ORIGINAL ARTICLE

Oman Medical Journal [2019], Vol. 34, No. 3: 238-243

Substantial Effect of Fenugreek Seeds Aqueous Extract on Serum Estradiol Level in Ovarian Hyperstimulation Syndrome Rat Model

Asma S. Ben Hameid*, Taha A. Al-Sindi, Ahmad K. Allow, Emad M. Nafie, Basma E. Alahmad and Ghasak G. Faisal

Basic Medical Science, International Islamic University Malaysia, Kuantan, Malaysia

ARTICLE INFO

Article history:
Received: 20 April 2018
Accepted: 1 November 2018

Online:
DOI 10.5001/omj.2019.45

Keywords:
Ovarian Hyperstimulation Syndrome; Fenugreek; Estradiol.

ABSTRACT

Objectives: Estradiol (E2) plays an important role in the pathophysiology of ovarian hyperstimulation syndrome (OHSS). This study aimed to evaluate the effect of fenugreek seed aqueous (FSA) extract on serum E2 levels in a rat model of OHSS. ***Methods***: A total of 34 female Sprague Dawley rats, aged 18 days old, weighing 40 to 45 g, were randomly divided into negative control, positive control, and treatment groups. A daily dose of 1500 mg/kg per body weight of FSA extract was administrated orally to rats in the treatment group for 13 days. On day eight of the study, OHSS was induced in both positive control and treated groups by subcutaneous injection of pregnant mare's serum gonadotropin 50 IU for four consecutive days, followed by human chorionic gonadotropin 25 IU on the fifth day. The effect of FSA extract was evaluated by measuring the concentration of serum E2 using the enzyme-linked immunosorbent assay. ***Results:*** FSA extract reduced serum E2 level significantly in the treated OHSS model (p-value < 0.050) compared to the positive control group. ***Conclusions:*** The finding has important implications on the development of female infertility adjuvant drugs for safe assisted reproduction technology cycles in terms of OHSS prevention.

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic, serious complication in women undergoing assisted reproductive technologies (ARTs) for fertility treatment.[1–3] It occurs due to exaggeration of ovarian response, which is characterized by high estradiol (E2) levels, enlarged ovaries with increasing numbers of large follicles, and enhancement in vascular permeability and shifting of intravascular fluids into third space.[4]

The high basal E2 level has a predictable value in high-risk patients who are young, underweight, have a medical history of OHSS or polycystic ovarian syndrome, and have high follicles numbers and sizes.[5–7] The incidence is estimated to be 0.6% to 5% in moderate to severe OHSS in vitro fertilization (IVF) cycles,[8] and reaches up to 20% in high-risk patients.[9] E2 plays an important role in the pathophysiology of OHSS by increasing vascular permeability (VP) and vascular endothelial growth factor (VEGF) and its receptors numbers which is, in turn, enhanced by gene stimulation over time.[10]

Signs and symptoms of OHSS are hypovolemia, and metabolic and thromboembolic complications, which are mainly observed in severe OHSS cases needing hospitalization.[11–13]

Fenugreek (*Trigonella foenum-graecum*) is an annual herb that belongs to the family Fabaceae. Approximately 90% of the world production comes from India, China, Iran, Pakistan, and Palestine.[14] It is a daily food component for several nations and countries, without any notable, unpleasant effect.[15,16] It has been used in folk medicine as lactation stimulant.[17] Recently, the various medical benefits of fenugreek have been extensively studied[18] with further emphasis on its effects on female gonadal hormones and their reproductive functions.[19,20] It reduces the serum levels of E2 and the number of ovarian follicles.[21,22] Moreover, recent studies found that fenugreek can reduce VP by decreasing VEGF expression in hepatocyte cytoplasm.[23]

Preventing OHSS requires interrupting the pathological process, such as controlling the levels of serum E2. Hence, this study aimed to evaluate the effect of fenugreek seed aqueous (FSA) extract on

*Corresponding author: ✉A.benhameid@gmail.com

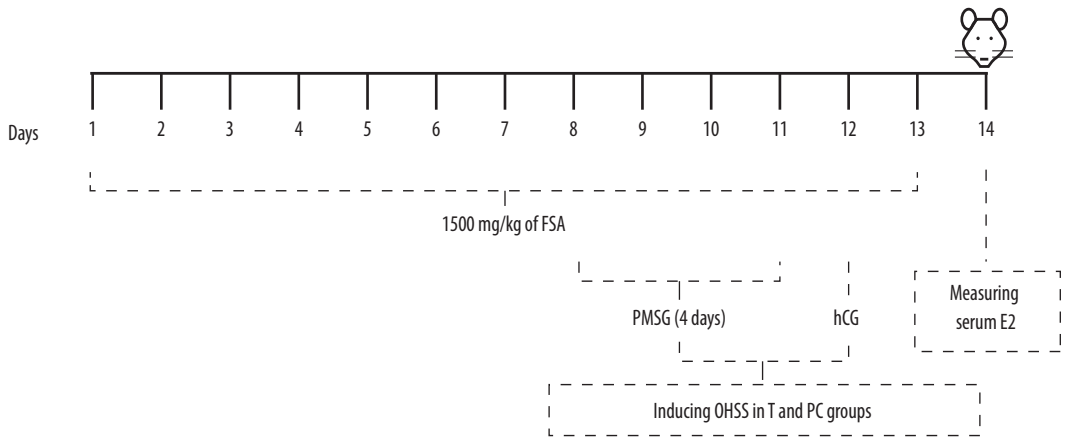

Figure 1: Duration of fenugreek seed aqueous (FSA) extract administration to the treated (T) group and the protocol of ovarian hyperstimulation syndrome (OHSS) induction in the positive control (PC) group and treated (T) group. On day eight of the experiment both PC ad T groups were injected with 50 IU/day of pregnant mares' serum gonadotropin (PMSG) for four consecutive days (25–28 days postnatal (DPN)), followed by subcutaneous injection of 25 IU of human chorionic gonadotropin (hCG) on 29 DPN.

serum E2 levels in an OHSS rat model. We assumed that the FSA extract prevents OHSS development in treated animals by decreasing E2 levels.

METHODS

This study was conducted over two weeks. The study protocol was approved by the Faculty of Medicine and the Integrated Center for Research Animal Care and Use.

A total of 34 immature Sprague Dawley female rats were used. The rats were obtained from an authorized supplier and kept for three days in the animal laboratory for acclimatization under maintained conditions on 12-hour cycles of light and darkness (lights on from 07:00–19:00) and at room temperature 24 °C. During the study period, the rats had free access to water and standard rat diet (rat food pellets). On the first day of the experiment, the rats were randomly divided into two control groups, negative (NC) and positive (PC), and a treated (T) group. The experiment started when the rats were 18 days postnatal (DPN), with a body weight 40.0±5.0 grams.

Fenugreek seeds were collected from a local market. The fenugreek seeds species were identified and deposited in Herbarium of Faculty of Pharmacy (voucher number PIIUM 0226-1). FSA extract was prepared following the method given by Khalki et al.[24] Dry, clean fenugreek seeds were ground into a fine powder and dissolved in distilled water at a ratio of 1:20 (g/mL). The suspension was stirred on a magnetic stirrer hot plate for 24 hours at room temperature. The mixture was then centrifuged at 5000 rpm for 15 minutes at room temperature. Eventually, the extract was freeze-dried for five days.

All rats were weighed daily and received a standard rat diet. In addition to the normal diet, the T group was given a daily oral dose of 1500 mg/kg body weight of FSA extract[25] at 09:00 a.m. from day one to day 13 of the experiment [Figure 1]. The following equation was used to calculate the doses:

$$\text{FSA extract dose} = \frac{\text{body weight of rat (g)} \times 1500}{1000}$$

The extract was given orally, using a metallic oral gavage (20 gauge).

In the second week of the experiment (day eight), OHSS was induced in both PC and T groups using the animal model published by Kitajima et al.[26] Rats in the PC and T groups were injected subcutaneously with pregnant mares' serum gonadotropin 50 IU/day (PMSG; Merck, US) for four consecutive days (25–28 DPN), followed by human chorionic gonadotropin 25 IU (hCG; N.V. Organon, Nederland) on 29 DPN. After 48 hours (31 DPN), blood samples were collected, and the rats were decapitated under general anesthesia.

Under general anesthesia (ketamine, tiletamine and xylazine + zolazepam; UKM, Malaysia),

Table 1: Concentrations of serum estradiol (pg/mL) in study groups.

Group	n	Mean ± SD	Median	Minimum	Maximum
NC	10	11.8 ± 7.8	9.94	3.66	25.67
PC	12	428.8 ± 758.4	127.42	28.35	2701.94
T	12	85.8 ± 131.9	47.48	9.28	491.36

NC: negative control; PC: positive control; T: treated; SD: standard deviation.

approximately 3 mL of blood sample were obtained from each rat from retro-orbital sinus using capillary tubes. The sera were separated by centrifugation of blood samples at 3000 rpm for 15 minutes and kept at -20 °C for subsequent E2 immunoassays analysis. It should be noted that pre-treatment blood sample collection as a baseline in this model (immature rats, body weight 40.0±5.0 grams) is impossible before FSA treatment. According to rodents' nonterminal blood collection guidelines,[27] blood collection is limited up to 10% of total circulating blood volume in healthy, normal, adult animals on a single occasion and collection may be repeated after three to four weeks. To measure the E2 level, at least 50 µL of serum from each rat was needed which was not practically possible in our model. Therefore, the T group was compared with the two control groups (PC and NC groups).

Serum E2 was assayed using rat's E2 enzyme-linked immunosorbent assay kit (ab108667-17 beta estradiol ELISA Kit, Abcam, USA). The detected concentration range for this kit is 20–2000 pg/mL. The 1X Washing Solution was prepared one day before the assay, while the other kit solutions were ready-to-use and stored at 4 °C. The serum samples, standards, and controls were equilibrated to room temperature one hour before use; 25 µL of standard and sample were added to their corresponding wells and 200 µL of 17 beta Estradiol-HRP conjugate. After incubation, the microplate wells were washed three times using 300 µL diluted washing solution, and 100 µL TMB substrate solution was added into each well and incubated for 30 minutes at room temperature in the dark. A blue color developed. Finally, 100 µL of Stop Solution was added into all wells and shook gently. The blue color turned yellow. All assays were done in duplicate.

The absorbance of the samples was detected at 450 nm wavelength within 30 minutes. E2 concentrations were calculated using an ELISA analysis online calculator[28] using optical density values against standard curves calibrated with known concentration values. The assays were done in duplicate. The absorbance of the samples was measured at 450 nm.

The results are presented as median. The significant differences between and among the groups were determined using the Kruskal-Wallis test. A p-value < 0.050 was considered statistically significant.

RESULTS

The effect of FSA extract on serum levels of E2 in the T group (median 47.48 pg/mL) was nearly three times lower than the PC group (median 127.42 pg/mL). Also, the maximum value of the concentration recorded in the T group (491.36 pg/mL) was five times lower than the PC group (2701.94 pg/mL) [Table 1].

In addition, we observed an increase in E2 concentrations in the OHSS model; PC and T groups compared to the NC group. The median E2 concentrations in the T group (47.48 pg/mL) was nearly five times higher than the NC group (9.94 pg/mL). While the PC group (127.42 pg/mL) was 13-times higher compared to the NC group. The

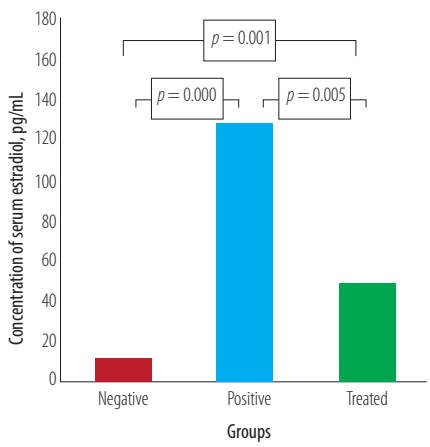

Figure 2: Enzyme-linked immunosorbent assay for the changes in serum estradiol between groups. Groups were compared with each other using the Kruskal-Wallis test. Values are presented as median.

change in E2 concentrations is considered to be a diagnostic value of the model success.

The Kruskal-Wallis test showed that there was a statistically significant difference in serum E2 levels between all groups (p-value < 0.050) and among groups when compared with each other; namely NC and PC (p-value < 0.001), PC and T (p-value = 0.005), and NC and T (p-value = 0.001) groups [Figure 2].

DISCUSSION

FSA extract significantly decreases serum E2 level, nearly three times in the OHSS model, which is consistent with findings of some experimental studies.[20,21] In these studies, the authors found a reduction in serum E2 level due to FSA extract effect.

E2 plays an important role in the pathophysiology of OHSS by increasing VEGF and VP. Controlling serum E2 levels is effective in decreasing the incidence of OHSS cases in IVF cycles.[29]

E2 was found to regulate VEGF through its effect on gene expression depending upon the length of exposure.[30] Also, E2 is an indicator of patients at high risk of developing OHSS.[31]

Normally, in premenopausal women, estrogens are synthesized in the ovaries, which are under the cyclic control of pituitary gonadotropins. Both cytochrome CYP17 (P-450 17α-hydroxylase) and cytochrome CYP19 (P-450 aromatase) are involved in estrogen biosynthesis. Estrogens are mainly catabolized by hydroxylation reactions.[32] Estrogen synthesis takes place in the ovarian granulosa, which is stimulated by the gonadotropins, luteinizing hormone (LH), and follicle stimulating hormone (FSH). Their response to FSH signaling increases enzymes responsible for estrogens synthesis.[33] Aromatase converts testosterone to E2.[34]

Another study demonstrated that herbs with estrogenic property could alter the action of pituitary gland through peripheral modulation of LH and FSH.[35] As a result, these actions decrease the serum concentration of E2. Another mechanism suggested that estrogenic property can affect E2 substrate supplementation during its synthesis. The production process is subsequently affected.[35]

A review addressed the biological role of phytoestrogens in the modulation of estrogen synthesis and metabolism. It stated that phytoestrogens are structurally similar to E2.[36] In vitro, in vivo, and human studies have proved that phytoestrogens may also control estrogen function by affecting estrogen synthesis and metabolism or hormonal signaling within the hypothalamus-pituitary-gonadal axis. Phytoestrogens can also affect estrogen levels in ovarian tissue by interfering with estrogen-synthesizing and metabolism enzymes such as aromatase and estrogen receptors signaling pathways.[36]

Similarly, such estrogenic properties have been found in fenugreek seeds extract.[37] Furthermore, the findings of a study by Bakrim,[20] found that fenugreek seed extract reduces FSH and LH, which may be the mechanism of reducing serum E2 level and could explain our results.

Testosterone is considered an E2 precursor in the aromatase pathway. Fenugreek extract is thought to act through aromatase inhibition, leading to decreased testosterone breakdown to estrogen,[38] which may also explain our results.

Although there is a significant increase in follicles numbers and size in FSA extract treated OHSS models, E2 levels are reduced. This may be due to the effect of FSA extract on the function of granulosa cell, where the E2 level is considered a marker of granulosa cell activity.[35,39]

CONCLUSION

We observed a significant decrease in serum E2 in treated OHSS model, which was almost three times compared to non-treated OHSS model. Thus, the FSA may have the ability to prevent OHSS. Findings in this study have important implications for the development of female infertility adjuvant drugs for safe ARTs cycles in terms of OHSS prevention.

Disclosure

The authors declared no conflicts of interest. This research was funded by Research Initiative Grant (RIG) from IIUM Research Management Center (RIGS– 15 -083).

Acknowledgements

Authors would like to thank IIUM Research Management Center. The authors gratefully acknowledge the services and facilities provided by Integrated Centre for Research Animal Care and Use IIUM. We are gratefully thankful to Professor Raul Gómez and Mr. Ahmed Ben Hameid for their assistance and guidance. Also, we are thankful for Mr. Suhaimi Bin Hashim the senior laboratory technician for his help and assistance.

REFERENCES

1. Pala Ş, Atilgan R, Ozkan ZS, Kavak SB, Ilhan N, Akpolat N, et al. Effect of varying doses of tamoxifen on ovarian

histopathology, serum VEGF, and endothelin 1 levels in ovarian hyperstimulation syndrome: an experimental study. Drug Des Devel Ther 2015 Mar;9:1761-1766.

2. Nouri K, Tempfer CB, Lenart C, Windischbauer L, Walch K, Promberger R, et al. Predictive factors for recovery time in patients suffering from severe OHSS. Reprod Biol Endocrinol 2014 Jul;12(1):59.
3. Al-Hussaini TK. OHSS-Free IVF practice: dream or reality. Middle East Fertil Soc J 2012;17(1):A1-A3.
4. Xu GF, Zhou CL, Xiong YM, Li JY, Yu TT, Tian S, et al. Reduced intellectual ability in offspring of ovarian hyperstimulation syndrome: a cohort study. EBioMedicine 2017 Jun;20:263-267.
5. Banker M, Garcia-Velasco JA. Revisiting ovarian hyper stimulation syndrome: Towards OHSS free clinic. J Hum Reprod Sci 2015 Jan-Mar;8(1):13-17.
6. Busso CE, Soares SR, Pellicer A, Barbieri RL. Prevention of ovarian hyperstimulation syndrome. 2016 [cited 2016 December]. Available from: https://www.uptodate.com/contents/prevention-of-ovarian-hyperstimulation-syndrome.
7. Elmahdy M, Abdelsalam EA, Maghraby HA. Combining several interventions to reduce the incidence of OHSS: A prospective cohort study. Eur J Obstet Gynecol Reprod Biol 2017 May;212:110-114.
8. D'Angelo A, Amso NN, Hassan R. Coasting (withholding gonadotrophins) for preventing ovarian hyperstimulation syndrome. Cochrane Database Syst Rev 2017 May;5(May):CD002811.
9. Nastri CO, Ferriani RA, Rocha IA, Martins WP. Ovarian hyperstimulation syndrome: pathophysiology and prevention. J Assist Reprod Genet 2010 Feb;27(2-3):121-128.
10. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev 2004 Dec;56(4):549-580.
11. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al; International Committee for Monitoring Assisted Reproductive Technology; World Health Organization. World Health Organization. The international committee for monitoring assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary on art terminology, 2009. Hum Reprod 2009 Nov;24(11):2683-2687.
12. Rajesh H, Lee WY, Fook-Chong S, Yu SL. Ovarian hyperstimulation syndrome: an analysis of patient characteristics in the Asian population. Singapore Med J 2011 Mar;52(3):168-174.
13. Mahajan N, Gupta S, Sharma S, Rani K, Naidu P, Arora PR. Early onset ovarian hyperstimulation syndrome despite use of segmentation approach and ovarian hyperstimulation syndrome prophylaxis. J Hum Reprod Sci 2015 Oct-Dec;8(4):234-238.
14. Shinde PM, Sancheti VP, Prajapati AD. Phytochemical screening and study of antioxidant activity of fenugreek seed. Int J. Phytother Res 2015;5(1):1-13.
15. Laila O, Murtaza I, Abdin MZ, Showkat S. Germination of fenugreek seeds improves hypoglycaemic effects and normalizes insulin signilling pathway efficiently in diabetes. Int J Pharm Sci Res 2016;7(4):1535.
16. Alfarisi H, Allow AK, Hamdan AH, Mohamed ZB. Assessment of acute liver toxicity of trigonella foenum-graecum (fenugreek) seeds aqueous extract in male mice. Elixir Hormo & Signal 2017;106:46697-46703.
17. Ghasemi V, Kheirkhah M, Vahedi M. The effect of herbal tea containing fenugreek seed on the signs of breast milk sufficiency in Iranian girl infants. Iran Red Crescent Med J 2015 Aug;17(8):e21848.
18. Nagulapalli Venkata KC, Swaroop A, Bagchi D, Bishayee A. A small plant with big benefits: Fenugreek (Trigonella foenum-graecum Linn.) for disease prevention and health promotion. Mol Nutr Food Res 2017 Jun;61(6).
19. Hilles AR, Allow AK, Mahmood S. Evaluation and comparison of the antifertility potential activity and adverse effects of trigonella foenum-graecum seeds and combined oral contraceptive pills in female rats. Int J Reprod Contracept Obstet Gynecol 2016;5(3):680-688.
20. Bakrim NM. Effects of trigonella foenum graecum (fenugreek) seeds aqueous extract on fertility of female rats. Unpublished Master's Dissertation, International Islamic University Malaysia, Kuantan 2015 [cited 2015 July]. Available from: https://lib.iium.edu.my/mom/services/mom/document/getFile/6iN8oV3VLF6fQAOGUKsgz1JCIKq9rXoZ20161118104908453.
21. Elfituri ZY. Study of the potential activity of aqueous extract of trigonella foenum-graecum (fenugreek) seeds on ovarian superovulation in female rats. Unpublished Master's dissertation, International Islamic University Malaysia 2015 [cited 2015 July]. Available from: https://lib.iium.edu.my/mom/services/mom/document/getFile/NQZDIaqMZnGYy98z2 bpBqfFBLDsxoc7d 20160216101218398.
22. Allaw AK, Belqees AK, Yousof Z, Al-Ani I, Bracamonte M, Sadeeq A, et al. Is there any effect of fenugreek seeds aqueous extract (FSA) on the quantity of ovarian follicles and estrus cycle of female rats? Journal of Medical Practice and Review 2016;1(1):55-63.
23. Vasant More S, Kim IS, Choi DK. Recent update on the role of Chinese material medica and formulations in diabetic retinopathy. Molecules 2017 Jan;22(1):E76.
24. Khalki L, M'hamed SB, Bennis M, Chait A, Sokar Z. Evaluation of the developmental toxicity of the aqueous extract from Trigonella foenum-graecum (L.) in mice. J Ethnopharmacol 2010 Sep;131(2):321-325.
25. Abdi MY. Anti-fertility effect of fenugreek seeds aqueous extract on female rats. Kulliyyah of Medicine, International Islamic University Malaysia, Kuantan; 2017.
26. Kitajima Y, Endo T, Nagasawa K, Manase K, Honnma H, Baba T, et al. Hyperstimulation and a gonadotropin-releasing hormone agonist modulate ovarian vascular permeability by altering expression of the tight junction protein claudin-5. Endocrinology 2006 Feb;147(2):694-699.
27. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 2010 Jul;1(2):87-93.
28. Analysis EL. [cited 2018 May 24]. Available from: https://elisaanalysis.com/app.
29. Lee TH, Liu CH, Huang CC, Wu YL, Shih YT, Ho HN, et al. Serum anti-Müllerian hormone and estradiol levels as predictors of ovarian hyperstimulation syndrome in assisted reproduction technology cycles. Hum Reprod 2008 Jan;23(1):160-167.
30. Sengupta K, Banerjee S, Saxena N, Banerjee SK. Estradiol-induced vascular endothelial growth factor-A expression in breast tumor cells is biphasic and regulated by estrogen receptor-alpha dependent pathway. Int J Oncol 2003 Mar;22(3):609-614.
31. Fisher S, Grin A, Paltoo A, Shapiro HM. Falling estradiol levels as a result of intentional reduction in gonadotrophin dose are not associated with poor IVF outcomes, whereas spontaneously falling estradiol levels result in low clinical pregnancy rates. Hum Reprod 2005 Jan;20(1):84-88.
32. Clemons M, Goss P. Estrogen and the risk of breast cancer. N Engl J Med 2001 Jan;344(4):276-285.
33. Ovarian steroidogenesis. Kyoto Encyclopedia of Genes and Genomes (KEGG). 2014 [cited 2018 March]. Available from: www.kegg.jp/dbget-bin/www_bget?hsa04913.
34. Mungenast F, Thalhammer T. Estrogen biosynthesis and action in ovarian cancer. Front Endocrinol (Lausanne) 2014 Nov;5:192.
35. Yakubu MT, Akanji MA, Oladiji AT, Olatinwo AO, Adesokan AA, Yakubu MO, et al. Effect of Cnidoscolous aconitifolius (Miller) I.M. Johnston leaf extract on reproductive hormones of female rats. Iran J Reprod Med 2008;6(3):149-155.
36. van Duursen MB. Modulation of estrogen synthesis and

metabolism by phytoestrogens in vitro and the implications for women's health. Toxicol Res (Camb) 2017 Sep;6(6):772-794.

37. Sreeja S, Anju VS, Sreeja S. In vitro estrogenic activities of fenugreek Trigonella foenum graecum seeds. Indian J Med Res 2010 Jun;131:814-819.
38. Wilborn C, Taylor L, Poole C, Foster C, Willoughby D, Kreider R. Effects of a purported aromatase and 5α-reductase inhibitor on hormone profiles in college-age men. Int J Sport Nutr Exerc Metab 2010 Dec;20(6):457-465.
39. Soares SR, Gómez R, Simón C, García-Velasco JA, Pellicer A. Targeting the vascular endothelial growth factor system to prevent ovarian hyperstimulation syndrome. Hum Reprod Update 2008 Jul-Aug;14(4):321-333.