http://www.cjmb.org
doi 10.34172/cjmb.2023.09
Open Access Original Article
Crescent Journal of Medical and Biological Sciences
Vol. 10, No. 2, April 2023, 56–60
eISSN 2148-9696

Effect of Anthocyanin on Oxidative Stress and Testis Structure in Streptozotocin-Induced Diabetic Rats

Nabiollah Ahmadi[1], Amir Afshin Khaki[2], Parvin Khodarahmi[1], Javad Baharara[3], Saeed Zakerbostanabadi[1*]

Abstract

Objectives: This study aimed to evaluate the effect of anthocyanin on oxidative stress, sperm, and testis structure in diabetic rats induced by streptozotocin (STZ).

Materials and Methods: In this experimental research, diabetes was induced by a single intraperitoneal injection of STZ (50 mg/kg). A total of 64 rats were assigned into four groups as follows: a control group, a diabetic control group, a diabetic group daily administrated with anthocyanin at a dose of 100 mg/kg, and a healthy group daily administrated with anthocyanin for 56 days. After intervention, all the rats were anesthetized, their blood samples were taken, and the serum levels of insulin, glucose, and oxidative stress markers were measured. Finally, the testicles were removed and histological parameters were assessed.

Results: Treating diabetic rats with anthocyanin significantly improved the testis tissue damage, glucose, and insulin plasma levels ($P=0.001$). Furthermore, the level of malondialdehyde (MDA) was up-surged and the serum levels of superoxide dismutase (SOD) and catalase (CAT) were reduced ($P=0.001$). Also, anthocyanin administration (100 mg/kg BW) significantly rectified these parameters ($P<0.05$).

Conclusions: Our results confirmed the antioxidant role of anthocyanin in improving the sperm parameters and testicular oxidative damage caused by diabetes.

Keywords: Anthocyanin, Testis, Oxidative stress, Diabetes

Introduction

Infertility is one of the problems of human society. According to the World Health Organization (WHO), 10-15% of couples have experienced infertility, 30-40% of which are related to the male factor (1). About 30-45% of abnormal semen parameters are idiopathic, even though male infertility can be treated for some other reasons(2). However, treatment for poor idiopathic semen quality is low (3).

Diabetes is a chronic and endocrine disease with widespread concern worldwide. It is a heterogeneous metabolic disorder caused by a lack of insulin production in the body or insulin resistance that impairs male sexual ability and fertility (4, 5). Testicular dysfunction leads to decrease in testicular weight, decreased sperm count and motility, change in the morphology of the seminiferous tubules (5), and decreased testosterone levels (6). Diabetes increases the rate of apoptosis (pro-apoptotic genes such as *Bax*) in germ cells and interrupts the process of spermatogenesis (7). In about 90% of diabetic patients, defects in sexual activity are seen as decreased libido and reduced fertility (6). Although the exact mechanism of diabetes is still unclear, increased production of free radicals and increased oxidative stress are its major damaging mechanisms (5, 7).

The presence of antioxidants such as vitamins or flavonoids in the diet can have protective effects in diabetic patients (8). The excessive production and increase of reactive oxygen species (ROS) can cause damage to the mitochondrial membrane and release of cytochrome C, resulting in induction of apoptosis in testicular tissue cells (7).

The most common anthocyanin in plants is cyanidin, which is found in 80% of colored leaves, 69% of fruits, and 50% of flowers. Cornflower blue and rose red are due to cyanidin. After cyanidin, the combination of delphinidin and pelargonidin is the most common one. Anthocyanins are found in all plant tissues, including leaves, stems, roots, flowers, and fruits. Animal studies showed that anthocyanins are rapidly absorbed and appear in the blood 6-20 minutes after ingestion and reach a maximum after 16-60 minutes. Plasma concentrated anthocyanin concentration varies slightly in nanomolar to micromolar (9, 10).

Glycosylation of anthocyanins reduces the activity of radical adsorption compared to aglycones, because it reduces the ability of anthocyanins to move electrons. In a human study by Ghosh et al, the researchers reported that consuming foods containing anthocyanins (such as blueberries and seedless cranberries) lowered the low-

Received 15 November 2021, **Accepted** 10 April 2022, **Available online** 13 January 2023

[1]Department of Biology, Islamic Azad University, Parand Branch, Parand, Iran. [2]Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran. [3]Department of Biology, Research Center for Animal Development Applied Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran.
*Corresponding Author: Saeed Zakerbostanabadi, Email: saeedzaker20@yahoo.com

density lipoprotein levels and increased plasma antioxidant capacity (11). Accordingly, this research aimed to investigate the effects of anthocyanin on spermatogenesis, testicular tissue damage, and blood biochemical factors in diabetic rats.

Key Messages

- Diabetes led to testis damage, oxidative stress and reduction in sperm quality.
- Treatment with anthocyanin increased the antioxidant enzyme activity and increased the sperm quality.

Materials and Methods

In this study, 64 male Wistar rats weighing 200-250 g were obtained from Razi Serum Center in Iran. The animals were kept in standard conditions with 12 hours of light/dark cycles and adequate humidity for two weeks. The rats were randomly assigned into four equal groups (n=8 each) as follows:

- Group 1: The control group only received water and food with standard conditions;
- Group 2: This group received 100 mg/kg anthocyanin by intraperitoneal (2) injection for eight weeks;
- Group 3: Diabetic control group that received one injection of streptozotocin (STZ) at a dose of 55 mg/kg intraperitoneally; and
- Group 4: Diabetic group that received 100 mg/kg anthocyanin by intraperitoneal injection (2) for eight weeks.

At first, the glucose levels of all rats in both intervention and control groups were determined. Then, STZ (50 mg/kg) was injected intraperitoneally. After 72 hours, blood glucose levels were measured by EasyGluco device (32). After confirming that the rats were diabetic (blood glucose above 250 mg/dL), the groups receiving anthocyanins for 56 days received anthocyanin once a day at a dose of 50 mg/kg by gavage (18). At the end of the treatment period (eighth week), ketamine (50 mg/kg) and xylazine (10 mg/kg) was used to anesthetize the rats, respectively. Then, 3-5 cc of blood was taken from the hearts of animals to measure biochemical factors related to diabetes.

Biochemical Assays

In order to assess the changes in the plasma level of insulin and glucose, blood samples were centrifuged immediately after sampling, and the serum samples were removed and stored at -80°C until analysis. The glucose condensation was measured using the Pars Azmoon kit, Iran. Plasma levels of insulin were measured using the Mercodia Rat Insulin ELISA kit (Uppsala, Sweden).

Histological Examination

Under sterile conditions, the right and left testicles were removed by creating an incision in the lower abdomen. After weighing the testes, the left testicle was used to evaluate the histological structure by the hematoxylin-eosin (H&E) staining method.

For histological studies, eight weeks after induction of diabetes, the abdominal area was opened and both testicles were removed from the animals. After opening both scrotum sacs, the testicles and epididim of the animals were carefully removed. Then, the right testis in each group was fixed in Bouin's fixative and tissue passage was performed. To study the changes in seminiferous tube diameter, germinal epithelium height was used. Also, to evaluate spermatogenesis process, 5-micron sections were prepared according to Johnsen Score and stained by H&E method. For this purpose, 50 nearly round seminiferous tubules were randomly examined in each tissue section.

Hormone Assay Method

Blood samples were taken directly from the hearts of rats and centrifuged at 3000 rpm for 10 minutes. The isolated sera were stored at -70°C for the analysis of serum testosterone level. The serum testosterone concentration was measured using the ELISA kits (Demeditec Diagnostics, Germany, Cat No: 4925-300A).

Measurement of Superoxide Dismutase and Glutathione Peroxidase Levels

The serum levels of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured by ELISA (Anthous, Austria) method according to the manufacturer's instructions (Randox, Ransod, UK).

Assessment of Serum Malondialdehyde Level

To measure plasma malondialdehyde (MDA) levels, 0.20 mL of serum was added to a microtube containing 3 mL of glacial acetic acid. Then, 1% thiobarbituric acid (TBA in 2% NaOH) was added to the microtube, and the tube was placed in boiling water for 15 minutes. After cooling, the adsorption of the resulting solution was read as pink at 532 nm.

Statistical Analysis

All the statistical analyses were performed in SPSS software version 19. The results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's range test were applied to analyze the data. A P value ≤0.05 was considered as statistically significant.

Results

Serum Glucose Level

There was a significant increase in the serum glucose level in the diabetic group compared to the control group (P=0.001). Additionally, a significant decrease was observed in the serum glucose levels in the diabetic rats treated with anthocyanin compared to diabetic control rats (Table 1).

Table 1. The Serum Level of Glucose in Study Groups

Groups	One Week Before Diabetes	One Week After Diabetes	At the End of Study
Control	94.5 ± 5.34	102.42 ± 7.25[b]	92.4 ± 9.34[b]
Diabetic	100.2 ± 2.6[a]	320.25 ± 4.07[a]	367.6 ± 38.73[a]
Diabetic + saponin	95.7 ± 5.89[a,b]	291.5 ± 4.03[a,b]	179.2 ± 3.2[b]
Saponin	96.25 ± 3.4[b]	93.8 ± 4.7[b]	92.3 ± 8.05[b]

Letter 'a' shows a significant difference between control and diabetic groups and Letter 'b' shows a significant difference between diabetic and treatment groups (P<0.05).

Serum Level of Insulin

We witnessed that diabetes caused a significant decrease in the level of serum insulin in intervention groups compared to the control group (P=0.001). Also, anthocyanin improved the decrease of serum insulin level compared to the diabetic group (P=0.03) (Figure 1).

Histological Findings

According to the results of germ cell counting, the number of germ cells notably declined in the diabetic group compared to the control group (P<0.05). However, in diabetic rats receiving anthocyanin (100 mg/kg), the number of germ cells was significantly higher than the diabetic control group (P<0.05). Histopathological examination showed that the seminiferous tubule in diabetic group was irregular with less diameter and thickness. Also in the seminiferous tubule was observed the hemorrhagia. Then again, in the other group treatment with anthocyanin improved this testicular damage (Figure 2 and Table 2).

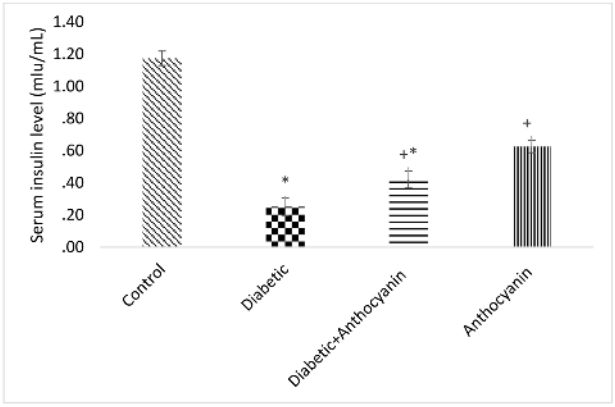

Figure 1. The Serum Level of Insulin in Study Groups. Note: Asterisk sign (*) shows a significant diffidence with control. Plus sign (+) shows a significance diffidence with diabetic

The Serum Level of Testosterone

The serum testosterone level was significantly decreased in diabetic group compared to control group (P=0.001). Treatment with anthocyanin increased the serum testosterone level in treated groups in comparison with diabetic group (P=0.001; Figure 3).

The Levels of Oxidative Stress Markers in the Testis Tissue

There was a strong significant increase in the MDA levels in the testis of diabetic rats compared to the control group (P=0.001). The diabetic rats treated with anthocyanin had a significantly decrease in testicular MDA level caused by diabetes (P=0.001). These findings indicate that diabetes significantly decreased the catalase (CAT) enzyme activity compared to the control group (P=0.001). Moreover, treating diabetic rats with anthocyanin made notable differences in comparison with untreated diabetic rats (P=0.001). The activity of SOD significantly declined in the diabetic group compared to the control group (P=0.001). Furthermore, comparing the results in the treatment and diabetic groups showed a dramatic increase in the activity of SOD enzyme in treated groups (P=0.001; Table 3).

Discussion

The present study examined the ameliorative effect of anthocyanin against injuries in the reproductive system of male diabetic rats. Diabetes produces testicular dysfunctions in the male reproductive organ and treatment with anthocyanin improves these functional deficiencies by antioxidant and anti-diabetic roles. Also, anthocyanin can regulate the oxidative stress markers and improve the antioxidant enzyme activity (12, 13). Our results showed

Table 2. The Count of Germ Cell in Study Groups

Groups	Mean Johnson's score	Seminiferous Tubule Diameter	Highest of Epithelium
Control	9.62 ± 0.36	262.42 ± 4.25[b]	64.5 ± 1.23[b]
Diabetic	4.35 ± 0.17[a]	140.11 ± 2.57[a]	33.5 ± 2.03[a]
Diabetic+ saponin	7.35 ±0.54[a,b]	190.5 ± 3.23[a,b]	54.5 ± 2.15[b]
Saponin	9.55 ± 0.24[b]	260.22 ± 1.70[b]	64.03 ± 1.05[b]

Letter 'a' shows a significant difference between control and diabetic groups and Letter 'b' shows a significant difference between diabetic and treatment groups (P<0.05).

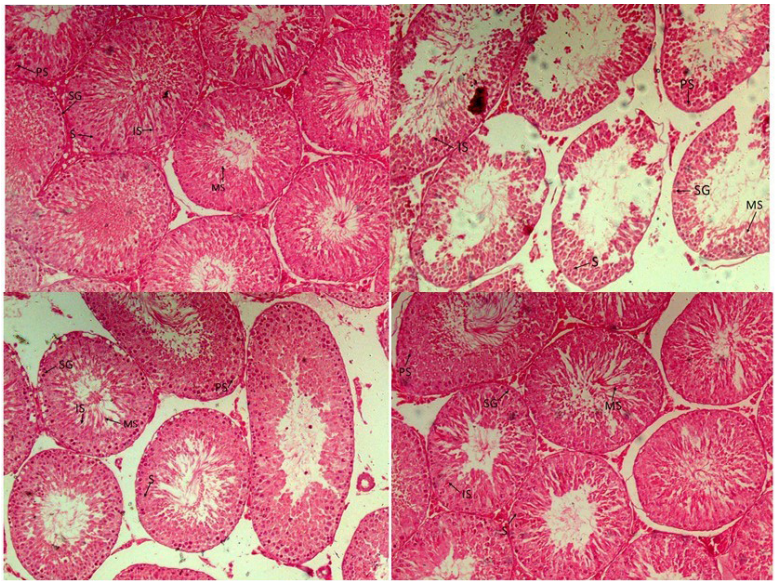

Figure 2. The Histological Assessment of Testes in the Study Groups. MS: mature spermatid, S: Sertoli cell, IS: immature spermatid, SG: Spermatogonia, PS: primary spermatocyte.

that anthocyanin could decrease the blood glucose levels and oxidative stress markers in the testis of diabetic rats. The hypoglycemic effect of anthocyanin is related to the increased sensitivity of tissues to insulin (14).

In diabetic patients, in addition to an enhanced amount of blood glucose, the balance between generation and resolution of free radicals is suspended. As a result, free radicals increment and cause oxidative stress (7, 15), which result in cell injury via mechanisms, such as lipid peroxidation and DNA and protein oxidative damage.

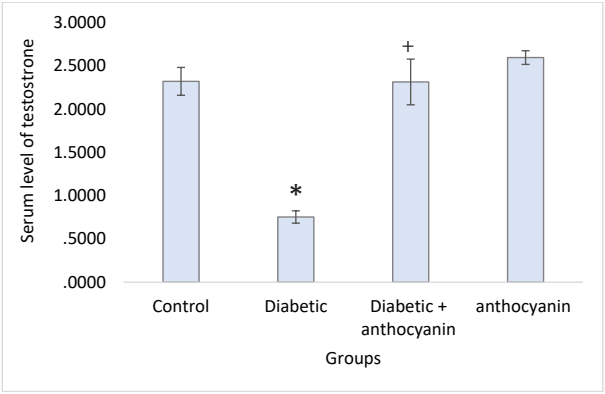

Figure 3. The Serum Level of Testosterone in the Study Groups. Note: Asterisk sign (*) shows a significant diffidence with control. Plus sign (+) shows a significance diffidence with diabetic group.

Our results showed that diabetes remarkably increased the MDA levels (as lipid peroxidation marker) in the testicles of diabetic rats, which is in line with the results of some previous studies on oxidative stress in the testis of diabetic rats (5,6). The increase in the MDA level in the testis of the diabetic rats emphasizes the increase of lipid peroxidation. In this research, anthocyanin significantly decreased MDA concentration in the testis tissue. Several studies reported that flavonoids in anthocyanin scavenge the free radicals generated during lipid peroxidation (16, 17). Thus, the decline in the MDA concentration in groups treated with anthocyanin may be related to its antioxidant effects.

We observed that the activity of SOD was extremely declined in diabetic rats, which is consistent with the results of some previous studies (15). SOD is known as one of the most important enzymes of the antioxidant system while its main action is the catalysis of superoxide anion radicals to H2O2. Through this procedure, the toxicity of superoxide decay and no free radicals from superoxide are produced (18). In our research, the SOD activity had a significant increase in the testes of diabetic rats treated

Table 3. The concentration of MDA, GPX, and SOD mice the serum of study groups

Groups	MDA, Mean ± SE	GPx, Mean ± SE	SOD, Mean ± SE
Control	0.63 ± 0.06	2.15 ± 0.057	1.63± 0.21
Diabetic	2.3 ± 0.05[a]	0.61 ± 0.048[a]	0.60 ± 0.06[a]
Diabetic + saponin	1.54 ± 0.17[a,b]	1.72 ± 0.072[b]	1.16 ± 0.18[b]
Saponin	0.80 ± 0.05[b]	2.43 ± 0.059[b]	1.55 ± 0.25[b]

SE, Standard error.
[a] In comparison with control group (P=0.001); [b] In comparison with diabetic group (P=0.001).

with anthocyanin.

CAT, as an antioxidant enzyme, is another enzyme that has detoxification effects against free radicals (19). In our research, while the CAT enzyme activity in diabetic rats was significantly reduced compared to the control group, it was significantly increased in the groups treated with anthocyanin. The decline in the activity of CAT in this study can be attributed to the increment in H2O2 generation because autoxidation of glucose and non-enzymatic protein glycation produce oxygen-free radicals (20). It is known that antioxidant therapy can increase the activity of CAT, as confirmed in our study.

Our findings indicated that while diabetes reduced the parameters of sperm (count, motility, and morphology), anthocyanin increased the count of sperm and ameliorated the sperm motility and morphology in diabetic rats. This may be related to the antioxidant capacity of anthocyanin and activation of antioxidant enzymes that can counteract free radicals. Similar to our results, some previous studies indicated that medical plants containing flavonoids components can improve sperm quality and testosterone levels (5,6,21).

Conclusions

Our findings revealed that diabetes had a negative effect on testis and sperm quality of rats, but anthocyanin reduced the oxidative stress induced by diabetes. Further studies are required to confirm our results.

Authors' Contribution

Conceptualization: NA, AAK, PK, JB, SZ. Methodology: SZ, NA, and AAK. Validation: NA, AAK, PK, JB, SZ. Formal Analysis: AAK. Investigation: NA. Data Curation: NA, AAK, SZ. Writing—Original Draft Preparation: NA, AAK, PK, JB, SZ. Writing—Review and Editing: NA, AAK, PK, JB, SZ. Visualization: NA, AAK. Supervision: SZ. Funding Acquisition: SZ

Conflict of Interests

None.

Ethical Issues

This study was approved by Islamic Azad University (Ethics No. IR.IAU.VARAMIN.REC.1399.043).

Financial Support

This study was funded by Islamic Azad University, Parand Branch, Parand, Iran.

References

1. Ameli M, Moghimian M, Saeb F, et al. The effect of clomiphene citrate and human chorionic gonadotropin on the expression of CatSper1, CatSper2, LHCGR, and SF1 genes, as well as the structural changes in testicular tissue of adult rats. Mol Reprod Dev. 2019;86(6):738-748. doi:10.1002/mrd.23151
2. Modaresi M, Mesripour M, Asadi MM, Hamedanian M. The effect of Saffron extract on testis tissue. Iranian Journal of Medicinal and Aromatic Plants. 2008;24(2):237-243.
3. Durairajanayagam D. Lifestyle causes of male infertility. Arab J Urol. 2018;16(1):10-20.
4. Abtahi-Evari S-H, Shokoohi M, Abbasi A, Rajabzade A, Shoorei H, Kalarestaghi H. Protective effect of *Galega officinalis* extract on streptozotocin-induced kidney damage and biochemical factor in diabetic rats. Crescent Journal of Medical and Biological Sciences. 2017;4:108-14.
5. Shoorei H, Khaki A, Shokoohi M, et al. Evaluation of carvacrol on pituitary and sexual hormones and their receptors in the testicle of male diabetic rats. Hum Exp Toxicol. 2020;39(8):1019-1030. doi:10.1177/0960327120909525
6. Shoorei H, Banimohammad M, Kebria MM, et al. Hesperidin improves the follicular development in 3D culture of isolated preantral ovarian follicles of mice. Exp Biol Med (Maywood). 2019;244(5):352-361. doi:10.1177/1535370219831615
7. Shoorei H, Khaki A, Khaki AA, Hemmati AA, Moghimian M, Shokoohi M. The ameliorative effect of carvacrol on oxidative stress and germ cell apoptosis in testicular tissue of adult diabetic rats. Biomed Pharmacother. 2019;111:568-578. doi:10.1016/j.biopha.2018.12.054
8. Fang J-Y, Lin C-H, Huang T-H, Chuang S-Y. In vivo rodent models of type 2 diabetes and their usefulness for evaluating flavonoid bioactivity. Nutrients. 2019;11(3):530.
9. Wallace TC, Giusti MM. Anthocyanins. Adv Nutr. 2015;6(5):620-2.
10. Castañeda-Ovando A, de Lourdes Pacheco-Hernández M, Páez-Hernández ME, Rodríguez JA, Galán-Vidal CA. Chemical studies of anthocyanins: A review. Food Chem. 2009;113(4):859-71.
11. Ghosh D, Konishi T. Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. sia Pac J Clin Nutr. 2007;16(2):200-208.
12. Liu Y, Li D, Zhang Y, Sun R, Xia M. Anthocyanin increases adiponectin secretion and protects against diabetes-related endothelial dysfunction. Am J Physiol Endocrinol Metab. 2014; 306(8):E975-E988. doi:10.1152/ajpendo.00699.2013
13. Moustaka J, Tanou G, Giannakoula A, et al. Anthocyanin accumulation in poinsettia leaves and its functional role in photo-oxidative stress. Environmental and Experimental Botany. 2020;175:104065.
14. Si X, Tian J, Shu C, et al. Serum ceramide reduction by blueberry anthocyanin-rich extract alleviates insulin resistance in hyperlipidemia mice.J Agric Food Chem. 2020;68(31):8185-8194. doi:10.1021/acs.jafc.0c01931
15. Shokri F, Shokoohi M, Niazkar HR, Kalarestaghi H, Ahin M. Investigation the spermatogenesis and testis structure in diabetic rats after treatment with *Galega officinalis* extract. Crescent Journal of Medical and Biological Sciences. 2019;6(1):31-36.
16. Yaribeygi H, Mohammadi MT, Sahebkar A. Crocin potentiates antioxidant defense system and improves oxidative damage in liver tissue in diabetic rats. Biomed Pharmacother. 2018;98:333-337. doi:10.1016/j.biopha.2017.12.077
17. Wang Y, Wang Q, Yu W, Du H. Crocin attenuates oxidative stress and myocardial infarction injury in rats. Int Heart J. 2018;59(2):387-393. doi:10.1536/ihj.17-114
18. Ameli M, Hashemi MS, Moghimian M, Shokoohi M. Protective effect of tadalafil and verapamil on testicular function and oxidative stress after torsion/detorsion in adult male rat. Andrologia. 2018;50(8):e13068.
19. Tremellen K. Oxidative stress and male infertility—a clinical perspective. Hum Reprod Update. 2008;14(3):243-258. doi:10.1093/humupd/dmn004
20. Perez-Gutierrez RM, Muñiz-Ramirez A, Gomez YG, Ramírez EB. Antihyperglycemic, antihyperlipidemic and antiglycation effects of Byrsonima crassifolia fruit and seed in normal and streptozotocin-induced diabetic rats. Plant Foods Hum Nutr. 2010;65(4):350-357. doi:10.1007/s11130-010-0181-5
21. Shokoohi M, Khaki A, Shoorei H, Khaki AA, Moghimian M, Abtahi-Eivary SH. Hesperidin attenuated apoptotic-related genes in testicle of a male rat model of varicocoele. Andrology. 2020;8(1):249-258. doi:10.1111/andr.12681