Serum bank	Standard	Reagent blank
Reagent	2 ml	2ml	2 ml	2ml
Incubate for 5 minutes at 37°C.				
Serum Reagent 2	50µl —	— —	— 5 0µl	— —
Incubate for exactly 15 min at 37°C.				
Reagent 3	0.5ml	0.5ml	0.5ml	0.5ml
Mixed well or preferably vortex.				
Reagent 4 Serum Distilled water	0.5ml — —	0.5ml 50µl —	0.5ml — —	0.5ml — 50µl
Mix. Let stand for 10 minutes in the dark. Measure.				

$$\text{Calculation} = \frac{\text{OD serum sample} - \text{OD serum blank}}{\text{OD standard}} \times n$$

4. LIPID PROFILE
Determination of Serum Total Cholesterol

Total cholesterol in the serum was measured by enzymatic method, with the biomerux kit, France.

Principle

The principle of this method was lysis of the cholesterol ester to produce cholesterol and fatty acids, then oxidized to produce the quinoemine:

$$cholesterol\ ester \xrightarrow{cholesterd\ esterase} cholesterol + fatty\ acid$$

$$cholesterol \xrightarrow{cholesterd\ oxidase} cholest-4-en-3-one + H_2O_2$$

$$2H_2O_2 + phenol + 4\cdot amino\ antipyrine \xrightarrow{peroxidase} quinoe\min e + 4H_2O_2$$

Reagents:

The reagent used in test is a mixture of:

1- phosphate buffer	0.1 mol /L
2- phenol	15 mol /L
3- sodium cholate surfaetante	3.74 mmol/L
4- 4 samino antipyrine	0.5 mmol/L
5- peroxidase	≥1000 u/L
6- cholesterol oxidase	≥ 200 u/L
7- cholesterol esterase	≥125 u/L

Procedure:

The procedure for this method is as follow:

	Reagent blank	Standard	Sample
Standard 200 mg/dL	-	10 µL	-
Sample	-	-	10 µL
Working reagent	1 ml	1 ml	1 ml

After addition, mixing the content of every tube.

Allow staying at room temperature for 10 minutes or incubating at 37^{o} C for 5 minuts and reading absorbance by spectrophotometer at 500 nm. The intensity of the produces color is directly proportional to total cholesterol concentration in the sample.

$$\text{Total cholesterol (mmol/L)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times 5.17$$

Determination of Serum Triglyceride

Total triglycerides in the serum were measured by enzymatic with the (biomerieux kit, France).

Principle

Total triglyceride determination depend on formation of quinonemine by using a group of enzymes as follows:

$$Triglycerides \xrightarrow{lipase} glycerol + fatty\ acids$$

$$Glycerol + ATP \xrightarrow{glyverokinase} glycerol - 3 - phosphate + ADP$$

$$glycerol - 3 - phsphate \xrightarrow{glycerol-3-phosphate} H_2O_2 + dihydroxy - acetone\ phosphate$$

$$H_2O_2 + parachlorophenol + \ amino\ antipyrine \xrightarrow{peroxidase} quinonei\min e + H_2O_2 + HCL$$

Reagents:

The reagent used in this test is a mixture of:

1- Buffer pH 7.6	100 mmol/L
2- p – Cholesterol	2.7 mmol/L
3- Magnesium	4 mmol/L
4- 4- Aminoantipyrine	0.4 mmol/L
5- Lipase	$\geq$ 1000 u/L
6- Glycerokinase	$\geq$ 200 u/L
7- Glycerol – 3- phosphate oxidase	$\geq$ 2000 u/L
8- Peroxidase	$\geq$ 200 u/L
9- ATP	0.8 mmol /L
10- Glycerol	2.29 mmol/L

Procedure:

	Reagent blank	Standard	Sample
Standard 200 mg/dl	-	10 µ L	-
Sample	-	-	10 µ L
Working reagent	1 ml	1 ml	1 ml

Gently mix the content of every tube after addition, allow staying at 20 – 25°C temperature for 10 minute or incubating at 37°C for 5 minutes and reading spectrophotometrically at 505 nm. The intensity of the produced color is proportional to total triglyceride in the sample.

Calculation:

Sample concentration = $\frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times n$ **(n= concentration of standard n= 2.29)**

Determination of Serum High Density Lipoprotein – Cholesterol (HDL – C):

HDL – Cholesterol the serum were measured by enzymatic method using biomeriex kit, France.

2.6.3.2 Reagents:

HDL – cholesterol precipitant	Phosphotungstic acid	**40 g/L**
	$MgCl_2$. $6H_2o$	100 g/L
	pH 6.2	1 g/L

Procedure:

	Reagent blank	Standard	Sample
Distilled water	50 µL	-	-
HDL – calibrator	-	50 µL	-
Supernatant	-	-	-
Working reagent	1 ml	1 ml	1 ml

The working solution is the cholesterol enzymatic solution gently mix with the contents of every tube; after the addition let it stay at 20 – 25 for 10 minutes or incubate it for 5 minutes at 37^{o}C and then read spectrophotometrically at 500 nm.

Calculation:

$$\text{HDL. Cho. (mmol/L)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times 1.42$$

(1.42 = the concentration of standard)

*STATISTICAL ANALYSIS

Statistical analysis was performed to compare two different groups by using ANOVA-test. Statistical significance was determined at $P<0.05$. (Al-Mohammed *et al*., 1986).

RESULTS AND DISCUSSION:-

The glucose level increased in serum of normal mice from that indicated to induced alloxan-diabetic mice as reported by(Anuradha and Ravikumar, 2001).(Table 1).

Alloxan diabetic mice treated with Origanum vulgare were shown reduced glucose level in serum from 196.50+3.06 to 163.41+2.84 (Table 1).They also conclude that Origanum vulgare improve insulin sensitivity and decrease insulin resistance in diabetic mice (Gupta *et al.,* 2002). The hypoglycaemia effects have been attributed to several mechanisms (Ethan Basch , *et al* 2003).The amino acid 4-hydroxyisoleucine in Origanum vulgare increased glucose-induced insulin release in pancreatic islet cells (Sauvairi *et al*.,1998). This amino acid appeared to act only on pancreatic beta cells, Origanum vulgare reduced the area under the plasma glucose curve and increased the number of insulin receptors (Raghuram *et al.,* 1994).

In humans, Origanum vulgare exert hypoglycaemia effect by stimulating glucose-dependent insulin secretion from pancreatic beta cells (Ajabnoor and Tilmisary, 1998) as well as by inhabiting the activities of alpha-amylase and sucrose to intestinal enzymes involved in carbohydrate metabolism (Amin, *et al* 1987).

The results showed significant increase ($P<0.05$) in body weight of alloxan diabetic mice treated with Origanum vulgare extract due to the Origanum vulgare extract contains tannins that lead to increase appetite (Hook , 1978 ، Yeomans *et al.*,1997) , and contain vitamins C and A (Ingram , 1995) (Table 2)

While the increased testes weight due to Origanum vulgare extract contain coumarin which cause accumulation of water in testes .(Table 2).

Testosterone , LH , FSH level no significant decrease in alloxine male diabetic mice compared with control male (1.46+0.08 , 162+0.34 , 0.23+0.32 , 0.34+0.9 , 0.14+0.82 , 0.25+0.8) respectively. Origanum vulgare extract also increased the testosterone , LH and FSH levels (1.60+0.27 , 0.33+0.56 and 0.23+0.41) respectively after 3 weeks from treated. This increase due to that the Origanum vulgare contain diosgenin which have important roles in sex hormone synthesis. (WWW. Allnutritionalis . Com / mardagosh . Htm , 2006).(Table 3).

The results obtained no significant decrease in viability , dead spermatozoa , abnormalities (60+2.24 , 33+0.83 and 24+3.38) respectively in alloxan diabetic male mice compared with control male (80+3.02 , 29+1.02 and 21+2.03)respectively. While Origanum vulgare extract increased (Table 4).

The increase fertility of spermatozoa due to increase the level of testosterone in serum (Vora,2000 and Dean,2004).

Table 5 showed increased in triglyceride total cholesterol and HDL-cholesterol 200.69+0.94 , 253.6+3.02 and 194.43+1.88 in alloxan-diabetic mice 170.51+3.32 , 204.91+4.33 and 166.22+3.41 respectively to compared with control 173.68+4.35 , 208.32+3.41 and 170.5+4.02 respectively. Origanum vulgare also lower serum TG, chol. and HDL-Chol. after 3 weeks. These effects may be due to saponins ,alkaloids, or to the high fibber content of the Origanum vulgare.(Hebel, *et al* 1997;Ethan Basch *et al.*, 2003).This study have ability of Origanum vulgare to significantly reduced of GOT, GPT, ALPH, in alloxan mice from 184.31+4.81 , 70.33+4.83 and 64.34+1.08 respectively to 190.03+1.44 , 72.23+4.11 and 68.82+4.05 respectively after 3 weeks from treated with Origanum vulgare.(Table 6) The mechanism of action is unknown but may be due to the saponine, alkaloids, tanin and rating or to the high fibre content of the Origanum vulgare.(Valent , 2003) Recent study showed that the Origanum vulgare act as antimicrobial and protect the liver cells from damage (Katzer , 2000).

Table (1):- Serum glucose levels in control and alloxan diabetic mice

Groups		Glucose mg/dl (mean +SE)
Control		A 163.41±2.84
A Treated with alloxan		B 196.50±3.06
B Treated with 0.1 ml of origanum extract after different period of alloxan treatment	B 1 week	C 186.41±2.03
	C 2 weeks	C 174.82±3.71
	D 3 weeks	A 168.84±2.55

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

Table (2):- Initial B.W. and Testes W. in control and alloxan diabetic mice

Groups		Initial B.W.(gm) (mean +SE)	Testes W.(mg/100mg B.W) (mean +SE)
Control		31.16±1.44	0.23±0.92
A Treated with alloxan		29.82±2.02	0.21±1.43
Treated with 0.1 ml of Origanum extract after different period of alloxan treatment	B 1 week	30.46±1.83	0.24±1.26
	C 2 weeks	33.56±2.92	0.25±1.08
	D 3 weeks	34.33±1.96	0.27±2.30

Table (3):- Serum Testosterone , LH , FSH levels in control and alloxan diabetic mice

Groups		Testosterone(ngm/ml) (mean +SE)	LH(ngm/ml) (mean +SE)	FSH(ngm/ml) (mean +SE)
Control		A 1.62±0.34	A 0.34±0.9	A 0.25±0.8
A Treated with alloxan		B 1.46±0.08	B 0.23±0.32	B 0.14±0.82
B Treated with 0.1 ml of Origanum extract after different period of alloxan treatment	B 1 week	C 1.51±0.09	C 0.26±0.43	C 0.19±0.70
	C 2 weeks	A 1.57±0.23	C 0.28±0.48	A 0.22±0.93
	D 3 weeks	A 1.60±0.27	A 0.33±0.56	A 0.23±0.41

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

Table (4):- Sperms activity , Dead sperms and Abnormalities sperms in control and alloxan diabetic mice

Groups		Sperms activity % (μ+SE)	Dead sperms %(μ +SE)	Abnormalities sperms(μ +SE)
Control		A 80±3.02	A 29±1.02	A 21±2.03
A Treated with alloxan		B 60±2.24	B 33±0.83	B 24±3.38
Treated with 0.1 ml of origanum extract after different period of alloxan treatment	B 1 week	C 70±3.63	C 38±1.33	A 21±2.22
	C 2 weeks	C 70±4.02	C 40±2.01	A 19±1.10
	D 3 weeks	A 75±2.94	A 31±1.92	A 21±2.20

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

Table (5):- Serum Triglyceride, Cholesterol and HDL-cholesterol levels in control and alloxan diabetic mice

Groups		Triglyceride mg/dl (µ+SE)	Cholesterol mg/dl (µ+SE)	HDL-cholesterol mg/dl (µ+SE)
Control		A 170.51±3.32	A 204.91±4.33	A 166.22±3.41
A Treated with alloxan		B 200.69±0.94	B 253.6±3.02	B 194.43±1.88
Treated with 0.1 ml of origanum extract after different period of alloxan treatment	B 1 week	C 186.42±3.45	C 236.61±4.05	C 186.32±2.45
	C 2 weeks	C 188.36±4.02	C 234.73±1.94	C 182.31±3.63
	D 3 weeks	A 173.68±4.35	A 208.32±3.41	A 170.5±4.02

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

Table (6):- Serum GOT , GPT and AL.Ph. levels in control and alloxan diabetic mice

Groups		GOT mg/dl (mean+SE)	GPT mg/dl (mean +SE)	AL.Ph. mg/dl (mean +SE)
Control		A 184.31±4.81	A 70.33±4.83	A 64.34±1.08
A Treated with alloxan		B 254.21±1.88	B 88.63±4.91	B 87.35±5.92
Treated with 0.1 ml of origanum extract after different period of alloxan treatment	B 1 week	C 221.82±3.61	C 76.86±1.34	C 82.44±1.93
	C 2 weeks	C 219.86±1.84	C 77.72±1.35	C 83.30±2.63
	D 3 weeks	A 190.03±1.44	A 72.23±4.11	A 68.82±4.05

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

References

1. Faleiro, D and Leonor;S. (2005). "Antibacterial and Antioxidant Activities of Essential Oils Isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L.". *J. Agric. Food Chem.* **53** (21): 8162–8168.
2. Dragland, F. and Steinar; P. (2003). "Several culinary and medicinal herbs are important sources of dietary antioxidants". *J Nutr.* **133** (5): 1286–1290. PMID 12730411. http://jn.nutrition.org/cgi/content/abstract/133/5/1286.
3. Benedict SR. 1911 The detection and estimation of glucose in urine. J. Am. Med. Assoc.: 57: 1193-1196.
4. Sasaki T.; matsui S.. Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for the blood glucose determination. Rinsho Kagaku, 1972; 1: 346-353
5. Reittman, S., &Frankel, S. (1957): Amer. J. Clin. Path., 28:56
6. Kind, P.R., & King, E.G. (1945):Clin. Path., 7:322.
7. Al-Mohammed, N.T.; Al-Rawi, K.M.; Younis, M.A. and Al-Morani, W.K. (1986). Principle of Statistics. J. Al-Mousl University (in Arabic).
8. Anuradha, CV. 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.
9. Gupta, A.; Gupta, R.; Lal, B. (2002). Effect of Trigonella foenum-graceum (fenugreek) seeds on glycaemic control and insulin resistance in type z diabetes mellitus: a double blind placebo controlled study. The Journal of the Association of Physicicans of India. 49: (1057-1061).
10. Ethan Basch, MD; Catherine Ulbricht, Pharm D.; Grace Kuo, Pharm D.; Philippe Szapory, MD; Michael Smith, MR Pharms, ND. (2003). Therapeutic Applications of fenugreek. Alternative Medicine Review, Volume 8, No.1 (20-27).
11. Sauvairey Y., Petit, P.; Broca C. (1998). 4-Hydroxisoleucine: a novel amino acid Potentiator of Insulin secretion. Diabetes. 47: 206-210.
12. Raghuram T.C.; Sharma R.D.; Sivakumar, P. (1994). Effect of foenugreek seeds on intravenous glucose disposition in non-insulin dependent diabetic patients. Phytother Res. 8: 83-86.
13. Ajabnoor, MA.and Tilmisany AK. (1998). Effect of Trigonella foenum graceum on blood glucose levels in normal and alloxan-diabetic mice. J., Ethnopharmacol. 22: 45-49.

14. Amin, R; Abdul-Ghani AS.; Suleiman MS. (1987). Effect of Trigonella foenum graceum on intestinal absorption. Proc. of the 47th Annual Meeting of the American Diabetes Association (Indiana Polis U.S.A.). 36: 211a.
15. Hook,EB. (1978). Dietary cravings and aversions during pregnancy . 1:AM.J. clin Nutr .Aug , 31(8) :62-1355.
16. Yeomans , M.R., Gray , R.W. , Mitchell , C.J. & True , S.(1997). Independent effects palatability and within –meal pauses on intake and appetite ratings in human volunteers. 1:Appetite . Ang, 92(1) :61-76.U.K. .
17. Ingram , G. (1995). Oregano :Nature's Healer .In interent file :http://www. Amazon. Com.
18. WWW. Allnutritionalis . Com / mardagosh . Htm , 2006
19. Vora, P. 2000. What every Man should know about Andropause. Natural Health & Beauty Care Center. 8 PP. San June Capistrano
20. Hebel SK eds (1997). Fenugreek, in the Lawrence Review of Natural Products. St. Louis: Facts and comparisons, 1996: 1-3.
21. Valent ,J. (2003) .Oregano oil .In interenet file :http://www.whole health Md. Com
22. Katzer , G. (2000). Oregano (origanum vulgarde L.). In interent file :http:// www.oregano floridata . com

Recived …….. (19/1/2009)
Accepted ……..…... (30/3 /2009)