The effect of Green tea aqueous extract *Camellia sinensis* and Vitamin E in infertile men in Tikrit city and It's Suburbs

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Abstract

This study investigated the effects of Green tea aqueous extract and Vitamin E effect on the fertility of men, and the antioxidant and anti-infertile activity for comparison. The study included examination of (281) samples of seminal fluid of men that were suffering from infertility caused by gonorrhea n :(144) , Varicose n: (47) , seminal vessels narrow n: (31) , different inflammations n: (59) samples , and (60) samples from normal individuals (subjects) as a control, for the period from March(2008) to April(2009) in Tikrit city and its surrounding. The results appeared that adminstratio of green tea extract alone or with Vit. E in an infertile men (Gonorrhea infections, Varicocele , Seminal vessels narrow and different sexual inflammations) lead to significant increase ($p<0.05$) in total numbers , the average of movement and normal forms of sperm. The treatment with Vit. E alone lead to increase in numbers and normal forms of spermatozoa , however , no significant variations were of served in the average of movement. The results indicated that using green tea aqueous extract , Vit.E or both together were effective to inducing significant increase in the concentrations of testosterone hormone. In normal cases the results showed no significant variation in total numbers of sperms after with green tea extract or Vit.E adminstration, whereas adminstration both together showed a significant increase , also an increase in the average of movement and normal forms when patints treated with green tea extract alone or green extract incombination with Vit.E but no significant varintions were observed when treated with Vit.E alone.The results emphasized that using Vit. E or green tea extract or both together were effective in inducing a significant increase in the concentration of testosterone hormone. The causes of the green tea extract effectivness relate to its consistence of antioxidants which reduced the free radicals in the infertile men and caused an improvement of their fertility.

دراسة تأثير المستخلص المائي للشاي الاخضر *Camellia sinensis* وفيتامين E في الرجال العقيمين في مدينة تكريت وضواحيها

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المستخلص

تضمنت الدراسة الحالية التعرف على تأثير المستخلص المائي للشاي الاخضر في خصوبة الرجال للاشخاص الذين تراوحت اعمارهم (٢٠-٤٠) سنة ومقارنة ذلك مع دور فيتامين E كمضاد للاكسدة وفي تحسين مستوى الخصوبة, للفترة من آذار (٢٠٠٨) لغاية شهر نيسان (٢٠٠٩) في مدينة تكريت وضواحيها .شملت الدراسة

فحص (٢٨١) عينة سائل منوي من الرجال الذي كانوا يعانون من حالات ضعف الخصوبة وعدم القدرة على الانجاب والاسباب متعددة منها (١٤٤) عينة لحالات الاصابة بمرض السيلان (٤٧) عينة لرجال مصابين بدوالي الخصية و (٣١) عينة لرجال مصابين بالتهابات جنسية مختلفة ، كما شملت الدراسة (٦٠) عينة لاشخاص طبيعيين (اصحاء) لا يعانون من ضعف الخصوبة. سبب التعامل بالشاي الاخضر (المستخلص المائي) لوحده او مع فيتامين E ارتفاعاً معنوياً ($p=0.05$) لدى الرجال المصابين بضعف الخصوبة بسبب مرض السيلان وضيق الانابيب المنوية والالتهابات العادية ، حيث ازداد العدد الكلي للنطف ومعدل الحركة ومعدل الاشكال الطبيعية ، وسبب فيتامين E تأثيراً معنوياً($p<0.05$) في معدل حركة النطف وكذلك زيادة في معدل تركيز هرمون الشحمون الخصوي ($p<0.05$) بعد المعاملة بمستخلص الشاي الاخضر وفيتامين E كلاً على حده او كليهما. وكذلك الحال للرجال المصابين بدوالي الخصية فكانت النتائج متشابهة لما ورد اعلاه باستثناء فيتامين E الذي لم يؤثر بشكل معنوي على معدل حركة النطف واشكالها. اما مجموعة الاصحاء الذين لايعانون من ضعف الخصوبة فالمعاملة بمستخلص الشاي الاخضر وفيتامين E لوحدهما لم تؤدي الى تغير معنوي في معدل اعداد النطف الكلي ، فيما حصل ارتفاعاً معنوياً($p<0.05$) في معدل اعداد النطف الكلي عند المعاملة بمستخلص الشاي الاخضر مع فيتامين E معاً وزادت حركة النطف في المجموعة المعاملة بمستخلص الشاي الاخضر فقط او المستخلص مع فيتامين E سوية وكذلك المعاملة المشتركة للشاي الاخضر مع فيتامين E زادت من معدل الاشكال الطبيعية اما هرمون الشحمون الخصوي فقد زادت معدلاته عند المعاملة بمستخلص الشاي الاخضر او فيتامين E او كليهما . ان من اسباب تأثير المستخلص المائي للشاي الاخضر هو لأحتوائه على مضادات الاكسدة متعددة وقوية تقوم باختزال الجذور الحرة لدى هؤلاء الرجال المصابين بضعف الخصوبة مما يحسن من مستواها لديهم.

Introduction

Infertility is the inability to fertile the ova in non-contraception couple to achieve pregnancy in one year(1). Reduced male fertility can be the result of congenital and acquired urogenital abnormalities , infection of the genital tract , increased scrotal temperature (varicocele) , endocrine disturbances , genetic abnormalities and immunological factors (2). These men present with no previous history associated with fertility problems and have normal findings on physical examination and endocrine laboratory testing , Semen analysis reveals decreased number of spermatozoa (oligozoospermia) , decreased motility (asthenozoospermia) and many abnormal forms on morphological examination (tertazoospermia).These abnormalities usually occur together and are described as the oligoasthenoteratozoospermia(OAT) syndrome (2,3). Unexplained forms of male infertility may be caused by several factors , such as chronic stress , endocrine disturbance due to environmental pollution , reactor oxygen species and genetic abnormalities .Also there are secondary factors like decrease protein-carboxy methylase (PCM) which play important role in sperm motion , decrease zinc (4,5) concentration disturbance in calcium use(4,5). Testosterone hormone is the major androgen responsible for sexual differentiation. In males,testosterone is made by the Leydig cells in the testicles and responsible for spermatogenesis and the development of scondary sexual characteretics. This hormone decrease in : An ovulation , Cryptorchidism, Delayed puberty , Down s Syndrome , Excessive alcohol intake , Hepatic insufficiency , Impotence , Klinefilter s syndrome , Malnutration , Myotonmic dystrophy and Orchiectomy (5). The human body is under constant attack from free radicals formed as a natural consequence of the body's normal metabolic activity and as part of the immune system's strategy for destroying invading microorganisms . It has been calculated that about 2×10^{10} molecules of reatille oxygen species (ROS) are generated per cell

per day (6). In stress condition this rate is substantially increased. During evolution , living organisms have developed specific antioxidant protective mechanisms to deal with ROS. There fore , the presence of natural antioxidants in living organisms is the major factor that enables their survival in an oxygen-rich environment (7). Using herbal extracts became one of the recomanded methods in treatment and improve the infertile condition for men .Because it has a high concentration of natural antioxidants like carotinoids , flavonoids , phenols and phenols . Green tea is one of that group which content Catechin (C) , gallaogatechin (EGC), epicatechin , (EC) and epigallocalechin gallate(EGCG) and flavonoids (Flavonols and theaflavins) successful medical benefits produced from green tea beverage include these antioxidants that act as scavengers of free radical to decrease oxidatione stress in the reproductive system of an infertile men(8). There are many studies emphasized on drinking green tea beverage (400 cc three times daily) for 4 weeks which reduced total cholesterol , triglycerides and light density lipoprotein –cholesterol(LDL-C) while increase High density lipoprotein –cholesterol(HDL-C) (9). Green tea has been known to prevent dental decay (cavities) by preventing the attachment of the bacteria associated with dental caries to the teeth(11) . Also green tea consumption can also benefit diabetics by improve glucose tolerance without change basal blood glucose levels (11). Although opidemiological studies have not shown a clear link between cancer prevention and green tea , animal studies have been very convincing . They have clearly demonstrated the preventative effects of green tea and EGCC against liver , stomach, breast , prostate , lung and skin cancer at any stage (initiation , promotion or progression)(12,13).Green tea protects against the development of parkinson's disease, Alzhemer's disease and stroke (neurological deficits)(11). The present research was designed to evaluate the effects of green tea beverage supplementation for 4 weeks on the infertile men including determination of the values of seminal fluid , and testosterone hormone conc. in blood serum of those patients.

Material and Methods

This research included examination of (281) samples of seminal fluid and the same number of samples of blood distributed as follow (144) patients were suffering from gonorrhea , 47 patients with varicocele , 31 patients with seminal vessels narrow , 59 patients with different inflammations and 60 individuals as control .Through a period that began from march 2008 to April 2009 in Tikrit city and its suburbs. All patients and subjects had been distributed to four groups as follow : 1st group treated with Vit. E (400 I.U/day) , 2nd group treated with green tea extract (beverage) (2 gm of green tea per 400 ml of water) repeated three times daily, 3rd group treated with the same dose in 2nd group beside Vit.E ,and the 4th group included control group which was not treated with any thing , the treatment period continued for one month, to all individuals. Seminal fluid analysis tests and some blood tests had been performed before and after the treatment above it included all test correlated with semen analysis (sperms count ,sperm motility and sperm morphology) .

Determination of testosterone hormone concentration :By using the ELISA technique kit made by VIDAS 09345 E-FR Co. and depending on the

Principle of mixing an Enzyme-Immunity test with influoresence detection , the samples of sera transfered to the wells which contained the conjugate (derived from labeled testosterone-alkaline phosphate).Testosterone had been competed with the same hormone(in the conjugate) to combine with antitestosterone specific antibody which covered the inside surface of solid phase receptacle(SPR). The last product 4-Methyl-umbelliferome had been measured at (450nm) and compared with the standard curve of the programe and with normal values of the testosterone hormone (2-10) ng/ml . The data were analyzed statistically using the ready (SPSS) , Analysis variance (ANOVA) Duncan test , differences were recorded as significant probability(p) was less than 0.05 .

The Results

1.Effect of green tea aqueous extract and Vitamin E treatments in fertile men (volunteers):

From table (1) the results showed that the total numbers of fertile men had been significantly increased ($p<0.05$) when the subjects treated with green tea extract and vitamin E(47±0.9) million/m l before treatment to (69±14)million/ml after treatment .While there were no significant increase happened in the others. The second column of the same table included sperm movement activity .The group who were treated with green tea extract only and another group with green tea extract plus vitamin E , caused significant increase of sperm movements at level($p<0.05$). In the third column a significant increase happened in the 4th treatment (59±2.2) million/m before to (80±1.6)million/ml after drinking green tea extract and vitamin E together . The results showed that testosterone hormone concentrations had been increased significantly at level ($p<0.05$) in all types of treatments except control group.

Table (1):- Effect of green tea aqueous extract and Vitamin E treatments on subjects (volunteers) fertile men as a control

S.	Types of the treatments	No. of cases	Total sperm numbers (million/ml) means±S.D		Sperm movement means(%) means±S.D		Normal morphological sperm means (million/ml) means±S.D		Testesterone conc. means (ng/ml) means±S.D	
			Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	Control	15	49±1.2	-	60±3.1	-	63±2.2	-	5.5±0.27	-
2	Vitamin E	15	48±1.1	50±1.8	62±2.7	63±1.9	65±2.6	70±2.0	53±0.41	8.1±0.62*
3	Green tea aqueous extract	15	50±1.5	58±1.1	60±2.1	75±2.0*	67±2.2	68±1.1	5.7±0.33	7.6±0.31
4	Green tea extract + Vitamin E	15	47±0.9	69±1.4*	59±2.2	80±1.6	63±3.0	79±1.4*	4.9±0.52	9.8±0.87

*:Significant variation at probability level : $p<0.05$.

2.Effect of green tea aqueous extract and Vitamin E treatments in fertile patients infected with *gonorrhea cocci* :

The results (table 2) showed a significant increase ($p<0.05$) in the total sperm numbers for the three types of the treatments (Vitamin E , Green tea extract and green tea extract + Vitamin E) the values before treatment were (5±1.5 , 4.8±1.9 , 5.5±1.7) million/ml respectively and (11±1.0 , 26±1.2 , 35±1.98) million/ml after treatment respectively too. While there were a significant increase ($p<0.05$) in the sperm movement had been revealed when comparing the results in the three types in the treatment (2 , 3 and 4 types) which were (20.5±1.8 , 20±1.6 and 25±1.1) million/ml before and after (35±2.0 , 57±1.1 and 60±1.4) million/ml respectively. The 3rd column of tables (2) showed a significant increase in the morphological sperms($p<0.05$) in the two types of treatment (vitamin E , Green tea extract) (30±2.3 , 27±1.5 %)million/ml before and (44±2.0 , 59±1.7 %) million/ml after that step. Also the values of testosterone hormone concentration were (3.2±0.6 , 2.6±1.0 , 2.8±0.5) ng/ml , the values of that groups were (5.6±0.8 , 6.3±0.6 , 7.9±0.33) ng/ml respectively when treated .This was a significant increase ($p<0.05$) in the concentration of that hormones.

Table (2):- Effect of green tea aqueous extract and Vitamin E treatments in an infertile patients who suffered of gonorrhea disease (infertile men)

S.	Types of the treatments	No. of cases	Total sperm numbers (million/ml) means±S.D		Sperm movement means(%) mean±S.D		Normal morphological sperm means (million/ml) means±S.D		Testesterone conc. means (ng/ml) mean±S.D	
			Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	Control	20	4.7±2.0	-	21±2.1	-	28±1.4	-	3.1±0.8	-
2	Vitamin E	44	5±1.5	11±1.0*	20.5±1.8	35±2.0*	32±1.0	39±1.5	3.2±0.6	5.6±0.8*
3	Green tea aqueous extract	38	4.8±1.9	26±1.2*	20±1.6	57±1.1*	30±2.3	44±2.0*	2.6±1.0	6.3±0.6*
4	Green tea extract + Vitamin E	42	5.5±1.7	35±1.98*	25±1.1	60±1.4*	27±1.5	59±1.7*	2.8±0.5	7.9±0.33*

*:Significant variation at probability level : $p<0.05$.

3.Effect of green tea aqueous extract and Vitamin E treatments in an infertile patients who were suffering of varicocele :

In table (3) the total sperm numbers had been increased significantly ($p<0.05$) in the three treated groups , and the results were (3.5±0.9 , 3.3±1.4 ,2.8±1.0 million/ml) before treatments and (14±1.0 , 18±1.3 , 20±1.0million /ml) respectively after treatments. The 2nd column of the same table reveal the changes which happened in the sperm movement activity acquired when the three groups treated with (vitamin E , green tea extract and green tea extract + vitamin E) . So , the effect of that treatments appeared in 3rd and 4th groups only as follow (14±1.4 , 12±1.1 %) before treatment , it increased to (34±2.0 , 37±1.7 %) respectively after treatments. The results in the 3rd and 4th columns of table(3) gave like resu.lts which happened in 2nd column , especially in 3rd and 4th groups. These results reflected the development of the normal morphological of sperms in significant increase ($p<0.05$) also the values of the testosterone hormone concentration had been significant increased ($p<0.05$) when compared with the values before treatments.

Table (3):- Effect of green tea aqueous extract and Vitamin E treatments in an infertile patients who suffered from varicocoele

S.	Types of the treatments	No. of cases	Total sperm numbers (million/ml) means±S.D		Sperm movement means(%) means±S.D		Normal morphological sperm means (million/ml) means±S.D		Testesterone conc. means (ng/ml) means±S.D	
			Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	Control	10	3.0±1.1	-	15±1.2	-	37±0.2	-	4.1±0.43	-
2	Vitamin E	12	3.5±0.9	14±1.0*	13±2.0	18±1.9	38±0.99	42±1.4	3.7±0.9	6.4±0.8*
3	Green tea aqueous extract	11	3.3±1.4	18±1.3*	14±1.4	34±2.0*	38±1.1	60±1.9*	3.4±1.1	7.0±0.56*
4	Green tea extract + Vitamin E	14	2.8±1.0	20±1.0*	12±1.1	37±1.7*	37.5±1.4	63±1.8*	3.2±0.7	7.4±0.7*

*:Significant variation at probability level : p<0.

4.Effect of green tea aqueous extract and Vitamin E treatments in an infertile patients who were suffering from seminal vessels narrow:

The results showed a significant increase 9p<0.05) in the total sperm numbers of the three groups (treated with vitamins E , green tea extract , and green tea extract + vitamin E) as follow (4±1.2 , 4.1±0.9 , 4.3±1.4) million/ml , before treatment , while the results after treatments (18±1.0 , 20±1.1 , 26±0.9) million/ml respectively (table 4) . The sperm movement acquired a significant increase ($p<0.05$) when compared before treatment in 3rd and 4th groups only (20±2.0 , 20±1.8 %) this activated after treatment to (47±1.9 , 50±1.1 %) respectively . Also the normal morphology of sperm and the concentration of testosterone hormone had been increase in both groups (3rd and 4th) when the patients treated with green tea extract or green tea extract and vitamin E together(table 4).

Table (4):- Effect of green tea aqueous extract and Vitamin E treatments in an infertile patients who sufferedfrom seminal vessels narrow

S.	Types of the treatments	No. of cases	Total sperm numbers (million/ml) means±S.D		Sperm movement means(%) means±S.D		Normal morphological sperm means (million/ml) means±S.D		Testesterone conc. means (ng/ml) means±S.D	
			Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	Control	5	4±1.0	-	21±2.0	-	29±3.2	-	2.9±0.66	-
2	Vitamin E	8	4±1.2	18±1.0	22±1.7	28±1.2	30±3.8	41±2.4*	3.3±0.9	5.7±0.8*
3	Green tea aqueous extract	8	4.1±0.9	20±1.1	20±2.0	47±1.9	25±2.7	53±3.0*	3.0±1.0	6.9±1.0*
4	Green tea extract + Vitamin E	10	4.3±1.4	26±1.4	20±1.8	50±1.1	28±2.9	58±3.6*	3.5±0.7	7.3±0.9*

*:Significant variation at probability level : $p<0.05$.

5.Effect of green tea aqueous extract and Vitamin E treatments in an infertile patients who were suffering from different inflammations:

From table (5) the first column show high significant ($p<0.01$) increase in total sperm number after treated (before 5.5±2.3 , 4.9±1.6 , 5.2±1.3) million/ml had been changed to (after 29±2.0 , 40±1.9 , 50±2.4) million/ml .While the activity of sperms had been increased with the level of sperms movement were a significant increased ($p<0.05$). The treatments improved the normal morphology of sperm in 3rd and 4th groups as follow (before 40±1.5 , 35±2.7 %) and (after 63±2.5 , 68±3.0 %) respectively. Testesterone hormone concentration had been increased significantly ($p<0.05$) in the 4th group when all groups treated with all types of the treatments.So the high increase was showed (8.1±1.0 ng/ml) of this hormone.

Table (5):- Effect of green tea aqueous extract and Vitamin E treatments in an infertile patients who suffered from different inflammations

S.	Types of the treatments	No. of cases	Total sperm numbers (million/ml) means±S.D		Sperm movement means(%) means±S.D		Normal morphological sperm means (million/ml) means±S.D		Testesterone conc. means (ng/ml) means±S.D	
			Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	Control	13	5.1±2.0	-	24±2.0	-	39±2.1	-	5.1±0.7	-
2	Vitamin E	15	5.5±2.3	29±2.0**	25±3.0	50±3.3*	38±2.4	42±2.0	4.7±0.7	5.0±0.5
3	Green tea aqueous extract	14	4.9±1.6	40±1.9**	23±2.5	58±3.0*	40±1.5	63±2.5*	5.4±1.0	5.8±1.1
4	Green tea extract + Vitamin E	17	5.2±1.3	50±2.4	27±2.8	66±3.0*	35±2.7	68±3.0*	5.4±0.9	8.1±1.0*

*:Significant variation at probability level : $p<0.05$.
**:High significant variation at probability level : $p<0.01$.

Discussion

The results showed that treatment of infertile men with vitamin E alone for all specimens which included in this study (gonorrhea , varicocele , seminal vessel narrow , different inflammations, low concentration of testosterone and control group) ,generally caused an increase in the total sperm count, its movements and normal morphology of sperms . This results agreed with (15) who used Vitamin E (100-200 I.U)daily to increase the fertility rate in infertile men . In another study found that given (200 LU daly) of vitamin E for three months to patients suffered of infertility .There had been significant increase ($p<0.05$) after one month of treatment by reducing the effects of free radicals which attached the sperm cells (16 , 17) . Vitamin E protect the polyunsaturated fatty acids form an oxidation (decreasing the perodixation of fats) , this trait is very important in reproductive system (18) In this study the results showed a significant increase in testesterone hormone in infertile men .This revealed the effective action of vitamin E to induce the production of testesterone , follicle stimulation hormone (FSH) and tautening hormone (LH) in men (19). The results showed the effective action of green tea aqueous extract (alone or with vitamin E) in the total numbers movement rate , normal morphology of sperms and testosterone hormone concentrations effect also this effect include the control group of subjects. The main reason of that related to the existence of arginine and some amino acid which were necessary to produce sperms. Supplied Arginine for few months has subsequently increased production and also green tea extract contains carinteine (amino acid) which participate in cell membrane structure of the sperms beside an energy to move it. Selenium another factors which increase the movement of sperms (21). Green tea extract contain vitamin E and Follic acid which participate in sperm formation , decrease in abnormal morphology ratio and an efficiency of cell division (22) .This agreed with this study when green tea extract increased the numbers , activity and movement of sperms. Green tea contained antioxidant like catechin , polyphenols , Epicatechin(EC) , Epicatechin gallate (ECG) , (EGCG) and Flavonoids which consider to prove to be superior to(10) times than vitamin E (23). All these compounds used to compensate injured or damaged sperms which are harmed by the free radicals found in natural condition in the body(22). Also green tea contain a lot of vitamin E and C , which both are antioxidants .The effective action of vitamin E increase when participate with another antioxidant (e.g vitamin C) vitamin E molecules when react with free radicals , but vitamin C can repeat the reduction of this molecules (vitamin B) to its origin (24). Green tea extract can protect the body from bacterial infections .Many studies indicated that antioxidants presented in green tea increased the activity of immune system to protects the body against bacteria(25). Catechain substance inhibit DNA gyrase , aprinciple bacterial enzyme and damage the bilayer lipids of Gram +ve and gram - ve stain bacteria(26 , 25). This study emphasized that green tea aqueous extracts are a strong antioxidants beside vitamin E and Vitamin C which had improved the results in the five groups (subjects and patients) to perfect situations by increase the total numbers of sperms , movement , normal morphology and increased the concentration of testestesron hormone.

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