JMedSci

Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 52, Number 4, 2020; 300-308
http://dx.doi.org/10.19106/JMedSci005204202002

Ethanolic extract of Dutch eggplants (*Solanum betaceum*) protects spermatozoa motility exposed to lead acetate

Rima Wirenviona[1]*, Reny I'tishom[2], Siti Khaerunnisa[3]

[1]Master of Reproductive Health, Postgraduate Program, [2]Department of Medical Biology, [3]Department of Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Submitted : 2020-05-02
Accepted : 2020-08-28

ABSTRACT

Lead is a toxic material that can have negative effects on reproductive organs. Lead exposure reduces the activity of endogenous antioxidant enzymes and increases the number of oxidants in the body. High free radicals will affect spermatogenesis and result in decreased motile spermatozoa. Antioxidants are known to protect the motility of spermatozoa, and adequate antioxidants can be found in Dutch eggplants (*Solanumbetaceum*). This study aimed to evaluate the effect of *S. betaceum* on spermatozoa motility after exposure to lead acetate. This study is a true experimental design with a randomized post-test-only control group design. Forty male Balb/C mice 12 weeks old were randomly divided into 5 groups: two control groups (C_0, C_1) and three treatment groups (T_1, T_2, T_3).The C_0 received distilled water, and the C_1 received 75 mg/kg BW lead acetate. The T_1, T_2 and T_3 received 100, 200 and 400 mg/kg BW of *S. betaceum*, respectively, an hour before exposed lead acetate. The data were analyzed using one-way ANOVA with a significant level of $p < 0.05$. A significantly increasein the mean total motility of spermatozoa in T_1, T_2, and T_3 was reported.This study indicates that *S. betaceum* have a protective effect on spermatozoa motility when exposed to lead acetate.

ABSTRAK

Timbal adalah bahan beracun yang dapat memberi efek negatif pada organ reproduksi. Paparan timbal mengurangi aktivitas enzim antioksidan endogen dan meningkatkan jumlah oksidan dalam tubuh. Radikal bebas yang tinggi akan mempengaruhi spermatogenesis dan mengakibatkan penurunan spermatozoa motil. Antioksidan telah diketahui dapat memberikan perlindungan pada motilitas spermatozoa dan antioksidan adekuat dapat ditemukan dalam terong Belanda (*Solanum betaceum*). Penelitian ini bertujuan untuk mengetahui pengaruh *S. betaceum* terhadap motilitas spermatozoa yang dipapar timbal asetat. Studi ini adalah eksperimental murni dengan desain *randomized post-test only control group*. Terdapat 40 mencit jantan berumur 12 minggu Balb/C yang secara acak dibagi menjadi 5 kelompok: dua kelompok kontrol (C_0 danC_1) dan tiga kelompok perlakuan (T_1, T_2, T_3). C_0 menerima aquades dan T_1 menerima 75 mg/kg BB timbal asetat. T1 menerima 100 mg/kg BB, T_2 menerima 200 mg/kg BB, dan T_3 menerima 400 mg/kg BB *S. betaceum* 1 jam sebelum pemberian asetat timbal. Analisis data menggunakan one way ANOVA dengan nilai (p=0,002). Terdapat peningkatan rerata total motil spermatozoa pada kelompok T_1, T_2, dan T_3. Penelitian ini menunjukkan bahwa *S. betaceum* dapat memberikan efek protektif pada motilitas spermatozoa yang dipapar timbal asetat.

Keywords:
Solanum betaceum;
motility;
spermatozoa;
lead acetate;
mice;

*corresponding author: rimawiren@gmail.com

INTRODUCTION

Infertility affects approximately 15% of couples trying to conceive, and a malefactor such as decreased quality of spermatozoa contributes to roughly half of these cases.[1] The incidence of infertility has been increasing in industrial countries from 7%-8% in 1960 to 20%-35% currently. Industrial countries have high environmental pollution. Environmental pollution is one of the factors that contributes to the decline in male fertility.[2] Environmental pollutant material that is often found daily, especially in industrial and developing countries, is lead (plumbum, Pb).[3]

Humans are exposed to many toxicants, including a heavy metal lead in the workplace and from the environment. Exposure to lead can result in significant adverse health effects on male reproductive systems.[4] The most frequent causes of male infertility are associated with spermatozoa motility. Lead can induce a prolonged liquefaction time, thereby reducing spermatozoa motility.[5] Lead induces lipid oxidation, especially in unsaturated fatty acid chains, which dominate the lipid architecture of the sperm plasma membrane.[6] These oxidized lipids undergo a chain reaction to form free radical products. Increasing the number of radicals will result in the decomposition of unsaturated fatty acids into lipid peroxide, which is very unstable. Lipid peroxidation is a process that commonly occurs following the production of high levels of reactive oxygen species (ROS).[7] High oxidants have a strong impact on spermatozoa motility. Spermatozoa are very susceptible to interference in ROS levels. Elevated ROS production can overwhelm the cell's limited antioxidant defenses, leading to dysfunction and loss of fertilizing potential.[8]

Adequate antioxidants are needed to increase male fertility.[9] One fruit that contains high antioxidants is *Solanum betaceum* or Dutch eggplants. *Solanum betaceum* is a subtropical non-climacteric fruit that produces fruit throughout the year.[10] *Solanum betaceum* fruit is very rich in nutrients and chemical compounds needed by the body.[11] Several previous studies indicate that lead exposure can be inhibited by administering exogenous antioxidants.[12] Antioxidants can bind to free radical compounds.[13]

Solanum betaceum is a natural antioxidant that has been shown to contain quite high antioxidants such as anthocyanin, flavonoids, and tannins.[14] Flavonoid compounds are secondary metabolite compounds that act as antioxidants because they are beneficial in preventing cell damage due to oxidative stress.[15] Flavonoids that function as antioxidants are flavonoids that have hydroxyl groups (-OH) because they can donate protons (H atoms) to free radicals so that free radicals become stable.[16] Anthocyanin is a natural source of antioxidants that can be used to minimize oxidation reactions and ward off free radicals.[17] Ellagic acid in tannins reacts with free radicals because of its ability to bind metal ions, which are powerful antioxidants against lipid peroxide.[18] *Solanum betaceum* extract acts as an antioxidant that can protect the biological membrane of sperm from damage due to free radicals and has the potential to protect the motility of spermatozoa from the influence of lead.

MATERIALS AND METHODS

Study design and site

This is true-experimental research with a randomized post-test only control group design. Forty male mice were obtained from Surabaya Veterinaria Center. Maintenance of mice and treatment were located at the Laboratory Animals Experiment,

Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya. The observation of the quality of the spermatozoa was located at the Medical Biology Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

Animal criteria, standard diet, and bedding

Forty clinically healthy male Balb/C mice, 12 weeks old, with an initial body weight of 25-30 g were used in this study. Acclimatization was performed before use for 7 days to adjust to the new environment. All mice were found to be in a clinically acceptable condition and were assigned to the study. Animals that are sick and die will be excluded from the study.

Animals were housed in five cages with a size of 500 x 300 x 150 mm for 35 days based on the spermatogenesis cycle. The cageswere well placed so that all experimental animals obtained good environmental conditions, such as light, temperature, and humidity, that were homogeneous and constant. There were five groups, namely, C_0, C_1, T_1, T_2, and T_3. C_0 was receiving distilled water. C_1 was receiving lead acetate. T_1, T_2, and T_3 were given lead acetate after receiving *S. betaceum*. All treatments were given orally. Each group (8 mice) received the same standard diet *ad libitum*. The sawdust used for the base of the cage was changed depending on needs or at least every 3 days to keep the cage clean and for animal health.

Lead acetate preparation

Lead acetate used a dose of 75 mg/kg BW, dissolved in distilled water and saved at room temperature. Lead acetate solution was given as much as 0.1 mL.

***Solanum betaceum* extract preparation**

The extracted material used in this study was the ethanolic extract of *S. betaceum.Solanum betaceum* fruit was dried by a fresh dryer. Dry powder was extracted by maceration 3 x 24 h 3 times at room temperature. The ethanol extract of *S. betaceum* was dissolved in distilled water before being given to mice orally. The extract was given in 3 different doses, namely, T_1 100 mg/kg BW, T_2 200 mg/kg BW, and P_3 400 mg/kg BW. Different doses were administered in the Medical Biochemistry Laboratory, Faculty of Medicine, UniversitasAirlangga. *Solanum betaceum* solution was given as much as 0.1 mL.

The instrument and measurement of spermatozoa motility

Mice were sacrificed by giving chloroform solution and then surgically performed. After sacrificing the animals, the spermatozoa was examined to determine their motility. The procedure for checking spermatozoa motility was carried out in each group. Spermatozoa suspension was obtained from the epididymis using a dissecting kit. The cauda epididymis was separated by cutting the proximal portion of the epididymal corpus and the distal portion of the vas deferens. Subsequently, the cauda epididymis was put into a petri dish containing 1 mL of NaCl 0.9%, and the proximal portion of the cauda was cut with scissors and then pressed gently until the secretion of epididymal fluid was released and suspended in NaCl 0.9%. Spermatozoa suspension from the cauda epididymis was used for observation approximately 30 min after collection.

Observation of the motility of

spermatozoa was performed by dripping approximately 10-15 µL into the glass object and then covered with a cover glass. Spermatozoa motility is observed under a microscope with a magnification of 200-400x. Evaluate at least 200 spermatozoa with acalculating machine and assess the movements of spermatozoa that occur by assessing 3 categories and calculating their percentage. Spermatozoa motility assessment criteria are spermatozoa moving forward (progressive), spermatozoa moving but not forward, only spinning, or moving in place (non progressive), and spermatozoa are silent or not moving (immotile).

Statistical analysis

The results are given as the means ± standard deviation (SD). Data were analyzed using one-way ANOVA and post hoc tests with a significance level of pvalue <0.05. The data were normally distributed.

Ethical consideration

This study was approved by the Research Ethics Committee of Faculty of Medicine, UniversitasAirlangga, Surabaya, with registration number: 30/EC/KEPK//FKUA/2020.

RESULTS

Spermatozoa motility assessment criteria, namely progressive, nonprogressive, and immotile are shown in FIGURE 1. The highest mean number of immovable or immotile spermatozoa in theC_1 group was given lead acetate. The lowest mean of progressive and nonprogressive motile spermatozoa was in the C_1 group that was given lead acetate.

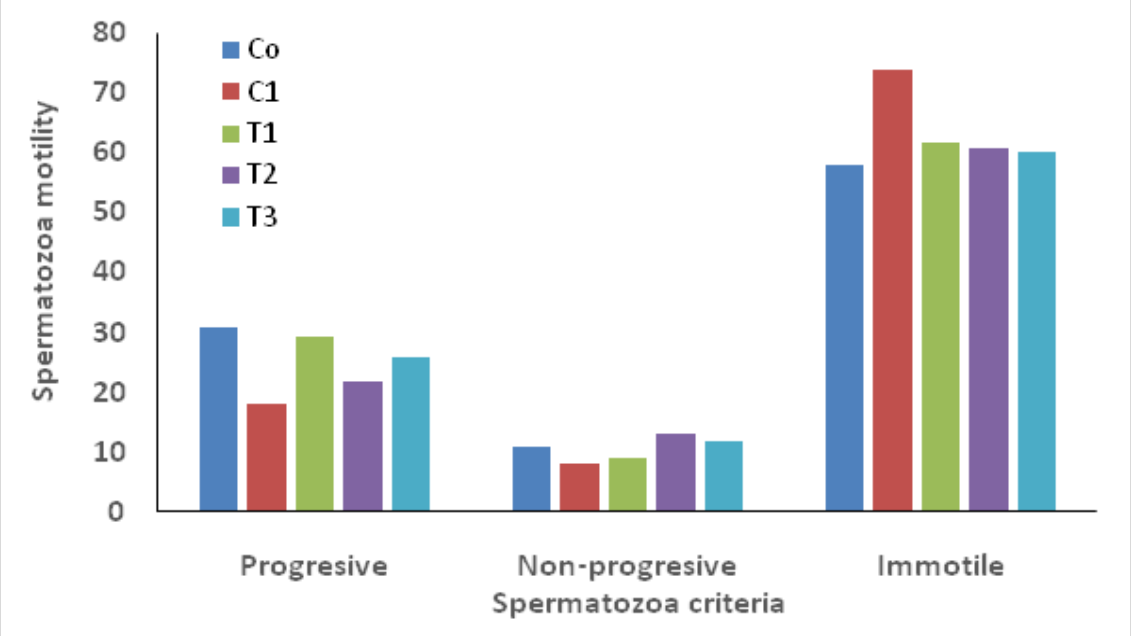

FIGURE 1. Average spermatozoa motility of 5 treatment groups (n=40).

Statistical analysis results of each group can be seen in TABLE 1. Each group consisted of 8 male mice. The average measurement results were obtained by combining progressive and nonprogressive motility as total motile. The mean of the total motile in each group was different. The highest mean in the

C_0 group was 45.13%, and the lowest in the C_1group was 25.88%. The mean value of total motile spermatozoa in groups T_1, T_2, and T_3 increased with increasing doses. To see the spermatozoa motility data normally distributed, a normality test with Shapiro-Wilk was performed because the data amounted to <50 data points. Thespermatozoa motility data distribution of each group was normal with a pvalue >0.05, and then the analysis was continued with a homogeneity test. The homogeneity test showed that the distribution of spermatozoa motility data in each group was homogeneous with a pvalue of 0.256 and then continued with the ANOVA difference test. ANOVA showed that there was a significant difference in spermatozoa motility with a p value of 0.002.

The post hoc LSD test showed that there were significant differences in total motile spermatozoa (p <0.05) as shown in TABLE 2. Superscript letters show significant differences, namely, C_0 and C_1 (0.000), C_1 with T_1 (0.004), T_1 with T_2 (0.003), and C_1 with T_3 (0.001).

TABLE 1. The difference in spermatozoa motility between the intervention and the control groups

Groups	Mean± SD	Normality	Homogeneity	p
C_0	45.13±5.489	0.631		
C_1	25.88± 10.092	0.732		
T_1	37.25± 5.574	0.347	0.256	0.002
T_2	37.88± 8.526	0.073		
T_3	38.75± 6.319	0.499		

C_0: normal mice; C_1: exposed to lead acetate only; T_1: 100 mg/kg BW + exposed to lead acetate; T_2: exposed to lead acetate + 200 mg/kg BW; T_3: exposed to lead acetate + 200 mg/kg; SD: standard deviation

TABLE 2.Post hoc LSD test for total motility of spermatozoa

Groups	C_0	C_1	T_1	T_2
C_1	0.000a	-	-	-
T_1	0.238	0.004b	-	-
T_2	0.265	0.003c	0.947	-
T_3	0.374	0.001d	0.766	0.817

DISCUSSION

In this study, the C_1 group that was only given lead acetate, showed the lowest total motile rate. These results showed that lead toxicity can reduce total motile spermatozoa. Lead-induced oxidative stress causes major damage to sperm quality by disrupting the antioxidant and reactive oxygen species (ROS) balance, thus resulting in abnormalities of spermatogenesis.[19] Increased ROS products cause oxidative stress (OS) due to an imbalance of antioxidants and oxidants.[20] Although ROS are necessary for the normal physiological function of sperm, excessive oxidative stress can cause increased susceptibility to DNA damage, potentially leading to infertility and reducing sperm

motility.[21] Oxidative stress has been identified as an area of great attention because ROS and their metabolites can attack enzymatic systems. Spermatozoa have minimal endogenous antioxidant defenses and are very dependent on exogenous enzyme sources during spermatogenesis.[22]

Spermatozoa are rich in mitochondria to supply much energy that is required for motility.[23] The movement of spermatozoa requires some ATP, which is used to move the flagellate apparatus.[24] ROS-induced damage to mitochondrial DNA leads to decreased ATP and energy availability, impeding sperm motility. The presence of dysfunctional spermatozoa in the semen significantly elevates the production of ROS, which in turn affects its mitochondrial function and sperm motility.[25] Disturbances in mitochondrial respiration function can result in decreased motility and fertility, which can be seen in the C_1 group. The cytology of animals that lead poisoning describes the occurrence of asthenozoospermia or a condition when sperm cannot move swiftly.[26]

The balance between ROS and antioxidants is necessary to maintain physiological oxidative mechanisms and to minimize the risk of cellular injury.[27] If ROS accumulate, endogenous antioxidant defenses will not be sufficient.[28] Exogenous antioxidants are needed to inhibit cell damage that occurs. *Solanum betaceum* fruit contains high antioxidants that the body needs.[29] The mean value indicates that there was a significant difference between the groups given lead (C_1) and the group's given extracts of *S. betaceum* and lead acetate (T_1, T_2, and T_3). The antioxidant content in the *S. betaceum* extract can function as a protective agent to prevent cell damage caused by free radicals, with the mean total motile values of the T_1, T_2, and T_3 groups increasing by 37.63, 37.88, and 38.75%, respectively, compared to the C_1 group. Spermatozoa motility significantly increases with increasing dose.[30] Administration of antioxidants can increase the body's metabolism and accelerate the formation of ATP so that spermatozoa motility will increase.[31] This happens because in the cell plasma membrane, there are many macromolecules in the form of proteins, lipoproteins, and glycoproteins that function as enzymes and receptors. Spermatozoa require several enzymes and receptors for their movement.[32]

The results of this study showed that the administration of ethanolic *S. betaceum* extracts significantly affected the total motility of spermatozoa. The highest percentage increase in the mean total motility is shown by giving the highest dose of 400 mg/kg body weight. The higher the dose of antioxidant administration, the more antioxidant content obtained will increase the motility of mouse spermatozoa.[33] Other studies have shown an improvement in mitochondrial function as a producer of ATP after exogenous antioxidants are given. ATP will be better synthesized as an energy source to drive flagella if given adequate antioxidants.[34]

CONCLUSION

The findings of this study shows that there is a significant difference in motility between the intervention and the control groups. *Solanum betaceum* extract has a protective effect by increasing the total motility of spermatozoa. The highest increase in the mean percentage of total motile spermatozoa was shown by giving the highest dose of 400 mg/kg BW in the T_3 group.

REFERENCES

1. Agarwal A, Virk G, Plessis SS. Effect of oxidative stress on male reproduction. The World J Mens Health 2014; 32(1):1-17.

https://doi.org/10.5534/wjmh.2014.32.1.1
2. Najafi TF, Roudsari RL, Namvar F, Ghanbarabadi VG, Talasaz ZH, Esmaeli M. Air pollution and quality of sperm. Iranian Red Crescent Med J 2015; 17(4):e26930 https://doi.org/10.5812/ircmj.17(4)2015.26930
3. Bierkens J, Smolders R, Van Holderbeke M, Cornelis C. Predicting blood lead levels from current and past environmental data in Europe. Sci Total Environ 2011; 409(23):5101–10. https://doi.org/10.1016/j.scitotenv.2011.08.034
4. Kumar S. Occupational and environmental exposure to lead and reproductive health ımpairment. Indian J Occup Environ Med 2018; 22(3):128-37. https://doi.org/10.4103/ijoem.IJOEM_126_18
5. Vigeh M, Smith DR, Hsu P. How does lead induce male infertility?. Iran J ReproMed 2011; 9(1):1-8.
6. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014; 1-31. https://doi.org/10.1155/2014/360438
7. Walters JLH, Gadella BM, Sutherland JM, Nixon B, Bromfield EG. Shining a light on lipids and lipid-modulating enzymes in the male germline. J Clin Med 2020; 9(327):1-25. https://doi.org/10.3390/jcm9020327
8. Nowicka-Bauer K &Nixon B. Molecular changes induced by oxidative stress that impair human sperm motility. Antioxidants 2020; 9(134):1-22. https://doi.org/10.3390/antiox9020134
9. Ross C, Morriss A, Khairy M, Khalaf Y, Braude P, Coomarasamy A, El-Toukhy T. A systematic review of the effect of oral antioxidants on male infertility. Reprod Biomed Online2010; 20(6): 711–23. https://doi.org/10.1016/j.rbmo.2010.03.008
10. Schotsmans WC, East A, Woolf A. Tamarillo (*Solanum betaceum* Cav.).In: Yahia EM ed. Postharvest Biology and Technology of Tropical and Subtropical Fruits. Cambridge UK: Wood Head Publishing, 2011. pp. 427–441. https://doi.org/10.1533/9780857092618.427
11. Durant AA, Rodríguez C, Santana AI, Herrero C, Rodríguez JC JC, Gupta MP. Analysis of volatile compounds from Solanumbetaceum cav. fruits from panama by head-space micro extraction. Rec Nat Prod 2013; 7(1):15-26.
12. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J 2016; 15(71):1-22. https://doi.org/10.1186/s12937-016-0186-5
13. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev 2010; 4(8):118-26. https://doi.org/10.4103/0973-7847.70902
14. Khaerunnisa S, Kusumastuti K, Mustika A, Aminah NS, Suhartati S. Mechanism of Solanum betaceum to prevent memory impairment in cigarette smoke-exposed rat. Int JApp Pharm 2019; 11(3):25-9. https://doi.org/10.22159/ijap.2019.v11s3.M1024
15. Ruiz-Cruz S, Chaparro-Hernández S, Hernández- Ruiz KL, Cira-Chávez LA, Estrada-Alvarado MI, Ortega LEG, *et al.* Flavanoids: important biocompounds in food. In: Justino G editors, Flavonoids: From Biosynthesis to Human Health. Intech Open 2017; 16:353-369. https://doi.org/10.5772/67864
16. Kaur, S. and Mondal, P. Study of total phenolic and flavonoid

content, antioxidant activity and antimicrobial properties of medicinal plants. J Microbiol Exp 2014; 1(1):23–8. https://10.15406/jmen.2014.01.00005
17. Diep T, Pook C, Yoo M. Phenolic and anthocyanin compounds and antioxidant activity of tamarillo (*Solanumbetaceum* cav.). Antioxidants 2020; 9(169):1-20. https://doi.org/10.3390/antiox9020169
18. Priyadarsini KI, Khopde SM, Kumar S, Mohan H. Free radical studies of ellagic acid, a natural phenolic antioxidant. J Agric Food Chem 2002; 50:2200-6. https://10.1021/jf011275g
19. Flora G, Gupta D, Tiwari A. Toxicity of lead: a review with recent up date. Interdiscip Toxicol 2012; 5(2):47-58. https://doi.org/10.2478/v10102-012-0009-2
20. Poljsak B, Šuput D, Milisav I. Achieving the balance between ros and antioxidants: when to use the synthetic antioxidants. Oxid Med Cell Longev 2013; 1-11. https://doi.org/10.1155/2013/956792
21. Alahmar AT. Role of oxidative stress in male infertility. J Hum Repro Sci 2019; 12(1):4-18. https://doi.org/10.4103/jhrs.JHRS_150_18
22. Sabeti P, Pourmasumi S, Rahiminia T, Akyash F, Talebi AR. Etiologies of sperm oxidative stress. Int J Repro Biomed 2016; 14(4):231-240.
23. Piomboni P, Focarelli R, Stendardi A, Freeamosca A, Zara V. The role of mitochondria in energy production for human sperm motility. Int J Androl 2012; 35:109-124. https://doi.org/10.1111/j.1365-2605.2011.01218.x
24. Mohri H, Inaba K, Ishjima S, Baba SA. Tubulin-dynein system in flagellar and ciliary movement. Proc Jpn Acad Ser B Phys Biol Sci 2012; 88(8):397-415. https://doi:10.2183/pjab.88.397
25. Wagner H, Cheng JW, Ko EY. Role of reactive oxygen species in male infertility. Arab J Urol 2018; 16(1):35-43. https://doi.org/10.1016/j.aju.2017.11.001
26. Gandhi J, Hernandez RJ, Chen A, Smith NL, Sheynkin YR, Joshi G, *et al.* Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning. Zygote 2017; 25(02):103-10. https://doi.org/10.1017/S0967199417000028
27. Cocuzza M, Sikka SC, Athayde KS, Agarwal A. Clinical relevance of oxidative stress and sperm chromatin damage in male infertility: an evidence based analysis. Int Braz J Urol 2007; 33(5):603-21. https://doi.org/10.1590/S1677-55382007000500002
28. Aguilar TAF, Navarro BCH, Pérez JAM. Endogenous antioxidants: areview of their role in oxidative stress. In: Moralez-Gonzales JA, Moralez-Gonzalez A, Madrigal-Santillan EO editors.A master regulator of oxidative stress the transcription factor Nrf2. In Tech Open 2016. http://dx.doi.org/10.5772/62743
29. Asih ARA, Manuaba IBP, Berata K, Satriyasa BK. The flavonoid glycosides antioxidant from terong belanda (*Solanum betaceum*). Biomed Pharmacol J 2018; 11(4). https://doi.org/10.13005/bpj/1593
30. Tu¨rk G, So¨nmez M, Aydin M, Yu¨ce A, Gu¨r S, Yu¨kselM, *et al.* Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clin Nutr 2008; 27:289-96. https://doi.org/10.1016/j.clnu.2007.12.006
31. Martin-Hidalgo D, Bragado MJ, Batista AR, Oliveira PF, Alves MG. Antioxidants and male fertility: from molecular studies to clinical evidence. Antioxidants 2019; 8(4):1-22.

https://doi.org/10.3390/antiox8040089
32. Dey S, Brothag C, Vijayaraghavan S. Signaling enzymes required for sperm maturation and fertilization in mammals. Front Cell Dev Biol 2019; 7(341):1-15. https:doi.org/10.3389/fcell.2019.00341
33. Ahmadi S, Bashiri Rm Ghadiri-Anari A, Nadjarzadeh A. Antioxidant supplements and semen parameters. Int J Reprode Biomed 2016; 14(12):729-36.
34. Dutta S, Majzoub A, Agarwal A. Oxidative stress and sperm function. Arab J Urol 2019; 17(2):87-97. https://doi.org/10.1080/2090598X.2019.1599624