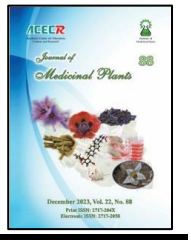
J. Med. Plants 2023; 22(88): 10-22

Institute of Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Research Article

The effects of hydroalcoholic extract of *Salvia sahendica* on sperm parameters quality and reproductive hormones in rats exposed to Aluminum

Arash Khorrami[1], Reza Vajdi-hokmabad[2], Mahdi Tavakolizadeh[3,4], Mojtaba Ziaee[1,5,*]

[1] *Medicinal Plants Research Center, Maragheh University of Medical Sciences, Maragheh, Iran*
[2] *Department of Veterinary Medicine, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran*
[3] *Department of Pharmacognosy, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran*
[4] *Department of Pharmacognosy and Pharmaceutical Biotechnology, School of Pharmacy, Iran University of Medical Sciences, Tehran, Iran*
[5] *Research Center for Integrative Medicine in Aging, Aging Research Institute, Tabriz University of Medical Sciences, Tabriz, Iran*

ARTICLE INFO

Keywords:
Aluminum
Salvia sahendica
Oxidative stress
Reproductive toxicity
Sex hormones
Male rats

ABSTRACT

Background: There is a growing focus on aluminum compounds due to their significant impact on fertility, and reproduction. Natural products offer a promising solution for treating heavy metal toxicity as they are effective, have fewer side effects, and are relatively affordable. **Objective**: This study aimed to investigate the effects of *Salvia sahendica* hydroalcoholic extract on reproductive toxicity induced by aluminum (Al) exposure in rats. **Methods**: Thirty-two adult male Wistar rats were divided into four groups: control, $AlCl_3$ orally administered group (40 mg/kg bw), *S. sahendica* hydroalcoholic extract gavaged group (100 mg/kg bw), and $AlCl_3$+ *S. sahendica* treated group. The rats were treated daily for 70 consecutive days. **Results**: Oral administration of $AlCl_3$ resulted in oxidative damage, indicated by an increase in malondialdehyde level and a decrease in total antioxidant content. Additionally, $AlCl_3$-intoxicated rats exhibited significant declines in serum levels of male reproductive hormones testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). However, daily administration of *S. sahendica* to rats before $AlCl_3$ was found to reduce testicular oxidative stress and improve reproductive markers in the serum, ameliorating the reproductive toxicity induced by Al administration. **Conclusion**: These findings suggest that *S. sahendica* could potentially be used as an alternative agent to minimize reproductive toxicity associated with Al exposure.

Abbreviations: $AlCl_3$, Aluminum Chloride; FSH, Follicle-stimulating hormone; LH, Luteinizing Hormone; MDA, Malondialdehyde; TAC, Total Antioxidant Capacity; VSCCs, voltage-sensitive calcium channels; RIA, Radioimmunoassay, WHO, World Health Organization.

*Corresponding author: ziaee.m@mrgums.ac.ir, mjziaee@gmail.com

doi:

Received 1 November 2023; Received in revised form 9 April 2024; Accepted 13 April 2024

1. Introduction

Infertility is a growing concern in the field of reproductive health, with research showing that factor infertility is now considered to be as significant as female factor infertility and accounts for almost 60 % of all infertility cases [1]. Globally, deficient sperm production is reported to be the primary contributor to the incidence rate of male factor infertility [2]. Infertility can be a major life crisis for couples who are unable to conceive, despite their many efforts [3]. Male infertility is on the rise and various factors such as genetics, lifestyle factors (such as tobacco and obesity), aging and environmental conditions play a role in this condition [4]. Unfortunately, environmental factors such as exposure to pollutants are largely attributed to male infertility, and in industrialized societies, such exposure is almost unavoidable due to lifestyle choices. Many environmental pollutants and pathogens can affect the sperm production process and sex hormones, leading to a reduction in the quality and quantity of sperm [5]. Exposure to chemicals and xenobiotics, such as pesticides, heavy metals, and industrial chemicals, can have detrimental effects on sperm quality and hormone balance [6]. While contributing to male infertility, recent studies have shown that exposure to Al may also play a significant role. In this context, the effect of Al on male infertility has become an area of interest for researchers. Human exposure to Al is unavoidable, and its long-term effects are still not fully understood. Al is the third most abundant element in the Earth's crust after oxygen and silicon, and both natural and human activities have contributed to its increased biological availability over time [7].

Aluminum exposure occurs through both dietary and non-dietary sources. It is added to certain foods as a salt and used in water treatment, packaging, and storage materials. Non-dietary Al is commonly used in the medical field for antacids, dialysis water, vaccines, solutions for parenteral nutrition, sunscreens, antiperspirants, and makeup products, which also contribute to aluminum exposure [8].

Additionally, they are utilized in water treatment as chelators. However, the increased exposure to this metal raises concerns about its potential toxicity to the human body. It is crucial to identify and understand the harmful effects of Al and explore strategies to mitigate these risks. The tolerable weekly intake of Al for humans is set at 1 mg Al/kg body weight [9]. However, individuals may still exceed these guidelines.

While the absorption of aluminum through the gastrointestinal tract is relatively low, chronic exposure can lead to an accumulation of this metal in the body's organs, potentially causing damage to various tissues, including the testicular tissues of both humans and animals [10]. In male rodents, the accumulation of Al in the gonads has been linked to necrosis of spermatocytes and spermatids, as well as a marked decrease in fertility [11]. Al may contribute to male reproductive toxicity through a variety of mechanisms, including the induction of oxidative stress, interference with spermatogenesis and steroidogenesis, disruption of cell signaling, damage to the blood test is barrier, and effects on the endocrine system [12]. Recent studies have focused on the impact of Al exposure at relatively high levels on the male reproductive system. Additionally, elevated levels of Al in human spermatozoa and seminal plasma have been associated with reduced sperm viability and motility [13]. The harmful effects of Al are mainly caused by the production of free radicals and an increase in oxidative stress. Al increases the production of free radicals, which deplete the body's antioxidants and cause damage to cells, including disruption of DNA structure [14].

Throughout history, plants have been utilized for their therapeutic properties as they contain a plethora of biologically active compounds [15]. Medicinal herbs are a diverse group of herbs renowned in numerous countries for their disease-treating properties and beneficial compounds that promote human well-being [16].

Genus *S.* belonging to the Lamiaceae family is widely recognized as a medicinal plant across the world. It serves various purposes, including as a flavoring agent and in fragrance compounds. With approximately 900 species, *S.* boasts a rich diversity. In Iran alone, there are 58 cultivated species, of which 17 are exclusive to the country [17]. *Salvia sahendica*, a species indigenous to the Sahand region of East Azerbaijan, is recognized for its antibacterial and antioxidant properties. Traditional medicine utilizes this species to address bacterial and fungal infections as well as indigestion [18]. The antioxidant activity of *S. sahendica* extract can be attributed to its rich content of phenolic compounds and flavonoids. The specific type and quantity of these compounds are closely associated with the antioxidant effect exhibited by the extract [19]. So this study was conducted to assess the possible protective effects of *S. sahendica* extract on male fertility damage induced by Al toxication.

2. Materials and methods

2.1. Preparation of Salvia sahendica hydroalcoholic extract

The aerial parts of *S. sahendica* samples were collected from Azarshahr city (Qirmizi Gol at 37.721518 E latitude, 46.084685 N longitude) in East Azerbaijan, Iran during the flowering season in June and July (2019). The plant material's authenticity was verified after an identification procedure conducted by a botanist from the medicinal research center at Zanjan University of Medicine. The voucher specimens were deposited in the Zanjan University of Medical Sciences (ZUMZ-1319). To avoid harming the natural population, representative samples of plant material were carefully selected. The samples were air-dried at room temperature and stored in a temperature-controlled environment (20 ± 2 °C) until extraction. After drying, the plant material was finely ground into a powder using a laboratory mill at 6,000 rpm for 1.5 minutes before being directly subjected to the extraction process. The herb was then ground into a fine powder to facilitate the extraction process [15].

The Macerate method was used for the extraction process. In this method, 100 g of the powdered herb was mixed with 70% ethanol in an Erlenmeyer flask [20]. To extract the compound, we started by crushing and grinding 100 grams of dried aerial parts of *S. sahendica*, then immersing them in 3 liters of ethanol 70 % for 3 days at room temperature. The ethanol was removed using a rotary vacuum evaporator (RE), and the remaining substance was concentrated and transferred to a different vessel. This process was repeated thrice to obtain the complete extract from the plant. The resulting solid materials were preserved in a dark place at 4 °C until needed [21].

After the extraction process was complete, the solution was filtered using Whatman No. 1 filter paper. The filtered solution was then subjected to rotary evaporation at 50°C to remove the solvent. The resulting extract was then placed in an oven at 50 °C to dry and convert to a dried extract [22].

2.2. Chemicals

The chemicals utilized in this study were of analytical grade and procured from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Aluminum chloride ($AlCl_3$) (CAS number 7446-

70-0), which was anhydrous, was dissolved in a 0.9% sodium chloride solution.

2.3. Animals

A total of 32 adult male Wistar rats, weighing between 280 and 300 g, were obtained from the animal facility of the Pasture Institute of Iran for the experiments. The rats were housed for one week in temperature-controlled rooms (22 ºC) with constant humidity (40-70 %) and 12-hour light and 12-hour dark conditions. They were given access to standard laboratory chow and tap water.

The rats were randomly divided into four groups, with eight animals per group. The control group was given normal saline solution (0.9 % NaCl) orally for 70 days, while the *S. sahendica* group was given 100 mg/kg body weight (BW) for 40 consecutive days. $AlCl_3$ group received 40 mg AlCl3 /kg body weight (BW) (1/20 LD_{50}) dissolved in saline solution for 70 days. The treatment group received *S. sahendica* + $AlCl_3$ group (100 mg *S. sahendica* /kg BW + 40 mg $AlCl_3$ /kg BW) received by gavage thirty days after the start of $AlCl_3$ administration and for 40 days.

This study was approved by the Committee of Research Ethics for Laboratory Animal Care, Maragheh University of Medical Sciences (approval no. IR.MARAGHEHPHC.REC.1395.2), and all experimental procedures were performed according to their guidelines.

2.4. Sampling

After the treatment period, the animals were euthanized by intraperitoneal injection of 30 mg/kg of thiopental sodium and blood was collected from the heart.[23] To obtain serum, blood samples were centrifuged at 1500g for 10 minutes at a temperature of 4 ˚C. Reproductive organs were also dissected for histopathological examination.

2.5. Serum Total Testosterone, LH, and FSH

To determine the serum concentrations of FSH and LH, duplicated samples were analyzed using radioimmunoassay (RIA) with kits obtained from Isotope Company Ltd. (Budapest, Hungary). The FSH/LH kits were used according to the provided protocol. The sensitivities of the assay were 0.2 ng/mL for FSH and 0.14 ng/mL for LH per assay tube. For the measurement of total testosterone, a double antibody RIA kit from Immunotech Beckman Coulter Company (USA) was used. The sensitivity of the assay was 0.025 ng/mL per assay tube.

2.6. Sperm characteristics analysis

To evaluate the weight of the tissues and the sperm's physical parameters, the testicles were removed after anesthesia, and the testicles, seminal vesicles, and epididymis were weighed. To analyze the characteristics of the sperm, the cauda epididymis was used. The sperm was released by cutting it into a medium (Hams F10) mixed with 0.5 % bovine serum albumin. After a 5-minute incubation at 37 ºC with 5 % CO2, the sperm reserves in the cauda epididymis were determined using the standard hemocytometry method [3]. Sperm motility was then assessed by observing them under a microscope and reported as the mean of motile sperm following WHO methods. To evaluate sperm abnormality, smears of the sperm suspension were made on clean glass slides and stained with periodic acid-Schiff's reaction hematoxylin. The stained smears were examined under a light microscope at 40× magnification, and the sperms were classified as normal or abnormal. The total percentage incidence of sperm abnormality was calculated and expressed as the result [6].

2.7. Total antioxidant capacity and malondialdehyde concentration measurement in serum

Total Antioxidant Capacity (TAC) detection kit was bought from Nanjing Jiancheng Bioengineering Institute, China. This kit measures the ability of the antioxidant defense system to reduce Fe3+ to Fe2+. TAC was measured by using a spectrophotometer at 520 nm to detect the reaction between phenanthroline and Fe2+. A Total Antioxidant Capacity (TAC) unit was defined as the amount of antioxidants required to make absorbance increase by 0.01 in 1 mL of serum at 37°C. Malondialdehyde (MDA) was used to specifically measure radical damage. MDA was produced as an end product of lipid peroxidation, which was measured in serum and testis homogenates using a spectrophotometric method at 532 nm. The results were expressed as nanomole MDA per mL of serum or gram of testis tissue.

2.8. Data analysis

The statistical analysis was conducted using SPSS statistical package version 23. The data were presented as mean ± standard error of the mean. To analyze the statistical differences between the treated and control groups, a Student's t-test was employed. Within the groups, repeated measures of one-way analysis of variance were used for analysis. A value of $P < .05$ was considered to be statistically significant.

3. Results

3.1. Tissue weight

Table 1 presents the tissue weights of rats in the experimental groups. The findings indicate at the beginning of the study, the rats' body weight was similar across all groups. However, by the end of the treatment, the AlCl3 group showed a significant decrease in body weight. The intake of water, Al, and feed were not different among the groups ($P > 0.05$), as shown in Table 1 using one-way ANOVA and t-test. Exposure to Al at 100 mg/kg bw/day resulted in a reduction in the weight of male reproductive organs, including the testis, vesicle seminal, epididymis, and prostate ($P < 0.05$). However, treatment with *S. sahendica* mitigated the detrimental effects of $AlCl_3$ on body and tissue weight, this effect did not reach statistical significance ($P > 0.05$).

Table 1. Effect of hydroalcoholic extract of *S. sahendica* on male sexual organs' weight.

Variable	Control	*S. sahendica*	$AlCl_3$	$AlCl_3$+*Saliva sahendica*
Initial Body weight (g)	291.2 ± 7.1	288.7 ± 7.7	288.54 ± 8.3	293 ± 8.9
Final Body weight (g)	352.6 ± 15.1	368.7 ± 10.7	338.54 ± 11.4*	344 ± 12.1
Testis (g)	1.9 ± 0.08	1.94 ± 0.05	1.74 ± 0.04*	1.83± 0.06
Testis (g/100 g)	0.4 ± 0.01	0.4 ± 0.03	0.38 ± 0.03*	0.4 ± 0.02
Epididymis (mg)	722 ± 28.4	754 ± 38.1	665 ± 19.3*	707 ± 23.02#
Seminal Vesicle (g)	1.48 ± 0.12	1.51 ± 0.11	1.23 ± 0.15*	1.35± 0.12#
Prostate (g)	437.8 ± 31.4	451.7 ± 33.1	409.8 ± 27.9*	428.8 ± 29.2#

Data are expressed as mean ± SD. (for each group, n = 8). Statistical differences between control and $AlCl_3$ groups: *P < 0.05 Statistical differences between $AlCl_3$ Vs $AlCl_3$ + *Saliva sahendica* groups #P < 0.05.

3.2. Evaluation of sperm count

While daily consumption of the hydroalcoholic extract of *S. sahendica* alone led to an increase in sperm count, this increase was not statistically significant when compared to the control group. In contrast, the $AlCl_3$ group exhibited a significant decrease in sperm count ($P < 0.001$) compared to the control group, indicating that the consumption of $AlCl_3$ for 70 days resulted in a substantial reduction in sperm

count in male rats. Furthermore, although the consumption of *S. sahendica* did not elevate the sperm count to the level of the control group, it did lead to a significant increase when compared to the control group ($P < 0.001$).

3.3. Evaluation of viability, motility, movement rate, and abnormal sperm count

Regarding the effect of $AlCl_3$ and *S. sahendica* hydroalcoholic extract on different sperm parameters, exposure to $AlCl_3$ significantly ($P < 0.01$) reduced sperm viability. Therefore, $AlCl_3$ administration significantly decreased the number of live sperms and treatment with *S. sahendica* hydroalcoholic extract increased this rate to the average rate of the control group ($P < 0.05$).

Likewise, regarding the number of motile sperms, exposure to $AlCl_3$ significantly reduced the number of motile sperms ($P < 0.001$). The rate or degree of mobility, which was measured based on a standard, showed a significant decrease in animals receiving $AlCl_3$ ($P < 0.001$). The number of abnormal sperms also increased significantly ($P < 0.001$). *S. sahendica* hydroalcoholic extract significantly improved the number of motile sperms and their degree of motility ($P < 0.01$) and reduced the number of abnormal sperms ($P < 0.001$). *S. sahendica* possibly affected the process of spermatogenesis by reducing the levels of free radicals and increasing the levels of antioxidants in the body and improved this process, which was confirmed by the results regarding the number, quality, and motility of sperms (Table 2).

3.4. Measurement of FSH, LH, and testosterone hormones

FSH and LH in $AlCl_3$ + *S. sahendica* rats were significantly ($P < 0.05$) higher than in the $AlCl_3$-treated rats, but significantly ($P < 0.05$) lower than in the control and *S. sahendica* rats.

$AlCl_3$ significantly reduced the levels of LH ($P < 0.001$) and testosterone ($P < 0.05$). Administering *S. sahendica* hydroalcoholic extract for 40 days significantly increased LH and testosterone levels ($P < 0.001$ and $P < 0.05$, respectively). These findings regarding the levels of sex hormones are in line with the previous findings regarding the quantity and quality parameters of sperms and show that the *S. sahendica* hydroalcoholic extract increases the quantity and quality parameters of sperms by increasing the levels of sex hormones, especially testosterone (Fig. 1).

Table 2. Protective effects of hydroalcoholic extract of *S. sahendica* on AlCl3-induced toxicity of sperms of the rats.

Variable	Control	*S. sahendica*	$AlCl_3$	$AlCl_3$+*Saliva sahendica*
Count (million/ML)	86.37 ± 5.83	88 ± 6.47	$49.6 \pm 4.46^{***}$	$84 \pm 5.93^{\#\#\#}$
Viability (%)	68.75 ± 4.24	66.5 ± 5.37	41.87 ± 3.97**	$63.25 \pm 4.87^{\#}$
Motility (%)	54.12 ± 3.68	53.12 ± 4.94	$29.25 \pm 2.83^{***}$	$50.37 \pm 4.35^{\#\#}$
Motility grade	3.37 ± 0.26	3.62 ± 0.18	$1.87 \pm 0.22^{***}$	$3.25 \pm 0.31^{\#\#}$
Abnormality	4.12 ± 0.66	4.12 ± 0.71	$11.75 \pm 1.26^{***}$	$3.875 \pm 0.54^{\#\#\#}$

Data are expressed as mean ± SD values (n = 8). Statistical differences between control and $AlCl_3$ groups: **P < 0.01 ***P < 0.001. Statistical differences between $AlCl_3$ Vs $AlCl_3$ + *Saliva sahendica* groups $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$

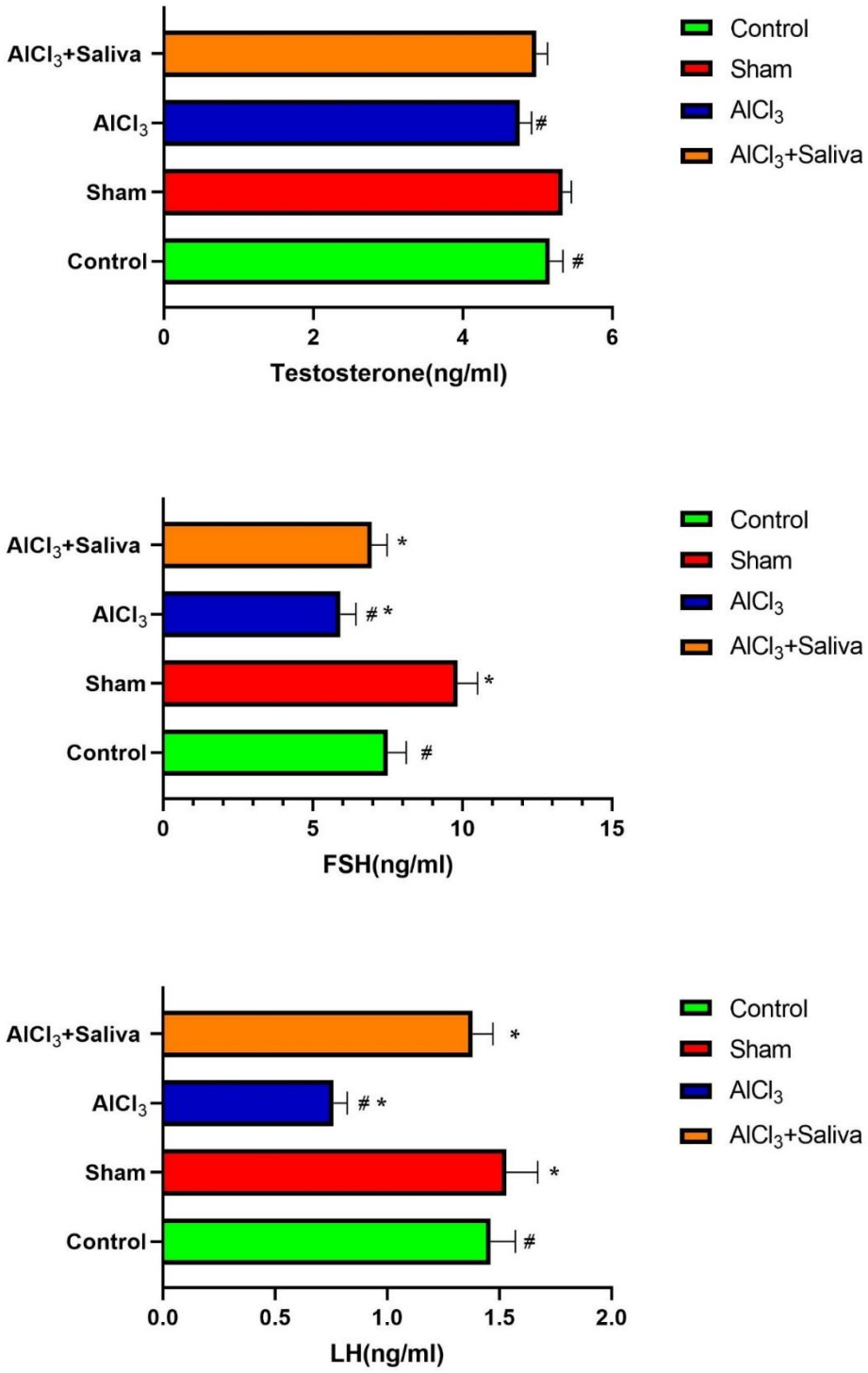


Fig. 1. Effects of *S. sahendica* on $AlCl_3$ -induced disturbance in male androgen hormones in rats. Data are expressed as Mean ± S.D. values (n=8). *P < 0.05 vs. the control group, # P < 0.05 vs. the $AlCl_3$ group, using the Tukey's post hoc test.

3.5. Measurement of total antioxidant capacity and malondialdehyde levels in serum and tissue

In tissues, such as epididymis tissue, seminal vesicle, and testis, where cell division occurs continuously with endocrine function, there is a need for high levels of energy, and oxidative stress resulting from exposure to $AlCl_3$ can cause cell damage and tissue dysfunction.

Table 3 shows the damaging effects of $AlCl_3$ and the protective effects of *S. sahendica* on total antioxidant capacity and MDA concentrations. The levels of MDA in the serum and testis tissue of animals received $AlCl_3$ increased significantly ($P < 0.001$, $P < 0.01$), whereas the total antioxidant capacity decreased ($P < 0.05$). Treatment with *S. sahendica* improved these conditions so that the MDA levels decreased significantly ($P < 0.001$ and $P < 0.01$) and the total antioxidant capacity increased to its levels in the control group ($P < 0.05$).

Table 3. Effect of *S. sahendica* on lipid peroxidation profile $AlCl_3$-treated rats.

Variable	Control	*S. sahendica*	$AlCl_3$	$AlCl_3$ + *Saliva sahendica*
Serum MDA	1.73 ± 0.15	1.55 ± 0.12	$2.58 \pm 0.13^{***}$	$1.61 \pm 0.15^{\#\#\#}$
Testis MDA	26.8 ± 1.89	24.1 ± 1.5	$38.5 \pm 2.1^{**}$	$28 \pm 2.4^{\#\#}$
Total antioxidant Capacity	1.38 ± 0.12	1.52 ± 0.13	$0.87 \pm 0.04^{*}$	$1.33 \pm 0.14^{\#}$

Data are expressed as mean ± SD values (n = 8). Statistical differences between control and $AlCl_3$ groups: $^{**}P < 0.01$, $^{***}P < 0.001$. Statistical differences between $AlCl_3$ Vs $AlCl_3$ + *Saliva sahendica* groups $^{\#}P<0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$

4. Discussion

In today's world, humans are increasingly exposed to environmental pollutants and chemical toxins due to lifestyle changes. This continuous exposure to toxic chemicals can have detrimental effects on various organs, particularly the reproductive system. Disturbingly, reports are suggesting that Al, a common chemical in modern life, may have toxic effects. This challenges conventional notions, as many commonly used chemicals have been linked to physiological disorders. Given the beneficial effects of various phytochemicals in mitigating toxicity and related disorders, herbal medicine is gaining attention as an alternative approach. The purpose of the recent study was to evaluate the positive impact of *S. sahendica* hydroalcoholic extract on the reproductive ability and hormone levels (testosterone, LH, and FSH), as well as on the quality of spermatogenesis and fertility rate in Wistar rats. The current study revealed that administering $AlCl_3$ led to a marked reduction in sperm count, as well as decreased motility and viability. Additionally, we observed changes in sperm morphology. These findings align with existing knowledge that $AlCl_3$ can induce degenerative alterations in the seminiferous tubules, culminating in impaired spermatogenesis. In addition, the body weight and weight of the testes were affected by the administration of $AlCl_3$. This finding is in line with previous literature that also reported a decrease in body weight gain and testes weight due to aluminum treatment [11, 24, 25]. Our experiment indicates that the administration of *S. sahendica* extract notably improved the body weight and sexual organs alongside sperm quality of rats that were exposed to $AlCl_3$ toxicity. Correspondingly, Al-Chalabi et al. found that *S. officinalis* (common sage) significantly boosts sperm motility and decreases both sperm mortality and deformities in diabetic male albino rats [24]. Such improvements may be due to the S. extract's compounds, like saponins and alkaloids, which are known to promote testicular growth and support the

proliferation, maturation, and differentiation of sperm cells [26].

In this study, the increase in serum and testes MDA levels following exposure to $AlCl_3$ can be attributed to an increased burden of oxidative stress. This is likely due to the production of reactive oxygen species (ROS), which in turn induce lipid and protein oxidation, as previously mentioned by recent studies [27-29]. Additionally, there was a reduction in TAC activity and sperm count. These findings are consistent with previous studies.[11, 30-32]The beneficial effects of *S. sahendia* align with our prior findings, which demonstrated its capacity to enhance oxidative stress enzyme levels while diminishing malondialdehyde (MDA) concentrations [26, 32, 33].

The results of this study demonstrated that the administration of $AlCl_3$ ($AlCl_3$) led to a significant reduction in the serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. This is in line with prior studies which also observed that $AlCl_3$ substantially lowered the concentrations of LH, FSH, and testosterone in rat blood [11, 29, 34]. These studies suggest that $AlCl_3$ acts as an endocrine disruptor, interfering with the synthesis and regulation of various hormones, including testosterone, FSH, and LH. Moreover, when S. was administered alongside $AlCl_3$, there was an increase in the serum levels of LH, FSH, and testosterone. This finding echoes previous research, which reported significant elevations in the levels of testosterone, LH, and FSH in the serum of albino rats following treatment with *S. officinalis*. $AlCl_3$ exposure is believed to reduce testosterone levels by blocking calcium channels, which in turn downregulates gonadotrophin secretion in the pituitary gland [35]. Additionally, $AlCl_3$ exposure can suppress steroidogenesis by increasing testicular NO concentrations and decreasing c-AMP. In a previous investigation by Cheraghi et al., it was suggested that $AlCl_3$ injection in rats decreased the rate of glutamate, which likely blocked voltage-sensitive calcium channels (VSCCs) in hypothalamic cells responsible for GnRH synthesis [11]. This affects calcium influx in those cells and decreases GnRH secretion. The authors concluded that since FSH and LH secretion are promoted by FSH-releasing hormone (FSHRH) and LH-releasing hormone (LHRH) factors produced in the hypothalamus, it is probable that $AlCl_3$ administration inhibited the production of releasing factors in the hypothalamus, ultimately reducing the secretion of LH and FSH from the pituitary gland.

Following the administration of $AlCl_3$, our study observed a notable elevation in malondialdehyde (MDA) levels within serum and testicular tissue, alongside a marked reduction in total antioxidant capacity (TAC). This phenomenon may stem from the neutralization of cellular antioxidants by lipid peroxides and reactive oxygen species (ROS), which are byproducts of $AlCl_3$ intoxication. Additionally, it has been suggested that $AlCl_3$ could interfere with the gene expression of these enzymes at the transcriptional level.

Conversely, the simultaneous administration of *S. sahendica* and $AlCl_3$ resulted in reduced MDA levels and an upsurge in total antioxidant activity. These findings are consistent with prior research, which attributes the mitigation of lipid peroxidation to the antioxidant capabilities of flavonoids and vanillin found in *S. officinalis* [36, 37]. These components are known for their efficacy in scavenging free radicals and chelating divalent cations. The bioactive compounds present in *S. sahendica* extract exhibit potent antioxidant and free radical neutralizing properties, which contribute to the extract's

protective effects. Budgetary constraints restricted the scope of histological tissue analysis, potentially influencing the results' interpretation. Additionally, the inability to prepare various extract fractions presented a further limitation. For future studies, it is recommended to prepare and examine different fractions of the plant. Additionally, histological tests and advanced techniques such as ELISA and histochemistry should be conducted to identify and analyze the active compounds and biological effects of the plant more precisely. This approach can provide a better understanding of the plant's functional mechanisms and therapeutic potential.

5. Conclusion

This research determines that exposure to $AlCl_3$ adversely impacts sperm quality and integrity, reproductive hormone levels, and testicular oxidative stress indicators. Conversely, *S. sahendica* counteracts the detrimental effects of $AlCl_3$ by significantly enhancing spermatogenesis and reducing oxidative stress, thereby boosting reproductive function. However, more and additional studies are needed to generalize the results.

Author contributions

Author contributions Conceptualization, A.K., M.T and R.V.H.; methodology, A.K, and M.Z.; software A.K.; validation A.K. and R.V.H.; investigation, A.K., R.V.H., and M.Z.; writing—original draft A.K, M.T and R.V.H.; writing—review and editing, M.Z and R.V.H.; visualization, M.Z; supervision, A.K; funding acquisition, A.K. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare there is no conflict of interest.

Acknowledgment

We appreciate the personnel of the Medicinal Plants Research Center of Maragheh University of Medical Sciences for guidance and help.

References

1. Babakhanzadeh E, Nazari M, Ghasemifar S and Khodadadian A. Some of the factors involved in male infertility: a prospective review. *Int. J. Gen. Med.* 2020; 13: 29-41. doi: 10.2147/IJGM.S241099.

2. Khorrami A, Ghanbarzadeh S, Ziaee M, Arami S, Vajdi R and Garjani A. Dietary cholesterol and oxidized cholesterol: effects on sperm characteristics, antioxidant status, and hormonal profile in rats. *Andrologia.* 2015; 47(3): 310-317. doi: 10.1111/and.12262.

3. Delashoub M, Ziaee M, Khorrami A and Banan-Khojasteh SM. Comparison of the effects of Clofibrate and Silafibrate on sperm parameters quality and sex hormones in male rats. *Urol. J.* 2018; 15(2): 38-43. doi: 10.22037/uj.v0i0.3954.

4. Osadchuk L V and Osadchuk A V. Individual lifestyle and male fertility. *Hum. Physiol.* 2023; 49(2): 196-207. doi: 10.1134/S0362119722600527.

5. Roychoudhury S, Chakraborty S, Choudhury AP, Das A, Jha NK, Slama P, Nath M, Massanyi P, Ruokolainen J and Kesari KK. Environmental factors-induced oxidative stress: Hormonal and molecular pathway disruptions in hypogonadism and erectile dysfunction. *Antioxidants.* 2021; 10(6): 837. doi: 10.3390/antiox10060837.

6. Hamidi M, Ziaee M, Delashoub M, Marjanid M, Karimitabar F, Khorami A and Ahmadi NA. The effects of essential oil of *Lavandula angustifolia* on sperm parameters quality and reproductive hormones in rats exposed to

cadmium. *J. Rep. Pharm. Sci.* 2015; 4(2): 135-142.

7. Rahimzadeh MR, Rahimzadeh MR, Kazemi S, Amiri RJ, Pirzadeh M and Moghadamnia AA. Aluminum poisoning with emphasis on its mechanism and treatment of intoxication. *Emerg. Med. Int.* 2022; 2022. 1480553. doi: 10.1155/2022/1480553.

8. Tietz T, Lenzner A, Kolbaum AE, Zellmer S, Riebeling C, Gürtler R, Jung C, Kappenstein O, Tentschert J, Giulbudagian M, Merkel S, Pirow R, Lindtner O, Tralau T, Schäfer B, Laux P, Greiner M, Lampen A, Luch A, Wittkowski R and Hensel A Aggregated aluminum exposure: risk assessment for the general population. *Arch. Toxicol.* 2019; 93: 3503-3521. doi: 10.1007/s00204-019-02599-z.

9. Lyons-Weiler J and Ricketson R. Reconsideration of the immunotherapeutic pediatric safe dose levels of aluminum. *J. Trace. Elem. Med. Biol.* 2018; 48: 67-73. doi: 10.1016/j.jtemb.2018.02.025.

10. Sjögren B, Iregren A, Montelius J and Yokel RA. Aluminum. In: *Handbook on the Toxicology of Metals*. Vol: Elsevier; 2015, 549-564. doi: 10.1016/B978-0-444-59453-2.00026-3.

11. Cheraghi E, Golkar A, Roshanaei K and Alani B. Aluminium-induced oxidative stress, apoptosis and alterations in testicular tissue and sperm quality in Wistar rats: Ameliorative effects of curcumin. *Int. J. Fertil. Steril.* 2017; 11(3): 166-175. doi: 10.22074/ijfs.2017.4859.

12. Pandey G and Jain GC. A review on toxic effects of aluminum exposure on male reproductive system and probable mechanisms of toxicity. *Inter. J. Toxicol. Appl. Pharmacol.* 2013; 3(3): 48-57.

13. Aghashahi M, Momeni HR and Darbandi N. Impact of aluminum toxicity on vital human sperm parameters—Protective effects of silymarin. *Andrologia.* 2020; 52(10): e13742. doi: 10.1111/and.13742.

14. Li H, Liu J, Nong W, Shen M, Dou S, Sun S and Wang J. Aluminum exposure impairs oocyte quality via subcellular structure disruption and DNA damage-related apoptosis in mice. *J. Environ. Sci.* 2024; 139: 308-319. doi: 10.1016/j.jes.2023.04.007.

15. Fallah Huseini H, Andalib S, Jasemi E, Khalighi-Sigaroodi F, Momtaz S, Mohammadi Savadroodbari R, Salehirad H and Ziaee M. Protective effect of *Citrullus colocynthis* (L.) Schard. fruit extract on high glucose-induced neurotoxicity in PC-12 cells. *J. Med. Plants* 2021; 20(80): 60-68. doi: 10.52547/jmp.20.80.60.

16. Tavakolizadeh M, Peyrovi S, Ghasemi-Moghaddam H, Bahadori A, Mohkami Z, Sotoudeh M and Ziaee M. Clinical efficacy and safety of okra (*Abelmoschus esculentus* (L.) Moench) in type 2 diabetic patients: a randomized, double-blind, placebo-controlled, clinical trial. *Acta Diabetol.* 2023; 60(12): 1685-1695. doi: 10.1007/s00592-023-02149-1.

17. Ghorbani A and Esmaeilizadeh M. Pharmacological properties of *S. officinalis* and its components. *J. Tradit. Complement. Med.* 2017; 7(4): 433-440. doi: 10.1016/ j.jtcme.2016.12.014

18. Mokhtari R, Kazemi Fard M, Rezaei M, Moftakharzadeh SA and Mohseni A. Antioxidant, Antimicrobial Activities, and Characterization of Phenolic Compounds of Thyme (*Thymus vulgaris* L.), Sage (*S. officinalis* L.), and Thyme–Sage Mixture Extracts. *J. Food. Qual.* 2023; 2023. doi: 10.1155/2023/2602454.

19. Dehghan GR, Torbati S, Mohammadian R, Movafeghi A and Talebpour AH. Essential oil composition, total phenol and flavonoid contents and antioxidant activity of *S. sahendica* at different developmental stages. *J. Essent. Oil-*

Bear. Plants. 2018; 21(4): 1030-1040. doi: 10.1080/0972060X.2017.1409656.

20. Esmaeili S, Asadi M, Mosaddegh M, Motamed SM and Hamzeloo-Moghadam M. Cytotoxic activity of *Centaurea albonitens* Turrill aerial parts in colon and breast cancer cell lines. *J. Med. Plants.* 2021; 20(78): 59-67. doi: 10.52547/jmp.20.78.59.

21. Bahramloo M, Moradkhani S and Sedaghat Hamedani M. Phytochemical evaluation and antioxidant effects of the essential oil and distillates of *Nepeta crispa* Willd. *J. Med. Plants* 2023; 22(86): 27-43. doi: 10.61186/ jmp. 22.86.27.

22. Mashhadi F, Ghorbani Nohooji M and Yaraee R. Immunomodulatory effects of *Origanum vulgare* L. and *Origanum majorana* L. ethanolic extracts in vitro. *Immunoregulat.* 2021; 4(1): 21-32. doi: 10.32598/Immunoregulation.4.1.1.

23. Ziaee M, Samini M, Bolourtchian M, Ghaffarzadeh M, Ahmadi M, Egbal MA, Khorrami A, Andalib S, Maleki-Dizaji N and Garjani AR. Synthesis of a novel siliconized analog of clofibrate (silafibrate) and comparison of their anti-inflammatory activities. *Iran. J. Pharm. Res.* 2012; 11(1): 91-95.

24. Salah M, Hussein M, Rana I and Khalid LB. Effect of *S. officinalis* L.(Sage) aqueous extract on liver and testicular function of diabetic albino male rats. *J. Babyl. Uni. Pure. Appl. Sci.* 2016; 24(4): 390-399.

25. Ogunlade B, Adelakun S and Iteire K. Sulforaphane response on aluminum-induced oxidative stress, alterations in sperm characterization and testicular histomorphometry in Wistar rats. *Int. J. Reprod. Biomed.* 2020; 18(8): 611. doi: 10.18502/ijrm.v13i8.7503.

26. Basal WT, Issa AM, Abdelalem O and Omar AR. *S. officinalis* restores semen quality and testicular functionality in cadmium-intoxicated male rats. *Scientific Reports.* 2023; 13(1): 20808. doi: 10.1038/s41598-023-45193-1.

27. Afolabi OK, Wusu AD, Ugbaja R and Fatoki JO. Aluminium phosphide-induced testicular toxicity through oxidative stress in Wistar rats: Ameliorative role of hesperidin. *Toxicol. Res. Appl.* 2018; 2: 2397847318812794. doi: 10.1177/2397847318812794.

28. Kalaiselvi A, Suganthy OM, Govindassamy P, Vasantharaja D, Gowri B and Ramalingam V. Influence of aluminium chloride on antioxidant system in the testis and epididymis of rats. *Iran. J. Toxicol.* 2014; 8(24): 991-997.

29. Lokman M, Ashraf E, Kassab RB, Abdel Moneim AE and El-Yamany NA. Aluminum chloride–induced reproductive toxicity in rats: the protective role of zinc oxide nanoparticles. *Biol. Trace Elem. Res.* 2022; 200(9): 4035-4044. doi: 10.1007/s12011-021-03010-8.

30. Salama EEA, El Fouhil AF, Alyahya KI, Shaheen SY, Alshaarawy SA and Mahmoud SA. Protective effect of propolis against aluminum chloride-induced reproductive toxicity in male rats. *GSC Adv. Res. Rev.* 2021; 9(2): 083-088. doi: 10.30574/gscarr.2021.9.2.0273.

31. Akinola BK, Olawuyi TS, Ukwenya VO, Daniel LD and Faleye BC. Protective effects of Aloe vera gel (Aloe baberdensis Miller) on aluminum chloride-induced reproductive toxicity in male Wistar rats. *JBRA Assist. Rep.* 2021; 25(2): 193-201. doi: 10.5935/1518-0557.20200082.

32. Boussadia A, Kharoubi O, Lahouel Z, Benglia A and Aoues A. Effect of aqueous *S. officinalis* extract on Aluminum chloride-induced neurotoxicity in female rats. *Int. J. Pharm. Res. Al. Sci.* 2020; 9(2): 139-150.

33. Rashwan HM, Mohammed HE, El-Nekeety AA, Hamza ZK, Abdel-Aziem SH, Hassan NS and Abdel-Wahhab MA. Bioactive phytochemicals from *S. officinalis* attenuate

cadmium-induced oxidative damage and genotoxicity in rats. *Envir. Sci. Poll. Res.* 2021; 28(48): 68498-68512. doi: 10.1007/s11356-021-15407-y.

34. Muselin F, Cristina RT, Igna V, Dumitrescu E, Brezovan D and Trif A. The consequences of aluminium intake on reproductive function in male rats: a three-generation study. *Turk. J. Med. Sci.* 2016; 46(4): 1240-1248. doi: 10.3906/sag-1501-101.

35. Olanrewaju JA, Akinpade TG, Olatunji SY, Owolabi JO, Enya JI, Adelodun ST, Fabiyi SO and Desalu AB. Observable protective activities of quercetin on aluminum chloride-induced testicular toxicity in adult male Wistar rat. *J. Hum. Reprod. Sci.* 2021; 14(2): 113-120. doi: 10.4103/jhrs.jhrs_190_20.

36. Zhao D, Sun J, Sun B, Zhao M, Zheng F, Huang M, Sun X and Li H. Intracellular antioxidant effect of vanillin, 4-methylguaiacol and 4-ethylguaiacol: Three components in Chinese Baijiu. *RSC Advances.* 2017; 7(73): 46395-46405. doi: 10.1039/C7RA09302K.

37. Brindisi M, Bouzidi C, Frattaruolo L, Loizzo MR, Cappello MS, Dugay A, Deguin B, Lauria G, Cappello AR and Tundis R. New insights into the antioxidant and anti-inflammatory effects of Italian *S. officinalis* leaf and flower extracts in lipopolysaccharide and tumor-mediated inflammation models. *Antioxidants* 2021; 10(2): 311. doi: 10.3390/antiox10020311.

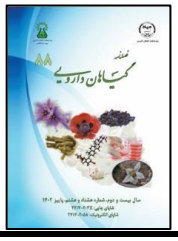
How to cite this article: Khorrami A, Vajdi-hokmabad R, Tavakolizadeh M, Ziaee M. The effects of hydroalcoholic extract of *Salvia sahendica* on sperm parameters quality and reproductive hormones in rats exposed to Aluminum. ***Journal of Medicinal Plants*** 2023; 22(88): 10-22.
doi:

فصلنامه گیاهان دارویی ۱۴۰۲؛ ۲۲(۸۸): ۱۰-۲۲

فصلنامه گیاهان دارویی

Journal homepage: www.jmp.ir

پژوهشکده گیاهان دارویی
جهاد دانشگاهی



مقاله تحقیقاتی

اثر حفاظتی عصاره هیدروالکلی گیاه مریم‌گلی سهندی (*Salvia sahendica*) بر مقابل اثرات سمی ناشی از کلرید آلومنیوم بر کیفیت پارامترهای اسپرم و هورمون‌های تولید مثلی در موش‌های صحرایی نر

آرش خرمی[۱]، رضا وجدی حکم آباد[۲]، مهدی توکلی‌زاده[۳،۴]، مجتبی ضیائی[۱،۵،*]

[۱] مرکز تحقیقات گیاهان دارویی، دانشکده علوم پزشکی مراغه، مراغه، ایران
[۲] دانشکده دامپزشکی، دانشگاه آزاد اسلامی واحد میانه، میانه، ایران
[۳] گروه فارماکوگنوزی، دانشکده داروسازی، دانشگاه علوم پزشکی زنجان، زنجان، ایران
[۴] گروه فارماکوگنوزی و بیوتکنولوژی دارویی، دانشکده داروسازی، دانشگاه علوم پزشکی ایران، تهران، ایران
[۵] مرکز تحقیقات طب تلفیقی در سالمندی، پژوهشکده سالمندی، دانشگاه علوم پزشکی تبریز، تبریز، ایران

اطلاعات مقاله

گل‌واژگان:
مریم‌گلی سهندی
آلومینیوم
استرس اکسیداتیو
سمیت تولیدمثل
هورمون‌های جنسی
موش‌های نر

چکیده

مقدمه: تماس با آلومینیوم و سمیت ناشی از آن چالش عمده عصر حاضر است. مطالعات اخیر نشان داده ترکیبات آلومینیوم تأثیر منفی قابل توجه بر باروری و تولیدمثل دارد. **هدف:** بررسی اثرات عصاره هیدروالکلی گیاه مریم‌گلی سهندی بر سمیت ایجاد شده با آلومینیوم در موش‌های صحرایی است. **روش بررسی:** در این مطالعه سی و دو سر موش نر بالغ نژاد ویستار به چهار گروه تقسیم شدند: گروه کنترل، گروه $AlCl_3$ (۴۰ میلی‌گرم/کیلوگرم وزن بدن)، گروه عصاره هیدروالکلی گیاه (۱۰۰ میلی‌گرم / کیلوگرم وزن بدن) و گروه درمان گیاه + $AlCl_3$ موش‌ها به مدت ۷۰ روز تحت مطالعه قرار گرفتند. **نتایج:** مصرف $AlCl_3$باعث آسیب اکسیداتیو شد، که با افزایش سطح MDA و کاهش TAC سمیت خود را نشان داد. علاوه بر این، موش‌های مسموم شده با $AlCl_3$ کاهش قابل توجهی در سطح سرم هورمون‌های جنسی نر از جمله تستوسترون، هورمون LH و هورمون FSH داشتند. با این حال، مصرف روزانه مریم‌گلی سهندی به موش‌ها قبل از تجویز $AlCl_3$ منجر به کاهش استرس اکسیداتیو در بیضه‌ها و بهبود نشانگرهای تولیدی در سرم شد، و اثرات سمیت ایجاد شده ناشی از مصرف کلرید آلومینیوم را بهبود بخشید. **نتیجه‌گیری:** این نتایج نشان می‌دهد که عصاره هیدروالکلی مریم‌گلی سهندی ممکن است به عنوان یک عامل جایگزین برای کاهش سمیت ایجاد شده در اثر تماس یا مصرف آلومینیوم استفاده شود.

مخففها: AlCl3، کلرید آلومینیوم؛ FSH، هورمون تحریک‌کننده فولیکول؛ LH ، هورمون لوتئینیزه‌کننده؛ MDA، مالون دی آلدئید؛ TAC، ظرفیت آنتی‌اکسیدانی تام؛ VSCCs، کانال‌های کلسیمی حساس به ولتاژ؛ RIA، رادیوایمونواسی؛ WHO، سازمان بهداشت جهانی.

* نویسنده مسؤول: ziaee.m@mrgums.ac.ir, mjziaee@gmail.com

تاریخ دریافت: ۱۰ آبان ۱۴۰۲؛ **تاریخ دریافت اصلاحات:** ۲۱ فروردین ۱۴۰۳؛ **تاریخ پذیرش:** ۲۵ فروردین ۱۴۰۳

doi: