



Protective Role of *Rosa damascena* Miller Hydroalcoholic Extract on Oxidative Stress Parameters and Testis Tissue in Rats Treated with Sodium Arsenite

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ABSTRACT

Regarding the strong antioxidant properties of *Rosa damascena* extract, this study aimed to investigate the protective role of *Rosa damascena* Miller hydroalcoholic petal extract on oxidative stress parameters and testis tissue in rats treated with sodium arsenite. To this end, 30 male rats were divided into five groups, including control, positive control (treated with arsenite), and three groups of patients affected by sodium arsenite with 150 mg/kg, 300 mg/kg, and 450 mg/kg *Rosa damascena* extract for 34 days by gavage. The animals were then anesthetized, and the blood samples were collected from the heart. The left testis was removed for histopathological studies. The findings revealed that Sodium arsenite in the positive group caused a significant reduction in TAC, testosterone, and serum Luteinizing hormone (LH) and a significant increase in serum Malondialdehyde. In addition, there was no statistically significant difference among the groups regarding the amount of Follicle-stimulating hormone (FSH). Moreover, the consumption of *Rosa damascena* extract with sodium arsenite caused a significant increase in testosterone, LH, and FSH compared to the positive control group. Histopathological results showed that in the experimental group receiving a dosage of 300 mg/kg b.w and the control group, the number of sperm tubes increased, and the germinal epithelium's thickness was appropriate. Daily treatment with *Rosa damascena* extract with a dosage of 300 mg/kg b.w for 34 days could improve the changes caused by sodium arsenite and reduce Malondialdehyde levels. Thus, it seems that *Rosa damascena* hydroalcoholic extract can effectively improve the male reproductive system's function.

Keywords: Oxidative stress, Rats, Rose petals, Sodium arsenite, Testis

INTRODUCTION

In their living and working environments, humans are exposed to agents that can be detrimental to the functioning of the reproductive system. Due to the rapid division of spermatogonia cells, the male reproductive system is very sensitive to many chemicals and physical agents produced by agricultural and industrial activities (Paul and Frazier, 2000).

Among chemical pollutants, arsenic is an element that can be present in mineral waters by dissolving from different soil layers. The concentration of arsenic in groundwater sources of drinking water is among the major global health problems. Inorganic arsenic is found in drinking water in the forms of arsenate (pentavalent) and arsenite (trivalent). The toxicity of arsenite compounds has been reported to be higher than that of arsenate (Mir et al., 2021). In addition, arsenic is used to manufacture herbicides, rodenticides, food preservatives, and even medicines. It is also a carcinogen that can be absorbed in various ways, including the skin, respiratory, and digestive systems, and threaten human and animal health (Wang et al., 2007).

One of the compounds of arsenic is sodium arsenite, which is an odorless and colorless substance. Sodium arsenite is an environmental pollutant that can induce male reproductive system abnormalities through oxidative stress. Arsenic poisoning in laboratory animals weakens Leydig cells' functioning, thereby negatively affecting spermatogenesis (Momeni and Eskandari, 2012). Sodium arsenite induces oxidative stress in various body tissues, including the testes (Akbari, 2022). Oxidative stress and reactive oxygen species (ROS) are considered among the major causes of male infertility. This compound is an oxidizing agent from the group of free radicals that can damage sperm if overproduced (Shi et al., 2004).

Oxidative stress is a pathological process that results from an imbalance between the body's antioxidant defense systems. In this case, the formation rate of free radicals in the body increases and paves the way for the peroxidation and oxidation of lipids, proteins, and nucleic acids (Yamakado et al., 2014).

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Lipid peroxidation (LP) is known as an indicator of OS. The increase in LP is associated with increased malondialdehyde concentration (Yousef et al., 2006). The oxidative stress induction causes fat oxidation and Malondialdehyde production as the main indicator of oxidative stress of lipids and sperm dysfunction (Kubiliene et al., 2021; Salkin et al., 2022). Due to the presence of fat tissue on the genitals and testes, Malondialdehyde is considered an indicator of oxidative stress (Ahmadi et al., 2011). Natural antioxidants have received more attention because of being safe and have favorable effects in dealing with oxidative stress (Kim et al., 2010). Antioxidants reduce oxidative stress and the amount of testicular damage (Salimnejad et al., 2014). Gole Mohammadi, with the scientific name of *Rosa damascena*, is a kind of Rose flower in Iran. Essential oil and rose water, as two important product of this plant, has been popular for many years regarding their mystical and economic properties (Shalit et al., 2003).

This plant's extract contains terpene, glycoside, flavonoid, anthocyanin, carboxylic acid, myrcene, vitamin C, kaempferol, quercetin, and geraniol. This plant is a rich source of phenolic compounds such as eugenol and granulol. These compounds have antioxidants. They also inhibit free radicals and have anti-cancer, anti-inflammatory, anti-genetic mutation, and anti-depressant properties. These phenolic compounds also have anti-epileptic properties (Aycı et al., 2005).

Rosa damascene has been long used for food and is considered its essential oil. For this reason, its medicinal uses have been disregarded. On the other hand, research has proven the adverse effects of sodium arsenite on the male reproductive system (Sarkar et al., 2003; Momeni and Eskandari, 2012). Considering the toxicity of sodium arsenite and its negative impact on the male reproductive system and the strong antioxidant properties of *Rosa damascene* extract, this study aimed to investigate the protective role of *Rosa damascene* on oxidative stress parameters and testis tissue of sodium arsenite-treated rats.

MATERIALS AND METHODS

Ethical approval

This research was conducted adhering to ethical principles and with the thesis code 162343080 at the Pet Breeding Center of Shahrekord University.

Study design

A total of 30 adult male Wistar rats aged 8 weeks with an average weight of 225 grams were purchased from the Pasteur Institute of Iran. To adapt to the environment, they were kept in standard conditions (that is the temperature of $22 \pm 2^{\circ}\text{C}$, a 12:12 light-dark (LD) cycle, and separate standard cages) for 2 weeks with ad libitum access to tap water and special food for laboratory animals (Royan Institute, Iran).

Extraction

Rosa damascene from the gardens of Shahrekord, Iran. Next, after identifying the desired species by a medicinal plant expert from the Research Center for Medicinal Plants. About 500 g of *Rosa damascene* was powdered using a mill and successively extracted three times with 6 L ethanol: water (70:30) at room temperature using a percolator. The extraction process took 72 hours. The obtained liquid extract was filtered through filter paper. Under a vacuum, the extract was evaporated at 35°C until dry using a rotary evaporator (Buchi, Switzerland). The 100-g gummy dark extract was kept in a refrigerator (4°C ; Khoshdouni Farahani, 2021).

MATERIALS AND METHODS

The rats were carefully weighed and marked into groups before the experiment started. For this purpose, the *Rosa damascene* petal extract has 150, 300, and 450 mg/kg b.w doses. Also, sodium arsenite with a dose of 5 mg/kg b.w were fed to the animals daily and through gavage for 34 days.

In this research, the animals were randomly divided into 5 groups (each with 6 rats):

The first group included healthy controls. The second group included the positive controls exposed to sodium arsenite (5 mg/kg body weight). The third group was treatment group 1 (sodium arsenite + 150 mg/kg body weight of *Rosa damascene* Miller hydroalcoholic petal extract). The fourth group included treatment group 2 (sodium arsenite + 300 mg/kg body weight of *Rosa Damascene* Miller Hydroalcoholic Petal Extract). Finally, the fifth group was treatment group 3 (sodium arsenite + 450 mg/kg body weight of *Rosa Damascene* Miller Hydroalcoholic Petal Extract).

After the end of the treatment period, the animals were euthanized with ketamine (Pfizer, Germany) at a dosage of 100 mg/kg, and a 2 mL blood sample was taken from their heart (right ventricle) immediately using a 25 gauge needle (Ganguly et al., 2018). The obtained blood was poured into sterile test tubes and centrifuged at a speed of 4000 rpm for 10 minutes. Blood serums were immediately separated, poured into Eppendorf microtubes, and returned to the laboratory to measure biochemical factors (that is Malondialdehyde and total antioxidant capacity). Testosterone, LH,

and FSH hormones of blood serum were measured using the ELISA method and special kits (Padgin Teb Co, Iran). After taking blood from the animals' hearts, their right testicle was removed from the body and weighed.

Histopathological examination of testis tissue

After washing the testes with physiological serum, they were fixed in 10% formalin for sectioning and histopathological studies. Next, Masson's trichrome staining was performed for samples. Then, the number of seminiferous tubules and spermatogenesis status were checked using an optical microscope (B-510BF, Optika, Italy) by a magnification of $\times 10$ (Kuntsal et al., 2023)

Statistical analysis

The results were analyzed through analysis of variance (ANOVA) and Tukey's tests in SPSS 25 at a significance level of 0.05 was chosen.

RESULTS

The weight of the rats in treatment groups 2 and 3 was almost close to each other and had a significant increase compared with other groups. Also, the weight of the rats in the positive control group and treatment group 1 were almost close to each other and had a significant decrease compared to the other groups ($p < 0.05$). These changes in the healthy control group had a significant decrease compared with the treatment groups 2 and 3 ($p < 0.05$).

The changes in the weight of the testis + epididymis in the treatment groups (2 and 3) had a significant increase compared with the treatment groups 1 and positive control ($p < 0.05$). Also, the testis + epididymis weight in the positive control group had a significant decrease compared with the other groups ($p < 0.05$).

The amount of Malondialdehyde in the positive control group (i.e., the group receiving only sodium arsenite) was higher than that in the healthy control group, indicating a significant difference ($p < 0.05$). There was also a significant difference between treatment groups 1, 2, and 3 ($p < 0.05$) such that treatment group 1 (sodium arsenite + 150 mg/kg body weight of the extract) had an increase in the amount of Malondialdehyde compared with the other two groups ($p < 0.05$).

Moreover, treatment group 2 (dose of 300 mg/kg body weight of the extract) had a decreasing trend in Malondialdehyde compared to the healthy control group, although the difference was nonsignificant. The TAC level significantly increased in the healthy control group compared with the other groups ($p < 0.05$). Moreover, the TAC level of the serum had a significant increase in treatment group 2, compared to treatment groups 1 and 3. However, it decreased significantly in the positive control group compared with the healthy control and treatment groups ($p < 0.05$).

The serum testosterone level significantly increased in the healthy control group, compared with the treatment groups ($p < 0.05$), and in treatment group 2 compared with treatment groups 1 and 3. In addition, the testosterone level significantly decreased in the positive control group compared with the other groups ($p < 0.05$).

As can be seen in Table 1, the serum LH level significantly increased in the healthy control group, compared to the treatment groups ($p < 0.05$). It also significantly increased in treatment group 2 compared with treatment groups 1 and 3 ($p < 0.05$). Notably, the serum LH level had a significant decrease in the positive control group compared with other groups ($P < 0.05$). The serum FSH level had no significant increase in treatment group 2 compared with treatment groups 1 and 3 ($p < 0.05$). However, the serum FSH level had no significant decrease in the positive control group compared with the other groups ($p > 0.05$).

Table 1. Comparison of the mean values of weight and hormonal status in rats treated with sodium arsenite

Traits Mean \pm SD	MDA (nmol/L)	TAC (nmol/L)	Testosterone (nmol/L)	LH (nmol/L)	FSH (nmol/L)	Rat's weight (g)	Testis and epididymis Weight (g)
Healthy Control	0.13 \pm 0.03 ^a	623.00 \pm 102.50 ^b	1.62 \pm 0.29 ^c	0.35 \pm 0.06 ^c	0.43 \pm 0.21 ^a	237.67 \pm 4.04 ^{bc}	1.77 \pm 0.13 ^{bc}
Positive Control	0.24 \pm 0.04 ^b	338.30 \pm 78.92 ^a	0.52 \pm 0.39 ^a	0.11 \pm 0.03 ^a	0.38 \pm 0.13 ^a	208.00 \pm 19.24 ^a	1.24 \pm 0.17 ^a
Treatment 1	0.19 \pm 0.06 ^{ab}	532.60 \pm 41.50 ^b	0.82 \pm 0.06 ^{ab}	0.17 \pm 0.04 ^{ab}	0.40 \pm 0.07 ^a	210.80 \pm 19.25 ^{ab}	1.35 \pm 0.17 ^a
Treatment 2	0.12 \pm 0.1 ^a	572.20 \pm 41.90 ^b	1.14 \pm 0.23 ^{bc}	0.23 \pm 0.06 ^b	0.46 \pm 0.15 ^a	248.80 \pm 7.79 ^c	1.89 \pm 0.19 ^b
Treatment 3	0.13 \pm 0.02 ^a	539.60 \pm 49.14 ^b	0.87 \pm 0.08 ^{ab}	0.16 \pm 0.03 ^{ab}	0.44 \pm 0.11 ^a	247.60 \pm 8.62 ^c	1.72 \pm 0.19 ^b
p-value	0.000*	0.000*	0.000*	0.000*	0.881	0.000*	0.000*

^{abc} Different superscript letters in a column denote a significant difference between the control group and treatment groups at a significance level of $p \leq 0.05$. TAC: Total antioxidant capacity, FSH: Follicle-stimulating hormone, MDA: Malondialdehyde, LH: Luteinizing hormone, SD: standard deviation

Pathology results

In the control group, seminiferous tubules have a high density, and germinal epithelium has an appropriate thickness (Figure 1). In the testes (sodium arsenite), seminiferous tubules have separation and ruptures, and the thickness of germinal epithelium has been reduced (Figure 2). In the testes in treatment group 1 (receiving 150 mg of the extract + sodium arsenite), seminiferous tubules have lost their normal shape, and the separation between the cells and the tubes can be seen. The thickness of the gem layer has also decreased compared with the control group (Figure 3). In treatment, group 2 (receiving 300 mg of the extract + sodium arsenite), seminiferous tubules have a relatively high density (Sertoli cells, spermatids, and mature sperm), and germinal epithelium has an appropriate thickness (Figure 4). In the testis in treatment group 3 (receiving 450 mg of the extract + sodium arsenite), seminiferous tubules have lost their normal structure to some extent, and separation between the tubes can be seen. The thickness of germinal epithelium has also been reduced compared with the control group (Figure 5).



Figure 1. The testis of a rat in healthy control group. Seminiferous tubules have a high density (spermatogonia, mature sperm, and spermatids), and germinal epithelium has an appropriate thickness (Masson's trichrome staining, 100x magnification).

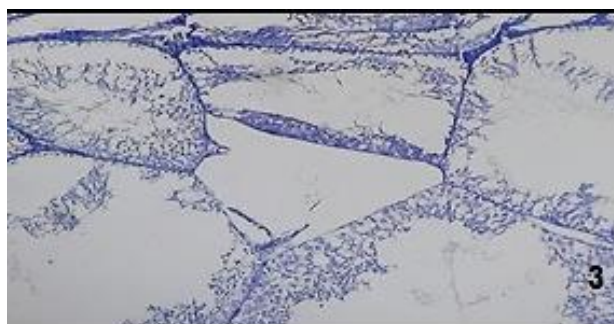


Figure 3. The testis of a rat in treatment group 1 (receiving 150 mg of the extract + sodium arsenite). Seminiferous tubules have lost their normal shape, and the separation between the cells and between the tubes can be seen. The thickness of the gem layer has also decreased compared with the control group (Masson's trichrome staining, 100x magnification).

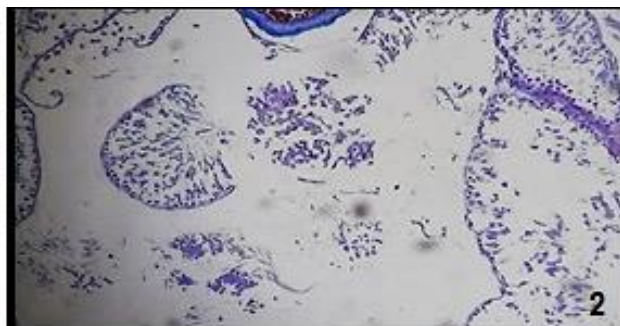


Figure 2. The testis of a rat in positive control group (sodium arsenite). Seminiferous tubules have separation and ruptures, and the thickness of germinal epithelium has been reduced (Masson's trichrome staining, 100x magnification).

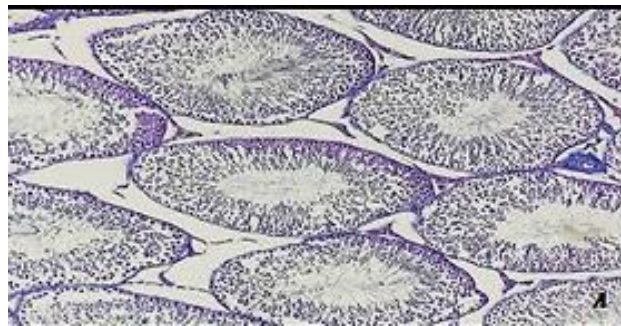


Figure 4. The testis of a rat in treatment group 2 (receiving 300 mg of the extract + sodium arsenite). Seminiferous tubules have a relatively high density (Sertoli cells, spermatids, and mature sperm), and germinal epithelium has an appropriate thickness (Masson's trichrome staining, 100x magnification).

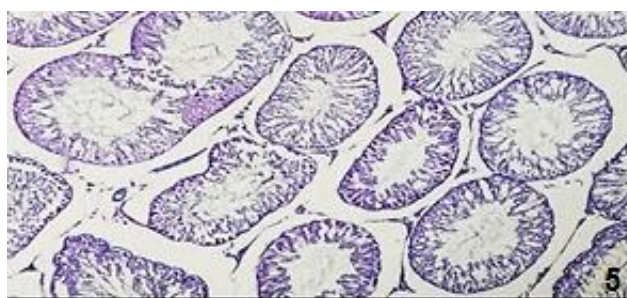


Figure 5. The testis of a rat in treatment group 3 (receiving 450 mg of the extract + sodium arsenite). Seminiferous tubules have lost their normal structure to some extent, and separation between the tubes can be seen. Thickness of germinal epithelium has also been reduced compared with the control group (Masson's trichrome staining, 100x magnification).

DISCUSSION

There is accumulating evidence regarding the enhanced free radicals generation and oxidative stress induction in animals exposed to inorganic arsenic and ROS-induced toxicity. Research has shown that sodium arsenite has toxic effects on the reproductive system, and its accumulation in the testes and prostate gland causes sperm dysfunction and imbalance in sex hormones (Sarkar et al., 2003; Jana et al., 2006). This effect of sodium arsenite is due to the creation of OS (Agarwal et al., 2004). So far, various studies have been conducted on the effect of plant extracts against LP, reduction of antioxidant defense, and imbalance of gonadotropins and testosterone in male rats. These studies are briefly discussed in the present study.

Hfaiedh et al. (2011) investigated the protective effects of garlic (*Allium sativum*) extract upon oxidative stress induced by lindane (a white crystalline powder used as an insecticide in agriculture) and related damages in the testes and brain of male rats. According to these authors, the daily injection of fresh garlic at the dose of 1 ml/kg body weight of rats for 30 days returns the lindane-induced changes to a normal state. It reduces the malondialdehyde level (Hfaiedh et al., 2011). These results are consistent with those of the present study and confirm the useful role of these plants as antioxidants. Raji et al. (2019) investigated the effect of hydroalcoholic garlic extract and N-acetylcysteine on fenvalerate-induced OS. The results revealed that fenvalerate in low doses induces OS, and injection of N-acetylcysteine and the hydroalcoholic extract of garlic alone and in combination can improve injuries caused by fenvalerate. The findings of this study are consistent with the present study.

Faraji et al. (2013) examined the effect of the extract of salep tubes on the formation of testis and sexual hormones in mice. They concluded that LH and testosterone expanded in the experimental group compared with the control group ($p < 0.05$). According to this study, the aqueous extract of the salep tubers increases the testosterone and LH hormones and the process of spermatogenesis and strengthens sexual powers. However, further research was recommended to find the mechanism and effect of this substance before it is used as an effective agent in increasing sexual activity and fertility. Jana et al. (2006) studied the effects of chronic exposure to sodium arsenite on testicular activities in adult rats. These authors concluded that treatment with sodium arsenite declined the weight of both testes, the number of epididymal sperm, LH and FSH hormones, and testosterone, and increased plasma corticosterone concentration (Jana et al., 2006).

Anwar and Qureshi (2019) investigated the effect of sodium arsenite on mice testis and epididymal organ cultures in initiating oxidative, biochemical, and genotoxic stress in testis and epididymal tissue cultures in mice. The results showed that testosterone concentrations are significantly reduced after 24 hours of incubation with 50 and 100 micro mols of sodium arsenite concentrations. The exposure of tissue pieces of the testis and epididymis to higher concentrations of arsenic in laboratory conditions induced rapid and immediate metabolic and genotoxic damage.

Shariati et al. (2009) examined the reaction of trifluralin on LH, FSH, and testosterone levels and testis histological changes in adult rats. They concluded that trifluralin passes through the cytoplasmic membrane of sensitive cells in the testis tissue, produces free radicals, and applies OS to them. Hence, they decline the testosterone serum concentration and disrupt the steroidogenesis and spermatogenesis processes. Askaripour et al. (2018) studied the effect of an aqueous extract of *Rosa damascena* on formaldehyde-get toxicity in mice testes. The results revealed that formaldehyde administration significantly reduced the serum testosterone level, testis volume and weight, tubule diameter, and sperm characteristics compared with the control group. Administration of *Rosa damascena* (40 mg/kg b.w) in rats treated with formaldehyde significantly improved serum testosterone level, weight, and histopathological structure of testis, tubule diameter, number of Leydig cells, and epididymal sperm characteristics compared with low doses and the control group (Askaripour et al., 2018).

According to the results of previous studies and the present study, sodium arsenite decreases body weight and testes, gonadotropin and testosterone hormones, and the number of cells in the testis tissue. The level of Malondialdehyde, one of the end products of LP, and a diagnostic tool for infertility analysis, increased after administering sodium arsenite (Zubair et al., 2016). It is worth mentioning that TAC, that is the total antioxidant activity of the body, had a significant decrease in this study in the positive control group compared with the other groups.

Testosterone serum concentration in treatment groups 1, 2, and 3 decreased significantly compared with the control group. Testosterone has direct anabolic effects on protein production in all organs and tissues of the body and increases muscle and bone mass in males (Ganong, 2001). In the groups treated with sodium arsenite, a decrease in testosterone may decline muscle volume and body weight by lowering protein production.

According to previous studies, the physiological concentrations of testosterone (FSH and LH) play an essential role in spermatogenesis (Ganong, 2001). In this study, the administration of sodium arsenite resulted in the reduction of these hormones, which is one of the main factors in the reduction of the number of germ, sertoli, and interstitial cells and degeneration of the testis tissue. Hence, the reduction of testicular weight is not unlikely.

A dose-dependent reduction in plasma and intratesticular testosterone concentration may lead to an increase in arsenic-treated rats due to the inhibition of testicular androgenic enzymes. The reason is that these enzymes are managed to regulate testosterone biosynthesis (Jana et al., 2006). Research has shown that arsenic-induced ROS affects testosterone production by reducing the expression of StAR and Cyp11a1 genes. These genes transfer cholesterol into the mitochondria, break the side chain of cholesterol, and convert it into pregnenolone, respectively (Wang et al., 2015; Hu et al., 2020). Additionally, the diffidence of testicular androgenic enzymes in arsenic-treated rats may result from low

plasma levels of LH, which is the primary regulator of testicular androgenic enzyme action. The secretion of large amounts of corticoids from the adrenal gland may cause low levels of gonadotropins. Elevated levels of corticosterone have been observed in arsenic-treated animals. Besides, arsenic activates the adrenocortical-hypophyseal stress signaling pathway and increases ACTH secretion from the pituitary gland. An increase in the plasma levels of corticosterone may suppress the sensitivity of gonadotrope cells to GnRH hormones, thereby inhibiting the secretion of FSH and LH hormones. Besides, high levels of ACTH and corticosterone directly inhibit the production and secretion of testosterone by lowering LH receptors on the surface of the testis. Therefore, the reduced process of spermatogenesis decreases the number of sperm (Akbari et al., 2022). In other studies, the sodium arsenite-dependent decrease in plasma gonadotropins has been attributed to low levels of dopamine and high amounts of noradrenaline in the hypothalamus and pituitary. The explanation is that catecholamines are important regulators of the secretion and synthesis of gonadotropins (Jana et al., 2006).

An increase in the malondialdehyde level and a decrease in the total antioxidant level indicate the generation of free radicals and OS. Therefore, the testicular changes in the present study may be associated with oxidative damage caused by sodium arsenite. The hydroalcoholic extract of *Rosa damascene* contains abundant antioxidants, including flavonoid and polyphenolic compounds, with the ability to absorb free radicals (Aycı et al., 2005). The antioxidant effects of the compounds found in this plant have been proven in many studies (Shahriari et al., 2007). Moreover, the other compounds of *Rosa damascene* hydroalcoholic extract include vitamin C, carboxylic acid, tannin, and especially flavonoid and polyphenolic compounds with high antioxidant power, including Kaempferol and Quercetin (Leenen et al., 2000; Loghmani-Khozani et al., 2007).

Based on the histopathological examination of the testis tissue in the different groups of the present study, in treatment group 2 (receiving 300 mg/kg b.w of the extract + sodium arsenite), seminiferous tubules were seen with high density, short distance, and very regular in the testis tissue. All the seminiferous tubules had a round abdomen, a high cell density, and an orderly arrangement. Therefore, a dose of 300 mg/kg b.w of the extract can partially reduce the destructive impacts of sodium arsenite on testis tissue. According to the present study, administrating a dose of 150 mg/kg b.w of the extract + sodium arsenite cannot neutralize the harmful impacts of sodium arsenite. Considering the destructive effects of using a dose of 450 mg/kg b.w of *Rosa damascene* extract + sodium arsenite on testis tissue, using such a dose seems dangerous. Therefore, by applying OS to the testis tissue, sodium arsenite reduces its function and spermatogenesis (Figures 1-5).

The present study indicated that the dose of 300 mg/kg b.w of *Rosa damascene* miller hydroalcoholic petal extract, compared with the samples treated with the dose of 450 mg/kg b.w, is more effective on the OS induced by sodium arsenite. Although the reason for this result is unknown, it seems that antioxidant substances act as prooxidants in certain conditions and cause OS, leading to tissue damage. This factor is the toxicity of the drug in this dose is a question that requires further research.

In the current study, the significant advance in serum testosterone degree and histopathological modification in testes by *Rosa damascene* miller hydroalcoholic petal extract in sodium arsenite-treated rats may be attributed to its antioxidant effect. However, further research is required to clarify the mechanism of action of these compounds on the human reproductive system.

CONCLUSION

The present study showed that the daily administration of *Rosa damascene* Miller hydroalcoholic petal extract at the rate of 300 mg/kg b.w for 34 days returns the sodium arsenite-induced changes to a normal state and reduces the malondialdehyde level. Accordingly, it seems that *Rosa damascene* hydroalcoholic extract can effectively improve the functioning of the reproductive system because of its high antioxidant properties.

DECLARATIONS

Authors' contributions

Shiva Mahdian experimental animals and dosed them with the materials throughout the experiment. Elham Moghtadaei designed the study and critically revised the manuscript. Moghtadaei and Mahdian contributed to the research of the notification data, analyses, and the writing of the final manuscript. The authors confirmed the statistical results and the final draft of the manuscript.

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Conflict of interests

The authors declare no conflict of interest.

Availability of data and materials

The authors can provide all necessary data to the editor upon request without delay.

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Ethical consideration

The authors checked the final draft of the manuscript to remove possible plagiarism and misconduct and prevent double publication/submission and redundancy.

REFERENCES

- Agarwal A, Nallella KP, Allamaneni SS, and Said TM (2004). Role of antioxidants in treatment of male infertility: An overview of the literature. *Reproductive Biomedicine Online*, 8(6): 616-627. DOI: [https://www.doi.org/10.1016/s1472-6483\(10\)61641-0](https://www.doi.org/10.1016/s1472-6483(10)61641-0)
- Ahmadi S, Jafari M, Asgari AR, and Salehi M (2011). Acute effect of diazinon on the antioxidant system of rat's heart tissue. *Trauma Monthly*, 16(2): 87-93. Available at: <http://eprints.bmsu.ac.ir/1281/>
- Akbari S, Talebpour Amiri F, Naderi M, Shaki F, and Seyedabadi M (2022). Sodium arsenite accelerates D-galactose-induced aging in the testis of the rat: Evidence for mitochondrial oxidative damage, NF- κ B, JNK, and apoptosis pathways. *Toxicology*, 470: 153148. DOI: <https://www.doi.org/10.1016/j.tox.2022.153148>
- Mir A, Butt HA, Yasmeen M, Mir UZ, Butt WA, Abbasi S, and Laique T (2021). Histomorphological effects of sodium arsenite on cervix of rats. *Pakistan Journal of Medical & Health Science*, 15(5): 1378-1381. DOI: <https://www.doi.org/10.53350/pjmhs211551378>
- Anwar N and Qureshi IZ (2019). *In vitro* application of sodium arsenite to mice testicular and epididymal organ cultures induces oxidative, biochemical, hormonal, and genotoxic stress. *Toxicology and Industrial Health*, 35(10): 660-669. DOI: <https://www.doi.org/10.1177/0748233719885574>
- Askaripour M, Hasanpour A, Hosseini F, Moshrefi M, Moshtaghi G, Hasannejad M, Rajabi S, and Nematollahi-Mahani SN (2018). The effect of aqueous extract of *Rosa damascena* on formaldehyde-induced toxicity in mice testes. *Pharmaceutical Biology*, 56(1): 12-17. DOI: <https://www.doi.org/10.1080/13880209.2017.1413663>
- Ayçi F, Aydınli M, Bozdemir ÖA, and Tutaş M (2005). Gas chromatographic investigation of rose concrete, absolute and solid residue. *Flavour and Fragrance Journal*, 20(5): 481-486. DOI: <https://www.doi.org/10.1002/ffj.1487>
- Faraji Z, Nikzad H, Parivar K, and Nikzad M (2013). The effect of aqueous extract of salep tubers on the structure of testis and sexual hormones in male mice. *Jahrom University of Medical Sciences*, 11(1): 71-76. DOI: <https://www.doi.org/10.29252/jmj.11.1.10>
- Ganguly S, Panetta JC, Roberts JK, and Schuetz EG (2018). Ketamine pharmacokinetics and pharmacodynamics are altered by P-glycoprotein and breast cancer resistance protein Efflux transporters in mice. *Drug Metabolism and Disposition*, 46(7): 1014-1022. DOI: <https://www.doi.org/10.1124/dmd.117.078360>
- Ganong WF (2001). *Review of medical physiology*, 20th Edition. McGraw-Hill, Philadelphia, pp. 383-438.
- Hfaiedh N, Murat JC, and Elfeki A (2011). Protective effects of garlic (*Allium sativum*) extract upon lindane-induced oxidative stress and related damages in testes and brain of male rats. *Pesticide Biochemistry and Physiology*, 100(2): 187-192. DOI: <http://journal.rums.ac.ir/article-1-2037-en.html>
- Hu Y, Li J, Lou B, Wu R, Wang G, Lu C, Wang H, Pi J, and Xu Y (2020). The role of reactive oxygen species in arsenic toxicity. *Biomolecules*, 10(2): 240. DOI: <https://www.doi.org/10.3390/biom10020240>
- Jana K, Jana S, and Samanta PK (2006). Effects of chronic exposure to sodium arsenite on hypothalamo-pituitary-testicular activities in adult rats: Possible an estrogenic mode of action. *Reproductive Biology and Endocrinology*, 4(1): 9. DOI: <https://www.doi.org/10.1186/1477-7827-4-9>
- Khoshdouni Farahani Z (2021). The effect of extraction method (ultrasonic, maceration, and soxhlet) and solvent type on the extraction rate of phenolic compounds and extraction efficiency of *Arctium lappa* L. roots and *Polygonum aviculare* L. grass. *Food & Health*, 4(2): 28-34. Available at: https://fh.srbiau.ac.ir/article_17920.html
- Kim MB, Park JS, and Lim SB (2010). Antioxidant activity and cell toxicity of 330amascene330d liquid extracts from 20 selected plant species in Jeju, Korea. *Food Chemistry*, 122(3): 546-552. DOI: <https://www.doi.org/10.1155/2017/7695605>
- Kubiliene A, Mickute K, Barauskaite J, Marksa M, Liekis A, and Sadauskiene I (2021). The effects of *Cannabis sativa* L. extract on oxidative stress markers *in vivo*. *Life*, 11(7): 647. DOI: <https://www.doi.org/10.3390/life11070647>
- Kuntsal E, Kadioğlu A, and Şirvancı S (2023). Testicular apoptosis and proliferation in relation to body mass index and m-TESE success in nonobstructive azoospermic men. *Andrologia*, 2023: 4720387. DOI: <https://www.doi.org/10.1155/2023/4720387>
- Leenen R, Roodenburg AJC, Tijburg LBM, and Wiseman SA (2000). A single dose of tea with or without milk increases plasma antioxidant activity in humans. *European Journal Clinical Nutrition*, 54: 87-92. DOI: <https://www.doi.org/10.1038/sj.ejcn.1600900>
- Loghmani-Khozani H, Sabzi Fini O, and Safari J (2007). Essential oil composition of *Rosa damascena* mill cultivated in central Iran. *Scientia Iranica*, 14(4): 316-319. Available at: <http://scientiairanica.sharif.edu/article>
- Momeni HR and Eskandari N (2012). Effect of vitamin E on sperm parameters and DNA integrity in sodium arsenite-treated rats. *Iranian Journal of Reproductive Medicine*, 10(3): 249. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4165969/>
- Paul M and Frazier L (2000). *Reproductive disorders*. Occupational health, 4th Edition. Lippincot Williams & Wilkns., pp. 590-593.

- Raji P, Seyed Alipour B, and Hajizadeh Moghadam A (2019). Effect of garlic extract and n-acetylcysteine against fenvalerate-induced oxidative stress in the serum and testis tissue of rat. *Journal of Gorgan University of Medical Sciences*, 21(2): 32-42. Available at: <http://goums.ac.ir/journal/article-1-3355-en.html>
- Salimnejad R, Jalali M, Nikravesh M, and Fazel A (2014). Effect of garlic aqueous extract on markers of oxidative stress in diabetic rats testes. *Journal of Rafsanjan University of Medical Sciences*, 13(4): 371-382. Available at: <http://journal.rums.ac.ir/article-1-2037-en.html>
- Salkin R, Haider AN, Akhter F, Billah M, Islam ME, and Islam KMD (2022). Lipid oxidation in pathophysiology of atherosclerosis: Current understanding and therapeutic strategies. *International Journal of Cardiology Cardiovascular Risk and Prevention*, 14: 3-16. Available at: <http://journal.rums.ac.ir/article-1-2037-en.html>
- Sarkar M, Chaudhuri GR, Chattopadhyay A, and Biswas NM (2003). Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. *Asian Journal of Andrology*, 5(1): 27-32. DOI: <https://www.doi.org/10.1186/1477-7827-4-9>
- Shahriari S, Yasa N, Mohammadirad A, Khorasani R, and Abdollahi M (2007). *In vitro* antioxidant potential of Rosa damascene extract from guilan, Iran comparable to α -tocopherol. *International Journal of Pharmacology*, 3: 187-190. DOI: <https://www.doi.org/10.3923/ijp.2007.187.190>
- Shalit M, Guterman I, Volpin H, Bar E, Tamari T, Menda N, Adam Z, Zamir D, Vainstein A, Weiss D et al. (2003). Volatile ester formation in roses. Identification of an acetyl-coenzyme A. Geraniol/citronellol acetyltransferase in developing rose petals. *Plant Physiology*, 131(4): 1868-1876. DOI: <https://www.doi.org/10.1104/pp.102.018572>
- Shariati M, Mokhtari M, and Asgari H (2009). The effects of trifluralin on LH, FSH, and testosterone levels and testis histological changes in adult rats. *Medical Sciences*, 19(2): 73-80. Available at: <http://tmuj.iutmu.ac.ir/article-1-193-en.html>
- Shi H, Shi X, and Liu KJ (2004). Oxidative mechanism of arsenic toxicity and carcinogenesis. *Molecular and Cellular Biochemistry*, 255(1): 67-78. DOI: <https://www.doi.org/10.1023/b:mcbi.0000007262.26044.e8>
- Wang JY, Lee YJ, Chou MC, Chang R, Chiu CH, Liang YJ, and Shin Wu L (2015). Astaxanthin protects steroidogenesis from hydrogen peroxide-induced oxidative stress in mouse leydig cells. *Marine Drugs*, 13(3): 1375-1388. DOI: <https://www.doi.org/10.3390/md13031375>
- Wang TC, Jan KY, Wang AS, and Gurr JR (2007). Trivalent arsenicals induce lipid peroxidation, protein carbonylation, and oxidative DNA damage in human urothelial cells. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 615(1-2): 75-86. DOI: <https://www.doi.org/10.1016/j.mrfmmm.2006.10.003>
- Yamakado M, Toda A, Tani M, and Ishizaka N (2014). Relationship between serum uric acid and serum oxidative stress markers in the Japanese general population. *Nephron Clinical Practice*, 128(1-2): 49-56. DOI: <https://www.doi.org/10.1159/000362456>
- Yousef MI, Awad TI, and Mohamed EH (2006). Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. *Toxicology*, 227(3): 240-247. DOI: <https://www.doi.org/10.1016/j.tox.2006.08.008>
- Zubair M, Ahmad M, Saleemi Mk, Gul ST, Ahmad N, and Umar S (2016). Protective effect of vitamin E on sodium arsenite-induced toxicity, testicular measurements and histopathological studies of testes in teddy goat bucks. *Andrologia*, 49(7): e12699. DOI: <https://www.doi.org/10.1111/and.12699>