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Effects of gallic acid on rat testopathy following morphine administration: an experimental study

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ABSTRACT

Introduction: Morphine (MOR) as a psychoactive agent in opium family causes free radicals accumulation which leads to failure in spermatogenesis. Gallic acid (GA), a polyphenolic acid, is found in various plants with antioxidant, anti-fungal, anti-viral, and anti-allergic activities. The purpose of this study was to evaluate the effects of GA against MOR-induced damage to the reproductive parameter of rats.
Methods: Sixty-four male Wistar rats (8 weeks, 220-250 g) were categorized into 8 groups by random (n=8/each); normal control and MOR control groups; GA groups (5, 10, 20 mg/kg) and MOR + GA groups (5, 10, 20 mg/kg). Treatments were administered intraperitoneally (i.p), daily for 4 weeks. The sperm parameters, spermatogenesis index (SI), total antioxidant capacity, testosterone level, and seminiferous tube diameter (STD) were assessed.
Results: All sperm parameters reduced significantly in MOR control group than to the normal control group ($P < 0.01$). All parameters were significantly improved in GA and GA + MOR treatment groups compared to the MOR control group ($P < 0.01$).
Conclusion: MOR caused a detrimental effect on male reproductive parameters. Also, no significant modifications were observed in all doses of GA treatments in comparison with the normal control group. GA compensates the toxic effect of MOR on reproductive parameters. Hence, GA administration is beneficial in MOR users.

Implication for health policy/practice/research/medical education:
Gallic acid can significantly reduce the morphine related damages in male reproductive parameters through antioxidant properties. Hence, its consumption is recommended in morphine consumers.

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Introduction

Male infertility is a complex disorder which affects human society. The fertility is a continuous process which any disruption in each stage leads to failure reproduction (1). Free radicals, by attacking to unsaturated fatty acids and launching of alkylation in the structure of the protein and other cellular macromolecules, result in cell membrane lipid peroxidation, change of enzyme activity, cellular damage, and finally the necrosis (2). The production of reactive oxygen species (ROS) can arrest cell cycle and increase the rate of apoptosis process, which in turn reduce daily sperm production and the total number of sperms (3-5).

Consumption of opium subsets in young people has been increased in the last decade. Opioids have oxidative properties and increase apoptosis in various cells through their free radical activities (6). Morphine (MOR, C16H19NO3) is an opioid drug with the analgesic feature. MOR is the main psychoactive substance in the opium family with high potential of addiction, tolerance and psychological dependence (7). MOR in long-term use leads to hypogonadism, hormonal imbalance, and histological changes in male germ cells (8). In a direct effect, MOR has detrimental impacts on spermatogenesis by inhibition of sperm or testicular function, and also impairs the hypothalamic-pituitary testicular axis by an indirect manner, causing male infertility (9). MOR increases the rate of apoptosis in various cells, including

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neurons, glial cells, hepatocytes, immune and epithelial cells (10). This material increases the level of free radicals by pro-oxidant activity. Lipid peroxidation increases the rate of anti-oxidant enzyme deformation. This process leads to accumulation of ROS. ROS has harmful effects on cell membrane and DNA (11). However, the cellular defense system is not fully capable of preventing free radical damage, especially under acute conditions. Thus, the use of antioxidant agents helps to reduce the damage and prevent the disease (12). Trihydroxybenzoic acid, or gallic acid (GA), is a phenolic acid with a molar mass of 170.12 g/mol. This compound is found in the form of red crystals and exists in various plants including oak, tea, *Rhus* (sumac), *Vitis* (grape) seeds and apples (13). The salts and esters of GA are known as Gallate. GA has an important place in traditional medicine due to the antioxidant, antifungal and antiviral properties. The biochemical group of ester available in GA prevents cellular damage by a reduction in cellular oxidative stress (14). GA (3,4,5-trihydroxy benzoic acid) also is a polyphenolic compound found in grape juice and green tea containing free acids, esters, catechin derivatives and hydrolyzable tannins (15). In addition, this compound has free radical scavenging activity. A study by Punithavathi et al has shown that GA has antihyperglycemic, antioxidant, peroxidative and anti-lipid properties in streptozotocin-induced diabetic Wistar rats (16). Therefore, the goal of this study was to evaluate the protective effects of GA on male reproductive dysfunction of rats induced by MOR administration. This preliminary study is the first investigation in protective effect of GA on male reproductive parameters induced by MOR in rats.

Materials and Methods

Animals

Sixty-four male Wistar rats (200-250 g, 8 weeks) were purchased from the Pasteur Institute (Tehran, Iran) and transferred to the animal house in medical school. The animals were kept under standard conditions including 12:12 hours light/dark cycle and 22 ± 2°C, in special cages and on a straw bed. Water and food (plate and treated municipal water) were freely available to all animals.

Study groups and treatment of animals

Sixty–four male Wistar rats were randomly divided into 8 groups (8 rats). The first group (normal control group) received normal saline equivalent to the amount of experimental groups. Animals in the second group (MOR control group) were administered by injection as follow; 10 mg/kg once daily in the first day, on the days of 2–28, the MOR doses increased to 20 mg/kg/d at 9.45 AM. Third to fifth groups (GA administration groups) in which each animal received 5, 10 and 20 mg/kg of GA daily (On days 1–28) for 4 weeks at 10 AM, respectively. In sixth to eighth groups (MOR + GA administration groups) each animal was treated with MOR in order to induce reproductive parameters damage at 9.45 AM, then they received 5, 10 and 20 mg/kg of GA i.p daily (On days 1–28) at 10 AM, respectively (7,13). All experimental treatments were applied i.p.

Animals dissection and sampling

After 28 days of treatment all rats were anesthetized by intraperitoneal injection of ketamine HCl (100 mg/kg) and xylazine (10 mg/kg). Blood was taken from the heart without thoracotomy. The samples were kept in a 37°C incubator for 20 minutes and then centrifuged at 255 g in 15 minutes. The blood serum was isolated and part of the serum was kept at -70°C for measuring total antioxidant capacity, nitric oxide, and testosterone levels. The tail epididymis was isolated and placed in DMEMF12/FBS 5% culture medium. The testicles were removed from the abdominal cavity and fixed in a 10% formalin solution (7).

Sperm cells collection

The caudal part of the epididymis was used for sperm cell parameters assessments and the left testis was applied for histological evaluations. Both cauda epididymis was crushed and conserved in a warmed petri dish (37°C) containing 10 ml Hank's balanced salt solution. After 15 minutes, the cauda was removed and the suspension was slightly shaken to be normalized and observed by a light microscope (400×) (10).

Sperm viability

The eosin staining was used to identify the living sperm from the dead cells based on the absorption of stain by dead cells and their disposals by the membrane of living cells. At the end of the given time, about 20 µL of the medium containing semen fluid was collected from each dish, and mixed with an equal volume (about 20 µL) of eosin stain solution. 2-5 minutes later, a part of the mixture was poured onto a neobar slide culture. The living sperm cells lacked color and dead sperm cells became pink. The prepared slide culture was examined with a magnification of 40×. At least 100 sperm cells were calculated from each random sample from the 10 fields of imagining and the percentage of live sperm cell was documented (8).

Sperm progressive motility

Four degrees of sperm motility were calculated according to WHO guidelines (2010), class A: progressive motility. The progressive motility of the sperm cells was examined by an optical microscope with a magnification of 40 in 10 fields of view. For this purpose, 50 µL of the semen liquid culture medium was prepared and placed on a slide culture cleaned with alcohol. Then, the slide culture was placed there and examined by the microscope. Sperm cell counting was performed through a cell count device (100 sperm cells were counted in each sample). The count was repeated in all experimental and control groups (12).

Sperm count

To analyze the sperm number, 400 µL of the sperm suspension was diluted through formaldehyde fixative (Sigma; USA). Approximately, 15 µL of which was placed on a hemocytometer which was located into a Petri dish with dampened filter paper and allowed to stand for 10 minutes. The stable sperms were counted and assessed per 250 small squares of the hemocytometer using a ×40 objective. The amount of sperm per mm^3 equated the number of sperm counted (10).

Sperm cells morphology

This parameter was assessed through sperm smears extracted from the right cauda epididymis. An aliquot of the sample was used to make the smears to appraise the malformations in the spermatozoa. Eosin/nigrosine stain was used to guesstimate the normal spermatozoa morphology. A drop of eosin was added to the suspension and was mixed slightly. The slides were then observed underneath a light microscope at 400× magnification. A total of 400 spermatozoa was studied on the respective slide (4000 cells in each group) for assessment of the head and tail irregularities (11).

Seminiferous tube diameter (STD) measurement

After testes fixation, the routine tissue processing was applied including dehydration, clearing and embedding. 5 µm sections were ready and hematoxylin and eosin staining was applied. Of each block, more than 30 segments were organized. A Motic camera and software (Moticam 2000; Spain) were hired for STD measurement (7).

The ferric reducing ability of plasma (FRAP) method

This method was hired to measure serum total antioxidant capacity. In this technique, the ability of the plasma to reinstate ferric ions was measured. This process requires a large amount of FeIII. A blue stain was formed when the FeIII-TPTZ in acidic pH is changed to the FeII and absorption at the maximum wavelength of 600 nm. Total antioxidant capacity values were strategized using the standard curve with diverse concentrations of iron sulfate (12).

Testosterone measurement

The collected blood was centrifuged (5000 g) in 23°C for 15 min. The serum samples were then kept in a freezer (-18°C). The serum testosterone level was examined through ELISA (Abcam 108666, USA) technique (9).

Spermatogenesis index (SI)

Via Johnson's score, the testis tubules were evaluated according to the SI value. Based on the previous score, the grades of 1 to 10 (no cell to complete spermatogenesis) were given to each cross-section tubule (12).

Statistical analysis

The Kolmogorov–Smirnov test was conducted to confirm data compliance of the normal distribution. One-way analysis of variance (ANOVA) and Tukey post hoc test were used for statistical analysis and determination of the differences. Statistical Package for the Social Sciences 16 (SPSS Inc., Chicago, IL) was used for data analysis, and the results were expressed as mean ± standard error, and $P < 0.05$ was considered as significant.

Results

Sperm viability, progressive motility, count and normal morphology

MOR caused a significant reduction in viability, progressive motility, count and normal morphology compared to the normal control group ($P < 0.01$). No significant variations were detected in GA groups compared to the normal control group ($P > 0.05$). Also, these parameters in whole GA and MOR + GA groups increased significantly compared to the MOR control group $P < 0.01$) (Table 1).

Seminiferous tube diameter

A significant reduction was seen in STD by MOR consumption compared to the normal control group ($P < 0.01$). No significant alterations were observed in

Table 1. Effect of gallic acid and morphine on sperm parameters in male rats

Groups	Mean of sperm count (10^6)	Sperm progressive motility (%)	Sperm viability (%)	Normal sperm morphology (%)
Normal control	85.37±1.06	19.6±1.32	75.53±1.16	81.37±2.46
MOR control	31.16±4.05*	1.87±1.40*	40.83±3.05*	36.53±3.1*
GA 5 mg/kg	85.75±2.43†	21.12±1.21†	76.62±2.09†	81.75±4.09†
GA 10 mg/kg	86.12±5.07†	20.87±1.74†	76.55±5.04†	81.37±4.54†
GA 20 mg/kg	85.25±4.07†	20.50±0.67†	75.05±1.07†	82.52±2.19†
GA + MOR 5 mg/kg	49.50±2.50¶	7.12±1.33¶	54.35±5.08¶	55.12±1.49†
GA + MOR 10 mg/kg	51.36±3.17¶	8.87±1.51¶	56.37±2.09¶	57.12±4.02†
GA + MOR 20 mg/kg	55.25±4.23¶	8.75±1.10¶	57.21±3.51¶	59.87±4.55†

Data are presented as mean ± SEM. n = 8 for each group. * $P < 0.01$ compared to the normal control group. † $P < 0.01$ compared to MOR control group. ¶ $P < 0.01$ compared to the MOR control group. MOR: morphine, GA: gallic acid.

GA groups compared to the normal control group ($P > 0.05$). STD in all GA and MOR + GA groups enhanced significantly compared to the MOR control group ($P < 0.001$) (Figure 1). The histological changes of seminiferous tubes are shown in Figure 2.

Total antioxidant capacity

Serum level of total antioxidant capacity was reduced significantly in the MOR control group compared to normal control group ($P < 0.001$). A significant increase

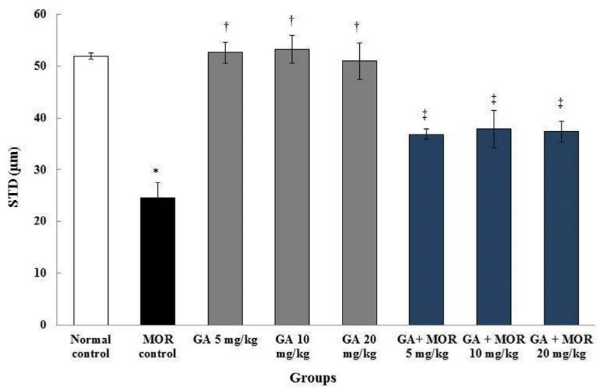

Figure 1. Comparison of seminiferous tube diameter in various groups. *Significant decrease in the MOR control group compared to the normal control group ($P < 0.01$). †Significant in all GA groups compared to the MOR control group ($P < 0.01$). ‡ Significant in all GA + MOR groups compared to the MOR control group ($P < 0.01$). MOR: morphine, GA: gallic acid, SDT: seminiferous tube diameter.

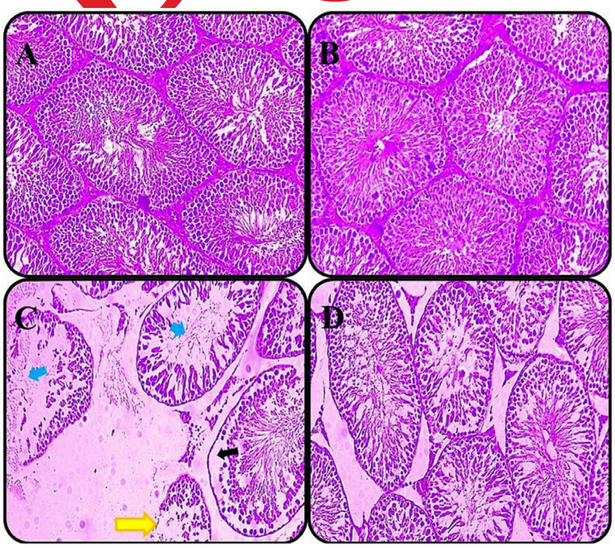

Figure 2. Effects of morphine and gallic acid on germinal layer of seminiferous tubules.
Normal ST structure was observed in the control group (A) and GA group (B). A decrease in germinal layer thickness and sperm cells in inside ST was observed in the MOR control group (C). Normal ST structure was observed in GA + MOR group (D). The black arrow identifies germinal layer (reduction in epithelial height and irregularities in the structure of the margin of ST), yellow arrow identifies destruction of the membrane ST structure and blue arrows identify sperm cells. MOR: morphine, GA: gallic acid, ST: seminiferous tubules.

caused by GA consumption was observed in total antioxidant capacity levels in treated rats of whole doses compared to the MOR control groups ($P < 0.01$). The total antioxidant capacity level was improved significantly in all MOR + GA groups compared to the MOR control group ($P < 0.001$) (Figure 3).

Testosterone

MOR caused a significant decrease in testosterone hormone level compared to normal control group ($P < 0.01$). No significant alterations were detected in GA groups compared to the normal control group ($P > 0.05$). Furthermore, the testosterone hormone level in all GA and MOR + GA groups improved significantly compared to the MOR control group ($P < 0.01$) (Figure 4).

Spermatogenesis index

MOR caused a significant decrease in the SI compared to the normal control group ($P < 0.01$). No significant changes were observed in all GA groups compared to the normal control group ($P > 0.05$). Moreover, SI in all GA and MOR + GA groups showed a significant increase compared to MOR control group ($P < 0.01$) (Figure 5).

Discussion

Present study showed that the values of viability, motility, count and normal morphology of sperms in MOR control group reduced significantly compared to normal control group. Also, it decreased the levels of total serum antioxidant capacity. MOR induced oxidative stress in testicular tissue. It was demonstrated as growth in the levels of ROS and lipid peroxidation and a reduction in antioxidant enzymes activity like total antioxidant capacity (17). In a study MOR-induced hepatocarcinogenesis in male rats and reduced serum levels of total antioxidant capacity significantly, which confirms the results of the

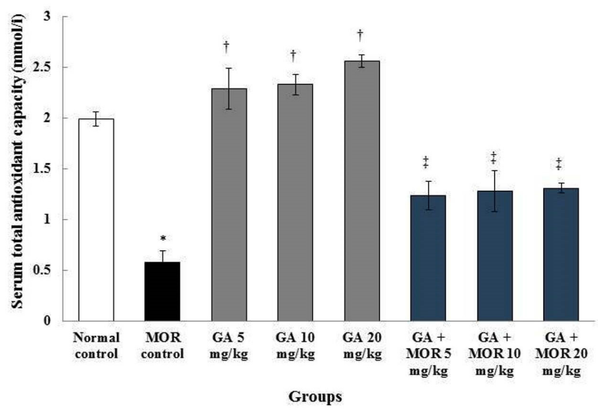

Figure 3. Comparison of total antioxidant capacity in treatment groups. *Significant decrease in the MOR control group compared to the normal control group ($P < 0.01$). †Significant increase in all GA groups compared to the MOR control group ($P < 0.01$). ‡Significant increase in all GA + MOR groups compared to MOR control group ($P < 0.01$). MOR: morphine, GA: gallic acid.

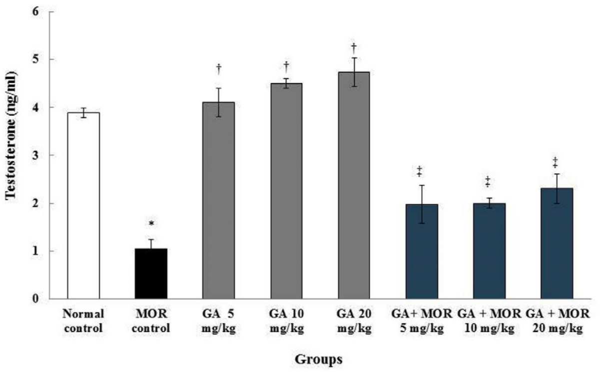

Figure 4. Comparison of testosterone hormone level in treatment groups. *Significant decrease in the MOR control group compared to the normal control group ($P < 0.01$). †Significant increase in all GA groups compared to MOR control group ($P < 0.01$). ‡Significant increase in all GA + MOR groups compared to MOR control group ($P < 0.01$). MOR: morphine, GA: gallic acid.

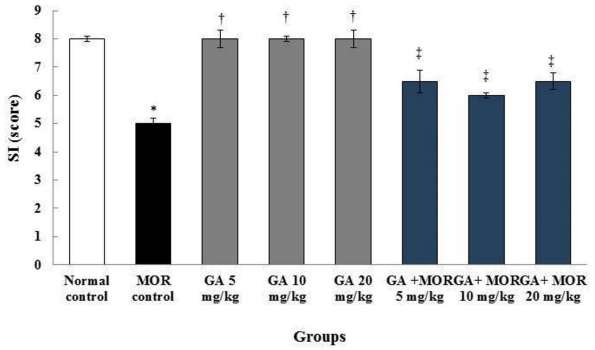

Figure 5. Comparison of SI in treatment groups.
*Significant decrease in the MOR control group compared to normal control group ($P < 0.01$). †Significant increase in all GA groups compared to MOR control group ($P < 0.001$). ‡Significant increase in all GA + MOR groups compared to MOR control group ($P < 0.01$). MOR: morphine, GA: gallic acid, SI: spermatogenesis index.

present study (18). It seems that the ROS affects the DNA and RNA synthesis and inhibits the mitochondrial function in sperms (19). It is also possible that the oxidative stress in germ cells also acts in the same way and disrupt the cellular divisions and differentiation. In this way, a number of spermatogonia available on the basement membrane may be affected.

Also, the number of primary and secondary spermatocytes, spermatids and adult sperms reduced significantly (20). Oxidative stress can disrupt spermatogenesis in which the defective gametes with remodeled chromatin will be formed. These are susceptible to the free radicals in the case of reduction in the number of spermatogonia, spermatocytes, spermatids, and spermatozoa (21). In agreement with the results of the present study, Khan et al showed that arsenic-induced oxidative stress in male rats, imposed an increase in ROS production, deformity of sperm and reduction in motility, number of sperms, and testosterone level (22). The first consequence of the ROS attack to the structure of membrane is occurrence of cell peroxidation within the cellular and organelles membrane. Since a large amount of sperm cytoplasm will be lost following the spermatogenesis, it is reported to be more sensitive to the presence of ROS than somatic cells due to the lack of antioxidant systems (23). It seems that high levels of ROS can reduce the spermatozoal motility because of the effect on Ca++ channels and reduction in spermatozoa ATP reserves (24). Also, the reduction in glutathione levels can reduce the spermatozoal motility (25). It seems that the plasma membrane of sperms is susceptible to oxidative damage due to the presence of large amounts of unsaturated fatty acids. This phenomenon can result in reduced motility and viability of sperms (7). Salahshoor et al showed that the oxidative stress in male rats caused by nicotine consumption induced ROS, sperm deformity, DNA fragmentation, reduced fertility index, motility, number of sperm and level of testosterone (26). Administration of MOR in male rats caused a significant reduction in sperm motility index and survival rate compared to control group, which confirms the results of the present study (10). In addition, the results of present study showed that GA or MOR + GA administration significantly increased the viability, motility, count and normal morphology of sperm cells compared to the MOR control group. GA is a methylxanthine derivative that can increase sperm motility by enhancement of intracellular calcium concentration and membrane penetration of cAMP analogs to inhibit phosphodiesterase (27). Due to low amount of cytoplasmic enzymes, no potential is found for regeneration of oxidative damage. Hence, antioxidants and antioxidant enzymes are highly necessary for the semen fluid to protect against oxidative damage (8). According to the studies by de Oliveira et al GA administration reveals the renal and hepatic anti-oxidative effects on biochemical and histological contents in diabetic mice (28). It has been shown that a number of GA derivatives has antioxidant effects to prevent neurodegenerative detrimental changes through free radicals and liposomes scavenging and by anti-apoptosis property in human SH-SY5-Y cell line (29). GA similarly exerts its anti-inflammatory activity by suppressing p65-NF-Kβ and IL-6/p-STAT3 pathways (30). Also, GA has anti-inflammatory properties, which seems to reduce the level of LPO and prevent damage to cells (31). It was shown that the proximity of sperm with GA induced motility in immobile sperm (32). Also, in the line of our study, Punithavathi et al found the per-oxidative and antioxidant effects of GA on streptozotocin-induced diabetic Wistar rats (16). Another study suggests that GA by initiation the pathway of receptor-alpha co-activator alpha has a crucial role in the Adenosine 3′,5′-monophosphate-activated protein kinase cellular pathway and regulation of mitochondrial function (33). However, we found that the serum level of total antioxidant capacity was improved significantly in the GA and GA + MOR groups compared to the MOR control group. The reduction in total antioxidant capacity level in this study shows the effects of oxidative stress of MOR on reproductive parameters. The present study showed that the STD and testosterone levels in the MOR control group were reduced significantly compared to the normal control group. In the GA and MOR + GA groups, a significant increment was observed in the normal morphology of sperm cells, the STD and testosterone level compared to the MOR control group. MOR can induce an increased level of oxidative stress, DNA damage, peroxidation lipid, as well as formation of additional protein compounds by producing ROS such as superoxide and hydrogen peroxide (8). An increased state of differentiation was reported in seminiferous tubules in the MOR control group in which the increased diameter of the tubules is probable (23). In addition, increasing state of ROS with increasing lipid peroxidation leads to induction of tubules' atrophy and apoptosis of the germ cells (9). It seems that a significant relationship is found among the production of oxygen species in sperms and disruption in morphology of sperms. The increased amount of free radicals results in destruction of surrounding cells, damage to Sertoli cells, the collapse of cytoplasmic bridges in which a reduction in the number of sperms and a decrease in sperm motility are probable (10). MOR administration causes a reduction in testosterone level due to the presence of oxidative stress (7). The study of Oyagbemi et al consistent with the present study showed that the administration of GA increased the STD and testosterone level significantly (32). It seems that, along with antioxidant properties, the vasodilatation and increased blood supply of GA can be represented as factors in increasing the production of testosterone in the present study (16). Moreover, the results of this survey showed that the MOR decreases the SI value. Similarly, a significant increase was detected in SI in all GA and GA + MOR groups compared to the MOR control group. Thus, the SI shifted from level 8 (few spermatozoa) to 5.5 (no spermatozoa and many spermatocytes) during the treatment by MOR, and also administration of GA increased the spermatozoa. Our results are supported by the findings of Roshankhah et al which showed that administration of crocin might increase SI in diabetic rats (12). ROS can affect the DNA and RNA synthesis in sperm cells (8). Therefore, it was assumed that GA raises the count and SI in the treated groups through improving the anti-oxidant protection of the body (34). A limitation of this study was the lack of information on the exact mechanism about the action of the protective effect of GA. Therefore, future studies are warranted to follow it.

Conclusion

The outcomes of this study demonstrated that MOR could generate defects in some male reproductive parameters and that GA has an antioxidant and defensive effect. GA was able to elevate quality of spermatozoa and improve normal morphology, SI, sperm viability, germinal layer seminiferous tubules height, TAC, motility, and sperm count. GA can be a valuable agent for treatment of infertile men and for enhancement of male fertility. The antioxidant properties of GA could be the main reason for its optimistic outcome on reproductive parameters. Supplementary studies are essential to explain its careful mechanism of action.

Authors' contribution

CJ conceived the research idea. MRS designed the work. SR wrote the first draft of the manuscript. AA and MRS carried out the literature search. CJ and SR carried out the statistical analysis. All authors read and approved final manuscript.

Conflict of interests

All authors declare no conflict of interest.

Ethical considerations

The protocol for this study was confirmed by Ethical Committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1397.500) and the authors of this manuscript observed ethical issues. Animals were handled according to the International Guidelines for Care and Handling of Experimental Animals.

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References

1. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, et al. European Association of Urology guidelines on Male Infertility: the 2012 update. Eur Urol. 2012;62(2):324-32. doi: 10.1016/j.eururo.2012.04.048.
2. Tremellen K. Oxidative stress and male infertility--a clinical perspective. Hum Reprod Update. 2008;14(3):243-58. doi: 10.1093/humupd/dmn004.
3. Agarwal A, Roychoudhury S, Sharma R, Gupta S, Majzoub A, Sabanegh E. Diagnostic application of oxidation-reduction potential assay for measurement of oxidative stress: clinical utility in male factor infertility. Reprod Biomed Online. 2017;34(1):48-57. doi: 10.1016/j.rbmo.2016.10.008.
4. Rostaei M, Fallah S, Lorigooini Z, Surki AA. The effect of organic manure and chemical fertilizer on essential oil, chemical compositions and antioxidant activity of dill (*Anethum graveolens*) in sole and intercropped with soybean (*Glycine max*). J Clean Prod. 2018;199:18-26.
5. Fallah S, Rostaei M, Lorigooini Z, Surki AA. Chemical compositions of essential oil and antioxidant activity of dragonhead (*Dracocephalum moldavica*) in sole crop and dragonhead-soybean (*Glycine max*) intercropping system under organic manure and chemical fertilizers. Ind Crops Prod. 2018;115:158-65.
6. Seiri L, Mokri A, Dezhakam H, Noroozi A. Using tincture of opium for treatment of opiate abusers in Iran.

Drug Alcohol Depend. 2014;140:e200. doi: 10.1016/j.drugalcdep.2014.02.558.

7. Jalili C, Ahmadi S, Roshankhah S, Salahshoor M. Effect of Genistein on reproductive parameter and serum nitric oxide levels in morphine-treated mice. Int J Reprod Biomed (Yazd). 2016;14(2):95-102.
8. Jalili C, Salahshoor MR, Jalili F, Kakaberaei S, Akrami A, Sohrabi M, et al. Therapeutic Effect of Resveratrol on Morphine-Induced Damage in Male Reproductive System of Mice by Reducing Nitric Oxide Serum Level. Int J Morphol. 2017;35(4):1342-7. doi: 10.4067/S0717-95022017000401342.
9. Roshankhah SH, Salahshoor MR, Aryanfar S, Jalili F, Sohrabi M, Jalili C. Effects of curcumin on sperm parameters abnormalities induced by morphine in rat. J Med Biomed Sci. 2017;6(2):1-10.
10. Salahshoor MR, Haghjoo M, Roshankhah S, Makalani F, Jalili C. Effect of thymoquinone on reproductive parameter in morphine-treated male mice. Adv Biomed Res. 2018;7:18. doi: 10.4103/abr.abr_69_17.
11. Jalili C, Sohrabi M, Jalili F, Salahshoor MR. Assessment of thymoquinone effects on apoptotic and oxidative damage induced by morphine in mice heart. Cell Mol Biol (Noisy-le-grand). 2018;64(9):33-8. doi: 10.14715/cmb/2018.64.9.5.
12. Roshankhah S, Jalili C, Salahshoor M. Effects of Crocin on Sperm Parameters and Seminiferous Tubules in Diabetic Rats. Adv Biomed Res. 2019;8(1):4.
13. Chhillar R, Dhingra D. Antidepressant-like activity of gallic acid in mice subjected to unpredictable chronic mild stress. Fundam Clin Pharmacol. 2013;27(4):409-18. doi: 10.1111/j.1472-8206.2012.01040.x.
14. Li W, Yue X, Li F. Gallic acid caused cultured mice TM4 Sertoli cells apoptosis and necrosis. Asian-Australas J Anim Sci. 2019;32(5):629-36. doi: 10.5713/ajas.18.0317.
15. Liu Z, Qi Y, Gui M, Feng C, Wang X, Lei Y. Sulfonated carbon derived from the residue obtained after recovery of essential oil from the leaves of *Cinnamomum longepaniculatum* using Brønsted acid ionic liquid, and its use in the preparation of ellagic acid and gallic acid. RSC Adv. 2019;9(9):5142-50. doi: 10.1039/C8RA08685K.
16. Punithavathi VR, Prince PS, Kumar R, Selvakumari J. Antihyperglycaemic, antilipid peroxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic Wistar rats. Eur J Pharmacol. 2011;650(1):465-71. doi: 10.1016/j.ejphar.2010.08.059.
17. Aitken RJ, Roman SD. Antioxidant systems and oxidative stress in the testes. Oxid Med Cell Longev. 2008;1(1):15-24. doi: 10.4161/oxim.1.1.6843.
18. Tuerxun H, Cui J. The dual effect of morphine on tumor development. Clin Transl Oncol. 2019;21(6):695-701. doi: 10.1007/s12094-018-1974-5.
19. Barroso G, Morshedi M, Oehninger S. Analysis of DNA fragmentation, plasma membrane translocation of phosphatidylserine and oxidative stress in human spermatozoa. Hum Reprod. 2000;15(6):1338-44. doi: 10.1093/humrep/15.6.1338.
20. Houston BJ, Nixon B, Martin JH, De Iuliis GN, Trigg NA, Bromfield EG, et al. Heat exposure induces oxidative stress and DNA damage in the male germ line. Biol Reprod. 2018;98(4):593-606. doi: 10.1093/biolre/ioy009.
21. Leisegang K, Henkel R, Agarwal A. Redox regulation of fertility in aging male and the role of antioxidants: a savior or stressor. Curr Pharm Des. 2017;23(30):4438-50. doi: 10.2174/1381612822666161019150241.
22. Khan S, Telang AG, Malik JK. Arsenic-induced oxidative stress, apoptosis and alterations in testicular steroidogenesis and spermatogenesis in wistar rats: ameliorative effect of curcumin. Wudpecker J Pharm Pharmocol. 2013;2(3):33-48.
23. Jalili C, Kamani M, Roshankhah S, Sadeghi H, Salahshoor MR. Effect of *Falcaria vulgaris* extracts on sperm parameters in diabetic rats. Andrologia. 2018;50(10):e13130. doi: 10.1111/and.13130.
24. Armstrong JS, Rajasekaran M, Chamulitrat W, Gatti P, Hellstrom WJ, Sikka SC. Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy metabolism. Free Radic Biol Med. 1999;26(7-8):869-80. doi: 10.1016/s0891-5849(98)00275-5.
25. Gomez E, Irvine DS, Aitken RJ. Evaluation of a spectrophotometric assay for the measurement of malondialdehyde and 4-hydroxyalkenals in human spermatozoa: relationships with semen quality and sperm function. Int J Androl. 1998;21(2):81-94. doi: 10.1046/j.1365-2605.1998.00106.x.
26. Salahshoor MR, Khazaei M, Jalili C, Keivan M. Crocin improves damage induced by nicotine on a number of reproductive parameters in male mice. Int J Fertil Steril. 2016;10(1):71-8.
27. Güngör Ş, İnanç ME, Ata A. Effect of gallic acid on ram semen spermatological parameters at +4°C storage. Eurasian Journal of Veterinary Sciences. 2019;35(2):87-92.
28. de Oliveira LS, Thome GR, Lopes TF, Reichert KP, de Oliveira JS, da Silva Pereira A, et al. Effects of gallic acid on delta - aminolevulinic dehydratase activity and in the biochemical, histological and oxidative stress parameters in the liver and kidney of diabetic rats. Biomed Pharmacother. 2016;84:1291-9. doi: 10.1016/j.biopha.2016.10.021.
29. Chou SF, Luo LJ, Lai JY. Gallic acid grafting effect on delivery performance and antiglaucoma efficacy of antioxidant-functionalized intracameral pilocarpine carriers. Acta Biomater. 2016;38:116-28. doi: 10.1016/j.actbio.2016.04.035.
30. Pandurangan AK, Mohebali N, Esa NM, Looi CY, Ismail S, Saadatdoust Z. Gallic acid suppresses inflammation in dextran sodium sulfate-induced colitis in mice: Possible mechanisms. Int Immunopharmacol. 2015;28(2):1034-43. doi: 10.1016/j.intimp.2015.08.019.
31. Tanaka M, Kishimoto Y, Sato A, Sasaki M, Kamiya T, Kondo K, et al. Molecular mechanisms underlying anti-inflammatory and antioxidant activities of terminalia bellirica extract and gallic acid in LPS-stimulated macrophages. Atheroscler Suppl. 2018;32:126. doi: 10.1016/j.atherosclerosissup.2018.04.388.
32. Oyagbemi AA, Omobowale TO, Saba AB, Adedara IA, Olowu ER, Akinrinde AS, et al. Gallic acid protects against cyclophosphamide-induced toxicity in testis and epididymis of rats. Andrologia. 2016;48(4):393-401. doi: 10.1111/and.12459.
33. Doan KV, Ko CM, Kinyua AW, Yang DJ, Choi YH, Oh IY, et al. Gallic acid regulates body weight and glucose homeostasis through AMPK activation. Endocrinology. 2015;156(1):157-68. doi: 10.1210/en.2014-1354.
34. Abarikwu SO, Akiri OF, Durojaiye MA, Alabi AF. Combined administration of curcumin and gallic acid inhibits gallic acid-induced suppression of steroidogenesis, sperm output, antioxidant defenses and inflammatory responsive genes. J Steroid Biochem Mol Biol. 2014;143:49-60. doi: 10.1016/j.jsbmb.2014.02.008.