

Possible Involvement Of L-Arginine-Nitric Oxide Pathway In The Antidepressant Activity Of Auraptene In Mice

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Research

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Abstract

Background: Depression is one of the most common mental illnesses around the world. Nitric oxide (NO) is involved in the pathophysiology of depression. Auraptene (a coumarin derivative) has been shown to have pharmacological effects on neurological diseases.

porpuse: The present study aimed to investigate the possible role of NO pathway in Auraptene antidepressant effects in male mice.

Methods: Behavioral tests were used to assess depression-like behaviors. The mice received Auraptene at 10, 30 and 100 mg/kg, the combination of the sub-effective dose of Auraptene (10 mg/kg) and L-NAME, and the combination of the effective dose of Auraptene (30 mg/kg) and L-arginine. Finally, brain and serum MDA level and antioxidant capacity, as well as hippocampus and serum NO level was measured.

Results: Auraptene (30 mg/kg) improved depression-like behaviors. Auraptene (30 mg/kg) also significantly reduced serum NO levels ($P < 0.05$) and significantly increased serum MDA (10 mg/kg, $P < 0.05$). Auraptene at 30 mg/kg also increased serum antioxidant capacity ($P < 0.01$). Co-administration of L-NAME and the sub-effective dose of Auraptene enhanced the effects of Auraptene. However, co-administration of the effective dose of Auraptene and L-arginine reduced the effects of Auraptene.

Conclusion: The results showed that Auraptene causes antidepressant effects in a dose-dependent manner and acts as a prooxidant at 100 mg/kg and exacerbates oxidative stress. The antidepressant effects of this active molecule are exerted by reducing the NO level in the hippocampus and serum, increasing the antioxidant capacity and reducing the MDA level in the serum.

Introduction

Depression is one of the most common psychiatric diseases with a prevalence of 15–25% and can cause a significant decline in patient performance in all occupational areas, and social and family relationships (Kandel et al., 2000). Decreased function of neurotransmitters such as serotonin (5-HT), norepinephrine or noradrenaline (NA) and dopamine (DA) is one of the causes of depression (Nutt, 2008).

Oxidative stress is an effective factor for various disorders of the central nervous system and can accelerate the ageing process and lead to behaviors related to depression and anxiety. During oxidative stress, the production of free radicals exceeds the capacity of the body's antioxidant defence system, including enzymatic components (such as catalase) and non-enzymatic components (such as vitamin C and vitamin E) to neutralize them and consequently, free radicals cause cell damage and death by attacking various cell components, including nucleic acids, proteins, enzymes, as well as cell membrane lipids (Pandya et al., 2013). Serum malondialdehyde (MDA) level is one of the markers of lipid peroxidation and the important indicators to evaluate oxidative stress. It has been shown that the total antioxidant capacity of serum is significantly reduced in people with major depressive disorder (Sarandol et al., 2007).

Nitric oxide (NO) is a free gas and messenger molecule that is involved in regulating the nervous and immune system. Studies have shown that NO is involved in depression and stress (van Amsterdam and Opperhuizen, 1999). There are three different genetic isoforms of nitric oxide synthase (NOS) for NO production, including neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), and induced nitric oxide synthase (iNOS). Several studies have shown the role of nNOS in the pathophysiology of depression, and antidepressants have been shown to reduce NO levels in patient's serum (Heiberg et al., 2002; Joca and Guimarães, 2006; Dhir and Kulkarni, 2007).

Pharmacotherapy for depression can be associated with unwanted side effects such as anticholinergic and arrhythmic effects, all of which increasingly intensify the need to identify active drug compounds (Sirois and Gick, 2002; Baldwin et al., 2011). Medicinal plants are a rich source of active secondary metabolites that are widely drawn the attention of researchers (Ho et al., 2007).

Auraptene (7-geranyloxycoumarin) is a coumarin derivative isolated from the skin of some citrus and is the most abundant form of natural geranyloxycoumarin. Auraptene is found in many plants of the citrus genus such as grapefruit and oranges (Curini et al., 2006) and has valuable medicinal properties including anti-cancer, antibacterial, antifungal, anti-inflammatory and antioxidant. Auraptene is also known to protect the nervous system (Abdel-Latif, 2005; Soltani et al., 2010; Genovese and Epifano, 2011; Curini et al., 2012).

Some coumarin compounds isolated from plant species exert inhibitory effects on monoamine oxidase. Monoamine oxidase A (MAO-A) selectively targets the catalysts of the neurotransmitters of serotonin and norepinephrine and is a pharmacological target in seeking out beneficial agents for the treatment of depression (Vergel et al., 2010). The evidence shows that coumarin compounds inhibit TNF- α or PGE2 secretion by affecting NF κ B nucleus transport and inhibiting the phosphorylation of P38, JNK1/2, PKC kinases in LPS-stimulated macrophages and mononuclear cell lines. Moreover, research has shown that all coumarin compounds affect NO production by reducing the expression and activity of the iNOS gene and its protein, indicating the anti-inflammatory activity of coumarin compounds, e.g., Auraptene (Stefani et al., 2012). Coumarin compounds have been shown to exhibit anti-cancer properties by downregulating the PI3K/Akt and MEK/ERK pathways and increasing P-gp expression (Guo et al., 2018).

Therefore, this study aimed to investigate the antidepressant effects of Auraptene in male mice with regards to the role of the NO pathway.

Material And Methods

Study design

80 male NMRI mice were divided into eight groups (n = 10) as follows:

1. The group received normal saline;
- 2, 3 and 4 Experimental groups received Auraptene at doses of 10, 30 and 100 mg/kg (Razavi et al., 2015);
5. The group received nitric oxide synthetase (NOS) inhibitor (L-

NAME) at 10 mg/kg (Haj-Mirzaian et al., 2016); 6. The group received L-arginine (L-arg) at 100 mg/kg 45 minutes before the test (Haj-Mirzaian et al., 2016); 7. The group received a sub-effective dose of Auraptene plus L-NAME and 8. The group received an effective dose of Auraptene plus L-arg.

Auraptene was injected acutely and simultaneously with NO mediators intraperitoneally. After injections, behavioral tests were performed and after that the brain and blood samples of mice under deep anaesthesia with chloroform, to minimize stress, was taken. Then malondialdehyde content, antioxidant capacity and NO in the brain and serum of mice were measured.

Behavioral tests

Open field test (OFT)

This test is used to evaluate the stress and emotional stability of rodents. The experimental groups were evaluated on PND 45 using an open field apparatus which is a box made of Plexiglas with dimensions of 60×50×40 cm. The floor area of this box is divided into 16 equal squares and movements of each mouse in this box was monitored for 5 minutes. This test is also used to assess anxiety based on the amount of vertical and horizontal movements and the amount of body scratching (Amiri et al., 2017).

Tail suspension test (TST)

In this method, metal bases with a height of 70 cm are used. A 50 cm string is stretched lengthwise between two metal bases. The mouse tail is closed and secured by a strap. The test was then started with strong stimulation of the completely suspended, inactive and unresponsive mouse, and then the duration of immobility was recorded. The total duration of the test was 6 minutes, 2 minutes for the animal's adaptation and 4 minutes for recording the duration of immobility (Sun, 2004).

Forced swim test (FST)

This test has been approved to investigate depression in rodents. According to the theory of helplessness of Martin Seligman, if the animal is exposed to constant stress and has no way out of it, it gradually loses hope of escaping and becomes immobile. The glass container (25×12×15 cm) was filled with 25 °C water and the animal was gently placed in the water from a height of 20 cm, the discontinuation of movements of the mouse was considered immobility. The total duration of the FST is 6 minutes, 2 minutes for the animal's adaptation and 4 minutes for measuring the immobility. Increasing the immobility duration is considered as depression and its decrease is considered as the effectiveness of antidepressant treatment (Amiri et al., 2015).

Biochemical tests

Measurement of the brain and plasma malondialdehyde levels

To measure the amount of malondialdehyde (MDA), a working solution was used, containing 0.5 g of thiobarbituric acid, 80 ml acetic acid 20% whose pH reached 5.3 by addition of sodium hydroxide (NaOH) using a pH meter and its final volume was increased to 100 ml by addition of 20% acetic acid. In the next step, 100 µl of the sample, 100 µl of 8.1% SDS, and 2.5 ml of the working solution were mixed in a glass test tube and then the tubes in boiling water for one hour. The tubes were then cooled and centrifuged at 4000 rpm. Then the optical absorbance of the supernatant was recorded at 523 nm (Kuloglu et al., 2002).

Determination of serum and brain total antioxidant capacity

The basis of the FRAP method is the ability of serum to reduce Fe + 3 ferric ions to ferrous Fe + 2 in the presence of an agent called TPTZ. In this method, the Fe + 2 reaction with the TPTZ reagent creates a blue Fe + 2-TPTZ complex with a maximum absorbance of 593 nm. The serum's reducing power was measured by increasing the concentration of the above complex using spectrophotometry (Benzie and Strain, 1999).

Determining the nitric oxide level of the hippocampus

To determine the NO level in the hippocampus, the level of nitrite, as a stable product of NO, was measured in different groups of animals after administration of different doses of Auraptene in the presence and absence of selective NOS inhibitor based on the Griess test. The basis of this reaction is the formation of the diazotization dye of a sulfonamide with the help of nitrite in an acidic medium and then its conjugation with an aromatic amine N-naphtyle ethylenediamine¹ (NEDD) (Granger et al., 1996; Haj-Mirzaian et al., 2016).

Data analysis

Statistical analysis was performed using Prism software and the results were expressed as Mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test. The results were presented as mean plus SEM. P < 0.05 was considered a significance level.

Results

The number of horizontal movements in the OFT

As illustrated in Fig. 1, co-administration of Auraptene (10 mg/kg) and L-NAME significantly increased the number of horizontal movements in the OFT in compared to the normal saline group (P < 0.05) and the group received Auraptene at 10 mg/kg (P < 0.001). However, no significant difference was observed between the groups received Auraptene and the normal group.

The number of vertical movements in the OFT

The results of one-way ANOVA and Tukey's post-hoc test (Fig. 2) showed that administration of Auraptene at 30 mg/kg significantly increased the number of vertical movements in the OFT in compared to the normal saline group (P < 0.01). Moreover, co-administration of Auraptene (10 mg/kg) and L-NAME

significantly increased this movement compared to the normal saline group ($P < 0.01$). Administration of 10 mg/kg Auraptene plus L-NAME significantly increased the number of vertical movements in compared to the group received 10 mg/kg Auraptene alone ($P \leq 0.001$). Administration of Auraptene at a dose of 30 mg/kg plus L-Arg significantly reduced the number of vertical movements in compared to the group received Auraptene alone ($P \leq 0.001$). However, administration of Auraptene at the doses of 10 and 100 mg/kg, as well as L-NAME and L-Arg alone, did not have any effect.

The amount of scratching in the OFT

The results of our study (Fig. 3) showed that although administration of Auraptene at doses of 30 and 100 mg/kg did not make any differences from the normal group; however, administration of Auraptene at a dose of 10 mg/kg significantly reduced the amount of scratching in the OFT in compared to the normal saline group ($P \leq 0.05$). Also, administration of Auraptene at a dose of 10 mg/kg plus L-NAME significantly increased the amount of this behavior is compared to the group received Auraptene alone ($P \leq 0.01$).

The duration of immobility in the TST

As illustrated in Fig. 4, Administration of Auraptene (30 mg/kg) significantly reduced the duration of immobility in the TST in compared to the normal saline group ($P < 0.01$), and administration of Auraptene at 100 mg/kg significantly increased the duration of immobility in compared to the normal saline group ($P < 0.05$). Also, administration of L-NAME (10 mg/kg) and administration of L-Arg (100 mg/kg) significantly reduced the duration of immobility in compared to the normal saline group ($P < 0.001$). Co-administration of Auraptene (30 mg/kg) and L-Arg significantly reduced the immobility in compared to the normal saline group ($P < 0.001$). Administration of Auraptene (10 mg/kg) plus L-NAME significantly reduced the duration of immobility in the TST in compared to the group received Auraptene alone ($P < 0.001$).

The duration of immobility in the FST

The results of this study (Fig. 5) showed that Administration of Auraptene at 30 mg/kg significantly reduced the duration of immobility in the FST in compared to the normal saline group ($P < 0.05$). Moreover, administration of L-NAME (10 mg/kg) significantly reduced this time compared to the normal saline group ($P < 0.01$). Co-administration of Auraptene (10 mg/kg) and L-NAME significantly reduced the duration of immobility in compared to the group received Auraptene alone ($P < 0.05$).

The serum and brain NO levels

As illustrated in Fig. 6, administration of Auraptene at 30 mg/kg significantly reduced serum NO level in compared to the normal saline group ($P \leq 0.05$), while doses of 10 and 100 mg/kg did not produce any significant effect. Injection of L-NAME at 10 mg/kg significantly reduced serum NO level in compared to

the normal saline group ($P < 0.001$). Co-administration of Auraptene (10 mg/kg) and L-NAME significantly reduced serum NO level in compared to the group received Auraptene at 10 mg/kg ($P < 0.05$).

According to the results (Fig. 7), administration of Auraptene at the doses of 10 and 100 mg/kg significantly increased brain NO level in compared to the normal saline group ($P < 0.01$ and $P < 0.001$). Besides, administration of L-Arg at 100 mg/kg significantly increased brain NO level in compared to the normal saline group ($P < 0.001$). Co-administration of Auraptene (10 mg/kg) and L-NAME significantly increased this level in compared to the normal saline group ($P < 0.001$).

The serum and brain MDA levels

The results of Tukey's post hoc test (Fig. 8) showed that administration of Auraptene at 10 mg/kg significantly increased serum MDA levels compared to the normal saline group ($P < 0.05$). Co-administration of Auraptene (10 mg/kg) and L-NAME significantly reduced serum MDA levels in compared to the normal saline group and the group received Auraptene alone ($P < 0.01$ and $P < 0.001$ respectively). Moreover, administration of Auraptene (30 mg/kg) plus L-Arg significantly reduced serum MDA levels compared to the normal saline group and the group received Auraptene alone ($P < 0.001$).

The results (Fig. 9) showed that administration of Auraptene at 10, 30 and 100 mg/kg did not make any significant effect in compared to the normal saline group. But the administration of L-Arg at 100 mg/kg significantly increased brain MDA levels compared to the normal saline group ($P < 0.01$). Co-administration of Auraptene (10 mg/kg) and L-NAME significantly reduced brain MDA levels compared to the group received Auraptene at 10 mg/kg alone ($P < 0.01$).

The serum and brain antioxidant capacity

Administration of Auraptene at 10 mg/kg significantly reduced serum antioxidant capacity compared to the normal saline group ($P < 0.001$, Fig. 10). Administration of Auraptene at 30 mg/kg significantly reduced the serum capacity of antioxidants in compared to the normal saline group ($P < 0.01$), but at the dose of 100 mg/kg, did not have any significant effect. Injection of L-NAME at 10 mg/kg significantly reduced serum antioxidant capacity compared to the normal saline group ($P < 0.05$). Administration of L-Arg at 100 mg/kg significantly reduced serum antioxidant levels compared to the normal saline group ($P < 0.05$). Co-administration of Auraptene (30 mg/kg) and L-Arg significantly increased serum antioxidant capacity compared to the normal saline group ($P < 0.001$). Co-injection of Auraptene (10 mg/kg) and L-NAME significantly increased serum antioxidant level compared to the group received Auraptene at 10 mg/kg alone ($P < 0.001$).

Figure 11 illustrates that there are no significant differences in the brain antioxidant capacity between the groups received Auraptene at doses of 10, 30 and 100 mg/kg and the normal saline group. But the administration of Auraptene (30 mg/kg) plus L-Arg significantly increased brain antioxidant capacity in compared to the normal saline group and the group received 30 mg/kg of Auraptene ($P < 0.001$ and $P < 0.05$ respectively). Besides, co-administration of Auraptene (10 mg/kg) and L-NAME significantly

increased the level of antioxidants in the brain is compared to the group received Auraptene alone ($P < 0.05$).

Discussion

This study aimed to investigate the antidepressant effect of Auraptene in male mice with regards to the NO pathway. In agreement with other studies (Sashidhara et al., 2015), our results showed that Auraptene reduced the immobility duration in the FST and TST and increased the amount of vertical movement in the open field test, which indicates the stimulant effect of this drug. Auraptene also reduced the amount of scratching in the open field test.

It can be argued that Auraptene has antidepressant action. Auraptene at 100 mg/kg increased the duration of immobility in the TST, which may indicate that antioxidants at high doses have prooxidant properties. But the effective dose of Auraptene exerted no significant effect on the amount of horizontal movement in the OFT. In the study by Sevgi et al., the role of acute and chronic stress in depression was investigated using the FST. They found that acute and chronic stress caused depression and anxiety (Sevgi et al., 2006). In one study, the neuroprotective and memory-boosting effects of Auraptene were studied in a mouse model of vascular dementia. The results showed the effectiveness of Auraptene in managing the nerve damage and memory-boosting following cerebral ischemia (Ghanbarabadi et al., 2016).

Auraptene has valuable medicinal properties including anti-cancer, antibacterial, antifungal, anti-inflammatory and antioxidant and is known as a protective agent for the nervous system (Soltani et al., 2010; Genovese and Epifano, 2011; Curini et al., 2012). We speculate that NO systems, antioxidant capacity and MDA levels of serum and brain may affect Auraptene function. The results of a study (2013) showed that, with increasing the dose of antioxidants, their antidepressant and antioxidant properties decrease, which can be attributed to the production of oxygen free radicals following an increase in the dose of antioxidant compounds from a certain limit, which leads to a decrease in antioxidant properties, and consequently a decline in the beneficial behavioral effects related to antioxidant properties. Antioxidants under certain conditions may act as prooxidants and therefore exacerbate oxidative stress (Rafieian-Kopaei et al., 2013). The results of this study are in agreement with our study.

Our study showed that NOS inhibitor (L-NAME) enhanced the effect of Auraptene on immobility, while L-arg, as an NO precursor, attenuated this effect. Therefore, the NO pathway is likely to contribute to the antidepressant effect of Auraptene.

The results of Sevgi et al. showed that acute and chronic stress-induced depression- and anxiety-like behaviors in mice and acute inhibition of NOS by L-NAME at 10 mg/kg prevented this stress-induced anxiety and depression. L-arg at 50 mg/kg also plays a role as a precursor of NO in inducing depression and anxiety (Sevgi et al., 2006). In a study on the mechanism of NO in the protective effects of naringin against depression after stroke in mice, the researchers reported that naringin treatment in mice

significantly reduced neurobehavioral changes and oxidative damage. Co-administration of naringin and attenuated L-arg, as well as the co-administration of naringin and L-NAME, enhanced its protective effect (Aggarwal et al., 2010). These results of these studies were in line with our study.

Gawali et al. (2017) reported that chronic treatment with Agmatine in FST and OFT triggered significant antidepressant behaviors. Agmatine also reduced levels of acetylcholinesterase and oxidative stress markers. They found that treatment with L-NAME had a strengthening effect, while L-arg counteracts anxiolytic, antidepressant and neuroprotective effects of Agmatine (Gawali et al., 2017). Research has shown that coumarin compounds inhibited secretion of TNF- α or PGE2 by affecting NF κ B nucleus transport and inhibiting the phosphorylation of P38, JNK1/2, PKC kinases in LPS-stimulated macrophages and mononuclear cell lines (Stefani et al., 2012).

The pattern of results obtained in our study is consistent with the above studies, and it seems that the NOS inhibitor (L-NAME) can be effective in the process of Auraptene action. Similarly, co-administration of Auraptene and L-arg reduced the amount of vertical movement in the OFT but increased the amount of immobility in the TST. Co-administration of Auraptene and L-arg in OFT and FST did not change the movements and behavior of mice. We obtained approximately contradictory results in FST and TST. The results of our study indicate that Auraptene has antidepressant properties and the discrepancy between the results in FST and TST may be due to measurement error during the study or indicate that Auraptene action in FST and TST is not related to L-arg.

In a study on the MDA levels in patients with depressive disorder and the relationship between plasma MDA levels and cognitive function, it was observed that an increase in MDA levels adversely affected the function of working, visual, auditory and verbal and short-term memory, and higher plasma MDA concentrations were associated with exacerbation of depressive symptoms (Talarowska et al., 2012). The researchers reported that major depressive disorder was associated with a decrease in antioxidant status and induction of oxidative and nitrosative pathways. They argued that injuries due to oxidative and nitrosative pathways were associated with increased MDA levels (Maes et al., 2011). Our results showed that Auraptene significantly increases serum MDA and antioxidant capacity and can thus be effective in reducing depression.

Since there is a significant relationship between Auraptene treatment and brain MDA and antioxidant capacity, it can be argued that the effect of this substance is probably due to the involvement of the NO pathway. Co-administration of Auraptene and L-NAME decreases serum MDA and increases serum and brain antioxidant capacity. Moreover, no dose of Auraptene affects brain MDA, but its co-administration with L-NAME reduces brain MDA. Therefore, the pattern of results obtained in our study is consistent with the above studies. Co-administration of Auraptene (30 mg/kg) and L-arg increases serum and brain antioxidant capacity and decreases serum MDA, while L-arg alone has the opposite effect, indicating that Auraptene at 30 mg/kg is effective in reducing depression possibly through the mechanism of increasing antioxidant capacity and reducing MDA level.

Research has shown that all coumarin compounds affect NO production by reducing the expression and activity of the iNOS gene and its protein, indicating the anti-inflammatory activity of coumarin compounds, including Auraptene (Stefani et al., 2012). Coumarin compounds have been shown to have anti-cancer properties by downregulating the PI3K/Akt and MEK/ERK pathways and increasing P-gp expression (Guo et al., 2018). Research also showed that plasma nitrite concentration was higher in depressed patients than in healthy individuals (Suzuki et al., 2001). The results of our study are consistent with the results of these studies. We also found that treatment with Auraptene at 30 mg/kg significantly reduced serum NO and therefore can be effective in reducing depression.

However, administration of Auraptene at 10 and 100 mg/kg significantly increases brain NO and at 30 mg/kg has no effect on brain NO, which may be due to a measurement error during the study, or indicate that the effect of Auraptene on the rate of depression is not related to the amount of NO in the brain or that with increasing the dose of Auraptene, its antidepressant and antioxidant properties are reduced. The cause of this phenomenon can be considered the production of oxygen free radicals following increasing the dose of antioxidant compounds from a certain limit, which leads to a decrease in the antioxidant properties of these compounds and thus reduces the beneficial behavioral effects of their antioxidant properties.

However, the mechanism of action of this drug in our study is different from the cited studies, so further studies are necessary to obtain information on all the mechanisms involved in the action of this drug.

Conclusion

The results of this study indicate that Auraptene triggers antidepressant activity in mice possibly by inhibiting the NOS pathway and reducing NO levels, increasing antioxidant capacity, and decreasing serum MDA. It should be noted that the action of this drug is not related to the amount of MDA, antioxidant capacity and NO in the mouse brain.

Abbreviations

NO, nitric oxide; MDA, malondialdehyde; L-NAME, L-Nitro arginine methyl ester; NA, noradrenaline; DA, dopamine; nNOS, neuronal nitric oxide synthase; iNOS, induced nitric oxide synthase; MAO-A, Monoamine oxidase A; OFT, Open field test; TST, Tail suspension test; FST, Forced swim test; NaOH, sodium hydroxide;

Declarations

Ethics approval and consent to participate

All procedures were carried out in accordance with the regulations of the University and the Guide for the Care and Use of Laboratory Animals of National Institutes of Health (Ethical Code: IR.SKUMS.REC.1397.70) and Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press). Full efforts were made to reduce the use of animals and to advance their welfare.

Consent for publication

All authors are agreed to publish this manuscript.

Availability of data and material

Data regarding the present study are available at Medical Plants Research Center, Shahrekord University of Medical Sciences.

Competing interests

The authors have no conflicts of interest to declare regarding the study described in this article and preparation of the article.

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Authors' contributions

ZLG and HAK contributed to the design of the study, supervised the research and manuscript editing, FM and EB helped the supervision and preparation of the manuscript. EB, FM and SHNB performed the experiments and data collection, and prepared manuscript drafting. MTM and ZLG revised the language and grammar of the manuscript. All authors have read and confirmed the final version of the manuscript for publication.

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Figures

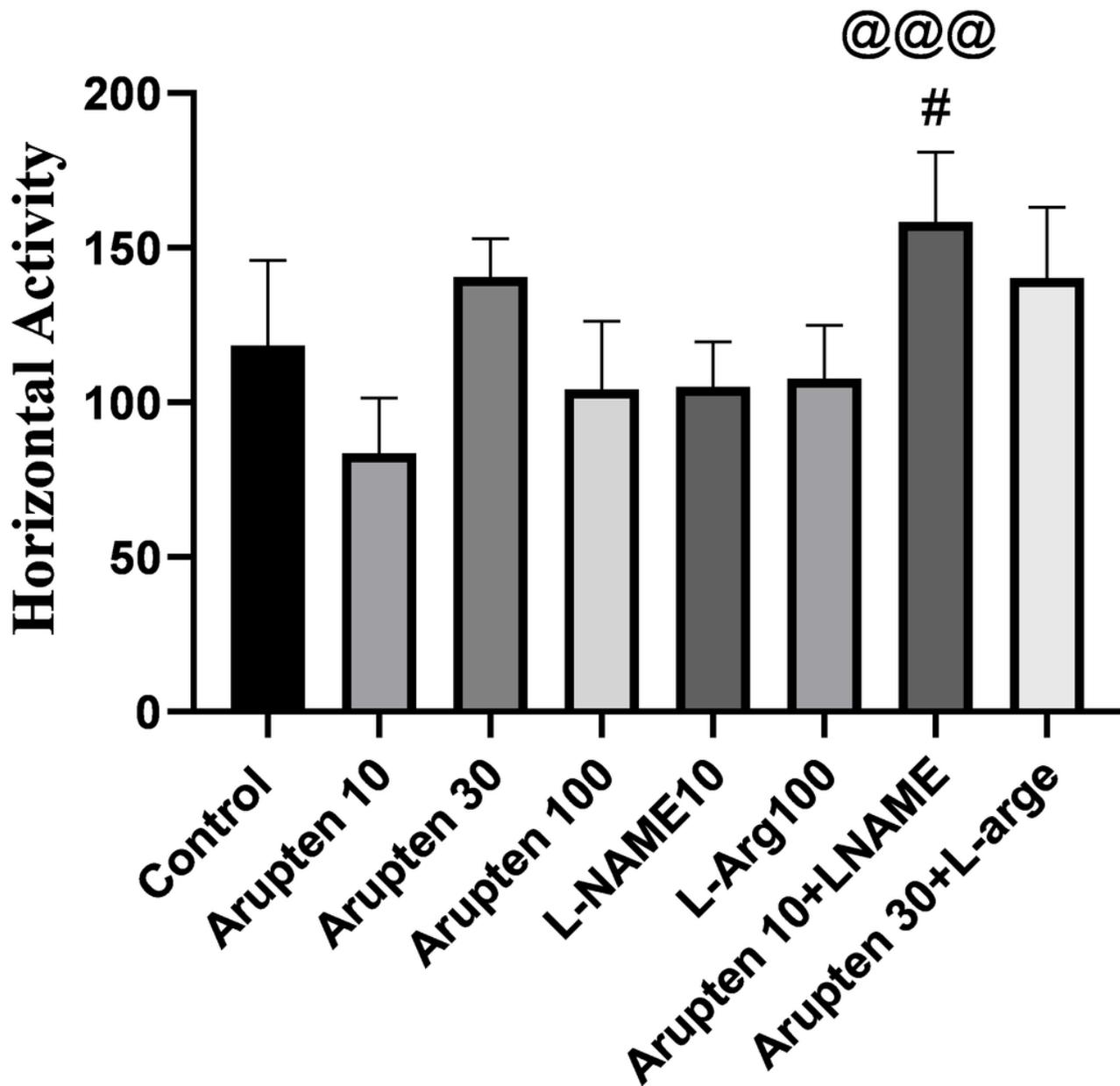


Figure 1

The amount of horizontal movements in OFT in the experimental groups. Data were reported as Mean ± SEM and analyzed using one-way ANOVA and Tukey post-hoc test. # $P \leq 0.05$ in compared to the normal group. @@@ $P \leq 0.001$ in compared to the group received Aaruptene at a dose of 10 mg/kg.

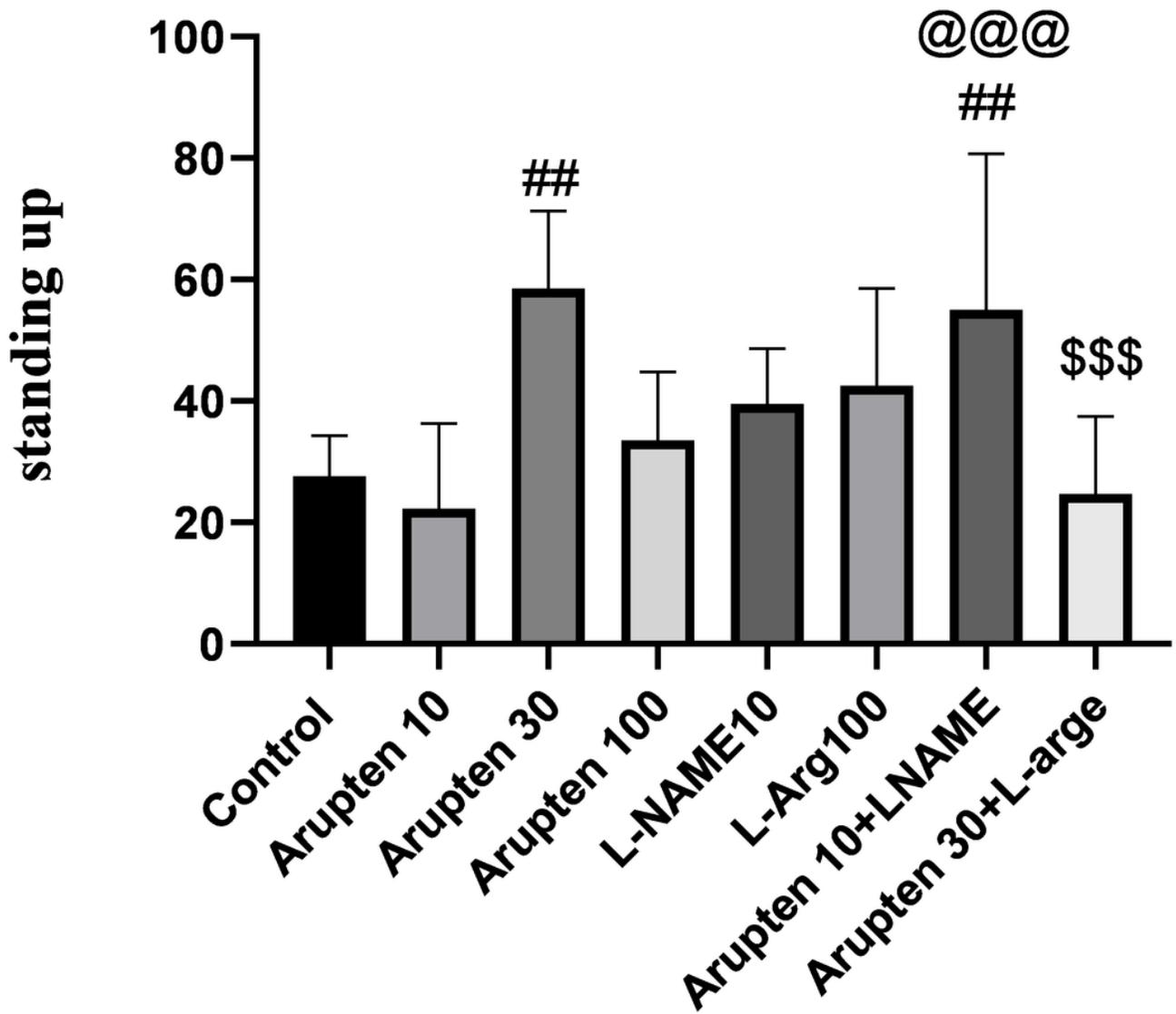


Figure 2

The amount of vertical movements in OFT in the experimental groups. Data were reported as Mean \pm SEM and analyzed using one-way ANOVA and Tukey post-hoc test. ## $P \leq 0.01$ in compared to the normal group. @@@ $P \leq 0.001$ in compared to the group received Auroptene at a dose of 10 mg/kg. &&& $P \leq 0.001$ in compared to the group received Auroptene at a dose of 30 mg/kg.

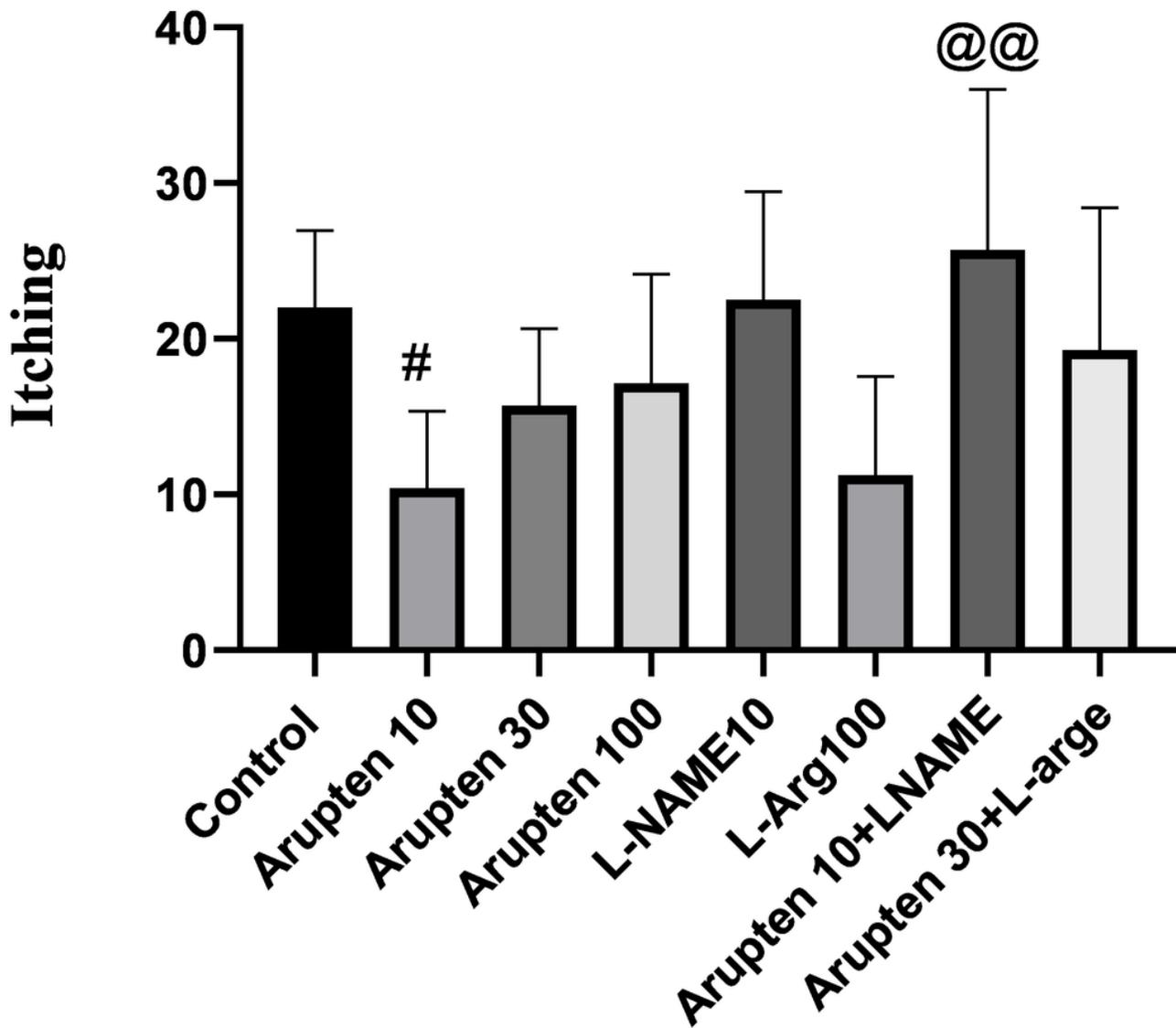


Figure 3

The scratching rate in OFT in the experimental groups. Data were reported as Mean \pm SEM and analyzed using one-way ANOVA and Tukey post-hoc test. # $P \leq 0.05$ in compared to the normal group. @@ $P \leq 0.01$ in compared to the group received Auroptene at a dose of 10 mg/kg.

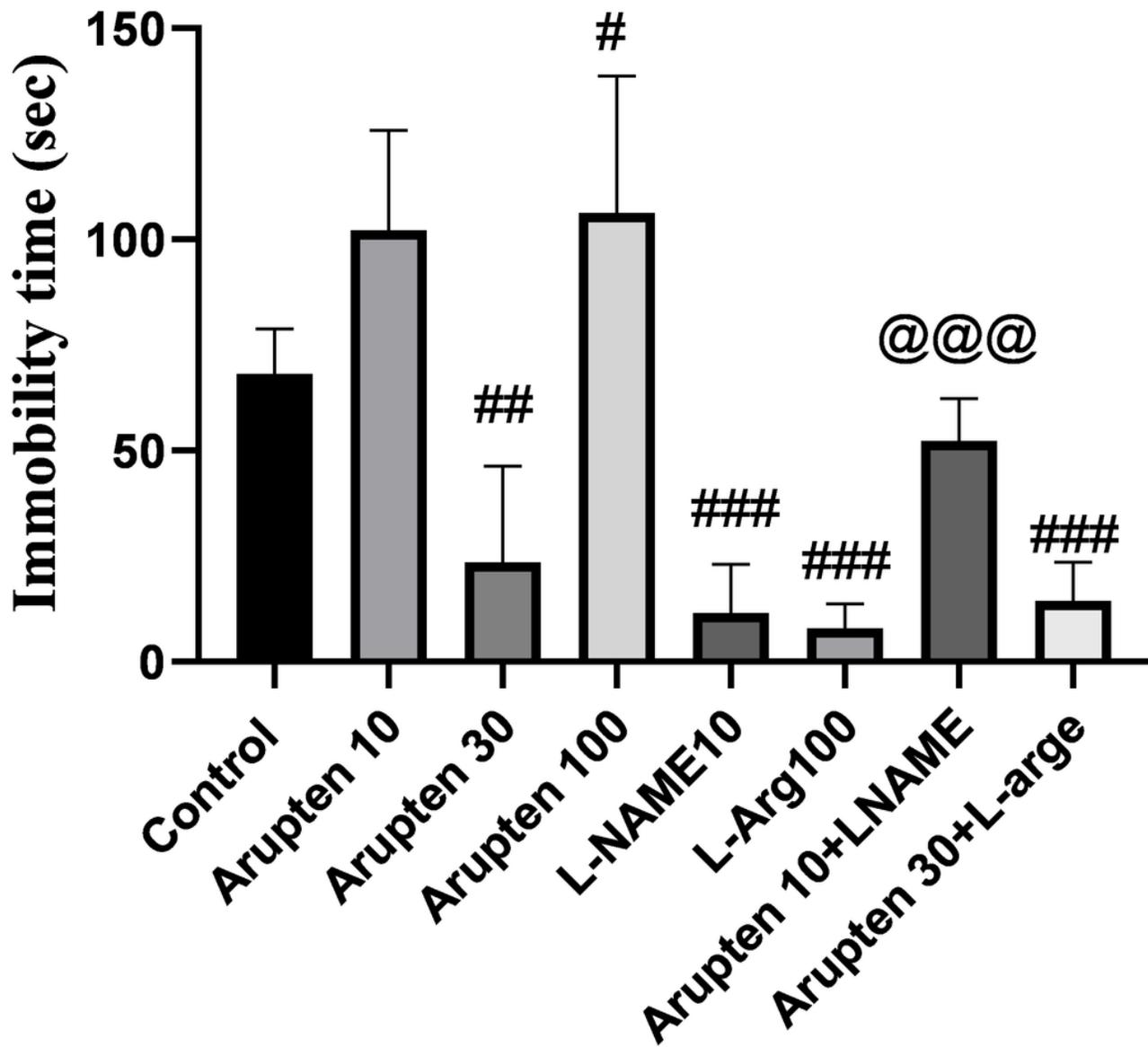


Figure 4

The duration of immobility in TST in the experimental groups. Data were reported as Mean \pm SEM and analyzed using one-way ANOVA and Tukey post-hoc test. # $P \leq 0.05$, ## $P \leq 0.01$ and ### $P \leq 0.001$ in compared to the normal group. @@@ $P \leq 0.001$ in compared to the group received Auraptene at a dose of 10 mg/kg.

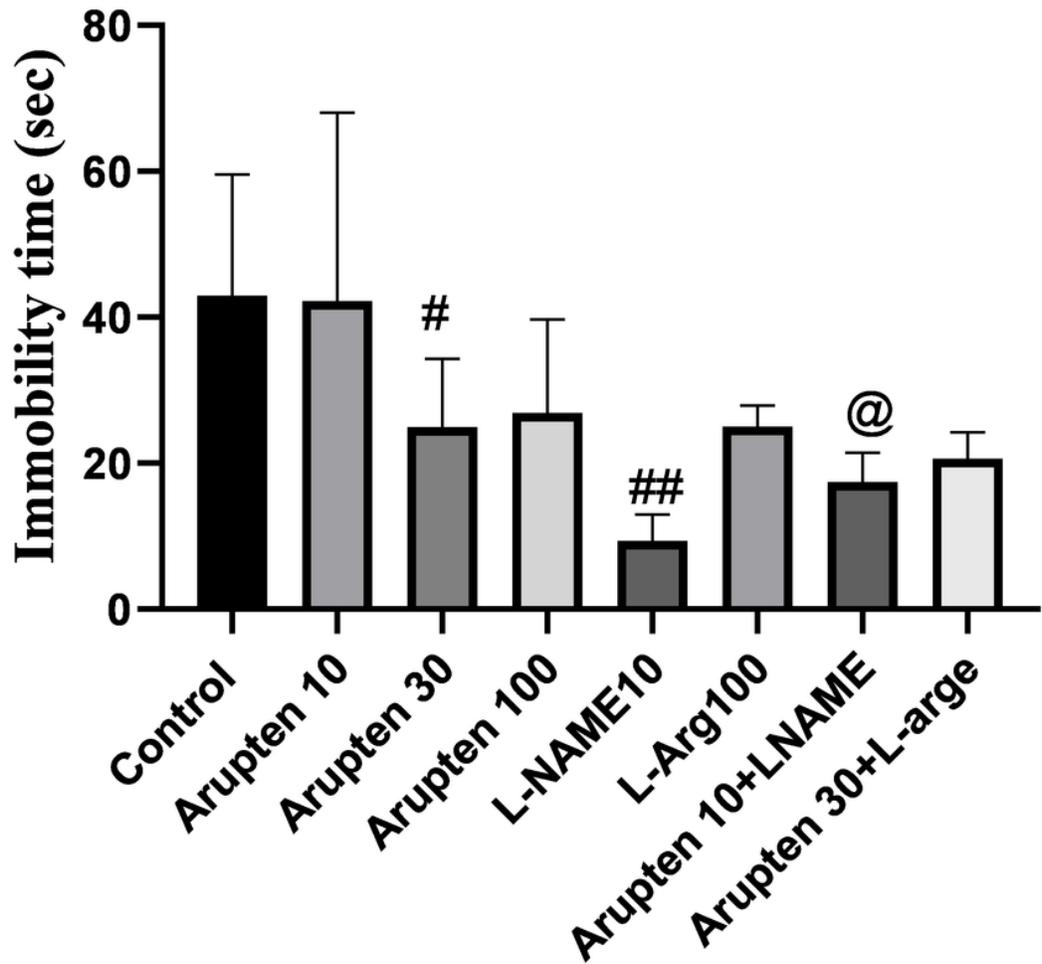


Figure 5

The duration of immobility in FST in the experimental groups. Data were reported as Mean \pm SEM and analyzed using one-way ANOVA and Tukey post-hoc test. # $P \leq 0.05$ and ## $P \leq 0.01$ in compared to the normal group. @ $P \leq 0.05$ in compared to the group received Auraptene at a dose of 10 mg/kg.

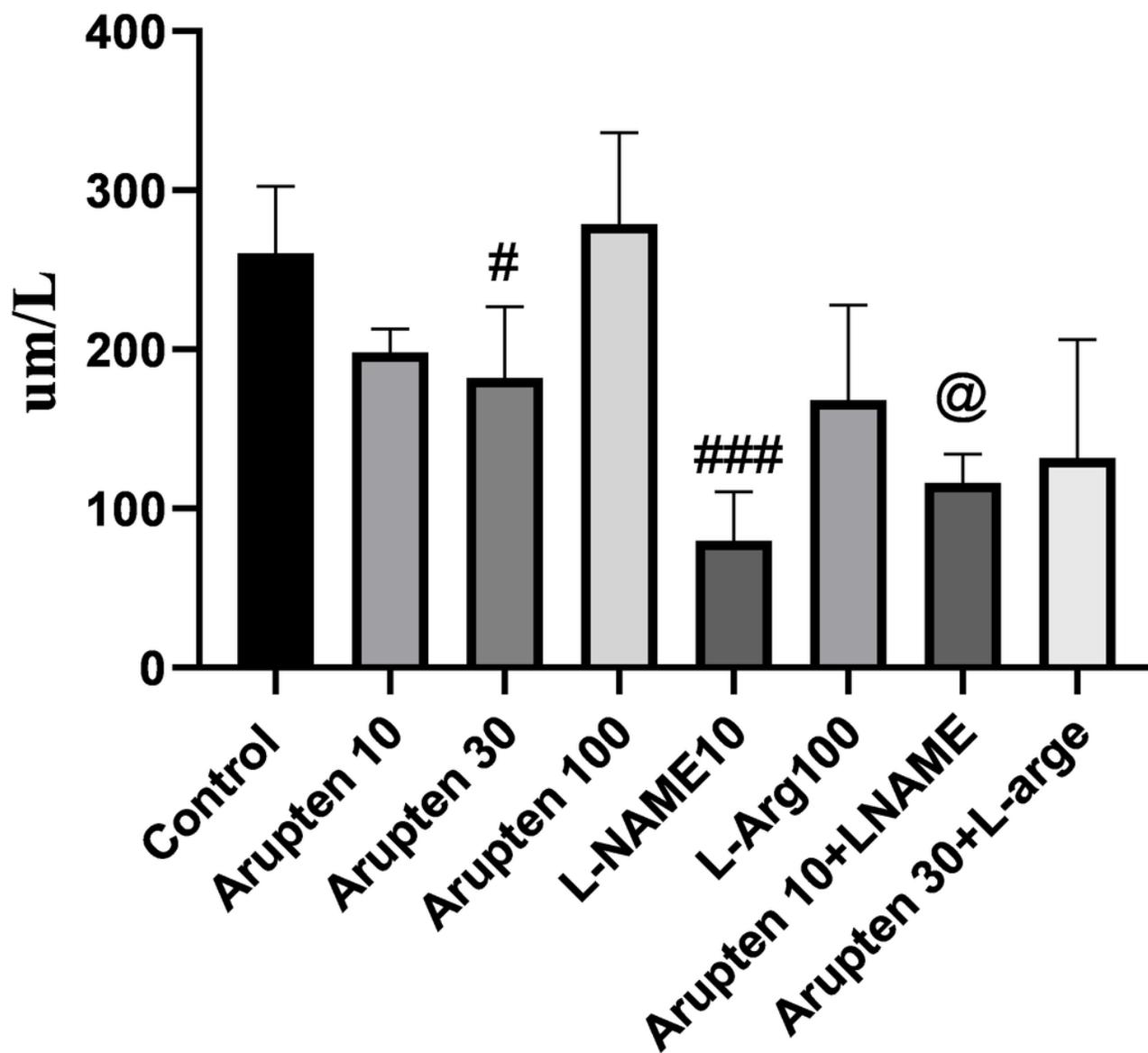


Figure 6

The serum nitric oxide levels in the different groups. Data were reported as Mean \pm SEM and analyzed using one-way ANOVA and Tukey post-hoc test. # $P \leq 0.05$ and ### $P \leq 0.001$ in compared to the normal group. @ $P \leq 0.05$ in compared to the group received Auraptene at a dose of 10 mg/kg.

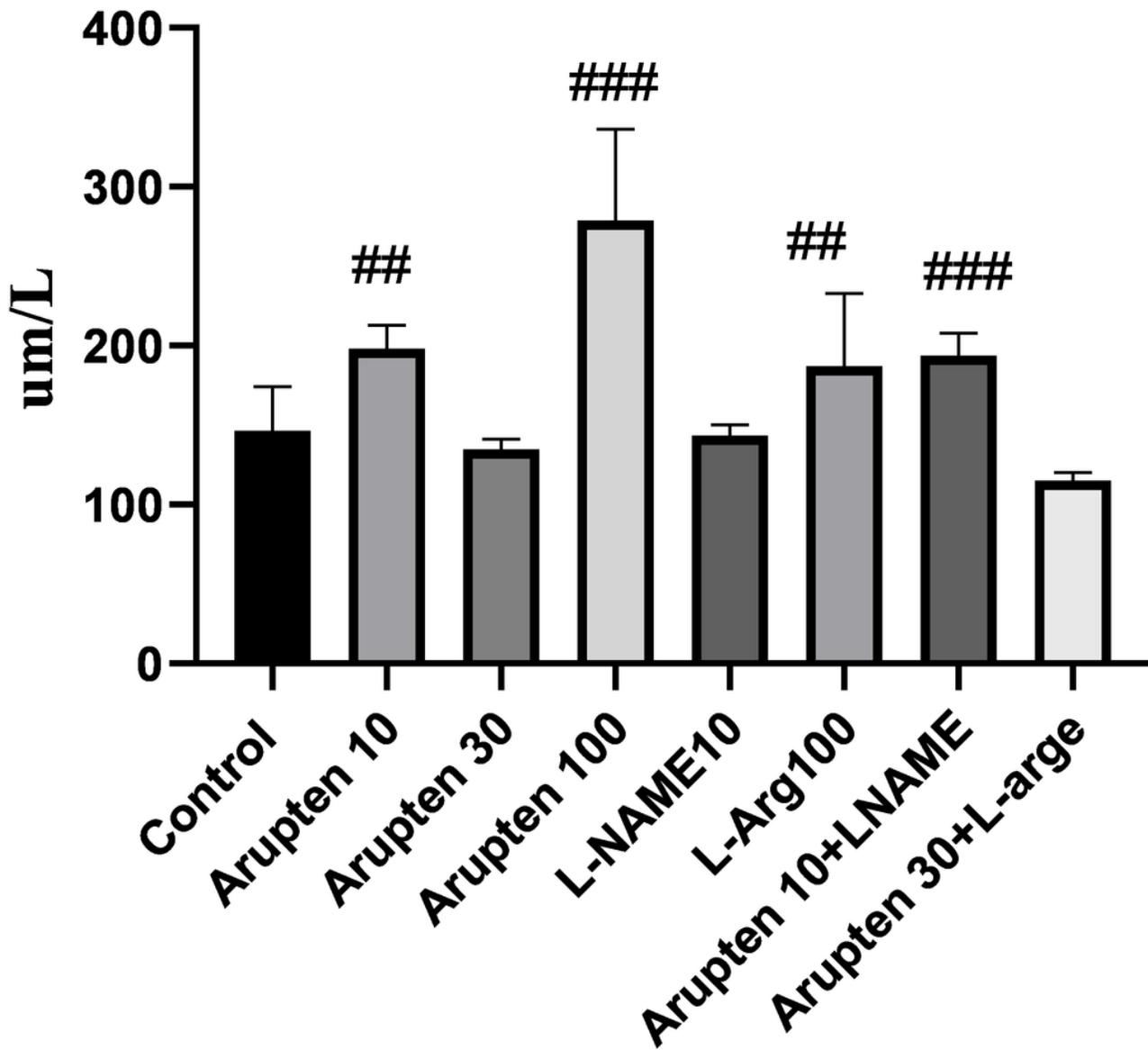


Figure 7

Brain nitric oxide content in the study groups. Data were reported as Mean \pm SEM and analyzed using one-way ANOVA and Tukey post-hoc test. ## $P < 0.01$ and ### $P < 0.001$ in compared to the normal group.

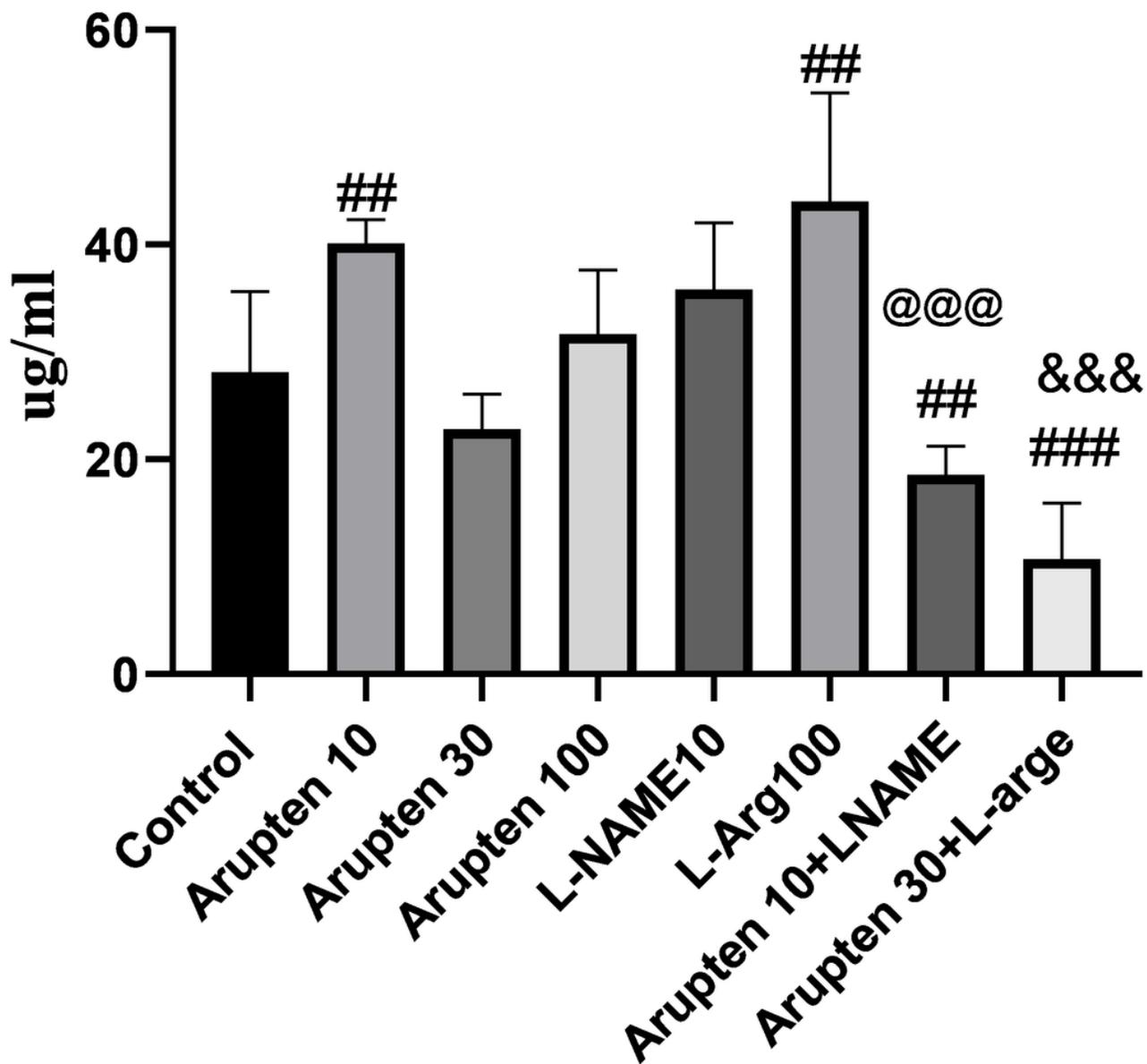


Figure 8

Serum MDA levels in the study groups. Data were reported as Mean \pm SEM and analyzed using one-way ANOVA and Tukey post-hoc test. ## $P < 0.01$ and ### $P < 0.001$ in compared to the normal group. @@@ $P < 0.001$ in compared to the group received Auraptene at a dose of 10 mg/kg. &&& $P < 0.001$ in compared to the group received Auraptene at a dose of 30 mg/kg.

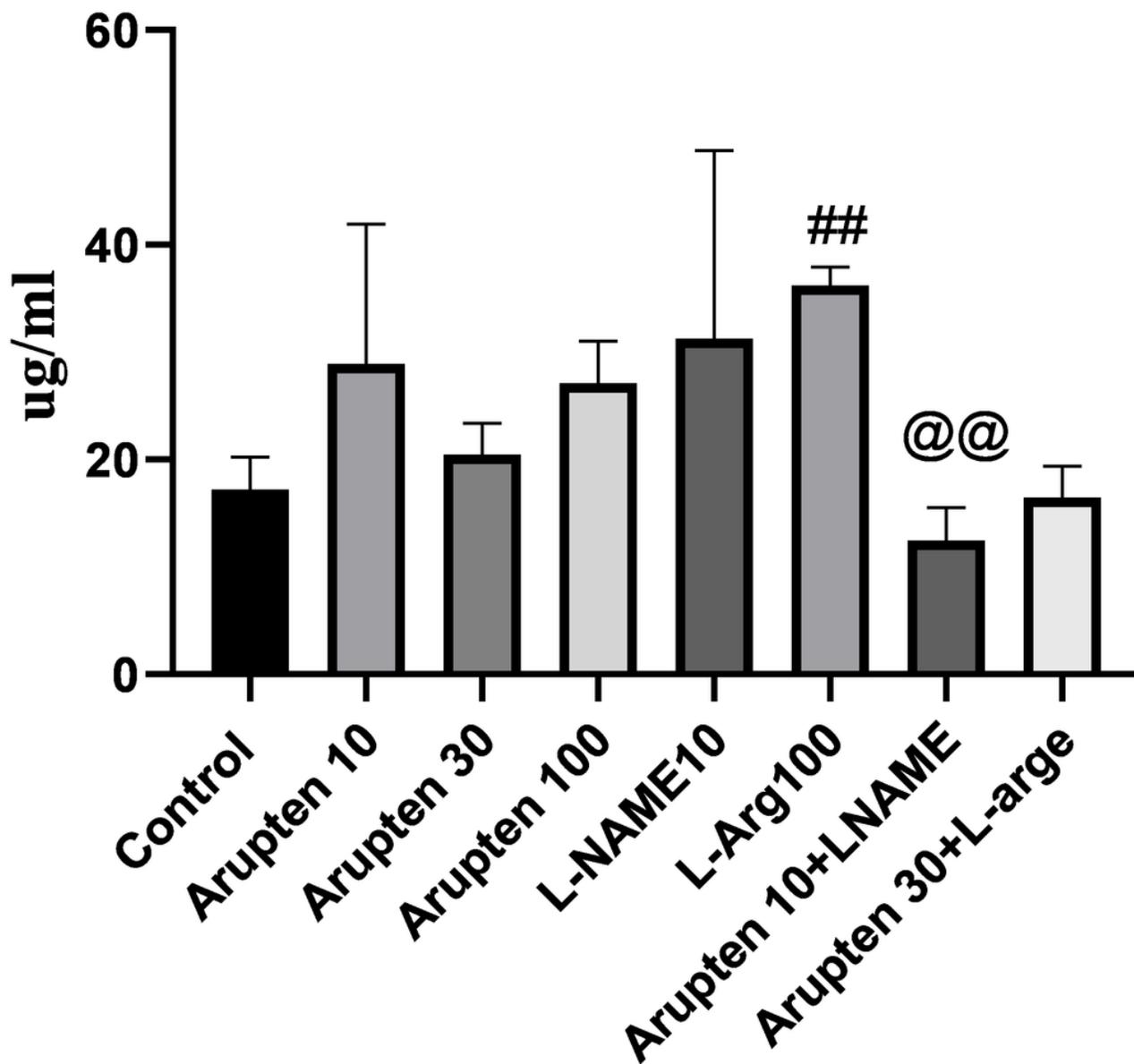


Figure 9

Brain MDA levels in the study groups. Data were reported as Mean \pm SEM and analyzed using one-way ANOVA and Tukey post-hoc test. ## $P < 0.01$ in compared to the normal group. @@ $P < 0.01$ in compared to the group received Auraptene at a dose of 10 mg/kg.

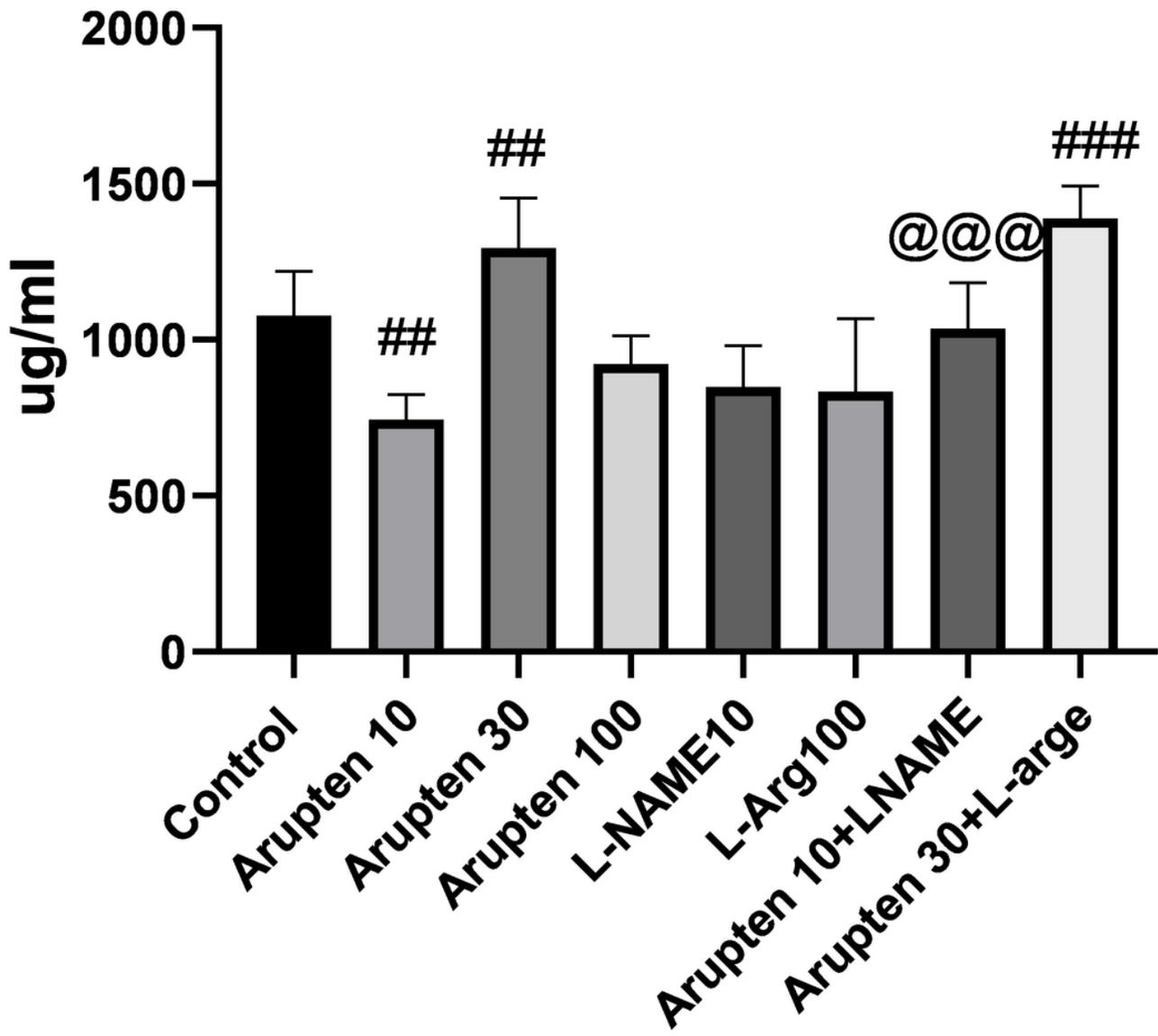


Figure 10

Serum antioxidant capacity in the experimental groups. Data were reported as Mean \pm SEM and analyzed using one-way ANOVA and Tukey post-hoc test. # $P \leq 0.05$, ## $P \leq 0.01$ and ### $P \leq 0.001$ in compared to the normal group. @@@ $P \leq 0.001$ in compared to the group received Auraptene at a dose of 10 mg/kg.

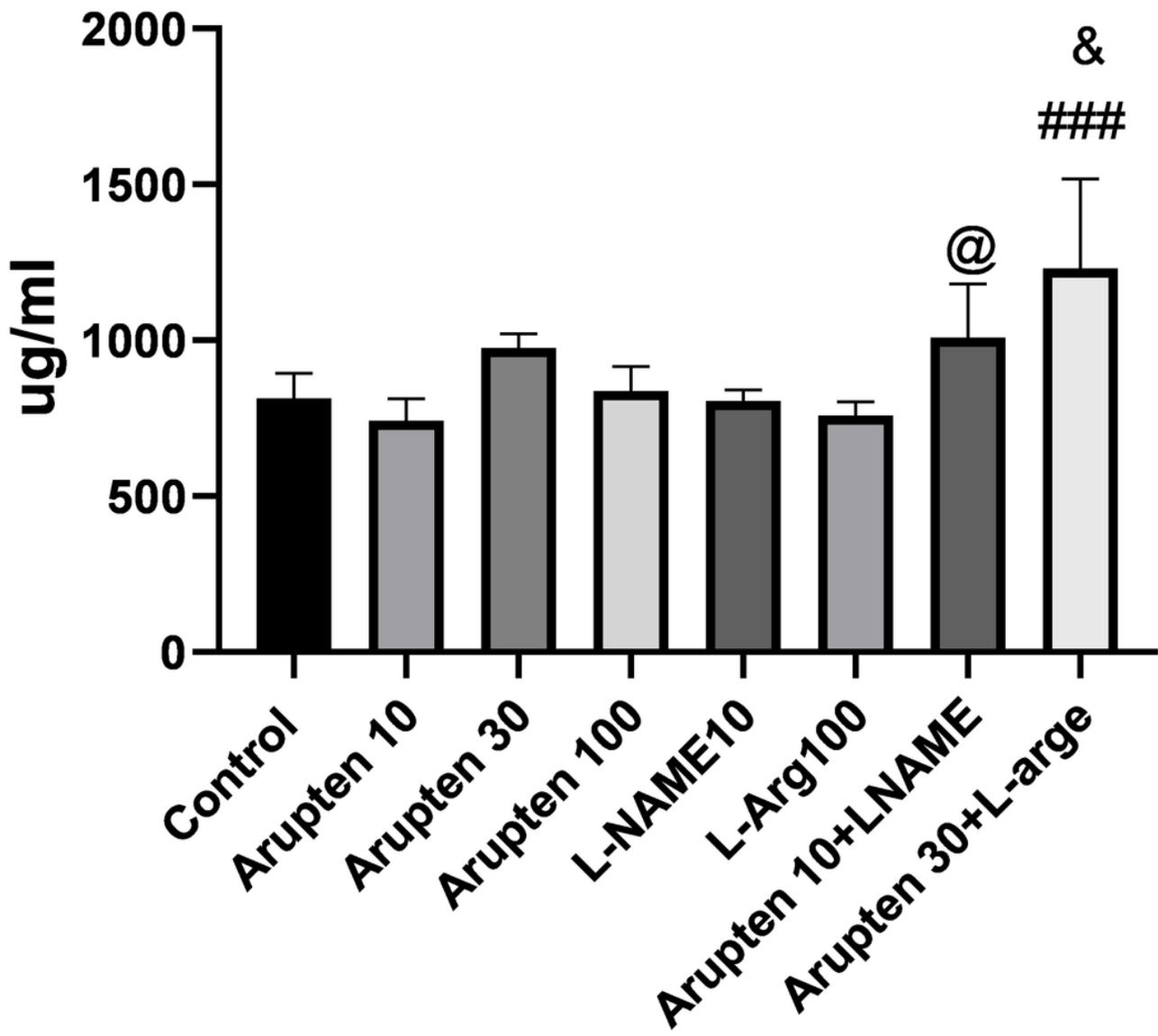


Figure 11

Antioxidant capacity of the brain in the experimental groups. Data were reported as Mean \pm SEM and analyzed using one-way ANOVA and Tukey post-hoc test. ### $P < 0.001$ in compared to the normal group. @ $P < 0.05$ in compared to the group received Auraptene at a dose of 10 mg/kg. & $P < 0.05$ in compared to the group received Auraptene at a dose of 30 mg/kg.