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Original Article

Positive Effects of β-Alanine Supplementation on Sperm Function in Mice

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Abstract

Introduction: Male fertility disorder is attributed to oxidative stress, and β-alanine has antioxidant activity. Thus, this study aimed to determine the effect of β-alanine on semen antioxidant activity and sperm functional parameters in mice. Thus, this study aimed to determine the effect of β-alanine on semen antioxidant activity and sperm functional parameters in mice.

Methods: Eighteen male NMRI mice were allocated to 3 experimental groups. The control group had received no treatment, while mice in group 2 were exposed to morphine (2 mg/kg, i.p) for 21 days. In group 3, mice were co-exposed to morphine (2 mg/kg, i.p) and β-alanine (30 mg/kg, i.p) for 21 days. At the end of the study, the animals were sacrificed, and semen antioxidant activity as well as sperm functional parameters underwent investigation. According to the results, exposure to morphine significantly increased semen malondialdehyde (MDA) production but decreased glutathione peroxidase (GPx), superoxide dismutase (SOD), and total antioxidant status (TAS levels) compared to the control group ($P<0.05$). Likewise, β-alanine substantially decreased the adverse effect of morphine on semen MDA levels, whereas it enhanced GPx, SOD, and TAS levels ($P<0.05$). Morphine exposure sharply decreased density while increasing immotile and non-progressive motility of the semen ($P<0.05$ β-alanine markedly increased density and sperm progressive motility ($P<0.05$). The percentage of sperm with normal morphology significantly decreased following morphine exposure ($P<0.05$), while supplementation of β-alanine remarkably increased percentage of the normal sperm ($P<0.05$). During morphine exposure, the percentage of sperm with abnormal head and tail considerably increased, while β-alanine decreased this percentage ($P<0.05$).

Results: According to the results, exposure to morphine significantly increased semen malondialdehyde (MDA) production but decreased glutathione peroxidase (GPx), superoxide dismutase (SOD), and total antioxidant status (TAS levels) compared to the control group ($P<0.05$). Likewise, β-alanine substantially decreased the adverse effect of morphine on semen MDA levels, whereas it enhanced GPx, SOD, and TAS levels ($P<0.05$). Morphine exposure sharply decreased density while increasing immotile and non-progressive motility of the semen ($P<0.05$ β-alanine markedly increased density and sperm progressive motility ($P<0.05$). The percentage of sperm with normal morphology significantly decreased following morphine exposure ($P<0.05$), while supplementation of β-alanine remarkably increased percentage of the normal sperm ($P<0.05$). During morphine exposure, the percentage of sperm with abnormal head and tail considerably increased, while β-alanine decreased this percentage ($P<0.05$).

Conclusion: These results confirmed that β-alanine supplementation improves mouse sperm due to its antioxidant activity.

Keywords: β-alanine, Sperm, Mouse

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Introduction

Infertility is a significant health issue for couples, with around 30% of cases attributed to males (1). Even with advances in science and increasingly sophisticated diagnostic techniques, the etiology and pathogenesis of male infertility remain unknown in some cases, resulting in idiopathic infertility. Male fertility disorder is related to environmental factors such as exposure to certain chemicals, heavy metals, pesticides, and heat, or electromagnetic radiation (2). Moreover, reduced male fertility may be associated with smoking, alcohol misuse, chronic stress, obesity, urogenital trauma, and inflammation within the male reproductive

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system, leading to oxidative stress. Oxidative stress arises when the production of reactive oxygen species (ROS) exceeds capacity of the antioxidant system to neutralize and eliminate them, resulting in a disruption of the redox balance (3).

Additionally, an overabundance of ROS leads to a harmful reaction, causing harm to cells and tissues. It should be noted that sperm cells are highly vulnerable to the harmful impact of ROS due to their high levels of unsaturated fatty acids in the cell membrane, making them susceptible to oxidation, and the low presence of the enzyme needed to counteract ROS in the cytoplasm (4). Furthermore, lipid oxidation results in the loss of membrane integrity, heightened permeability, enzyme inactivation, DNA damage, and cellular apoptosis. Oxidative stress can adversely affect sperm quantity and quality, leading to impaired mortality and abnormal morphology (5). Recently, plant-based pharmaceutical products have been increasingly used in traditional medicine due to their positive effects (6). Plants contain numerous secondary metabolites, including flavonoids, tannins, terpenoids, alkaloids, and more, serving as a valuable resource (7). Currently, there is a growing global interest in traditional medicine, and people wish to have a greater intake of medicinal plants because of their healing attributes (8). β-alanine, found in the human central nervous system, acts as a neuromodulator and has a strong attraction to both normal and reactive astrocytes. In addition, oral β-alanine supplementation enhances exercise performance, inhibits tumor growth, and improves glucose sensitivity in Parkinson's disease patients (9). Many physiological roles have been linked to β-alanine, such as anticancer and antioxidant effects, along with improvements in muscle function and exercise performance (10). Likewise, β-alanine supplementation (300 mg/kg) decreases glutathione peroxidase (GPx) and superoxide dismutase (SOD) levels in cerebral cortex and cerebellum of rats (11).

Due to antioxidant activity of β-alanine and lack of previous reports on the effects of β-alanine on male fertility, this study aims to determine the effect of β-alanine supplementation on semen antioxidant activity as well as sperm functional parameters in mice.

Materials and Methods

Animals and Treatment

A total of 18 male NMRI mice (weighing 28–30 g and aged 8–10 weeks) were included in 3 experimental groups and kept in standard plastic cages within controlled laboratory conditions, maintaining a temperature of 22 ± 2°C and following a 12-hour light/dark cycle. Additionally, access to standard chow pellets and fresh water was provided to the mice. The control group had no any treatment, while mice of the morphine group were exposed to morphine (2mg/kg, i.p) for 21 days. Similarly, in the β-alanine + morphine group, mice were co-exposed to morphine β-alanine (30 mg/kg, i.p) and (2 mg/kg, i.p) for 21 days. The experimental procedures were followed according to the Zimmermann (12).

Semen Antioxidant Activity

At the conclusion of the experiment, mice were administered pentobarbital via intraperitoneal injection at a dosage of 40 mg/kg. An incision was made in the peritoneum of each animal to remove the testes. Semen samples were then obtained from the cauda epididymis and mixed in 10% (W/V) cold buffer (0.1 M phosphate buffer, pH: 7.4 + 150 mM KCl). Subsequently, the mixture was spun at 9,000 rpm for 20 minutes to separate a liquid layer called supernatant, which was then analyzed for SOD, malondialdehyde (MDA), GPx, and total antioxidant status (TAS) levels (13). Next, the animals were put down using CO_2 gas over a 2-hour time frame. Then semen SOD, MDA, GPx, and TAS levels were determined using commercial kits (Gelbio, Germany).

Sperm Functional Parameters

Sperm Density

Sperm concentration was evaluated using the method reported in previous research with slight modifications. The tail end of the epididymis was disrupted in Petri dishes with Modified Tyrode's medium (MT6) for 30 minutes at 36°C in order to allow sperm to be released into the medium (14). The medium consisted of 125 mM NaCl, 2.7 mM KCl, 0.5 mM $MgCl_2 \cdot 6H_2O$, 0.36 mM $NaH_2PO_4 \cdot 2H_2O$, 5.56 mM glucose, 25 mM $NaHCO_4$, 1.80 mM $CaCl_2$, 100 unit's penicillin, and 4 mg/mL bovine serum albumin. The number of cells on a haemocytometer was counted to determine sperm concentration. Additionally, the sperm cell count in two larger squares (1 mm^2) was measured, excluding the cells on the top and right boundaries, followed by determining the average value. Finally, sperm cells per mL were calculated by multiplying the average value by 10^4 (14).

Sperm Morphology

Sperm morphology was evaluated according to Monographs (15) with slight modifications. A slide was coated with a drop of sperm suspension in MT6 medium and allowed to dry in the air. Specimens were preserved in 95% ethanol and then stained with haematoxylin-eosin. Afterward, sperm morphology was assessed using bright field microscopy and categorized based on the Wyrobek and Bruce (16) criteria. Likewise, sperm cells were evaluated for normal morphology as well as abnormalities in the head, neck/mid-piece, and tail. A minimum of 200 sperm cells were observed on every slide when viewed at a magnification of 1000×.

Sperm Motility

A portion of sperm was placed on a microscope slide in MT6 medium. Sperm motility was assessed by counting the total number of progressively moving sperm as well as the non-progressively moving and immotile sperm, within a single field (15). A minimum of 100 sperm cells were tallied on every slide. Furthermore, sperm motility evaluation was conducted again using a fresh preparation

Table 1. Effect of Morphine and β-Alanine Supplementation on Sperm Antioxidant Activity and Parameters in Mice

	Antioxidant Activity		
	Control	***Morphine***	***Morphine + B-Alanine***
MDA (µg/mg protein)	6.34 ± 0.61 [c]	12.98 ± 0.65 [a]	8.45 ± 0.56 [b]
SOD (U/g/mg protein)	32.34 ± 2.12 [a]	19.45 ± 1.87 [c]	26.67 ± 2.56 [b]
GPx (U/mg protein)	18.13 ± 0.87 [a]	9.31 ± 0.71 [c]	12.63 ± 0.54 [b]
TAS (U/mg protein)	7.12 ± 0.42 [a]	3.29 ± 0.32 [b]	6.65 ± 0.11 [a]
	Sperm parameters		
	Control	***Morphine***	***Morphine + β-alanine***
Density (×10^6)	5.1 ± 0.12 [a]	2.43 ± 0.09 [b]	4.65 ± 0.11 [a]
Progressive motility (%)	41.23 ± 5.23 [a]	16.54 ± 3.98 [c]	29.56 ± 4.56 [b]
Non-progressive motility (%)	25.99 ± 2.76 [c]	43.70 ± 3.17 [a]	35.86 ± 2.54 [b]
Immotile sperm (%)	32.78 ± 2.75 [b]	39.76 ± 2.35 [a]	35.60 ± 2.67 [b]
Normal sperm (%)	85.76 ± 3.13 [a]	32.65 ± 3.76 [c]	56.76 ± 3.89 [b]
Abnormal head sperm (%)	7.32 ± 1.12 [c]	38.96 ± 5.42 [a]	22.76 ± 3.87 [b]
Abnormal tail sperm (%)	6.92 ± 1.11 [c]	28.39 ± 1.90 [a]	18.92 ± 1.65 [b]

Note. MDA: Malondialdehyde; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; TAS: Total antioxidant status; Data are shown as means ± standard errors. There are significant differences between groups with different superscripts (a, b, and c; $P<0.05$).

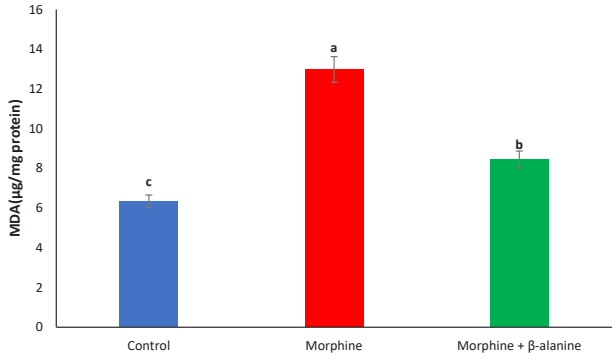


Figure 1. Effect of Morphine and β-Alanine Supplementation on Semen MDA Levels in Mice
Note. MDA: Malondialdehyde. Data are expressed as means ± standard errors. There are significant differences between groups with different superscripts (a, b, and c; $P<0.05$)

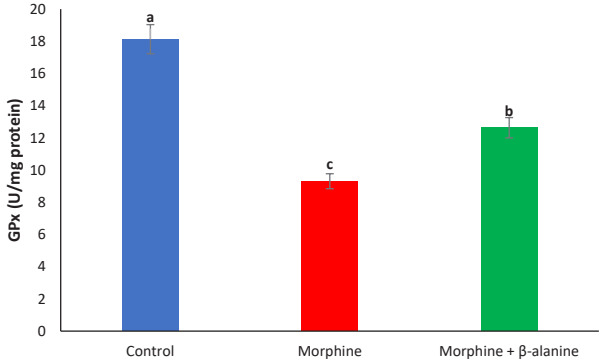


Figure 3. Effect of Morphine and β-Alanine Supplementation on Semen GPx Levels in Mice
Note. GPx: Glutathione peroxidase. Data are presented as means ± standard errors. Significant differences are observed between groups with different superscripts (a, b, and c; $P<0.05$)

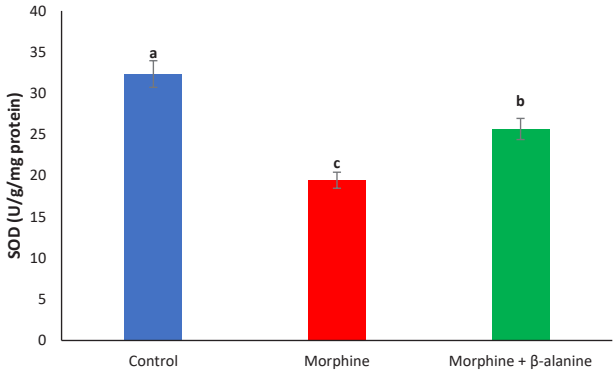


Figure 2. Effect of Morphine and β-Alanine Supplementation on Semen SOD Levels in Mice
Note. SOD: Superoxide dismutase. Data are shown as means ± standard errors. Marked differences are notable between groups with different superscripts (a, b, and c; $P<0.05$)

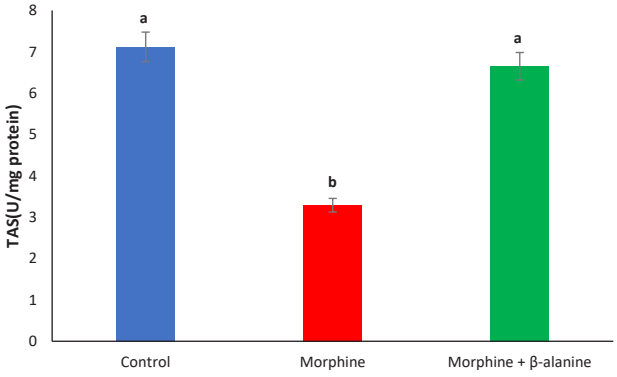


Figure 4. Effect of Morphine and β-Alanine Supplementation on Semen TAS Levels in Mice
Note. TAS: Total antioxidant status. Data are provided as means ± standard errors. The results indicate remarkable differences between groups with different superscripts (a and b; $P<0.05$)

of the identical sample. Initially, the proportion of sperm cells within each mobility category was determined, and subsequently, the mean for each category from the two evaluations of the two slides was evaluated in accordance with Monographs (15).

Statistical Analysis

The collected data underwent statistical analysis using one-way analysis of variance and were expressed as means ± standard errors (SE). Post hoc analysis using the

Tukey's honestly significant difference test was performed for treatments displaying significant differences. Differences between treatments with P values less than 0.05 were considered statistically significant.

Results

The results related to the effects of morphine, β-alanine, and their combination on semen antioxidant activity are presented in Table 1 and Figures 1–4. As shown (Figure 1), exposure to morphine significantly increased semen MDA levels ($P<0.05$), while supplementation of β-alanine decreased the adverse effect of morphine on semen MDA levels ($P<0.05$). Additionally, morphine exposure markedly decreased ($P<0.05$) semen SOD levels, whereas β-alanine supplementation could substantially increase ($P<0.05$) semen SOD levels (Figure 2). Based on the results (Figure 3), exposure to morphine considerably decreased semen GPx levels ($P<0.05$). However, β-alanine supplementation could remarkably improve semen GPx levels compared to the morphine group ($P<0.05$). According to Figure 4, morphine administration sharply decreased semen TAS levels ($P<0.05$), while supplementation of β-alanine alleviated the adverse effect of morphine on semen TAS levels compared to the morphine group ($P<0.05$).

Table 1 and Figures 5–8 show data associated with the effects of exposure to morphine and β-alanine supplementation on sperm functional parameters. Based on the findings (Figure 5), morphine exposure significantly reduced semen density ($P<0.05$), while supplementation of β-alanine markedly increased semen density ($P<0.05$). The results (Figure 6) revealed that morphine administration substantially decrease sperm progressive motility ($P<0.05$). Contrarily, β-alanine enhanced sperm progressive motility ($P<0.05$). According to Figure 7, morphine administration remarkably increased non-progressive motility of the sperm ($P<0.05$), whereas β-alanine decreased the percentage of the non-progressive motility of the sperm ($P<0.05$). The highest percentage of immotile sperm was observed following morphine administration ($P<0.05$). However, β-alanine supplementation significantly decreased the corresponding percentage ($P<0.05$). Related data are illustrated in Figure 8.

Figures 9–11 display the effects of morphine, β-alanine, and their combination on semen physiological parameters. As shown (Figure 9), the percentage of sperm with normal morphology substantially decreased following morphine exposure ($P<0.05$), while this percentage markedly increased after β-alanine supplementation ($P<0.05$). The results (Figure 10) further demonstrated that morphine exposure considerably increased the percentage of abnormal head sperm ($P<0.05$), while β-alanine decreased this percentage ($P<0.05$). Ultimately,

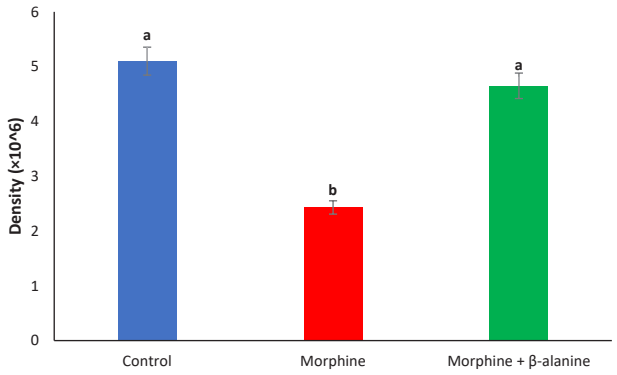


Figure 5. Effect of Morphine and β-Alanine Supplementation on Semen Density in Mice
Note. Data are displayed as means ± standard errors. There are substantial differences between groups with different superscripts (a and b; $P<0.05$)

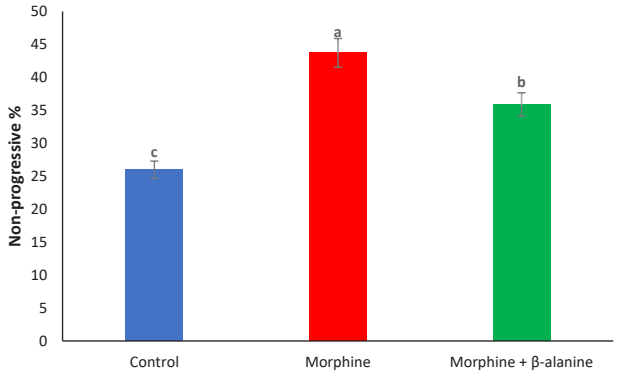


Figure 7. Effect of Morphine and β-Alanine Supplementation on Sperm Non-Progressive Motility in Mice
Note. Data are shown as means ± standard errors. There are considerable differences between groups with different superscripts (a, b, and c; $P<0.05$)

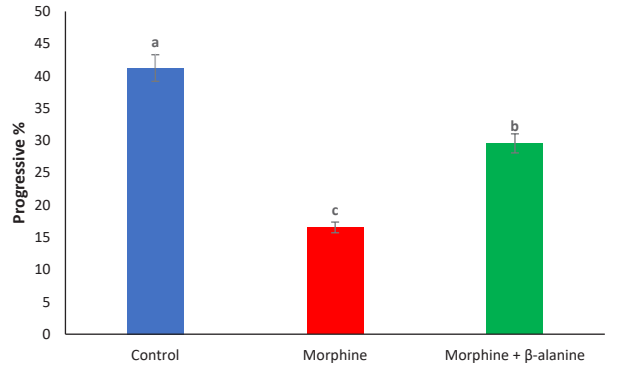


Figure 6. Effect of Morphine and β-Alanine Supplementation on Sperm Progressive Motility in Mice
Note. Data are expressed as means ± standard errors. Significant differences can be found between groups with different superscripts (a, b, and c; $P<0.05$)

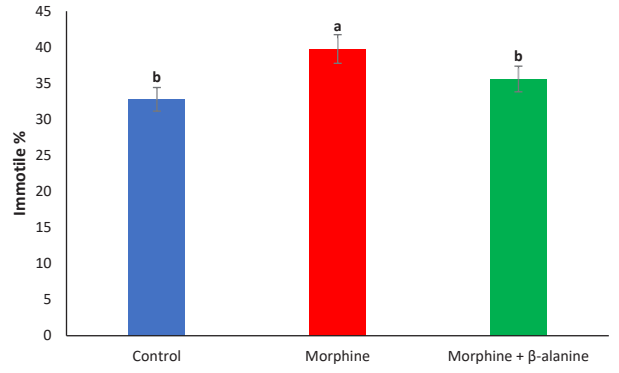


Figure 8. Effect of Morphine and β-Alanine Supplementation on Immotile Sperm in Mice
Note. Data are expressed as means ± standard errors. Remarkable differences are observed between groups with different superscripts (a and b; $P<0.05$)

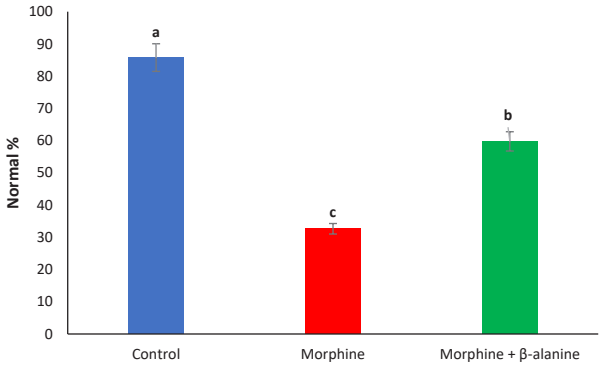


Figure 9. Effect of Morphine and β-Alanine Supplementation on Normal Sperm Morphology in Mice
Note. Data are shown as means ± standard errors. There are significant differences between groups with different superscripts (a, b, and c; $P<0.05$)

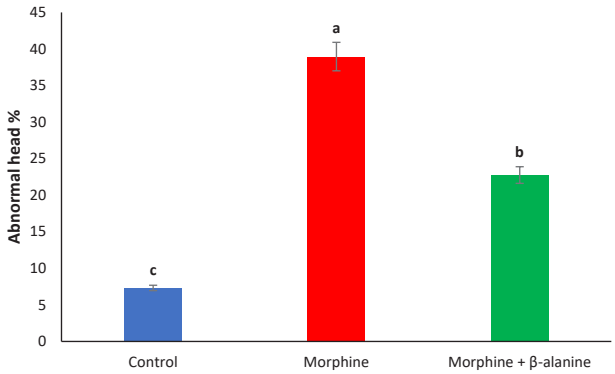


Figure 10. Effect of Morphine and β-Alanine Supplementation on Abnormal Head Sperm Morphology in Mice
Note. Data are presented as means ± standard errors. Substantial differences are found between groups with different superscripts (a, b, and c; $P<0.05$)

morphine administration substantially increased the percentage of abnormal tail sperm ($P<0.05$), whereas β-alanine decreased this percentage ($P<0.05$), the results of which are depicted in Figure 11.

Discussion

Based on the main findings of the current study, β-alanine supplementation improved semen antioxidant activity of the semen following morphine administration. Further, β-alanine supplementation increased sperm density and progressive motility while decreasing the percentage of sperm with abnormal heads and tails in mice receiving morphine. The sperm membrane contains high levels of polyunsaturated fatty acids, which are plentiful and readily susceptible to oxidation by ROS. Additionally, MDA, which is a product of lipid peroxidation, can be more detrimental to sperm than ROS because it has a longer half-life, leading to reduced motility, DNA damage, and difficulties in binding to oocytes, ultimately causing infertility (17). The findings of a prior investigation revealed that MDA is significantly more harmful to equine sperm function when seminal plasma is absent, as opposed to ROS alone (18).

β-alanine is a beta amino acid that occurs naturally and has its amino group positioned at the β-position from the carboxylate group. It is not involved in the production of key proteins or enzymes through biosynthesis. Moreover,

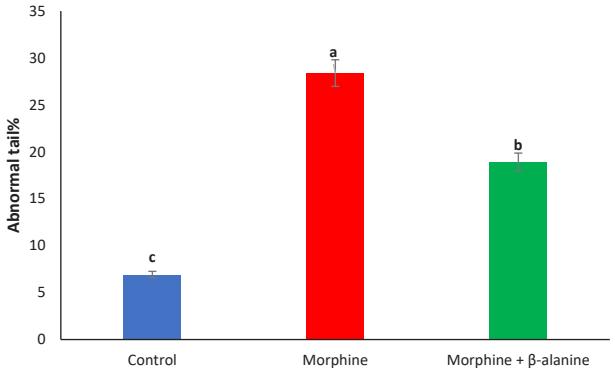


Figure 11. Effect of Morphine and β-Alanine Supplementation on Abnormal Tail Sperm Morphology in Mice
Note. Data are expressed as means ± standard errors. The results demonstrate marked differences between groups with different superscripts (a, b, and c; $P<0.05$)

β-alanine is one of the components of carnosine, alongside histidine. It was found in epididymal lumen fluids and was more concentrated in the cauda region (19). Likewise, β-alanine metabolism was found to be the most important pathway in the fluid of the cauda epididymal lumen. β-alanine is also present in goat epididymal lumen fluids (20). Regrettably, the physiological roles of β-alanine in the reproductive system have yet to be documented. It has been reported that β-alanine administration decreased lipid peroxidation and ROS levels while increasing SOD, CAT, GPx activities in myocardial ischemia and reperfusion-induced rats (21). The antioxidant potential of β-alanine against exercise-induced oxidative stress has been confirmed in women (22).

The antioxidant effect of β-alanine supplementation against exercise-induced oxidative stress has been reported in previous research (23). β-alanine injection elevated the activities of SOD and CAT but decreased MDA production in mussel tissues (24). The exact mechanisms underlying brain damage in this disorder remain poorly known; nonetheless, it is postulated that high concentrations of β-alanine produce direct and/indirect effects. Wang et al (24) demonstrated that β-alanine exhibits antioxidant effects through various methods such as metal ion binding, scavenging ROS, and peroxyl radicals. Further, β-alanine was found in epididymal lumen fluids and showed higher concentrations in the cauda area. In addition, the metabolism of β-alanine was the most important pathway in the fluid of the cauda epididymal lumen (19). Similarly, Patel et al documented the presence of beta-alanine in the fluids of goat epididymal lumen (20). Regrettably, the biological roles of β-alanine in the reproductive system are yet to be documented. Accordingly, further research is needed to clarify the role of beta-alanine in sperm development and preservation. Free carnitine is extracted from blood plasma and accumulated in the epididymal lumen in mammals, including humans. In one study, carnitine concentration within the epididymal lumen distally rose, peaking in the cauda in both humans and various animals, such as boar, ram, rat, hamster, rabbit, bull, dog, horse, and monkey (19).

Limitations of the Study

Based on limitations, we were not able to determine in carnosine levels in epididymal tissues or seminal fluids.

Conclusion

In general, our findings confirmed that β-alanine supplementation improves mouse sperm due to its antioxidant activity. It is suggested that future studies determine carnosine levels in epididymal tissues or seminal fluids. Finally, further research should determine the protective activity of β-alanine against male infertility caused by abuse morphine administration.

Authors' Contribution

Conceptualization: Shahin Hassanpour
Data Curation: Hooman Ghamari, Fatemeh Mahmoudi Ashlaghi
Formal Analysis: Shahin Hassanpour
Funding: Hooman Ghamari, Fatemeh Mahmoodi Ashlaghi
Investigation: Hooman Ghamari, Fatemeh Mahmoudi Ashlaghi
Methodology: Shahin Hassanpour
Project Administration: Shahin Hassanpour
Resource: Shahin Hassanpour
Software: Shahin Hassanpour
Supervision: Shahin Hassanpour
Validation: Shahin Hassanpour
Visualization: Shahin Hassanpour
Writing–Original Draft: Hooman Ghamari, Fatemeh Mahmoudi Ashlaghi
Writing–Review & Editing: Shahin Hassanpour

Competing Interests

None.

Ethical Approval

This study was conducted in line with the principles of the Declaration of Helsinki. Moreover, written informed consent forms were obtained from all the participants. Approval was also granted by the Ethics Committee of Department of Veterinary Basic Sciences, SR.C., Islamic Azad University, Tehran, Iran (IR.IAU.SRB.REC.1403.037).

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