Anti-nociceptive role of the β-alanine on hot plate-induced pain in mice

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Abstract

Pain is a distressing sensation that arises as a consequence of damage to bodily tissues, while β-alanine is an amino acid that is both nonessential and non-proteogenic, functioning as an inhibitory neurotransmitter. Hence, the purpose of this investigation was to ascertain the analgesic role of β-alanine in the context of pain induced by exposure to a hot plate in mice. A total of 85 male NMRI mice were employed in five separate experiments. In the initial experiment, the mice were administered saline, β-alanine at doses of 15mg/kg, 30mg/kg, and 45mg/kg, as well as morphine at a dose of 5mg/kg. In the second experiment, the mice were given saline, β-alanine at a dose of 30mg/kg once daily for a duration of 7 days, naloxone at a dose of 2mg/kg, and naloxone in combination with β-alanine at a dose of 30mg/kg. For experiments 3-5, the mice were subjected to flumazenil (5mg/kg), L-NAME (10 mg/kg), and 6-hydroxydopamine (100mg/kg) instead of naloxone. Importantly, the mice received β-alanine once daily for a period of 7 days, whereas all the antagonists were administered 30 minutes prior to the respective tests. Subsequently, the hot plate test was employed to measure pain responses, both prior to and at 10, 15, 20, 25, and 30

minutes following the injections, by recording the response latency time. At the conclusion of the experiments, blood samples were obtained and used to determine the levels of serum malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant status (TAS), and nitric oxide (NO). The findings revealed that morphine led to a significant increase in the latency time ($P<0.05$), while β-alanine also resulted in a notable increase in the latency time ($P<0.05$). Interestingly, pretreatment with β-alanine followed by naloxone resulted in a decrease in the latency time ($P<0.05$). Similarly, pretreatment with β-alanine followed by flumazenil significantly reduced the latency time on the hot plate ($P<0.05$). Furthermore, pretreatment with β-alanine followed by L-NAME led to an increase in the latency time ($P<0.05$). Administration of β-alanine resulted in a decrease in serum NO and MDA levels, and an increase in SOD, GPx, and TAS levels ($P<0.05$). Collectively, these findings suggest that the antinociceptive activity of β-alanine is mediated through GABAergic mechanisms and NO production, and possibly through its antioxidant properties, in the context of pain induced by exposure to a hot plate in mice.

Keywords: Anti-nociceptive, β-alanine, Hot plate, Mice

1. Introduction

The sensation of pain serves as a defensive mechanism that has evolved in order to safeguard tissues against actual or potential harm, thereby playing a crucial role in the survival of various animal species. Moreover, pain also manifests as a symptom in numerous diseases, serving as an indicator that there is an underlying issue within the organism (1). Nevertheless, the persistence of pain can potentially lead to the development of chronic conditions and result in alterations to both the central nervous system and peripheral tissues (2). Dysfunction in nociceptive signaling at different levels of the nervous system can be identified as the primary cause of pathological pain. Extensive documentation has confirmed that the opioidergic, GABAergic, dopaminergic, and nitrergic systems are responsible for providing relief from pain within the spinal cord (3). Despite the widespread prescription of opioids and nonsteroidal anti-inflammatory medications for pain relief, reports of side effects associated with their long-term usage have surfaced (3).

β-alanine, a nonessential and non-proteogenic amino acid, is produced as the end product of carnosine in the human body. This amino acid plays a critical role in intracellular buffering and

effectively delays the accumulation of lactic acid (4). Numerous physiological functions have been attributed to β-alanine, including anticancer and antioxidant activity, as well as the enhancement of muscle function and exercise performance (5). While the mechanisms through which β-alanine exerts its positive effects on the brain have not been fully elucidated, its structural similarities to γ-aminobutyric acid (GABA) and glycine enable it to bind to their receptors and function as an inhibitory neurotransmitter (6). Furthermore, β-alanine has been found to positively influence spatial memory, as evidenced by its ability to increase levels of this amino acid in the hippocampus following the administration of the Morris water maze test (7). In individuals diagnosed with schizophrenia, β-alanine supplementation has been shown to improve cognitive function through its antioxidant properties (5).

Supplementation with β-alanine has been found to decrease lipid peroxidation and the production of reactive oxygen species (ROS), while simultaneously enhancing levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), reduced glutathione (GSH), and catalase in rats with myocardial ischemia and reperfusion-induced injury (4). Despite an extensive review of the literature, no reports have been found regarding the anti-nociceptive properties of β-alanine. Therefore, the objective of this study is to investigate the potential anti-nociceptive role of β-alanine and its potential interaction with the opioidergic, GABAergic, dopaminergic, and nitrergic systems in the context of hot plate-induced pain.

2. Material and Methods

2.1. Animals and grouping

In this investigation, a total of 85 male NMRI mice with a weight range of 25−30 g were housed in a controlled environment with a room temperature of 23 ± 1 °C and a 12-hour light/dark cycle. The mice were kept in standard cages, with six mice in each cage. They had unrestricted access to fresh water and chow pellets. The first test involved the intraperitoneal injection of different substances into the mice. These substances included saline, β-alanine at three different doses (15 mg/kg, 30 mg/kg, and 45 mg/kg), and morphine (5 mg/kg). It should be noted that the mice received β-alanine once a day for a period of 7 days, while the morphine was injected only 30 minutes before the test. In the second test, the mice were again intraperitoneally injected with different substances. These substances included saline, β-alanine (30 mg/kg) once a day for 7 days,

naloxone (2 mg/kg), and a combination of naloxone and β-alanine (30 mg/kg). The third test followed a similar pattern, with the mice receiving different injections. These injections included saline, β-alanine (30 mg/kg) once a day for 7 days, flumazenil (5 mg/kg), and a combination of naloxone and β-alanine (30 mg/kg). In the fourth test, the mice were injected with saline, β-alanine (30 mg/kg) once a day for 7 days, L-NAME (10 mg/kg), and a combination of naloxone and β-alanine (30 mg/kg). The fifth test involved the intraperitoneal injection of saline, β-alanine (30 mg/kg) once a day for 7 days, 6-hydroxydopamine (100 mg/kg), and a combination of 6-hydroxydopamine and β-alanine (30 mg/kg). Similar to the first test, the mice received β-alanine once a day for 7 days, while all the antagonists were injected 30 minutes before the test.

2.2. Hot Plate Test

The thermal noxious stimuli anti-nociceptive activity of β-alanine was evaluated using Harvard's hot plate, which was set at a temperature of 52 ± 2°C (8). The mice were placed on the heated surface, and the time elapsed between placement and specific responses, such as jumping, withdrawal of paw(s), or licking of the forepaws, was recorded as the response latency time. These recordings were made before the injection and at 10, 15, 20, 25, and 30 minutes after the injections. A cutoff time of 20 seconds was used to determine analgesia and inhibition of tissue damage (8) (image).

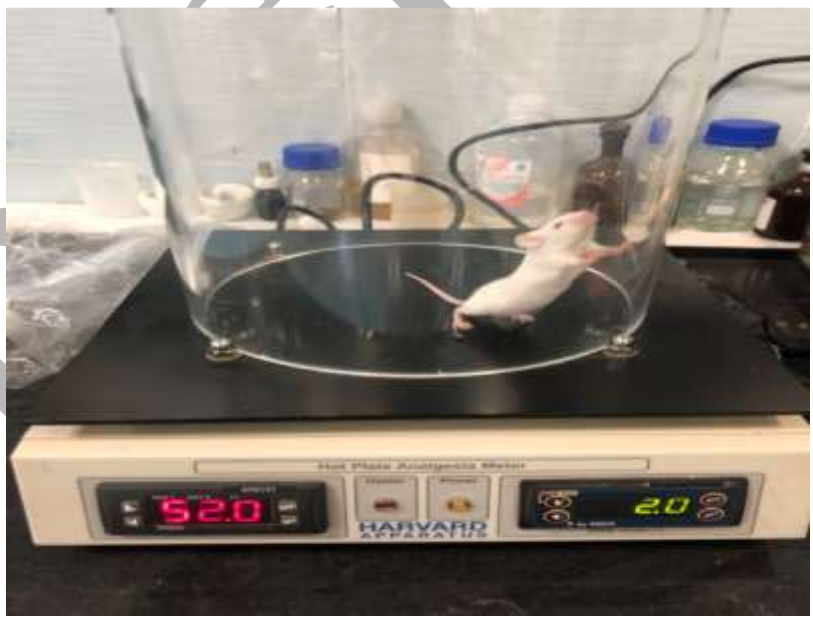

Image. The hotplate procedure

2.3. Biochemical evaluations

At the end of the tests, blood samples were collected and serum MDA, SOD, GPx, and TAS were determined using Zell Bio GmbH (Germany) assay kits. Also, the Greiss colorimetric method determined NO concentration in the blood serum. An ELISA reader measured samples' optical density (OD) at the wavelength of 540 nm (9).

2.4. Statistical Analysis

SPSS 22 was used for data analysis using a one-way analysis of variance (ANOVA) and were shown as the mean ± standard error. Tukey post hoc test was applied for the main effect by ANOVA ($P < 0.05$).

3. Results

The figures presented in the study demonstrate the anti-nociceptive properties of β-alanine as measured by the hot plate test. In figure 1, it can be observed that morphine significantly increased the latency time in the hot plate test compared to the control group ($P < 0.05$). On the other hand, β-alanine at a dosage of 15 mg/kg had no significant effect on latency time ($P > 0.05$). However, at dosages of 30 and 45 mg/kg, β-alanine significantly increased the latency time in the hot plate test compared to the control group ($P < 0.05$).

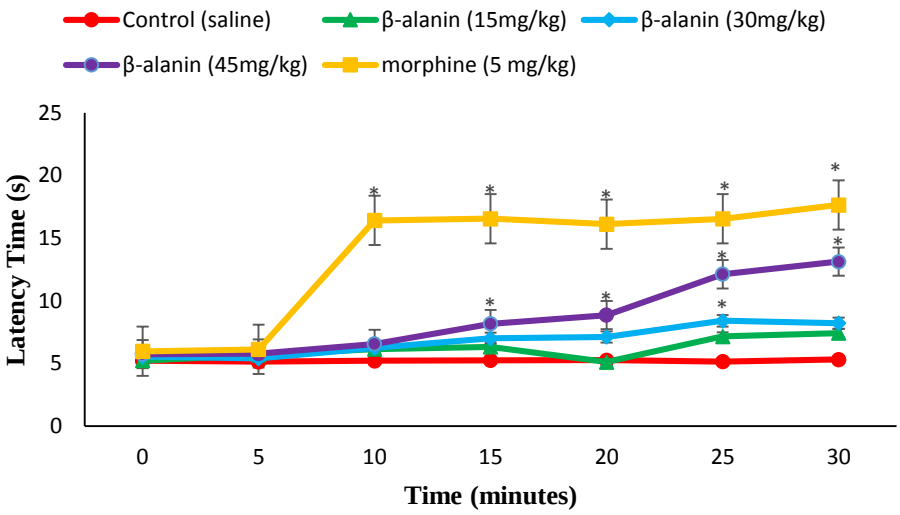

Figure 1. Effect of the β-alanine on Latency Time in the Hot Plate Test in Mice (mean ± SE, P<0.05).

Moving on to figure 2, it is evident that naloxone at a dosage of 2 mg/kg did not elicit an anti-nociceptive response in the hot plate test compared to the control group ($P > 0.05$). Conversely, β-alanine at a dosage of 30 mg/kg significantly increased the latency time in the hot plate test compared to the control group ($P < 0.05$). Moreover, when β-alanine was administered prior to naloxone (2 mg/kg), the latency time in the hot plate test was significantly decreased ($P < 0.05$).

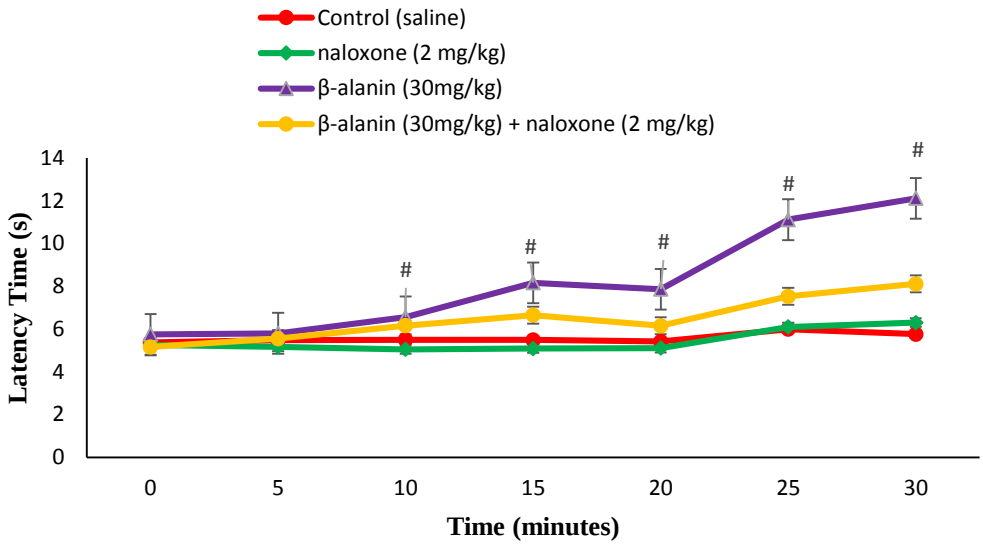

Figure 2. Effect of the β-alanine, Naloxone and their Co-Injection on Latency Time in the Hot Plate Test in Mice (mean ± SE, $P<0.05$).

Figure 3 reveals that flumazenil at a dosage of 5 mg/kg did not produce any anti-nociceptive response in the hot plate test compared to the control group ($P > 0.05$). In contrast, β-alanine at a dosage of 30 mg/kg significantly increased the latency time in the hot plate test compared to the control group ($P < 0.05$). Furthermore, when β-alanine was administered prior to flumazenil (5 mg/kg), the latency time in the hot plate test was significantly reduced ($P < 0.05$).

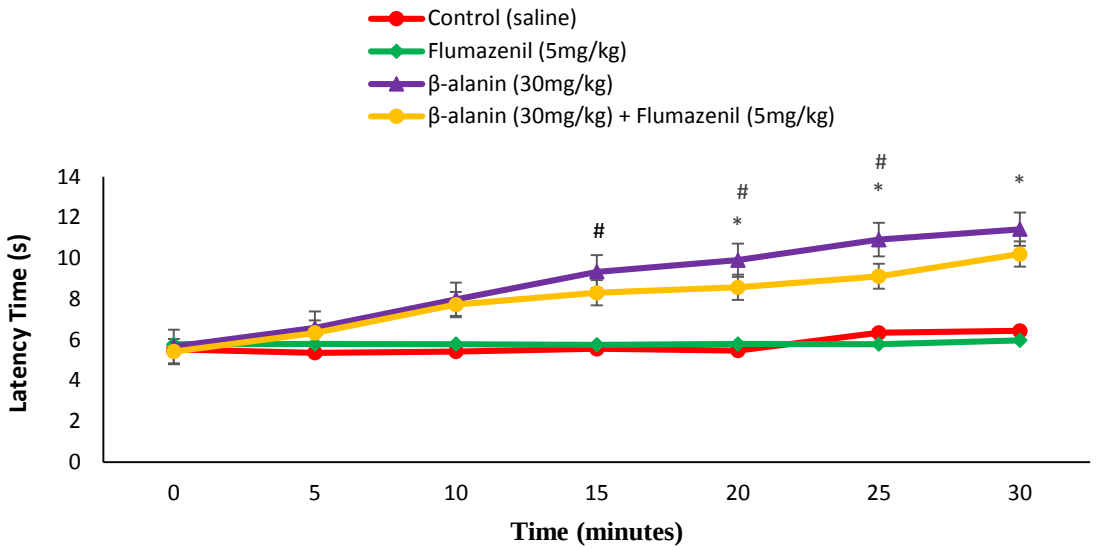

Figure 3. Effect of the β-alanine, Flumazenil and Their Co-Injection on Latency Time in the Hot Plate Test in Mice (mean ± SE, P<0.05).

Examining figure 4, it can be observed that the administration of L-NAME at a dosage of 10 mg/kg had no effect on the latency time in the hot plate test compared to the control group ($P > 0.05$). However, β-alanine at a dosage of 30 mg/kg significantly increased the latency time in the hot plate test compared to the control group ($P < 0.05$). Additionally, when β-alanine was administered prior to L-NAME (10 mg/kg), the latency time in the hot plate test was significantly increased ($P < 0.05$).

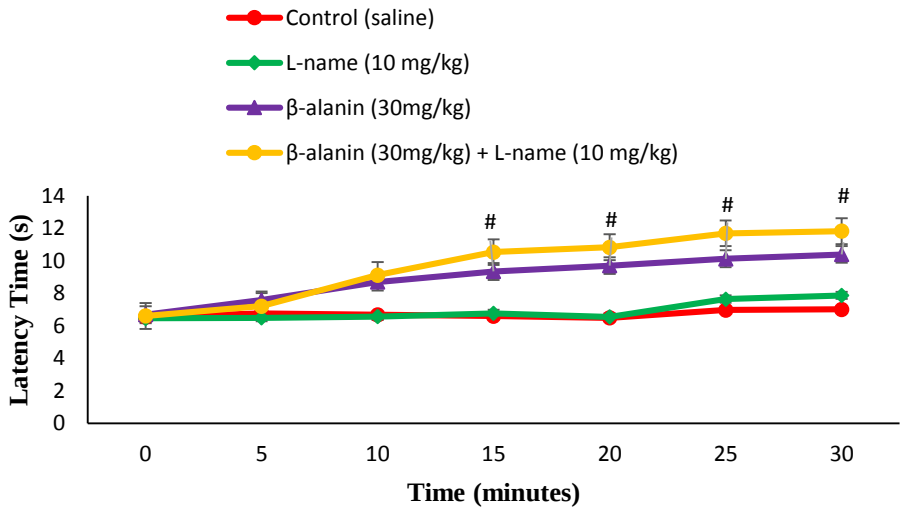

Figure 4. Effect of the β-alanine, L-NAME and Their Co-Injection on Latency Time in the Hot Plate Test in Mice (mean ± SE, P<0.05).

Figure 5 demonstrates that 6-hydroxydopamine at a dosage of 100 mg/kg did not elicit an anti-nociceptive response in the hot plate test compared to the control group (P > 0.05). Conversely, β-alanine at a dosage of 30 mg/kg significantly increased the latency time in the hot plate test compared to the control group (P < 0.05). Notably, no significant effect was observed on the latency time in mice pretreated with β-alanine (30 mg/kg) followed by 6-hydroxydopamine (P > 0.05).

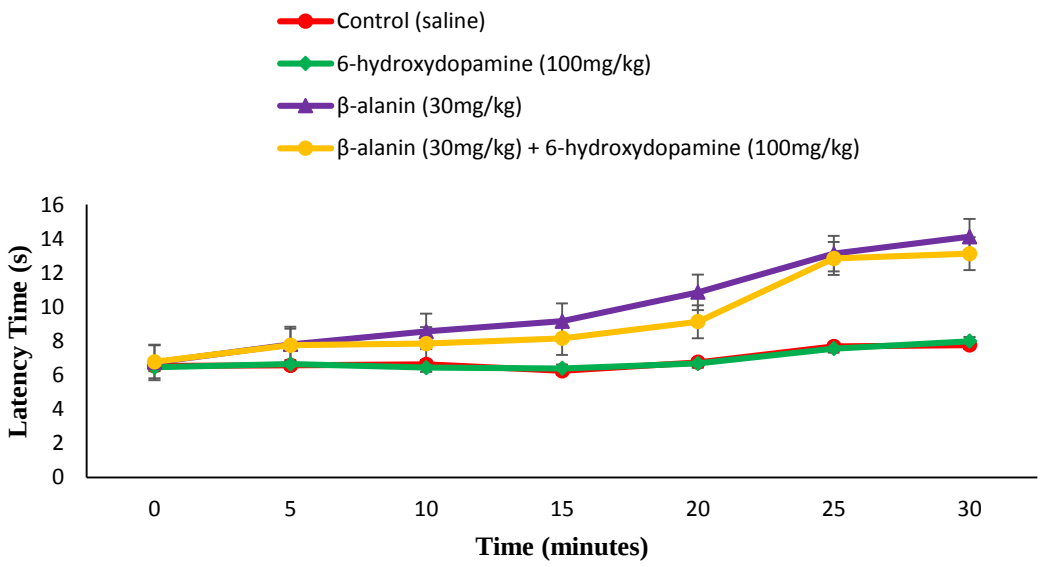

Figure 5. Effect of the β-alanine, 6-hydroxydopamine and Their Co-Injection on Latency Time in the Hot Plate Test in Mice (mean ± SE, P<0.05).

Shifting focus to the effects of β-alanine on serum biochemicals, figures 6-10 present the results. In figure 6, it can be seen that a single dose of morphine had no significant effect on serum MDA levels compared to the control group (P > 0.05). However, administration of β-alanine significantly decreased serum MDA levels compared to control mice (P < 0.05). There was no significant difference between the dosages of 30 and 45 mg/kg of β-alanine (P > 0.05).

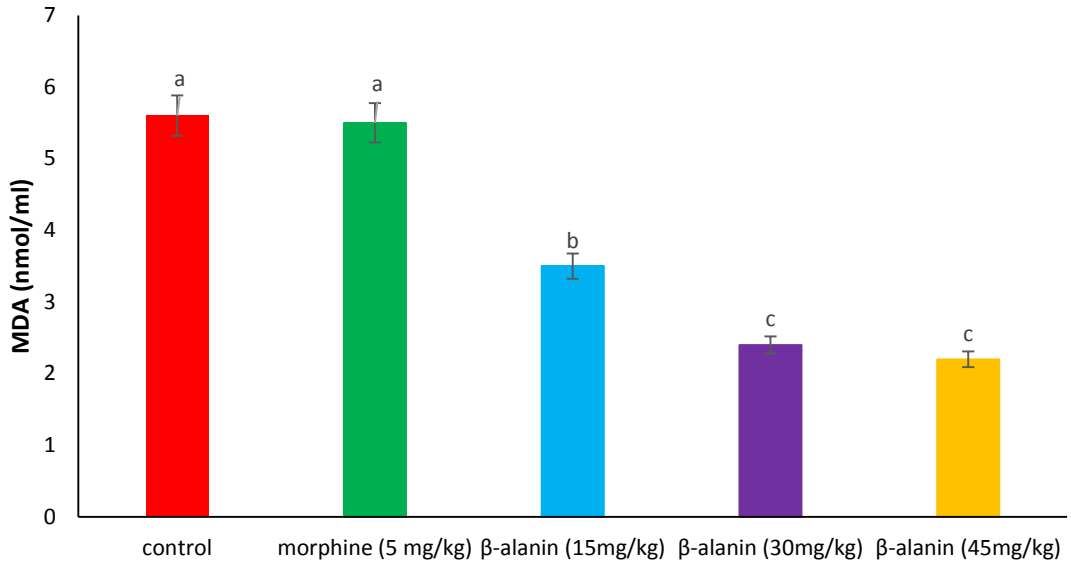

Figure 6. Effect of the β-alanine on serum malondialdehyde levels in Mice (mean ± SE, P<0.05).

Examining figure 7, it is evident that morphine had no significant effect on serum SOD levels compared to control mice (P > 0.05). Conversely, treatment with β-alanine significantly increased serum SOD levels compared to control mice (P < 0.05). There was no significant difference between the dosages of 30 and 45 mg/kg of β-alanine (P > 0.05).

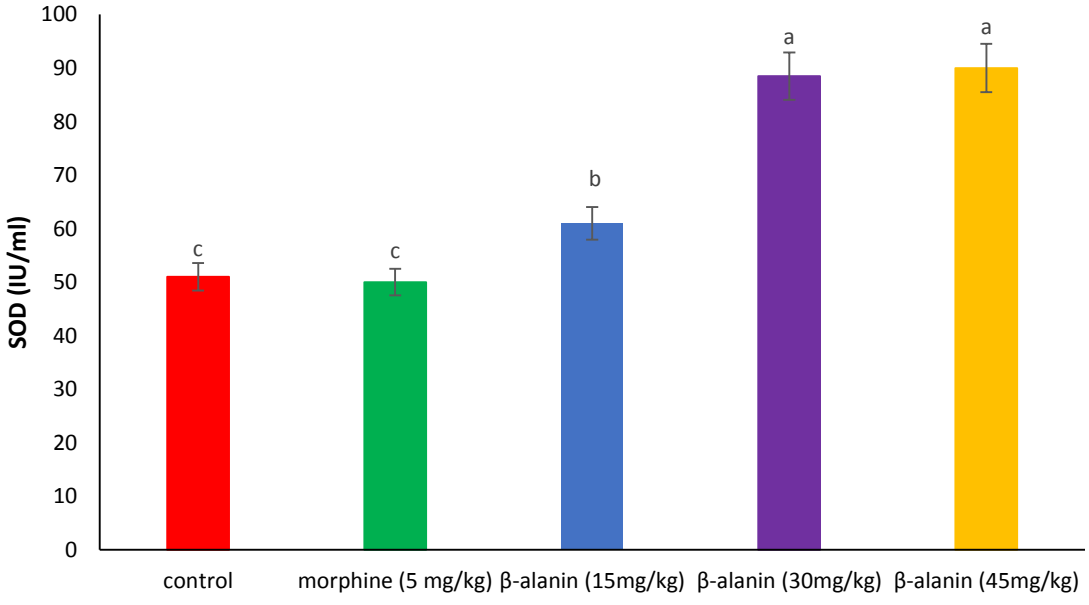

Figure 7. Effect of the β-alanine on serum superoxide dismutase levels in Mice (mean ± SE, P<0.05).

In this particular study, it was observed that the administration of morphine did not have any discernible impact on the levels of serum glutathione peroxidase (GPx) when compared to the control group of mice (P > 0.05). On the other hand, it was found that the treatment involving the use of β-alanine led to a significant increase in the levels of serum GPx in comparison to the control group (P < 0.05). Furthermore, there was no notable distinction between the levels of the 30 and 45 mg/kg doses of β-alanine (P > 0.05) (figure 8).

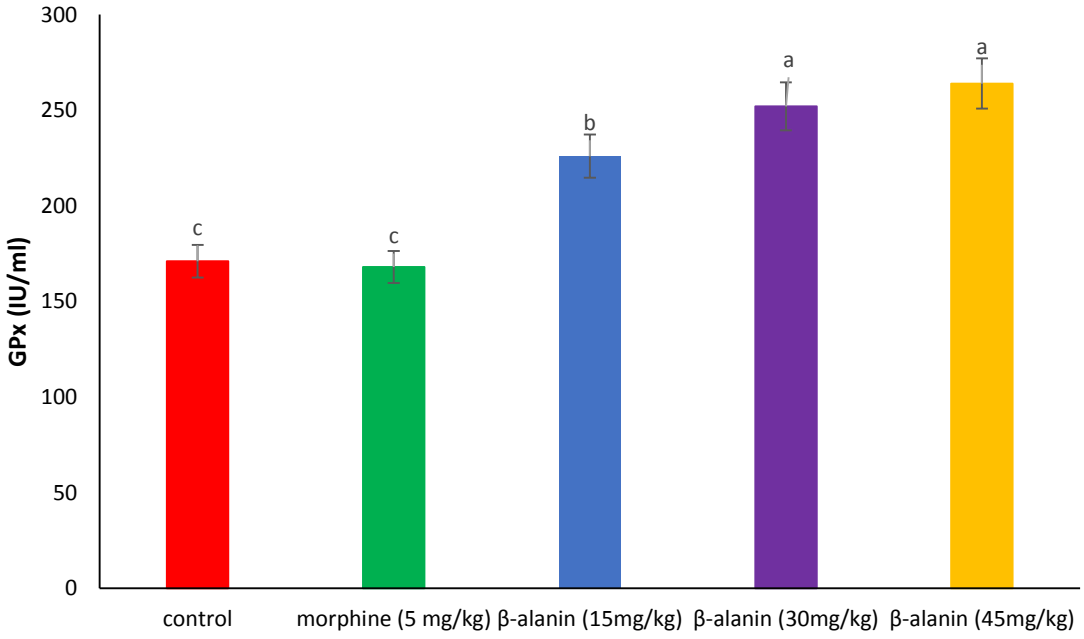

Figure 8. Effect of the β-alanine on serum glutathione peroxidase levels in Mice (mean ± SE, P<0.05).

Upon examination, it was determined that the administration of morphine did not exert any influence on the levels of total antioxidant status (TAS) in the serum, as compared to the control group of mice (P > 0.05). Conversely, it was observed that the treatment involving the use of β-alanine resulted in a significant increase in the levels of serum TAS (P < 0.05). Moreover, there was no significant distinction between the doses of 30 and 45 mg/kg of β-alanine (P > 0.05) (figure 9).

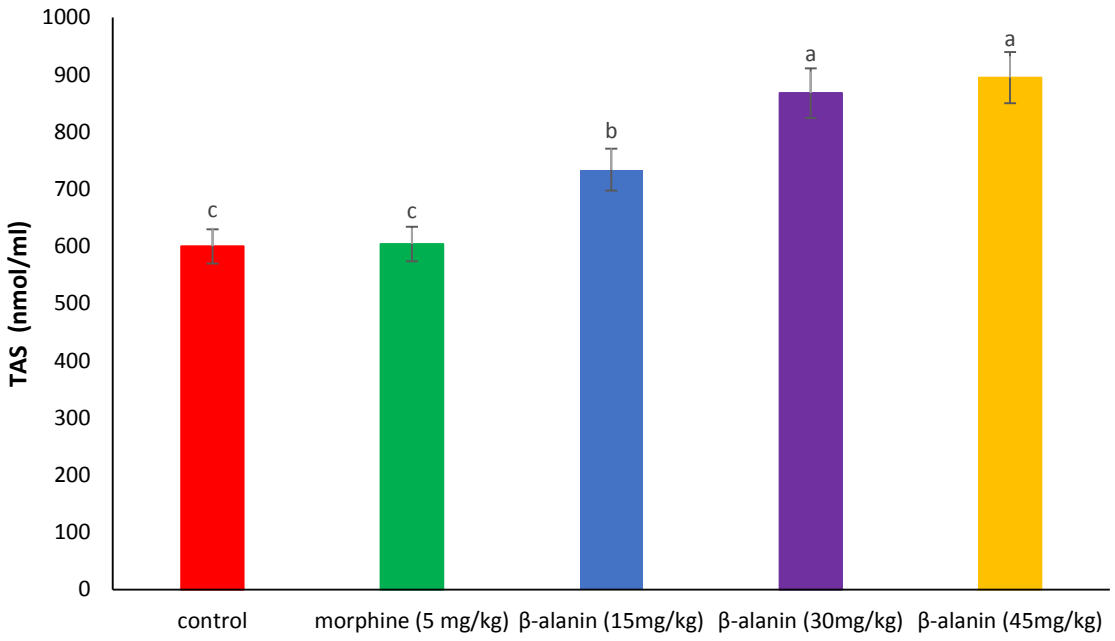

Figure 9. Effect of the β-alanine on serum total antioxidant status in Mice (mean ± SE, P<0.05).

Based on the findings illustrated in figure 10, it was observed that a single dose of morphine did not have any noticeable impact on the concentration of nitric oxide (NO) in the serum, as compared to the control group (P > 0.05). Conversely, it was found that the treatment involving the use of β-alanine led to a significant decrease in the concentration of serum NO, in comparison to the control group of mice (P < 0.05). Furthermore, there was no significant distinction between the levels of the 30 and 45 mg/kg doses of β-alanine (P > 0.05).

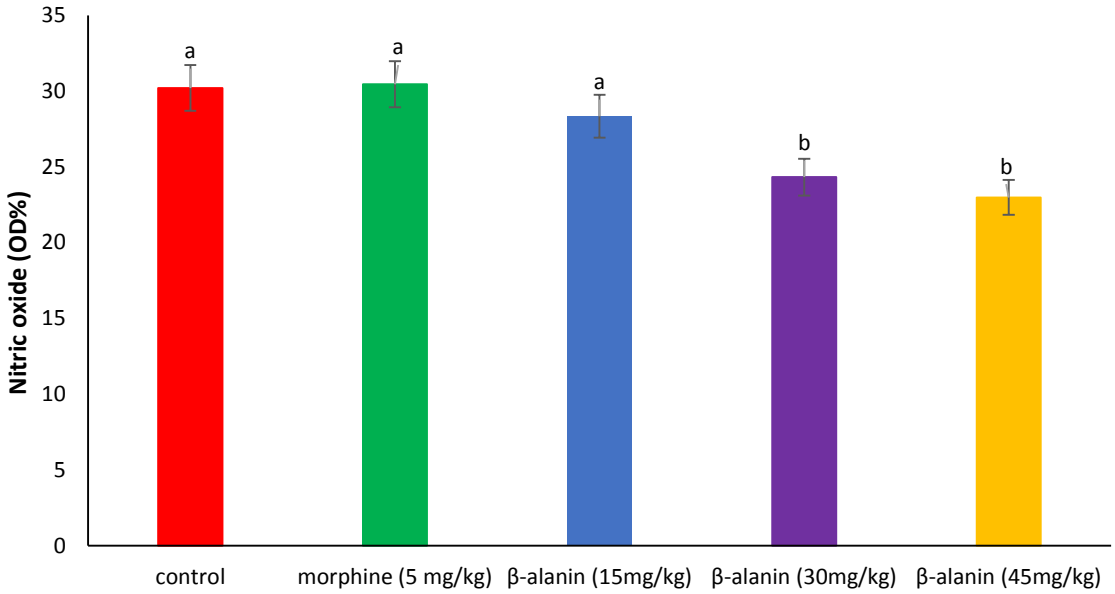

Figure 10. Effect of the β-alanine on serum nitric oxide concentration in Mice (mean ± SE, P<0.05).

4. Discussion

Based on the principal finding of the present investigation, the administration of β-alanine resulted in an increase in the duration of latency. Subsequent pretreatment with β-alanine, followed by the administration of an opioid antagonist, led to a decrease in the latency time. Furthermore, pretreatment with β-alanine, followed by a GABA antagonist, resulted in a reduction in the latency time on the hot plate. Additionally, pretreatment with β-alanine, followed by an inhibitor of NO synthesis, caused an increase in the latency time. β-alanine is classified as an endogenous β-amino acid and, although it is not utilized in the synthesis of proteins or enzymes, it does demonstrate physiological significance. β-alanine is present within the central nervous system as a neuromodulator (10). It has been reported that pretreatment with naloxone blocked the anti-nociceptive effects during the threshold period in hot plate tests (11). There exists a correlation between the synthesis of β-alanine and the GABA system facilitated by malonate semialdehyde. GABA-T exhibits comparable reactivity towards both β-alanine and GABA. Consequently, the endogenous concentration and release of β-alanine are significantly influenced by GABA-T, suggesting that this enzyme is also involved in regulating the synthesis of β-alanine within the central nervous system (12).

NO can induce peripheral hyperalgesia by modulating the expression of cyclooxygenase (13). The sub-plantar injection of formalin results in an elevation of NO levels at the site of injection, and pretreatment with L-NAME mitigates pain in mice (14, 15). β-alanine has the capability to bind to GABA and glycine receptors, as well as the N-methyl-D-aspartate (NMDA) complex. Within the central nervous system, β-alanine is localized in both neurons and glia, specifically within the brain stem and spinal cord (16). Electrical stimulation triggers the release of β-alanine, which then interacts with binding sites and inhibits neuronal excitability (17). Activation of N-methyl-D-aspartate receptors by glutamate leads to an increase in the release of NO and PGE2, subsequently enhancing glutamate levels within the dorsal horn neurons and promoting central sensitization (18). Conversely, glycine receptors suppress neuronal firing within the spinal cord, and pretreatment with cyclooxygenase inhibitors reduces pain (19). Presumably, the antinociceptive activity of β-alanine is mediated through the involvement of the NO and GABAergic systems.

As observed, the administration of β-alanine resulted in a decrease in serum levels of NO and MDA, while simultaneously increasing levels of SOD, GPx, and TAS. These findings suggest that the antinociceptive activity of β-alanine is mediated through the GABAergic and NO production pathways, and potentially through its antioxidant activity in the context of hot plate-induced pain in mice. The antioxidant properties of β-alanine have been reported in previous studies. Supplementation with β-alanine (at doses ranging from 300-1200 mg/kg) resulted in reduced serum levels of immunoglobulin G and immunoglobulin M, as well as an up-regulation of SOD and GPx expression in weaned piglets (20). Furthermore, β-alanine has been demonstrated to alleviate oxidative stress by decreasing MDA levels, increasing SOD levels, and mitigating muscle fatigue in mice (21).

Based on novel discoveries from the recent investigation, β-alanine exhibits an anti-nociceptive function and interacts with the opioidergic, GABAergic, and nitrergic systems in relation to hot plate-induced pain in mice. Furthermore, β-alanine ameliorates oxidative stress through the reduction of MDA levels and the augmentation of SOD, GPx, and TAS levels in the context of hot plate-induced pain in mice.

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Authors' Contribution

Study concept and design: S.H.

Acquisition of data: A.A.

Analysis and interpretation of data: S.H.

Drafting of the manuscript: A. A.

Critical revision of the manuscript for important intellectual content: S. H.

Statistical analysis: S.H.

Administrative, technical, and material support: S.H., A. K.

Conflict of Interests

No potential conflict of interest was reported by the authors.

Ethics

This study was approved by the Biomedical Research Ethics Committee of Islamic Azad University, Tehran, Iran (IR.IAU.SRB.REC.1402.141).

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Consent for publication

None

Informed consent

none

Author s contributions

Study concept and design: S.H

Acquisition of data: A.A

Analysis and interpretation of data: S.H

Analysis and interpretation of data: A.A. E.K

Critical revision of the manuscript for important intellectual content: S.H

Statistical analysis: S.H

Administrative, technical, and material support: A.A, S.H, E.K

Data availability

Data is available by request

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