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Therapeutic Effects of Sesame-Derived Arginine on Inflammation, Oxidative Stress, Muscle Atrophy, and Motor Function in Stroke-Induced Wistar Rats

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ABSTRACT

The present study was aimed at analyzing the biochemical, proximate and physiochemical Stroke-induced muscle atrophy and inflammatory response pose significant challenges in post-stroke recovery. This study investigated the therapeutic potential of sesame-derived arginine supplementation in reducing inflammation and oxidative stress, enhancing antioxidant enzyme activity, and preserving motor function in Wistar rats with experimentally induced ischemic stroke. The methodology included arginine extraction from sesame seeds, stroke induction using bilateral common carotid artery occlusion with reperfusion (BCCAO/R), and assessments of serum markers and motor function. Results showed that arginine supplementation led to a significant decrease ($p<0.05$) in pro-inflammatory cytokines, including TNF-α and IL-6, in stroke-induced rats compared to the control group. Additionally, antioxidant enzyme activities—SOD, CAT, and GPX—were significantly elevated ($p<0.05$) in the arginine-supplemented group, indicating a reduction in oxidative stress. Serum levels of malondialdehyde (MDA), an indicator of lipid peroxidation, were significantly reduced ($p<0.05$), while creatine kinase levels, indicative of muscle atrophy, also showed a marked decrease ($p<0.05$) with arginine treatment. However, no significant improvement in motor function and coordination was observed in the rotarod test.

Keywords: *Arginine, muscle atrophy, oxidative stress, stroke, wistar rats*

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INTRODUCTION

Stroke is a leading cause of acquired physical disability worldwide, with often substantial muscle atrophy, that can result from a gradual loss of muscle mass and strength (Murphy and Werring, 2020; Azzollini *et al*., 2021). The pathophysiology of Post-stroke muscle atrophy involves intricate processes including inflammation and free radical toxicity that ultimately lead to neuromuscular deficits (Gelderblom *et al*., 2009; Deb *et al*., 2010; Woodruff *et al*.,2011). Alterations in hormonal balance, has also been proven to can contribute to muscle wasting (Baraugh *et al*, 2014).

Neurogenic atrophy is as a result of damage to motor neurons in the brain that can lead to reduced muscle activity and subsequent atrophy while disuse atrophy due to paralysis or weakness also contribute to muscle wasting (Greenberg and Amin-Hanjani, 2012; Diringer and Levy, 2015). All these can significantly affect post-stroke recovery and as such, there is a growing need to explore pharmacological alternatives to complement established protocols in stroke management. Arginine, a semi-essential amino acid, is considered as a major regulator of muscle protein synthesis and degradation

and has drawn a lot of interest in the treatment of diseases characterized by muscular atrophy (Kalliokoski *et al*., 2006; Ham *et al*., 2014). The isomer, L- arginine serves as a precursor for nitric oxide (NO), a molecule involved in vasodilation and neurotransmission, potentially improving blood flow and oxygen delivery to the brain and skeletal muscles (Tong and Barbul, 2004). L- arginine also helps improve muscle health by providing substrate for the production of creatine, and can as well assist in the control of muscle growth through direct stimulation of the synthesis of muscle proteins (Wang *et al*., 2018).

Additionally, arginine has the ability to stimulate release of hormones, reduction of oxidative stress and modulation of immunological response (Tong and Barbul, 2004).While the evidence for arginine's efficacy in mitigating post-stroke muscle atrophy is still developing, several studies have shown promising results. For example, a study by Ham *et al*., (2014) found that arginine supplementation improved muscle strength and function in individuals with stroke. Another study by (Lee *et al*., 2022) demonstrated that arginine supplementation reduced muscle atrophy and improved functional outcomes in a rat model of stroke. It also has potential anti-inflammatory effects (De Palma *et al*., 2009; Bologna *et al*., 2020).

Sesamum indicum (sesame seed) is a plants specie that have been studied for its bioactive compounds and potent pharmacological activities Recent studies has shown that a sesame contains a significant amount of arginine compared to its overall protein content (Akeem *et al.,* 2023). High protein content might be beneficial for muscle repair and recovery. Therefore, sesame seeds can be considered as a good sources and a natural alternative to synthetic arginine supplement.

MATRIALS AND METHODS

Experimental animals

Fifteen (15) apparently healthy Wistar rats weighing between 150-250g was obtained from the Animal House of the Department of Pharmaceutical Sciences, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The animals were housed in clean plastic cages with soft wood shavings as bedding to allow them acclimatise to the environment. They were maintained under a 12 hour light/dark cycle at room temperature and provided with grower‘s mash (chikun®) and water ad-libitum.

Experimental design

Rats received oral supplementation of arginine at a dosage of 9 grams per day for 14 days post-I/R.

Acquisition and identification of sesame seeds

The sesame seeds that was used for the experiment was purchased from Sokoto fish and vegetable market, sokoto town.

Sample preparation

The samples were thoroughly washed with clean water and then sun dried. After drying, it was grinded into a fine powder using a blender to ensure homogenous samples for accurate analysis, and thereafter stored in separate air tight container for further investigation

Solvent extraction

About 30g of the ground sample was weighed and introduced into a thimble made of filter paper. The open end of the thimble was closed with a cotton wool and placed in a Soxhlet extractor. The Soxhlet extractor was connected with the round-bottomed flask and the other end of the extractor was connected to a water condenser. The Soxhlet was filled with the solvent (n-hexane) until some of it overflows into the flask and heated at 65 -70^{0}C for 4 hours. The heat caused the solvent to circulate through the extraction chamber, extracting the oil from the sesame seeds. After the extraction process is completed, the Soxhlet extractor was removed from the heat source and allowed to cool. The solvent was then removed by placing the content in a rotary evaporator until the extracted oil is obtained. The extracted oil is then collected and then transferred into a clean amber coloured bottle. The solid residue in the thimble is then stored in an air-tight container for further analysis.

Extraction of arginine from sesame seeds

The salt/alkaline extraction method was employed to determine arginine levels in sesame seeds.

Assessment of motor function and coordination

Pre-supplementation baseline measures of neurological test were obtained for comparison with post-supplementation data.

Rotarod test

This test assesses motor coordination, balance, and endurance. The rotarod apparatus consists of a horizontal rotating rod with lanes for individual rats and fall sensors. On the testing day, the rats were brought to the testing room and allowed to acclimatize to the environment for at least 15 minutes prior to the commencement. The rats were marked with non-toxic ink for individual identification. Each rat was weighed using a

weighing scale and the weight recorded. The rotarod was set to a slow initial speed (5RPM). A rat was gently placed on the rotating rod facing forward. Then, the timer was started and the latency (time), until the rat falls off the rod or a predetermined cut-off time (60 seconds) was recorded. The rotarod was cleaned with a disinfectant solution between trials to eliminate residues from previous rats. Multiple trials were ran (4 trials) per rat and the average latency to fall was calculated.

Ischaemic Stroke (IS) induction

Experimental ischaemic stroke (IS) was induced using bilateral common carotid artery occlusion with reperfusion (BCCAO/R). Ketamine and Xylazine at the doses of 80mg/kg and 5mg/kg body weight respectively was used to anaesthesize the rats. Once the rats were unconscious, the fur on their necks was shaved, and cleansed with 70 % ethanol and Chlorhexidine, the rats were thereafter placed in the supine position on the operating table with adhesive tape to hold their paws and tails to the surgical table. The upper margin of the sternum was cut with surgical scissors to create a shallow midventral cervical incision. A pair of ocular forceps was used to delicately relocate the submandibular gland. This made it easier to reach the common carotid artery, which is encased with the vagus nerve in the carotid sheath between the sternohyoid and sternocleidomastoid muscles. The vagus nerve was carefully preserved, and then, both the right and left common carotid arteries were carefully separated. The carotid arteries were clamped for 30 minutes using a non-traumatic artery forceps. The clamp was released from both arteries to allow for reperfusion after 30 minutes of cerebral ischemia. Visual inspection of the arteries was done to ensure that the blood flow stopped when the clamp was applied and resumed after it was removed. After cleaning with 70 % ethanol and applying an antibiotic ointment, the skin was sutured and lidocaine was applied as a local analgesic agent. During the surgery, the rat's rectal temperature was monitored with a rectal thermometer and maintained at 37±1 ^{o}C with a thermostat-controlled heating pad to avoid hypothermia. A corneal protectant was also applied to each eye to prevent the eyes from drying. The animals were returned to their cages with access to food and water after being kept warm until they had fully recovered from the anaesthesia.

Euthanasia and brain tissue preparation

Following completion of the experiment, the animals were anaesthetized with chloroform soaked cotton in a glass jar. The anaesthetized rats were decapitated and the brains were removed from the calvarium. After gentle removal, the brain was then transferred to a 60 mm petri dish, and was rinsed with a saline phosphate buffered (PBS) to remove any red blood cells and clots. The harvested whole brain was dissected at the coronal plane at 2 mm thickness in a brain matrix on ice. The brain tissues were then transferred to a second petri dish in ice chilled10% PBS solution.

Blood sample collection

At the end of the experiment, blood sample was collected from anaesthetized rats by cardiac puncture into appropriate bottles for serum separation. Samples in plane tubes were centrifuged at 3000rpm for 5 minutes using bench top centrifuge, the supernatant was harvested and stored at -20^{o}C until required for further analyses.

Biochemical assays

Oxidative stress markers

Colorimetric assays were used for the determination of Oxidative Stress Markers; Superoxide dismutase (SOD), Catalase and glutathione peroxidase. These were assayed using Cayman's superoxide dismutase assay kit, Cayman's catalase assay kit and Cayman's glutathione peroxidase assay kit respectively, following the manufacturer's instruction.

Estimation of Malondialdehyde (MDA)

Malondialdehyde as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was measured following the manufacturer's instructions.

Estimation of Tumor necrosis factor α (TNF-α)

Tumor necrosis factor α was assayed using Elab Science's ELISA (TNF-α) assay kit following the manufacturer's instruction.

Estimation Interleukin-6 (IL6)

Interleukin-6 was assayed using Elab Science's (IL6) assay kit following the manufacturer's instruction.

Statistical analysis

Data are expressed as mean ± SD. statistical significance was evaluated by one-way ANOVA, and $P < 0.05$ is considered significant.

RESULTS

Effect of Arginine on pro-inflammatory cytokine (TNF-α) in stroke-induced Wistar rats

The effect of Arginine on pro-inflammatory cytokine (TNF-α) in Wistar rats with induced stroke is presented in (Figure 1). The result indicated that serum TNF-α decreased significantly ($p<0.05$) after supplementation with arginine when compared with the TNF-α levels of rats in non-supplemented group.

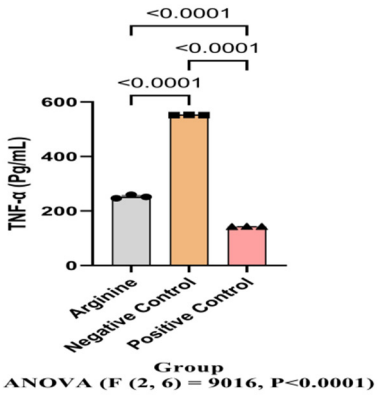

Figure 1: Effect of arginine on pro-inflammatory cytokine (TNF-α) in wistar rats with induced stroke.

Effect of arginine on interleukin-6 (IL-6) in stroke-induced wistar rats

The effect of arginine supplementation on serum IL-6 in stroke-induced Wistar rats is presented in (Figure 2). The result shows that stroke induction, caused significant ($p<0.05$) increase in inflammation in control group in contrast to the supplemented group where IL-6 decreased significantly ($p<0.05$).

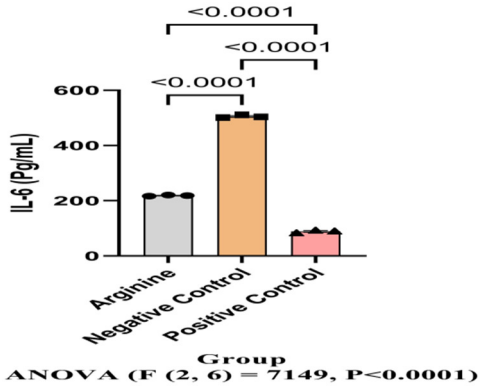

Figure 2: Effect of Arginine on pro-inflammatory cytokine (IL-6) in Wistar rats with induced stroke.

Effect of arginine on serum SOD activity in stroke-induced wistar rats

The result of serum SOD activity of stroke-induced Wistar rats on supplementation with arginine is presented in (Figure 3). SOD activity increased significantly ($p<0.05$) in rats given arginine supplementation when compared to the SOD activity of rats in the control group.

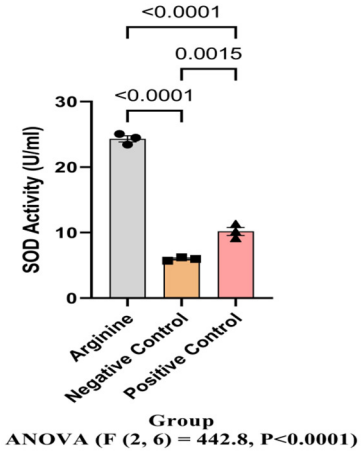

Figure 3: Effect of Arginine on SOD Activity in Wistar rats induced with stroke.

Effect of Arginine on CAT activity in stroke-induced wistar rats

The effect of arginine supplementation on serum CAT activity in Wistar rats induced with stroke is presented in (Figure 4). The result indicated that stroke induction, caused significant ($p<0.05$) decrease in the activity of the enzyme CAT, while CAT activity increased significantly ($p<0.05$) in the arginine supplemented group.

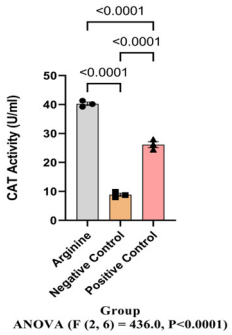

Figure 4: Effect of Arginine on CAT Activity in Wistar rats induced with stroke.

Effect of Arginine on GPX Activity in stroke-induced Wistar rats

The serum GPX activity of stroke-induced Wistar rats that were supplemented with arginine increased significantly ($p<0.05$) in contrary to the control group where stroke induction without supplementation, caused significant decrease ($p<0.05$) in the activity of the enzyme GPX as shown in (Figure 5).

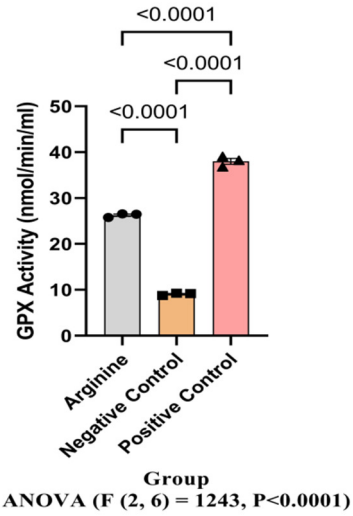

Figure 5: Effect of arginine on GPx activity in wistar rats induced with stroke.

Effect of Arginine on MDA concentration in stroke-induced Wistar rats

The effect of arginine supplementation on the serum MDA concentration in stroke-induced Wistar rats is presented in (Figure 6). The result shows that induction of stroke, caused a significant increase ($p<0.05$) in the concentration of MDA. After supplementation with arginine, MDA concentration serum MDA concentration 05).

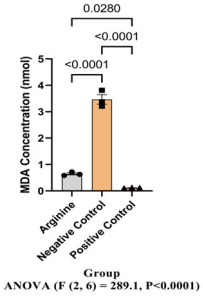

Figure 6: Effect of Arginine on MDA conc. in Wistar rats induced with stroke**.**

Effect of arginine on creatine kinase level in stroke-induced Wistar rats

The effect of arginine supplementation on creatine kinase level in stroke-induced Wistar rats is presented in (Figure 7).The result shows a significant decrease ($p<0.05$) in level of creatine kinase in the arginine supplemented group when compared to the control group.

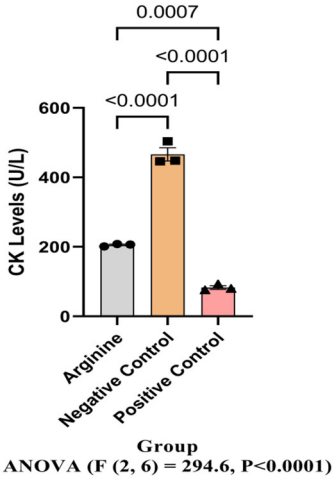

Figure 7: Effect of Arginine on Creatine kinase level in Wistar rats induced with stroke.

Effect of Arginine on Motor function and coordination in stroke-induced Wistar rats

Figure 8 below shows the result of the effect of arginine supplementation on motor function and coordination in stroke-induced Wistar rats. There was no significant difference in the level of rotarod latency between arginine supplemented and the control group.

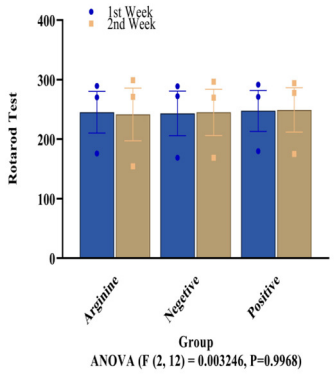

Figure 8: Effect of Arginine on Motor function and coordination in Wistar rats induced with stroke with stroke.

DISSCUSION

In this study, the potential role of sesame-derived arginine supplementation in enhancing blood flow, reducing inflammation, and preventing muscle atrophy post-stroke, has been evaluated. Arginine supplementation has shown anti-inflammatory potentials, affects the release of nitric oxide (NO) and contributed to improved functional outcomes post stroke. The result of the effect of arginine on TNF-α in stroke-induced Wistar rats indicated that serum TNF-α decreased significantly ($p<0.05$) after supplementation with arginine. This finding suggests that arginine has an inhibitory effect on TNF-α, since arginine is known to increase NO production, which can inhibit the production of TNF-α. The activation of nitric oxide synthesis by arginine and its association with the inhibition of TNF-α production is also supported by the reports of Joshi *et al*. (2007).

The result of the effect of arginine on TNF-α in stroke-induced Wistar rats indicated that serum TNF-α decreased significantly ($p<0.05$) after supplementation with arginine. This finding suggests that arginine has an inhibitory effect on TNF-α, since arginine is known to increase NO production, which can inhibit the production of TNF-α. The activation of nitric oxide synthesis by arginine and its association with the inhibition of TNF-α production is also supported by the reports of Joshi et al. (2007). Moreover, nitric oxide not only modulates the immune response by limiting the production of inflammatory cytokines such as TNF-α, but also helps prevent vascular dysfunction and reduce oxidative stress, which are critical factors in post-stroke pathology *(Moncada & Higgs, 2006)*. These findings align with studies by Miller *et al*. (2019) indicating that nitric oxide generated by arginine supplementation can reduce pro-inflammatory responses, thereby enhancing neuroprotection and promoting vascular stability in ischemic conditions.

The significant decrease in serum IL-6 levels observed in arginine-supplemented group aligns with findings from various studies highlighting L-arginine's impact on inflammation. L-arginine's anti-inflammatory effects are primarily associated with its role in nitric oxide (NO) production, which regulates immune responses and influences cytokine production. Specifically, L-arginine has been shown to modulate inflammatory pathways, including reducing pro-inflammatory cytokines like IL-6. By stimulating NO synthesis, arginine can act as an anti-inflammatory agent, helping to mitigate excessive inflammatory responses, as documented in inflammatory disease research. Studies demonstrate that NO can inhibit NF-kB, a transcription factor involved in cytokine production, leading to reduced IL-6 levels. For example, Cui et al. (2000) highlighted L-arginine's potential to lower inflammatory markers in various physiological settings. These findings underscore L-arginine's therapeutic promise in managing conditions with elevated inflammation, such as ischemic events, by down regulating cytokines like IL-6, which plays a role in inflammation and tissue damage.

The observed significant ($p<0.05$) increase in SOD (superoxide dismutase) activity in the arginine-supplemented group is significant, given SOD's role as a critical antioxidant defense against superoxide radicals. Nitric oxide (NO) produced from arginine can induce SOD synthesis, a mechanism known to attenuate oxidative stress by dismuting superoxide radicals into less reactive molecules like hydrogen peroxide, which is further neutralized by enzymes such as catalase (CAT) and glutathione peroxidase (GPx) (Lee et al., 2010). This cascade supports cellular resilience against the heightened oxidative stress associated with ischemic events like stroke, where reactive oxygen species (ROS) accumulation contributes to cell and tissue damage. Moreover, arginine's impact on antioxidant enzymes extends beyond SOD, as it has been linked to increased activities of both CAT and GPx. These enzymes work synergistically to maintain redox homeostasis: CAT decomposes hydrogen peroxide into water and oxygen, while GPx reduces peroxides and protects lipid membranes from oxidative damage.

The significant increase ($p<0.05$) in CAT and GPx activities observed in the arginine-supplemented group of this study underscores arginine's potential as a potent antioxidant agent in stroke contexts, as supported by previous studies (Pigeolet *et al*., 1990; Tripathi *et al*., 2007). These enzymes' upregulation may provide a cumulative protective effect, shielding neural tissues from oxidative damage and supporting recovery from stroke-induced oxidative stress. The significant reduction in serum malondialdehyde (MDA) concentration following arginine supplementation observed in this study highlights arginine's protective role against lipid peroxidation. MDA is a well-recognized marker of lipid peroxidation and is generated when reactive oxygen species (ROS) attack polyunsaturated fatty acids in cell membranes, leading to membrane destabilization and cellular damage. High MDA levels are often associated with severe oxidative stress, as seen in conditions like stroke, where ROS generation is heightened due to ischemic damage and subsequent reperfusion injury (Saifaddin et al., 2023; Ayala et al., 2014). By decreasing MDA, arginine demonstrates an ability to reduce lipid peroxidation, which not only protects cell membrane integrity but may also support improved neurological outcomes after stroke. Arginine's effects in reducing oxidative markers like MDA likely result from its ability to enhance nitric oxide (NO) production. NO, apart from its vasodilatory and neuroprotective properties, plays an indirect role in mitigating lipid peroxidation by scavenging free radicals and reducing overall oxidative stress. This function is especially beneficial in stroke, where

mitigating oxidative damage can limit cell death and promote tissue recovery. Other studies have confirmed similar findings, showing that antioxidant compounds capable of lowering MDA levels contribute positively to neuroprotection and recovery in models of ischemic injury (Andersen, 2004; Shi *et al*., 2005). The result of this study shows a significant decrease ($p<0.05$) in creatine kinase (CK) levels in the arginine-supplemented group highlights the protective role of arginine against cellular injury associated with ischemic events. CK is a well-established biomarker of cellular damage, especially in muscle and neural tissues, where its elevated presence in serum indicates membrane permeability changes and cell necrosis. Arginine appears to stabilize cell membranes and mitigate CK release, likely through its role in enhancing nitric oxide (NO) production. NO can reduce oxidative stress and support vascular integrity, which in turn minimizes cellular injury and CK leakage (Delwing *et a*l., 2007). Additionally, NO has been shown to influence mitochondrial function and limit oxidative phosphorylation, helping to conserve cellular energy under stress conditions, which further supports cell survival during ischemia (Forstermann and Sessa, 2012). Other studies corroborate these findings, suggesting that arginine's NO-mediated effects reduce oxidative and inflammatory cascades, ultimately protecting cellular structure and function (Miller, 2006). This protective effect has profound implications for managing tissue damage following ischemic injury, where therapies that stabilize cell integrity and limit CK release can improve recovery outcomes (Zhou *et al*., 2011).

Although no significant differences were observed in motor function and coordination between the arginine-supplemented and control groups in this study, previous research by Schmidt et al. (2014) highlights the potential for arginine to improve neuromuscular function under certain conditions. Schmidt et al. reported that arginine supplementation enhanced functional recovery in ischemic models, likely due to its role in nitric oxide (NO) production, which supports vascular dilation and improved oxygen delivery to recovering tissues. This discrepancy may stem from variations in arginine dosage, model types, or duration of supplementation.

While the findings on motor function in this study were not statistically significant, the impact of sesame-derived arginine on post-stroke muscle atrophy was evident, suggesting its potential role in preserving muscle mass during recovery. The protective effect on muscle may be attributed to arginine's ability to reduce inflammation and oxidative stress, thus limiting the cellular damage that often contributes to muscle wasting post-stroke (Zhang *et al*., 2017). These antioxidant and anti-inflammatory properties may not only aid muscle health but also indirectly support neural repair by creating a less hostile environment for neural cells (Miller *et al*., 2006). However, additional research is warranted to explore the optimal dose, duration, and precise mechanisms by which arginine affects neuro-muscular and functional recovery in post-stroke settings. Future studies could aim to clarify the interaction between arginine's antioxidative properties and neuromuscular recovery, as well as examine whether prolonged or higher doses yield more substantial improvements in coordination and motor outcomes.

Conclusion

The findings suggest that arginine supplementation from sesame seeds may mitigate post-stroke inflammation, reduce oxidative stress, and decrease muscle atrophy markers in stroke-induced Wistar rats, although it did not significantly improve motor coordination. These results highlight arginine's potential as a supportive therapy for post-stroke recovery by targeting inflammatory and oxidative pathways. Further studies are needed to explore its long-term effects and impact on functional recovery.

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