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Original Article

Efficacy of *Salvia officinalis* Extract against Infertility in Oxidative Stress Conditions

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ABSTRACT

This study aimed to investigate the effects of *Salvia officinalis* L. extract on infertility of male rats. In this experimental study, 40 rats were used in 8 groups and cadmium chloride was also used to induce infertility. *S. officinalis* extract was fed to mice daily for 28 days with the help of gavage needle. Animals were anesthetized with ether and dissected, then epididymal sperm were examined for morphology, viability and motility. we analyzed their right testis for H/E staining and counting cell lines and their left testicles were analyzed to evaluate superoxide dismutase (SOD) and malondialdehyde (MDA) enzymes.

According to the results of three sperm quality analyses indicated a significant increasing of viability, motility and the number of sperm cells in infertile groups treated with 100, 200, and 400 mg/kg *S. officinalis* extract (P<0.001). Our SOD analysis revealed *Salvia Officinalis* extract caused to produce more enzymes in the healthy experimental groups, even compared to the healthy control group (P<0.0001). our analysis indicated that increasing the dosage of the *S. officinalis* extract resulted in increasing enzyme production in infertile groups. MDA analyses showed that the healthy experimental groups produced a lower amount of MDA enzyme, which is almost the same level as the healthy control group (p<0.0001). Due to the usage of *S. officinalis* extract, the level of lipid peroxidation and MDA significantly decreased. In this study, *S. officinalis* extract can have significant positive impacts on the viability, motility, number of sperms in oxidative stress conditions.

Running title: *Salvia officinalis* Extract against Infertility

INTRODUCTION

Infertility is defined as failure to achieve pregnancy for more than 1 year without contraceptive methods and disable couples start looking for medical treatment for infertility. Infertility affects both sexes and in about 50% of childless couples, male infertility is an associated factor. According to the World Health Organization, 35% of infertility cases come from men, 40% of cases come from women, 15% of cases come from both of them and in 5% of cases infertility comes from unknown causes [1].

Infertility poses a significant challenge to couples worldwide, affecting their aspirations for parenthood and overall quality of life. While numerous factors contribute to infertility, the role of oxidative stress has emerged as a critical determinant in the reproductive health of both men and women. Oxidative stress, characterized by an imbalance between the production of reactive

oxygen species (ROS) and the body's ability to neutralize them with antioxidants, can detrimentally impact gametes, reproductive tissues, and hormonal balance [1-3].

While in almost 40% of infertile men there is no clear etiology, infertility in men can occur from various reasons which mainly include physical causes, hormonal deficits, sexually transmitted problems, genetic factors, and environment and lifestyle. In order to better understand the issues and problems associated with male infertility, we first discuss some of the key elements involved in the male reproductive system [2]. The testicles are the primary male reproductive organs enclosed by the tunica albuginea capsule in the testicle sack. There are two functionally and morphologically separated compartments in the testis. The tubular part includes seminiferous tubules and intercellular portions between seminiferous tubules which are involved in providing blood and immune responses [3].

Many medicinal plants were used for the enhancement of male fertility and are increasingly recognized globally as an alternative source of inexpensive and efficacious medications to synthetic chemotherapeutic compounds, and a high proportion of the world's population relies on plants for their primary health care [4]. *S. officinalis* is a perennial round bush in the Labiatae/Lamiaceae is the largest genus in this family with almost 900 species. Most of its species are common and native to the Mediterranean and the Middle East [5]. *S. officinalis*, commonly known as sage, has long been recognized for its therapeutic properties, including potent antioxidant and anti-inflammatory effects. Historically, it has been used in traditional medicine to address various health concerns, and its potential in mitigating oxidative stress-related disorders has garnered scientific interest [5]. Various studies have been conducted on the various biological effects and drug-related activities of *S. officinalis* such as anti-microbial, anti-inflammatory, anti-cancer, hypoglycemic, and hypolipidemic [6]. Although, several investigations focused on the chemical components of *salvia* such as carbohydrates, fatty acids, alkaloids, phenolic compounds, glycosidic products (flavonoid, cardiac glycosides, saponins), steroids, polyacetylene, terpenes/terpenoids (monoterpenoids, diterpenoids, triterpenoids) and waxes [7].

S. officinalis L. consists of isoflavonoids and phytoestrogen steroids and *S. officinalis* extract has been used for several pharmaceutical and therapeutic purposes [8]. For instance, it has been known that the signs of pregnancy decreased by the phytoestrogens component of *S. officinalis* and also are effective in recovery of infertility in women and also the clinical effects of *S. officinalis* were observed in postmenopausal women [9]. But there are limited studies that investigate the efficacy of *S. officinalis* extract on male infertility; In addition, understanding the potential benefits of *S. officinalis* extract in mitigating infertility in oxidative stress conditions has both clinical and societal implications. If proven effective, this herbal extract could offer a safe and accessible complementary approach to infertility management, potentially reducing the need for more invasive and costly treatments. So, in the present investigation, we analyze the impacts of *S. officinalis* on infertile male rats induced by cadmium chloride.

MATERIALS AND METHODS

Experimental Animals

In this experimental study, forty male rats of the Wistar species were used for this study, weighing 200±20g. The male rats were kept in a climate-controlled environment and were exposed to natural light for 12 h a day. The animals were kept in suitable cages and were subjected to standard laboratory processes for one week at temperature 25±2 ºC. In The current study, animal experiments were approved by the Ethical Committee of Islamic Azad University of Tehran- Science and Research Branch (IR.IAU.SRB.REC.1401.332).

Groups

The male rats were separated into eight groups (the 2 control groups for fertile (GI) and infertile (G II) groups and 6 treated groups), each group contained 5 male rats. The infertile control group (GII) received 3 mg/kg CdCl2 for 28 days daily. The first three experimental groups (GIII, GIV, GV) were given the *S. Officinalis* extract at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg respectively every day for 28 days. The second three experimental groups (GVI, GVII, GVIII) in addition to CdCl2, were given the *S. Officinalis* extract at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg respectively every day for 28 days.

Preparation of *S. officinalis* Extract

To mimic the extracts traditionally prepared by the soaking method. The dried plant was powdered, weighed (477 g) and mixed with 80% methanol. After adding the solvent, the mixture was stirred in order to completely wet the plant powder and increase the extraction efficiency as much as possible. After 72 hours, the green solution was extracted. A paper filter was used to separate plant particles. The colored solution was returned to the percolator by rotary evaporator at a temperature of 40 ºC and low pressure, concentrated and recycled methanol solvent. After stirring for better penetration of methanol into the plant tissues and dissolving the compounds in the solvent, it remained for 48 to 72 hours. Extraction of extract from the plant was repeated by 80% methanol until the solvent exiting gets colorless. After concentration, the extract obtained from the plant was poured into the crystallizer and placed under the hood to evaporate the remaining solvent and a complete extract was obtained. Finally, after a quality control of the extract, we used it for further aims.

Administration of Cadmium Chloride

The cadmium chloride pure powder was obtained, and distilled water was used as a solvent for the preparation of cadmium chloride administrable soluble. Then, the obtained soluble was given as a single dose injection intraperitoneally (3mg/kg) in the experimental groups. The health of the experimental subjects was closely monitored throughout the study. Vital signs, including heart rate, respiratory rate, and body temperature, were recorded at regular intervals following the intraperitoneal injection of cadmium chloride. Additionally, behavioral observations were conducted to assess any signs of distress or abnormal behavior in the experimental groups. Any unusual symptoms or adverse reactions were promptly noted and addressed by the research team.

Testis Extraction

All rats from each group were weighed and then anesthetized by ether solution at the end of the 28th day. Testicles and epididymis were removed from euthanized rats and weighed.

Sperm Count and Motility

The sperm quality outcome measurements were taken for sperm characteristics such as motility, morphology, viability and sperm count. To investigate the quality of the sperms, the caudal part of the epididymis of the left testis was sliced in Hank's buffer solution with a scalpel blade in a Petri dish. The suspension was kept at 37ºC for a minimum of 10 minutes to allow the sperms for dispersing in the medium. Further sample analyses included in counting motile (fast and slow), light microscopy analysis, and immotile sperms in a total of sperm samples, and the results were expressed in a percentage.

Histological Study

Testicular tissues were fixed in Bouin solution for 4 hours, then processed by dehydrating in ascending grades of ethanol alcohols, cleared in xylol, cast, embedded and cut at 6 µm thickness by microtome and stained with hematoxylin-eosin for microscopic examination. Johnson's score was carried out to study the maturity and quality of seminiferous tubules. The tubules were rate 1 to 10 based on the following criteria 1. The atrophic tubules were defined as seminiferous tubules with no epithelial (neither germ cells nor Sertoli cell), 2. There were no germinal cells and only Sertoli cells were recognized, 3. Only spermatogonia were presented, 4. No spermatozoa or spermatid were observed, 5. No spermatozoa or spermatids were seen. 6. No spermatozoa or spermatids pulp was seen, but a few primary round spermatids were presented, 7. No adult spermatozoa and spermatid were seen, but a large number of primary spermatids was seen, 8. Less than five spermatozoa were seen in each tube, and few mature spermatids were seen, 9. There were a large number of mature spermatids ,but the epithelium was degraded and the rounded and regular lumen was not seen, 10. Complete spermatogenesis and perfect tubules with the presence of a large number of spermatozoa was located on the round ,regular lumen were seen.

Assessment of the Oxidative Stress Status

First, 40 mg of tissue and 100mm of potassium dihydrogen phosphate and 1 mm EDTA (pH=7.4) were homogenized. Then, the solution was centrifuged at 12000 rpm for 30 min at 4ºC. Supernatant used for enzymatic analysis. For measuring superoxide dismutase (SOD), we added 30 µL to 2 ml Tris-HCl (0.05mM/L, pH=8.2) and 20 µL Pyrogallol solution (10 mM/L, pH=7.4). Then we measured the oxidation rate at 420 nm.

Statistical Analysis

Statistical analysis of the obtained data will be performed using analysis of variance followed by the Tukey-Krammer test and data sets were analyzed by ANOVA and the value of P<0.0001 is considered as the difference.

RESULTS

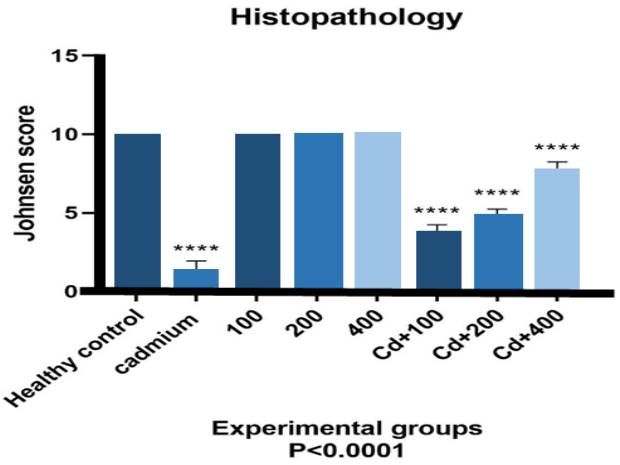

Fig. 1 Histopathology analysis based on the Johnsen score (P<0.0001).

Our histopathology analysis showed significant difference between *Taraxacum Officinalis* extracts treated infertile groups with infertile control group. Also, our analysis presented an equal amount (based on Johnsen Score) in fertile experimental groups and healthy control group (P<0.0001) (Fig. 1). According to the results, we observed an upward growth in the infertile experimental groups that received Taraxacum Officinalis extract 100, 200 and 400 ml/kg respectively (P<0.0001) (Fig. 1). Our histological investigations clearly showed severe destruction of the seminiferous tubules in infertile control group with no cell masses (Fig. 2B). Also, in the infertile group treated by 100 mg/kg *S. officinalis* extract, we observed a small number of spermatocytes (Fig 2F), which this small cell population in the seminiferous tubule rises to a large population in 400mg/kg dosage (Fig 2H). In addition to spermatocytes, we observed round spermatids and spermatozoa in group 8.

Sperm viability analysis clearly indicated the highest viability rate in healthy experimental groups that received 400 ml/kg doses of *S. officinalis* extract and healthy control group respectively. In groups 6, 7, and 8, which are infertile experimental groups treated with doses of 100, 200, and 400 ml/kg of *S. officinalis* extract, we observed an increasing viability respectively (p<0.0001) (Fig 3A).

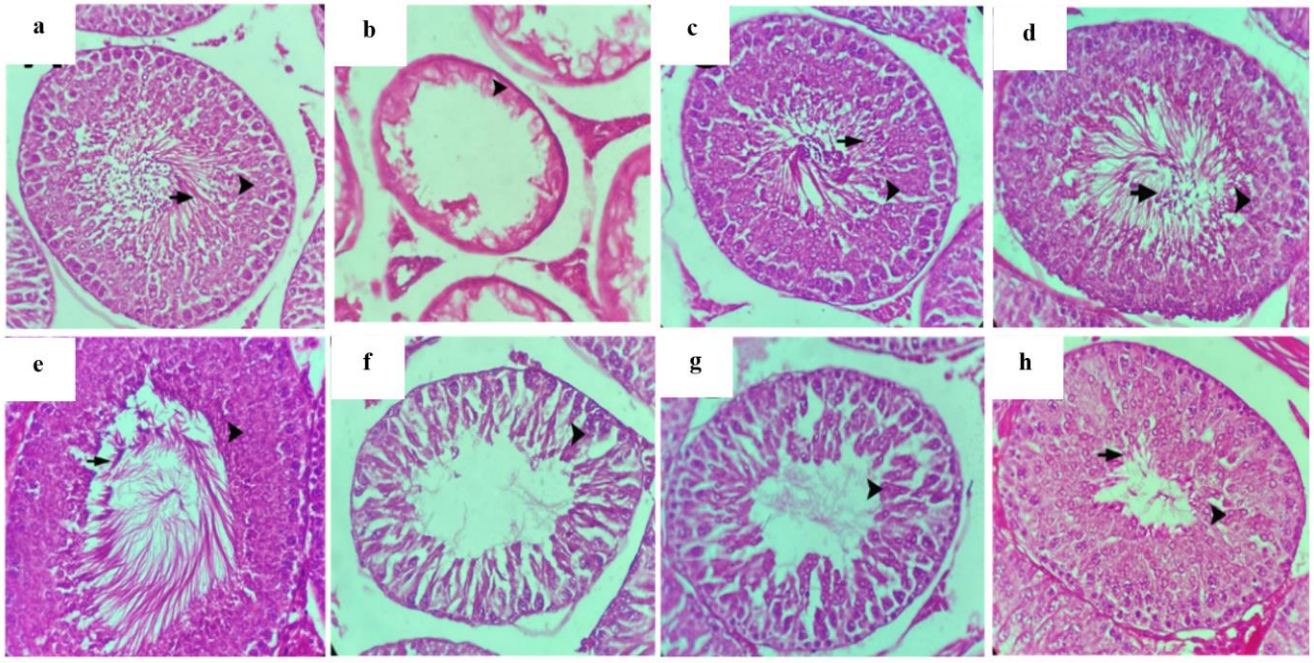

Fig. 2 A section of the testicular seminiferous tubules in the healthy control group, spermatocyte (arrowhead) and spermatozoa (arrow) (a). The section of the testicular seminiferous tubules in the infertile control group (induced by cadmium chloride) (b). The sections of the testicular seminiferous tubules in the normal groups only treated with *S. officinalis* extract (c, d, and e). The sections of the testicular seminiferous tubules in the infertile groups treated with *S. officinalis* extract (f, g and h) (scale bar: 50 µM).

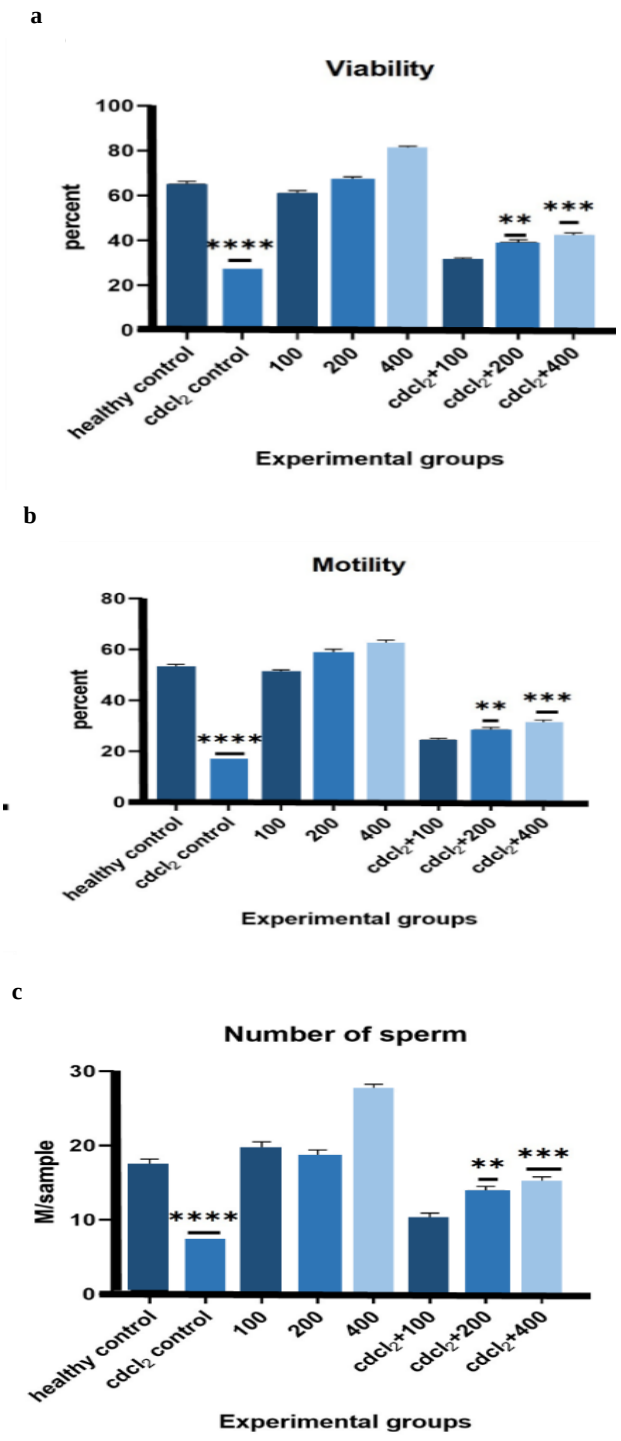

Fig. 3 Sperm quality analyses. Sperm viability (a), sperm motility (b) and the number of sperm (million/ml) (c) (p<0.001).

Analysis of the sperm motility showed significant difference between *S. officinalis* extracts treated infertile groups with infertile control group. Also, our analysis indicated a similar motility in fertile experimental groups and healthy control group (P<0.0001) (Fig. 3B). According to our results, we can see the highest number of sperm in the healthy experimental group that received a dose of 400 ml/kg *S. officinalis* extracts, which is more than the healthy control group. Although, we observed significantly higher sperm population infertile experimental group that received 400 ml/kg of *S. officinalis* extract in comparison to infertile control group (p<0.0001) (Fig 3C). superoxide dismutase (SOD) analysis showed, healthy experimental groups that received the 100-400 ml/kg doses of *S. Officinalis* extract, produced the highest amount of enzyme even more than the healthy control group. In addition, our analysis indicated a significant impact of higher doses of *S. officinalis* extract on enzyme production (p<0.0001) (Fig. 4A).

malondialdehyde (MDA) enzyme production analyses indicated a significant positive effect of 400 ml/kg concentration of *S. officinalis* extract in comparison to other concentrations (p<0.0001) (Fig. 4B). In addition, our analysis showed a similar amount of MDA production in healthy control group with 3 normal experimental groups.

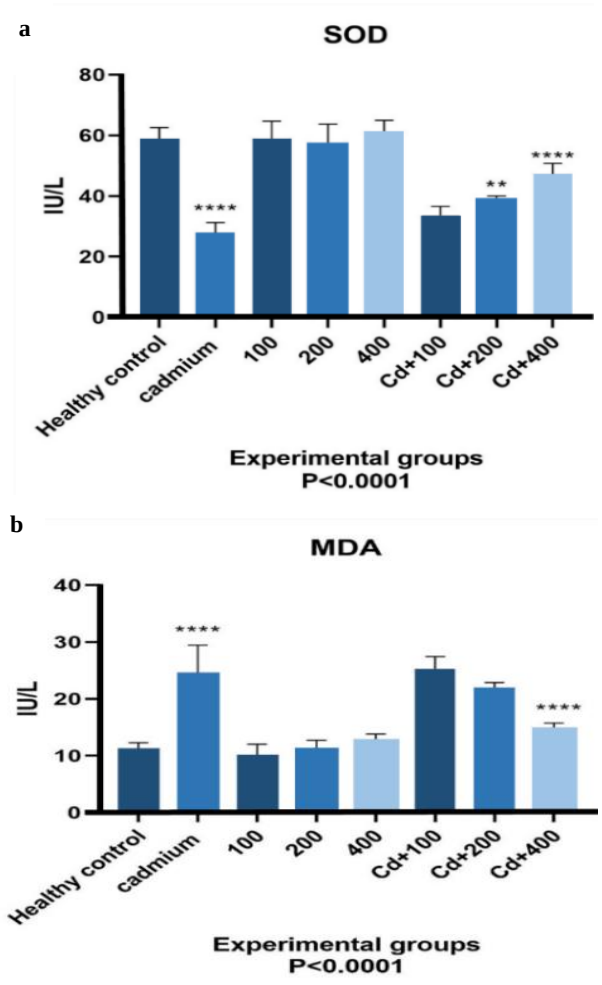

Fig. 4 Analysis of oxidative stress in experimental groups. Superoxide Dismutase (SOD) analyses (a). Malondialdehyde (MDA) analyses (b) (p<0.0001).

DISCUSSION

Male infertility continues to be a worldwide challenge with several contributing factors such as genetics, epigenetics, anatomy, biochemistry, hormones, infections, immunology, lifestyle or environmental exposure [10]. Because produced pharmaceuticals used to treat infertility are so expensive and have many side effects, many people turn to alternative approaches such as herbal therapy [11, 12]. *S. officinalis* is one of the plants that has recently been studied to treat infertility, but most of the investigations related to this family focused on its effects on female reproductive problems and there are limited investigations that analyze the role of *S. officinalis* on male infertility [11, 13]. In our study, we investigated the in vitro effect of *S. officinalis* extract on infertile rats (induced by cadmium chloride). Based on our histological analyses, induction with cadmium chloride result in the massive destruction of seminiferous tubules. Then, in-vivo treatment with *S. officinalis* extracts help to improve spermatogenesis and result in production of new spermatocytes and spermatids in the seminiferous tubules. Our histological results clearly indicated the positive impacts of *S. officinalis* extract on male infertility.

It has been reported that *S. officinalis* has a wide range of biological activities such as antibacterial, antifungal, antiviral, anticancer, anti-toxic and antioxidant. The beneficial effects of *S. officinalis* are probably related to the antioxidant properties of this plant [14-16]. The antioxidants present in it are effective in strengthening of the blood-testis barrier, protecting and repairing sperm DNA in infertile men by reducing the damage caused by radicals [17, 18].

According to the results of three sperm quality analyses including viability, motility and the number of sperm, the lowest amount in all of these factors was the infertile control group (induced by cadmium chloride) which grew significantly in all of these criteria treating by *S. Officinalis* extract. So, we detected high viability, motility and population of sperm in infertile group which treated by 400 mg/kg *S. officinalis* extract. Sperm viability, in the healthy control group has the highest viability rate, followed by the healthy experimental group with a dose of 400 mg/kg *S. officinalis* extract, which indicates the effect of *S. officinalis* antioxidants on the process of sperm production and survival.

In groups 6, 7, and 8, which are experimental infertile groups with doses of 100, 200, and 400 mg/kg *S. officinalis* extract, with the protective effect of this plant extract, these percentages have increased compared to the infertile experimental control group who only received cadmium chloride.

According to the graphs, we can see the highest number of sperm in the healthy experimental group that received a dose of 400 mg/kg *S. officinalis* extracts, which is more than the healthy control group, which shows the effect of *S. officinalis* extracts in improving spermatogenesis. While the lowest number of sperm was observed in the infertile control group which only received cadmium chloride, we see the greatest effect of *S. officinalis* extracts in the infertile group which are treated with 400 mg/kg *S. officinalis* extracts infertile control group.

The sperm motility in the healthy control group and then the healthy experimental group with doses of 100, 200 and 400 mg/kg *S. officinalis* extracts has the highest percentage. The lowest motility is related to the infertile control group, which only received cadmium chloride.

Previous studies have been shown that the superoxide dismutase (SOD) enzyme increased by the presence of the antioxidants [19]. According to our present research, *S. Officinalis* extract caused to produce more enzymes in the healthy experimental groups, even compared to the healthy control group. Also, our analysis indicated that increasing the dosage of the *S. officinalis* extract resulted in increasing enzyme production in infertile groups.

Several studies have been indicated the effect of curcumin on membrane integrity and oxidative stress indices of testis and serum of rats treated with cadmium chloride, they pointed out that cadmium chloride causes a significant decrease in sperm plasma membrane integrity and a significant increase in serum and testis malondialdehyde (MDA) and a significant decrease in the antioxidant power of the total serum and testis [20-22]. Also, it has been reported that mormomin as an antioxidant, can improve the destructive effects of cadmium chloride on the integrity of the sperm membrane, lipid peroxidation, and the antioxidant power of the whole serum and testis [23, 24].

MDA enzyme produced as a result of lipid peroxidation, then we observed at the highest level in the infertile control group [7, 25]. Due to the

usage of *S. officinalis* extract, which contains flavonoids and antioxidants, the level of lipid peroxidation and MDA significantly decreased. Although, the healthy experimental groups produced a lower amount of MDA enzyme, which is almost the same level as the healthy control group. Our results, could offer a safe and natural therapeutic option for individuals and couples struggling with infertility linked to oxidative stress. This could complement existing treatments, providing a more holistic approach to infertility management.

CONCLUSION

According to the results of the research, *S. officinalis* extract can have an improving effect on the process of spermatogenesis, the antioxidant conditions of testicular tissue against oxidative stress, motility and survival, as well as the number of sperm cells in oxidative stress conditions.

ACKNOWLEDGMENTS

No applicable.

Authors' Contributions

Conflict of Interest

The authors declare that they have no competing interests.

REFERENCES

1. Mbaye M.M., El Khalfi B., Addoum B., Mar P.D., Saadani B., Louanjli N., *et al*. The effect of supplementation with some essential oils on the mobility and the vitality of human sperm. The Scientific World J. 2019;2019.
2. Mbaye M.M., El Khalfi B., Ouzamode S., Saadani B., Louanjli N., Soukri A. Effect of Origanum vulgare essential oil supplementation on the advanced parameters of mobility and on the integrity of human sperm DNA. International J. Reproductive Medicine. 2020;2020.
3. Djerrou Z., Benyezzar-Kenana H., Maameri Z., Benhamza L. An ethnopharmacological survey of medicinal plants used in the traditional treatment of human infertility in eastern Algeria. Asian Pacific J. Reproduction. 2022;11(2):77.
4. CHOUABIA A., DJEMLI S., ABDENNOUR C., MALLEM L., KAHALERRAS L., ARKOUB FZ, *et al*. Protective effect of *Salvia officinalis* against cypermethrininduced reprotoxicity in male Wistar rats. Pharmacognosy Journal. 2021;13(6).
5. Mohsin A.S., Eidan S.M. Effect of adding antioxidants to Tris extender on cooling and post-Cryopreservative semen characteristics of Holstein bulls 2. *Salvia Officinalis* L Aqueous extract. Biochem Cell Arch. 2019;19(1):1157-64.
6. Pezo F., Yeste M., Zambrano F., Uribe P., Risopatrón J., Sánchez R. Antioxidants and their effect on the oxidative/nitrosative stress of frozen-thawed boar sperm. Cryobiology. 2021;98:5-11.
7. Davoodi F., Taheri S., Raisi A., Rajabzadeh A., Ahmadvand H., Hablolvarid M.H., *et al*. Investigating the sperm parameters, oxidative stress and histopathological effects of *Salvia miltiorrhiza* hydroalcoholic extract in the prevention of testicular ischemia reperfusion damage in rats. Theriogenology. 2020;144:98-106.
8. Greifova N.L. Investigation of the properties and effects of *Salvia officinalis* L. on the viability, steroidogenesis and reactive oxygen species (ROS) production in TM3 Leydig cells in 2 vitro 3.
9. Bresm M.H.A., Habeeb H.M.H., editors. Effect of Adding Different Vitamin D3 Concentrations on Chilled Storage of Awassi Ram Semen. IOP Conference Series: Earth and Environmental Sci. 2022: IOP Publishing.
10. Abarikwu SO, Onuah CL, Singh SK. Plants in the management of male infertility. Andrologia. 2020;52(3):e13509.
11. Alafid F., Edrah S.M., Meelad F.M., Belhaj S., Altwair K., Maizah N.R. Evaluation of phytochemical constituents and antibacterial activity of thymelaea hirsuta (l.) Endl, and that utilised as a conventional treatment of infertility and diabetic in Libya. World J. Pharmaceutical Res. 2019;8(11):72-88.
12. Zhang Q., Fan L., Li F., Sun Z., Zhang C., Chen R. Yishentongluo decoction in treatment of idiopathic asthenozoospermia infertility: Study protocol for a randomized controlled trial. Medicine. 2020;99(43).
13. Das S., Roychoudhury S., Dey A., Jha NK, Kumar D., Roychoudhury S., *et al*. Bacteriospermia and Male Infertility: Role of Oxidative Stress. Oxidative Stress and Toxicity in Reproductive Biology and Medicine: Springer. 2022. p. 141-63.
14. Diab K.A., Fahmy M.A., Hassan Z.M., Hassan E.M., Salama A.B., Omara E.A. Genotoxicity of carbon tetrachloride and the protective role of essential oil of *Salvia officinalis* L. in mice using chromosomal aberration, micronuclei formation, and comet assay. Environmental Sci and Pollution Res. 2018;25(2):1621-36.
15. El-Wakf A.M., El-Habibi E.S.M., Ali D.A., Abd El-Ghany E., Elmougy R. Marjoram and sage oils protect against testicular apoptosis, suppressed Ki-67 expression and cell cycle arrest as a therapy for male infertility in the obese rats. J. Food Biochemistry. 2020;44(1):e13080.
16. El-Defrawy SH.S., Sobhy H.M., El-Kholy T.A., El-Hashash S.A., Sultan H.A. Protective Effect of Moringa and Sage on Fertility Impairment Induced by Synthetic Pyrethroids in Male Rats. Egyptian Journal of Chemistry and Environmental Health. 2020;6(2):13-27.
17. Fahmy M.A., Abd-Alla H.I., Hassan E.E., Hassan Z.M., Heba-tollah M.S. Genotoxicity and sperm defects induced by 5-FU in male mice and the possible protective

role of Pentas lanceolata-iridoids. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2020;850:503145.

18. KARAYEL H.B. Effects of natural boron mineral on the essential oil ratio and components in medicinal sage (*Salvia officinalis* L.). Cumhuriyet Science J. 2021;42(2):269-75.
19. Adeldust H., Farzinpour A., Farshad A., Rostamzadeh J., Béjar M.L. Effect of orally administrated letrozole on reproduction performance and gene expression of FOXJ1, LPR2 and PVRL3 in reproductive tract in aged roosters. Theriogenology. 2021;161:131-9.
20. Krepkova L.V., Bortnikova V.V., Babenko A.N., Mizina P.G., Mkhitarov V.A., Job K.M., *et al*. Effects of a new thyrotropic drug isolated from Potentilla alba on the male reproductive system of rats and offspring development. BMC Complementary Medicine and Therapies. 2021;21(1):1-9.
21. Duracka M., Lukac N., Kacaniova M., Kantor A., Hleba L., Ondruska L., *et al*. Antibiotics versus natural biomolecules: The case of in vitro induced bacteriospermia by enterococcus faecalis in rabbit semen. Molecules. 2019;24(23):4329.
22. Juárez-Rojas L., Casillas F., López A., Betancourt M., Ommati M.M., Retana-Márquez S. Physiological role of reactive oxygen species in testis and epididymal spermatozoa. Andrologia. 2022;54(4):e14367.
23. Shi Y-Q., Wang Y., Zhu X-T., Yin R-Y., Ma Y-F., Han H., *et al*. The Application of Complementary and Alternative Medicine in Polycystic Ovary Syndrome Infertility. Evidence-Based Complementary and Alternative Medicine. 2022;2022.
24. Darestani A. Effect of Vitamins C and E on Some Sperm Characteristics in Varamini Rooster in Cold Conditon. Res. On Animal Production (Scientific and Res). 2019;10(24):66-75.
25. Raisi A., Davoodi F., Mohammadi R. Protective Effects of Rosmarinic Acid on Testicular Torsion-Detorsion in an Animal Model. Iranian J. Veterinary Surgery. 2022:80-6.