Original Article

Role of Oxidative Stress and Antioxidants in Male Infertility: An Interventional Study

Ganeswar Barik, Latha Chaturvedula[1], Zachariah Bobby

Departments of Obstetrics and Gynaecology and [1]Biochemistry, JIPMER, Puducherry, India

Abstract

Aims: The study aims, in infertile men, (i) to assess oxidative stress parameters in semen plasma and (ii) to study the effect of antioxidants in those with abnormal semen parameters. **Settings and Design:** This was an interventional study. **Population:** Ninety men attending and infertility clinic in a tertiary center were enrolled in the study. **Materials and Methods:** The present study was conducted in the departments of obstetrics and gynecology and biochemistry in a tertiary center. Ethical approval was obtained from the institute ethics subcommittee, and the study was conducted between July 2014 and July 2016. The study was conducted on two groups of 45 men with normal semen parameters in group 1 and 45 men with abnormal semen parameters in group 2. **Results:** Malondialdehyde (MDA) value was higher in men with abnormal semen parameters, which was statistically significant. The total antioxidant assay was higher in men with abnormal semen parameters, which was not statistically significant. Oxidative stress index (OSI) value was higher in men with normal semen parameters, which was not statistically significant. After 90 days of antioxidants therapy to men with abnormal semen parameters, MDA value decreased, total antioxidant assay increased, and OSI value decreased, which were statistically significant. Semen parameters such as sperm concentration, motility, and normal morphology improved after 90 days of antioxidant therapy, which were statistically significant. **Conclusions:** Oxidative stress is increased in men with abnormal semen parameters. Antioxidant therapy improves sperm concentration and motility and decreases oxidative stress in the semen plasma.

Keywords: *Antioxidants, male infertility, oxidative stress*

Introduction

In modern era, there are multiple, cost-effective medical treatment options available for female infertility; however, in cases of male infertility, the option of medical management is limited. In this regard, antioxidants seem to be promising options for the management of male infertility. Among young married couples, approximately 85%–90% conceive within 1 year that too mostly within 6 months.[1] Infertility affects 10%–15% of married couples. Male factor infertility contributes to 20%–30% of cases, both male and female factors together contribute to around 40%, female factor alone contributes 40%–55%, and remaining 10% of the factors are unexplained.

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Superoxide anion, hydroxyl radicals, and hydrogen peroxides are the major reactive oxygen species (ROS) in the seminal plasma. In physiological amount, ROS help in sperm capacitation, acrosomal reaction, and sperm oocyte fusion. However, excessive amount of ROS causes infertility in two ways; first, by damaging sperm plasma membrane that leads to loss of sperm motility and ability of sperm to fuse with oocytes,[2] and second, ROS can alter the sperm DNA, resulting in passage of defective

Address for correspondence: Dr. Ganeswar Barik, Department of Obstetrics and Gynaecology, JIPMER, Puducherry - 605 006, India. E-mail: drganes86@gmail.com

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paternal DNA to the conceptus that leads to implantation failure. To counter excessive amount of ROS, human body has natural antioxidants such as glutathione peroxidase and superoxide dismutase. In healthy male, delicate balance is maintained between ROS and antioxidant mechanism in the reproductive system.[3]

Seminal oxidative stress results from imbalance between ROS production and antioxidant defense in the seminal plasma. Seminal oxidative stress is one of the main factors in pathogenesis of male infertility. Oxidative stress can be quantified by measuring individual biomarkers such as malondialdehyde (MDA) and total antioxidant assay of the semen plasma. MDA is a stable lipid peroxidation product, which reflects total oxidative status of semen plasma. Oxidative stress index (OSI) is calculated as (total oxidative stress/total antioxidant status) ×100.

Several methods have been proposed for the management of infertility caused by oxidative stress. Positive lifestyle changes such as maintaining normal weight, reducing smoking or alcohol intake, and taking diet rich in fruits and vegetables would have beneficial effect on sperm health. Antioxidants are most commonly used as defense against oxidative stress-induced infertility.[4] Several clinical studies demonstrated beneficial effect of oral antioxidant in male infertility. Antioxidants augment scavenging capacity of seminal plasma by reducing ROS within semen plasma. The low cost and minimal toxicity of antioxidants are main contributing factors for its use in male infertility.

There are few studies on the role of oxidative stress and antioxidants in male infertility. In our study, we have assessed oxidative stress parameters in infertile men with both normal and abnormal semen parameters at the start of study, and then, we have assessed oxidative stress parameters and semen parameters after giving antioxidants to infertile men with abnormal semen parameters, which is one of the first types of study in male infertility.

Materials and Methods

The present study was conducted in the departments of obstetrics and gynecology and biochemistry in a tertiary center. Ethical approval was obtained from the institute ethics subcommittee, and the study was conducted between July 2014 and July 2016. The sample size was calculated by nMASTER software (CMC, Vellore, Tamil Nadu, India), with α error = 5%, power 80%, and dropout 20%. The sample size was 90 that were divided into 45 each in two groups. Men with normal semen parameters were included in group 1 and men with abnormal semen parameters were included in group 2.

Inclusion criteria for our study were infertile men with oligozoospermia (<15 million/ml) or asthenozoospermia (<40% motile spermatozoa) or teratozoospermia (<4% of normal shape and form spermatozoa) or combination of these and men with normal semen parameters. Exclusion criteria in our study were infertile men with testicular atrophy and other congenital testicular diseases such as cryptorchidism as antioxidants have no role in management of these conditions.

Outcome parameters we studied were baseline characteristics such as age, body mass index (BMI), and duration of infertility and laboratory parameters such as MDA, total antioxidant status, OSI, sperm count, sperm motility, and sperm morphology.

Brief procedure

Men who attended infertility clinic were recruited after fulfilling the inclusion criteria. Detailed history was taken and physical examination was done. Semen analysis was performed. Based on semen analysis, these men were divided into two groups – men with normal semen parameters and men with abnormal semen parameters. Baseline characteristics such as age, height, weight, and BMI were documented. The semen samples were collected from the subjects, centrifuged for separation of plasma, and stored at −80°C until further analysis was done for MDA and total antioxidant capacity. From this sample, semen plasma MDA level was measured by colorimetric method and expressed as micromole (μM) while total antioxidant capacity was measured by colorimetric method and expressed as millimole (mM). At start of the study, semen plasma MDA and total antioxidant capacity tests were done on both men with normal and abnormal semen parameters. Then, infertile men with abnormal semen parameters were treated with antioxidants for 90 days. Repeat semen analysis and oxidative stress test were done after 90 days on men with abnormal semen parameters only.

Estimation of malondialdehyde and total antioxidant assay

1. Semen plasma MDA was estimated by colorimetric method using QUANTIchrom thiobarbituric acid reactive substances (TBARS) assay kit, and the values were expressed as μM
2. Semen plasma total antioxidant assay was estimated by colorimetric method using Cayman's antioxidant assay kit (Product Number: 709001), and the values were expressed as mM.

Principle of the assay for malondialdehyde

TBARS are low-molecular weight end-products (mainly MDA) that are formed during the decomposition of lipid

peroxidation. TBRAS assay is based on the reaction of TBRAS with TBA to form a pink-colored product. The color intensity at 535 nm is directly proportional to TBRAS concentration in the sample.[5,6]

Principle of the assay for total antioxidant capacity

Total antioxidant capacity assay depends on the ability of antioxidants in the sample to inhibit the oxidation of 2,2-azino-di-3-ethylbenzthiazoline sulfonate (ABTS®) to ABTS®·+ by metmyoglobin. The amount of ABTS produced can be monitored by reading the absorbance at 750 or 405 nm. Under the reactive conditions used, the antioxidants in the sample cause suppression of the absorbance at 750 or 405 nm to a degree which is proportional to their concentration. The capacity of the antioxidants in the sample to prevent ABTS®·+ oxidation is compared with that of Trolox, a water-soluble tocopherol analog and is quantified as millimolar Trolox equivalents.[7,8]

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Medications used in study

Antioxidants containing per tablet:

- L- Carnitine - 500 mg
- Lycopene - 2500 mcg
- L-Glutathione - 10 mg
- Coenzyme Q10-50 mg
- Selenium - 75 mcg
- Zinc - 60 mg
- Twice daily doses - for 3 months.

Statistical analysis

Data were analyzed using software SPSS for Windows 20.0 (IBM Company, Illinois, USA).

Following parameters between men with normal and abnormal semen were compared using appropriate statistical tests. Age, BMI, and duration of infertility were tested using unpaired *t*-test. Semen plasma MDA and total antioxidant capacity were compared using independent *t*-test. OSI expressed as median using Mann–Whitney U test.

Before and after values of MDA and total antioxidant assay were compared using paired *t*-test. Before and after values of OSI values were compared using Wilcoxon signed-rank test. Before and after values of semen parameters were compared using paired *t*-test. All statistical analyses were carried out at 5% level of significance ($P < 0.05$).

Results

- Group 1: Men with normal semen parameters
- Group 2: Men with abnormal semen parameters.

Semen plasma MDA, total antioxidant capacity, and OSI were done in both two groups. Forty-five men with abnormal semen parameters were given antioxidant for 3 months. After which semen analysis, semen plasma MDA, total antioxidant assay, and OSI were repeated. Only two men dropped out from our study.

In our study, there was no significant difference in demographic variables such as age, BMI, and duration of infertility between the two groups [Table 1].

MDA value was higher in men with abnormal semen parameters, which was statistically significant. The total antioxidant assay was higher in men with abnormal semen parameters, which was not statistically significant. OSI value was lower in men with normal semen parameters, which was not statistically significant [Table 2].

We found reduction in the seminal plasma MDA values in men with abnormal semen parameters after giving antioxidants with values of 6.46 ± 1.93 μM before and 5.14 ± 2.29 μM after treatment, which was statistically significant. Improvement of total antioxidant capacity was

Table 1: Demographic factor of both the groups (*n*=45)

Factors	Mean±SD		*P* (<0.05 is significant)
	Group 1	Group 2	
Age (years)	29.40±4.95	28.51±3.29	0.31
BMI (kg/m^2)	20.84±1.33	21.18±3.19	0.51
Duration of infertility (years)	4.42±1.43	4.31±1.98	0.76

SD=Standard deviation, BMI=Body mass index

Table 2: Values of malondialdehyde, total antioxidant assay, and oxidative stress index of two groups (*n*=45)

Factors	Mean±SD		*P* (<0.05 is significant)
	Group 1	Group 2	
MDA (μM)	4.98±2.84	6.45±1.90	0.006
Total antioxidant capacity (mM)	2.78±1.90	3.52±1.77	0.060
Oxidative stress index*	0.16 (0.13-0.34)	0.22 (0.1-0.36)	0.900

*Median (IQR). MDA=Malondialdehyde, SD=Standard deviation, IQR=Interquartile range

also statistically significant with values 3.52 ± 1.81 mM before and 5.14 ± 2.29 mM after treatment. In our study, reduction in OSI was observed with median values of 0.16 before and 0.09 after treatment [Table 3].

There was significant difference in the semen volume in men with abnormal semen parameters after giving antioxidant with values before 3.28 ± 1.84 ml and after 2.81 ± 1.91 ml. However, the semen volume was decreased after antioxidant therapy. There was significant improvement in the sperm concentration after giving antioxidant with values 29.10 ± 23.58 million and 43.33 ± 30.34 million, respectively. After antioxidant therapy, there was statistically significant improvement in total motility (27.90% ± 14.21% to 48.48% ± 23.73%). There was significant improvement in sperms with normal morphology after giving antioxidant with values of 75.71% ± 17.09% before and 82.32% ± 17.33% after the treatment. In addition, there was decline in sperm with abnormal morphology with values 24.29% ± 17.09% and 15% ± 11.23%, respectively, before and after treatment [Table 4].

Discussion

Baseline characteristics such as age and BMI were comparable between two groups and did not have significant difference. The mean age was similar between two groups with 28.51 ± 3.29 years in Group 1 and 29.40 ± 4.95 years in Group 2. Our study result was comparable to other studies done by Kumar *et al*., Colagar *et al*., and Sheikh *et al*.[9-11] The mean BMI was also comparable between the groups with 20.84 ± 1.33 kg/m^2 in Group 1 and 21.18 ± 3.19 kg/m^2 in Group 2. Our study result was similar to studies done by MacDonald *et al*. and Nadjarzadeh *et al*.[12,13] The duration of infertility was also similar in both the groups, 4.42 ± 1.43 years in Group 1 and 4.31 ± 1.98 years in Group 2, and it was similar to the study done by Nadjarzadeh *et al*. and Verit *et al*.[13,14]

In the present study, seminal plasma MDA level was higher in men (6.42 ± 1.90 μM) with abnormal semen parameters than that in normal semen parameters (4.98 ± 2.84 μM), which was statistically significant. Our study result was similar to the studies done by Colagar *et al*., Fazeli and Salimi, and Hosen *et al*., and Asbagh *et al*.[10,15-17] Excessive oxidative stress in the semen of men with abnormal semen parameters was the reason for higher MDA value.

The total antioxidant capacity was higher in men with abnormal semen parameters than that in men with normal semen parameters with values 3.52 ± 1.77 and 2.78 ± 1.90 mM, respectively. Our study result was not similar to the studies done by Fazeli and Salimi and Hosen *et al*.[15,16] A case–control study was done by Fazeli and Salimi[15] on 35 infertile and 34 fertile men where total antioxidant capacity was measured by ferric reducing ability of plasma method. They found total antioxidant capacity more in the fertile men than that in infertile men. Hosen *et al*.[16] did a study on 25 fertile men and 41 infertile men using ABTS method to assay total antioxidant assay. They found total antioxidant assay value higher in the fertile men than infertile men. We got total antioxidant capacity higher in men with abnormal semen parameters as we have compared infertile men in both the groups unlike other studies, and also, our result was not statistically significant.

In our study, we found OSI value (0.16) was lower in men with normal semen parameters than in men with abnormal semen parameters (0.22), which was not statistically significant. In a study done by Verit *et al*.,[14] (prospective study) on 32 infertile men and 30 fertile men, the OSI value was higher in the infertile men than fertile men.

We had studied oxidative stress parameters after giving 90 days of antioxidants to men with abnormal semen parameters. We found significant reduction of the semen plasma MDA level after treatment with antioxidant with values 4.053 μm before and 3.073 μm after treatments, which was statistically significant. This was

Table 3: Comparison of malondialdehyde, total antioxidant capacity, and oxidative stress index before and after treatment in Group 2 (*n*=43)

Factors	Mean±SD		*P* (<0.05 is significant)
	Before	After	
MDA (μM)	6.46±1.93	5.32±2.01	<0.001
Total antioxidant capacity (mM)	3.52±1.81	5.14±2.29	<0.001
Oxidative stress index*	0.16 (0.13-0.34)	0.09 (0.6-0.13)	0.05

*Median (IQR). MDA=Malondialdehyde, SD=Standard deviation, IQR=Interquartile range

Table 4: Comparison of semen parameters before and after treatment in Group 2 (*n*=43)

Factors	Mean±SD		*P* (<0.05 is significant)
	Before	After	
Volume (ml)	3.28±1.84	2.81±1.91	0.050
Concentration (×10^6/ml)	29.10±23.58	43.33±30.34	0.003
Total motility (%)	27.90±14.21	48.48±23.73	<0.001
Normal morphology (%)	75.71±17.09	82.32±17.33	0.020
Abnormal morphology (%)	24.29±17.09	15±11.23	<0.001

similar to other studies done by Suleiman *et al*. and Singh *et al*.[18,19] In a study done by Suleiman *et al*.,[18] on 52 infertile patients giving antioxidants, they found significant reduction of MDA level and improvement of sperm motility. Singh *et al*.[19] did a study on 40 infertile men who received antioxidants for 3 months. They found significant reduction of MDA levels after 3 months of antioxidant therapy. The level of MDA which is marker of oxidative stress was decreased after antioxidant therapy in the present study.

The present study showed significant improvement of total antioxidant capacity after treatment with antioxidant, with mean levels being 3.52 ± 1.81 and 5.14 ± 2.29 mM before and after, respectively. This was similar to studies done by Singh *et al*. and Yadav *et al*.;[19,20] however, in a study done by Kumar *et al*.,[9] on 21 infertile men, antioxidant for 3 months found no significant improvement in total antioxidant capacity. This difference may be due to less sample size of 21 infertile men. Total antioxidant capacity which reflects total antioxidant power of the semen plasma was improved after antioxidant therapy. Antioxidant therapy improves infertility outcomes by supplying synthetic antioxidants to seminal plasma and by reducing MDA level in semen plasma.

In our study, we found significant reduction in OSI before and after antioxidant treatment, with mean levels of 0.16 before and 0.09 after treatments. We did not find any study comparing OSI before and after antioxidant therapy.

We had studied semen parameters after giving antioxidants to men with abnormal semen parameters for 90 days. There was a significant difference in semen volume (3.28 ± 1.84 vs. 2.81 ± 1.91 ml) after 3 months of antioxidant therapy. This decrease in semen volume may be due to incomplete collection or a short abstinence interval. Our study result was not similar to studies done by Kacem *et al*. and Kobori *et al*.[21,22] In the study done by Kacem *et al*.,[21] on 48 infertile couples after giving antioxidant therapy for a variable period of 3–5 months, they found no significant difference in semen volume. Kobori *et al*.[22] conducted a study on 169 infertile men after giving antioxidants; they observed semen parameters after 3 and 6 months and found no improvement of semen volume after antioxidant therapy.

Sperm concentration is an important parameter in the management of male infertility. With antioxidant therapy, we observed a significant improvement in sperm concentration which can help in change in the management plan from *in vitro* fertilization to intrauterine insemination. In our study, there was a significant improvement in sperm concentration (29.10 ± 23.58 × 10^6/ml vs. 43.33 ± 30.34 × 10^6/ml). Our study result was similar to the studies done by Singh *et al*., Khani *et al*., and Muneshwar *et al*.[19,23,24] Kacem *et al*.[21] did another study on 48 infertile men after giving antioxidant for 2-month duration; they found no increase in sperm volume, concentration, and motility. The length of treatment for 2 months is less than the life cycle of sperm to show any effect of antioxidant in their study.

Antioxidants improve sperm motility which is one of the important parameters in the management of male infertility. In our study, there was a significant improvement in motility after antioxidants in men with abnormal semen parameters (27.90% ± 14.21% vs. 48.48% ± 23.73%). Our study was similar to the studies done by Suleiman *et al*., Singh *et al*., Khani *et al*., Muneshwar *et al*., Comhaire *et al*., and Piomboni *et al*.[18,19,23-26] All the above studies used either single or combination of antioxidants for 3 months and observed improvement in sperm motility, except Suleiman *et al*.,[18] who observed improvement in sperm motility after giving Vitamin E alone for 6 months.

In our study, we found significant improvement in sperm morphology after antioxidant therapy. Our result was similar to the studies done by Piomboni *et al*. and Heidary *et al*.[26,27] as both studies observed improvement in the sperm morphology after 3 months of antioxidant therapy; however, Khani *et al*.[23] performed a study on 25 infertile men to determine the effect of antioxidant therapy on sperm morphology, and they found no significant improvement in sperm morphology. This difference may be due to less sample size in their study.

In our study, we got significant improvement in semen parameters such as sperm count and motility after 3 months of antioxidant therapy, and also, biochemical parameter such as MDA value was significantly decreased and total antioxidant capacity was increased significantly after treatment. As spermatogenic cycle completes in 3 months, our idea was to give antioxidants for 3 months to cover sperm damage at any level of spermatogenesis and to analyze the results. Our study of antioxidants use in male infertility is supported by many studies.

Conclusions

From the present study, it may be concluded that oxidative stress is increased in men with abnormal semen parameters. Antioxidant therapy improves sperm concentration and motility and decreases oxidative stress in the semen plasma.

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Conflicts of interest

There are no conflicts of interest.

References

1. Rowe PJ, Comhaire FH, Hargreave TB, Mahmoud AM. WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male. New York: Cambridge University Press; 2000.
2. Aitken RJ, West KM. Analysis of the relationship between reactive oxygen species production and leucocyte infiltration in fractions of human semen separated on percoll gradients. Int J Androl 1990;13:433-51.
3. Agarwal A, Saleh RA. Role of oxidants in male infertility: Rationale, significance, and treatment. Urol Clin North Am 2002;29:817-27.
4. Agarwal A, Sekhon LH. The role of antioxidant therapy in the treatment of male infertility. Hum Fertil (Camb) 2010;13:217-25.
5. Rao B, Soufir JC, Martin M, David G. Lipid peroxidation in human spermatozoa as related to midpiece abnormalities and motility. Gamete Res 1989;24:127-34.
6. Bharadwaj S, Bhat VB, Vickneswaran V, Adhisivam B, Zachariah B, Habeebullah S. Oxidative stress in preeclamptic mother – Newborn dyads and its correlation with early neonatal outcome – A case control study. J Matern Fetal Neonatal Med 2018;31:1548-53.
7. Mahfouz R, Sharma R, Sharma D, Sabanegh E, Agarwal A. Diagnostic value of the total antioxidant capacity (TAC) in human seminal plasma. Fertil Steril 2009;91:805-11.
8. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem 1996;239:70-6.
9. Kumar R, Saxena V, Shamsi MB, Venkatesh S, Dada R. Herbo-mineral supplementation in men with idiopathic oligoasthenoteratospermia: A double blind randomized placebo-controlled trial. Indian J Urol 2011;27:357-62.
10. Colagar AH, Pouramir M, Marzony ET, Jorsaraei SG. Relationship between seminal malondialdehyde levels and sperm quality in fertile and infertile men. Braz Arch Biol Technol 2009;52:1387-92.
11. Sheikh N, Amiri I, Farimani M, Najafi R, Hadeie J. Correlation between sperm parameters and sperm DNA fragmentation in fertile and infertile men. Int J Reprod Biomed 2008;6:13-8.
12. Macdonald AA, Stewart AW, Farquhar CM. Body mass index in relation to semen quality and reproductive hormones in New Zealand men: A cross-sectional study in fertility clinics. Hum Reprod 2013;28:3178-87.
13. Nadjarzadeh A, Sadeghi MR, Amirjannati N, Vafa MR, Motevalian SA, Gohari MR, *et al.* Coenzyme Q10 improves seminal oxidative defense but does not affect on semen parameters in idiopathic oligoasthenoteratozoospermia: A randomized double-blind, placebo controlled trial. J Endocrinol Invest 2011;34:e224-8.
14. Verit FF, Verit A, Ciftci H, Erel O, Celik H. Paraoxonase-1 activity in subfertile men and relationship to sperm parameters. J Androl 2009;30:183-9.
15. Fazeli F, Salimi S. Correlation of seminal plasma total antioxidant capacity and malondialdehyde levels with sperm parameters in men with idiopathic infertility. Avicenna J Med Biochem 2016;4:e29736.
16. Hosen MB, Islam MR, Begum F, Kabir Y, Howlader MZ. Oxidative stress induced sperm DNA damage, a possible reason for male infertility. Iran J Reprod Med 2015;13:525-32.
17. Akbari Asbagh F, Mostafavi E, Hamdi K, Azmodeh O, Ghasemynejad A, Moshtaghi J, *et al*. Relation of serum and semen malondialdehyde and total anti-oxidants with sperm parameters in infertile men. Am J Immunol 2010;6:43-9.
18. Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: Protective role of Vitamin E. J Androl 1996;17:530-7.
19. Singh A, Jahan N, Radhakrishnan G, Banerjee BD. To evaluate the efficacy of combination antioxidant therapy on oxidative stress parameters in seminal plasma in the male infertility. J Clin Diagn Res 2016;10:QC14-7.
20. Yadav SB, Suryakar AN, Huddedar AD, Durgawale PP, Shukla PS. Antioxidant treatment a new therapeutic approach to reversible male infertility. Biomed Res 2006;17:175-8.
21. Kacem O, Harzallah M, Zedini C, Zidi I, Meddeb S, Fékih M, *et al*. Beneficial effect of an oral antioxidant supplementation (Fertima×2) on IVF-ICSI outcomes: A preliminary clinical study. Adv Reprod Sci 2014;2:47-56.
22. Kobori Y, Suzuki K, Iwahata T, Shin T, Sadaoka Y, Sato R, *et al.* Improvement of seminal quality and sexual function of men with oligoasthenoteratozoospermia syndrome following supplementation with L-arginine and pycnogenol®. Arch Ital Urol Androl 2015;87:190-3.
23. Khani B, Bidgoli SR, Moattar F, Hassani H. Effect of sesame on sperm quality of infertile men. J Res Med Sci 2013;18:184-7.
24. Muneshwar JN, Baig MS, Kaderkar D, Khan ST, Deshmane S. A qualitative analysis of efficacy of multivitamin and micronutrient supplementation on semen parameters in patients with primary infertility: A prospective randomized control study. Int J Recent Trends Sci Technol 2013;1:124-6.
25. Comhaire FH, El Garem Y, Mahmoud A, Eertmans F, Schoonjans F. Combined conventional/antioxidant "Astaxanthin" treatment for male infertility: A double blind, randomized trial. Asian J Androl 2005;7:257-62.
26. Piomboni P, Gambera L, Serafini F, Campanella G, Morgante G, De Leo V, *et al.* Sperm quality improvement after natural anti-oxidant treatment of asthenoteratospermic men with leukocytospermia. Asian J Androl 2008;10:201-6.
27. Heidary M, Vahhabi S, Reza Nejadi J, Delfan B, Birjandi M, Kaviani H, *et al.* Effect of saffron on semen parameters of infertile men. Urol J 2008;5:255-9.