

# Efficacy of *Salvia Officinalis* Extract Against Infertility in Oxidative Stress Conditions

**Running title:** Salvia Officinalis extract against infertility

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## ABSTRACT

**Objective:** This study aimed to investigate the effects of *Salvia Officinalis* extract on infertility of male rats.

**Material and methods:** In this experimental study, 40 rats were used in 8 groups and cadmium chloride was also used to induce infertility. *Salvia 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.

**Results:** 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 *Salvia 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 *Salvia 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 *Salvia Officinalis* extract, the level of lipid peroxidation and MDA significantly decreased.

**Conclusion:** In this study, *Salvia officinalis* extract can have significant positive impacts on the viability, motility, number of sperms in oxidative stress conditions.

**Keyword:** *Salvia officinalis*, Infertility, Cadmium chloride, Oxidative stress

## 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].

*Salvia 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]. *Salvia 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 *Salvia 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].

*Salvia officinalis* consists of isoflavonoids and phytoestrogen steroids and *Salvia 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 *Salvia officinalis* and also are effective in recovery of infertility in women and also the clinical effects of *Salvia officinalis* were observed in postmenopausal women [9]. But there are limited studies that investigate the efficacy of *Salvia officinalis* extract on male infertility; In addition, understanding the potential benefits of *Salvia 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 *Salvia officinalis* on infertile male rats induced by cadmium chloride.

## **MATERIAL AND METHODS**

### **Experimental Animals**

In this experimental study, forty male rats of the Wistar species were used for this study, weighing  $200 \pm 20$ g. 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  $25 \pm 2$  °C. 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 CdCl<sub>2</sub> 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 CdCl<sub>2</sub>, 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 *Salvia 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 °C 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 37°C 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**

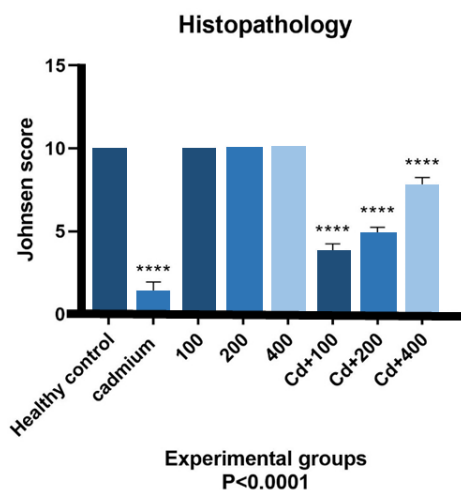
First, 40 mg of tissue and 100mm of potassium dihydrogen phosphate and 1 mm EDTA (pH=7.4) were homogenized. Then, the solution was centrifuged at 12000 rpm for 30 min at 4°C. Supernatant used for enzymatic analysis. For measuring superoxide dismutase (SOD), we added 30 µL to 2 ml Tris-HCl (0.05mM/L, 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-Kramer test and data sets were analyzed by ANOVA and the value of  $P < 0.0001$  is considered as the difference.

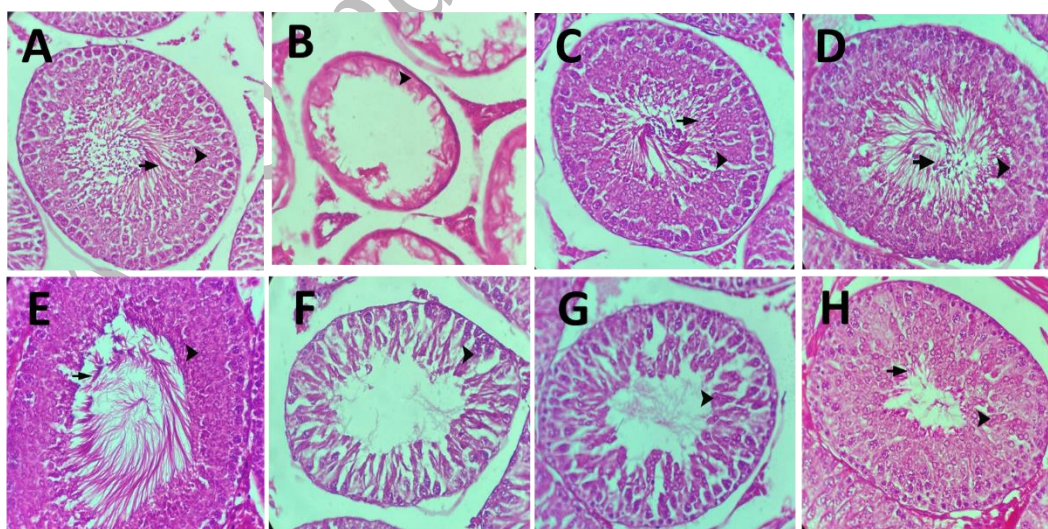
## RESULTS

Our histopathology analysis showed significant difference between *Taraxacum Officinale* 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 Officinale* extract 100, 200 and 400 ml/kg respectively ( $P < 0.0001$ ) (Fig 1).



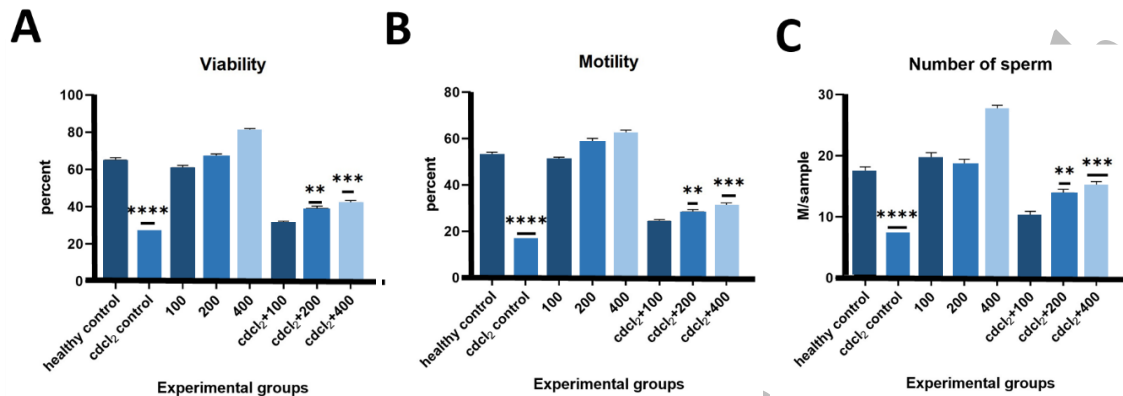
**Fig. 1** Histopathology analysis based on the Johnsen score ( $P < 0.0001$ ).

Our histological investigations clearly showed severe destruction of the seminiferous tubules in infertile control group with no cell masses (Fig2.B). Also, in the infertile group treated by 100 mg/kg *Salvia officinalis* extract, we observed a small number of spermatocytes (Fig2. F), which this small cell population in the seminiferous tubule rises to a large population in 400mg/kg dosage (Fig2. H). In addition to spermatocytes, we observed round spermatids and spermatozoa in group 8.



**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 *Salvia Officinalis* extract (C, D, and E). The sections of the testicular seminiferous tubules in the infertile groups treated with *Salvia Officinalis* extract (F, G and H) (scale bar: 50  $\mu$ M).

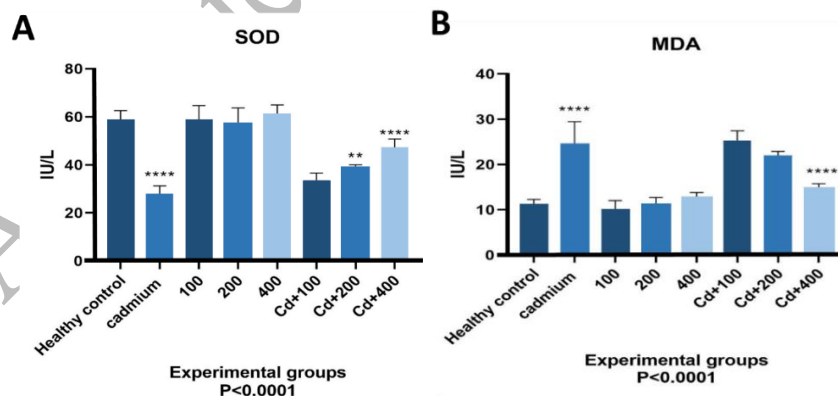
Sperm viability analysis clearly indicated the highest viability rate in healthy experimental groups that received 400 ml/kg doses of *Salvia Officinalis* extract and healthy control group respectively. In groups 6, 7, and 8, which are infertile experimental groups treated with doses of 100, 200, and 400 ml/kg of *Salvia Officinalis* extract, we observed an increasing viability respectively ( $P < 0.0001$ ) (Fig3.A). Analysis of the sperm motility showed significant difference between *Salvia Officinalis* extracts treated infertile groups with infertile control group. Also, our analysis indicated a similar motility in fertile experimental groups and healthy control group ( $P < 0.0001$ ) (Fig3.B). 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 *Salvia 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 *Salvia Officinalis* extract in comparison to infertile control group ( $P < 0.0001$ ) (Fig3.C).



**Fig. 3** Sperm quality analyses. Sperm viability (A), sperm motility (B) and the number of sperm (million/ml) (C) ( $P < 0.001$ ).

superoxide dismutase (SOD) analysis showed, healthy experimental groups that received the 100-400 ml/kg doses of *Salvia 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 *Salvia Officinalis* extract on enzyme production ( $P < 0.0001$ ) (Fig 4.A).

malondialdehyde (MDA) enzyme production analyses indicated a significant positive effect of 400 ml/kg concentration of *Salvia Officinalis* extract in comparison to other concentrations ( $P < 0.0001$ ) (Fig 4.B). 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]. *Salvia 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 *Salvia officinalis* on male infertility [11, 13]. In our study, we investigated the in vitro effect of *Salvia 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 *Salvia 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 *Salvia officinalis* extract on male infertility.

It has been reported that *Salvia officinalis* has a wide range of biological activities such as antibacterial, antifungal, antiviral, anticancer, anti-toxic and antioxidant. The beneficial effects of *Salvia 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 *Salvia Officinalis* extract. So, we detected high viability, motility and population of sperm in infertile group which treated by 400 mg/kg *Salvia 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 *Salvia Officinalis* extract, which indicates the effect of *Salvia 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 *Salvia 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 *Salvia Officinalis* extracts, which is more than the healthy control group, which shows the effect of *Salvia 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 *Salvia Officinalis* extracts in the infertile group which are treated with 400 mg/kg *Salvia 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 *Salvia 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, *Salvia 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 *Salvia 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 *Salvia 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, *Salvia 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.

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