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Nitric oxide synthase mediates the antioxidant effect of L-Arginine in the brain of mice subjected to chronic restrain stress

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Abstract

Chronic restraint stress (CRS) immobilize animal and induces the production of oxidative radicals as a preliminary cause of various health complications. CRS-induced oxidative stress led to pathological changes in brain tissue and consequently the general body condition. Thus, we elucidated the potential protective impact of L-Arginine (L-Arg), conditional amino acid and essential source of nitric oxide, on oxidative stress and brain tissue. BALB/c mice were randomly divided into five groups (n = 6) that received either 1) daily intraperitoneal (I.P) injection of phosphate-buffered saline (PBS) (Control group) (0.5ml), 2) I.P injection of PBS in combination with CRS (2h/day for 10 consecutive days) (CRS group), 3) I.P injection of L-Arg (500 mg/kg b.wt) with CRS (L-Arg + CRS group), 4) L-Arg alone (L-Arg group), or 5) pretreatment with I.P injection of $L-N^G$ –nitro-L-arginine methyl ester (l-NAME) (30 mg/kg b.wt), a potent common inhibitor of nitric oxide synthase (NOS), 30 minutes before L-Arg injection and CRS application (L-Arg + L-NAME + CRS group). Total antioxidant capacity (T-AOC), catalase, and histopathological status of brain tissue were evaluated. CRS significantly suppressed the T-AOC compared to the control. Furthermore, L-Arg elevated the T-AOC whereas L-NAME significantly reversed the action of L-Arg on CRS-induced T-AOC. L-Arg could not rescue CRS-decreased catalase. It is concluded that NOS is an essential mediator of the L-Arg ameliorative effect on CRS-induced oxidative stress in serum and brain tissue. Moreover, L-Arg antioxidant action is not mediated by catalase.

Keywords:

Chronic restraint stress, L-Arginine, L-NAME, Nitric oxide synthase, Oxidative stress.

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Introduction

Continuous exposure to stressors induces physical, behavioral, and neuropsychiatric symptoms (Lin and Scott, 2009). Furthermore, chronic stress can activate the sympathoadrenal-medullary system and causes hyperactivity of the hypothalamic-pituitary-adrenal axis, leading to the release of glucocorticoids and activation of the hypothalamic-pituitary-adrenal (HPA) axis (Stamper et al., 2017; Ter Horst et al., 2019) and consequently the elevation of serum corticosterone (Spiga et al., 2014; Niraula et al., 2018). A high level of serum corticosterone triggers glutamate release in the brain (Musazzi et al., 2010) which consequently cause mitochondrial malfunction and a rise in metabolic rate (Hardingham et. al. , 2002) which in turn induces an imbalance between the formation of reactive oxygen species (ROS) and the antioxidant system resulting production of free radicals (Samarghandian et al., 2016). The brain is more susceptible to stress due to high levels of intracellular mediators that are involved in the stress response (Kumar and Pandey, 2013). However, improving the antioxidant system may be useful in combating excessive ROS production (Samarghandian et al., 2015).

L-Arginine (L-Arg) is a conditional amino acid for adult mammals and essential amino acid for birds that exerts a crucial role in immune response (Kim and Won, 2017), wound healing (Debats et al., 2009), growth hormone release (Forbes et al., 2014) and cell proliferation (Fujiwara et al., 2014). Supplementation of L-Arg stimulates glutathione (GSH) synthesis and activates the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, leading to the up-regulation of antioxidant response element (ARE)-driven antioxidant expressions. So that, the availability of L-Arg is a critical factor to suppress oxidative stress, inducing an endogenous antioxidant response (Liang et al., 2018) reducing inflammatory reactions (Tan et al., 2014),

amelioration of cognitive decline, and counteracting shortened lifespan in mice subjected to chronic stress (Pervin et al., 2021). Moreover, l-Arg enhances catalase activity (El-Missiry, et al. 2004) and recovers superoxide dismutase, glutathione, and catalase activity suppressed by restraint stress (Pal et al., 2020).

L-Arg combats obesity through the reduction of fat intake and adipose white tissue (McKnight et al., 2010). Additionally, it down-regulates insulin and enhances blood flow to the insulin-sensitive tissue which helps lipogenesis and fat loss. However, induction of body weight loss by L-Arg is not dependent on NO activity (Thams and Capito, 1999; Wu et al., 2012).

Nitric oxide (NO), which is a product of nitric oxide synthase (NOS) action on L-Arg (Albaugh et al., 2017) , acts as a precursor of signaling molecules (Förstermann and Sessa, 2012) and is involved in a variety of functions, including blood vessel vasodilation (Albaugh et al., 2017) , learning and memory processing (Paul and Ekambaram, 2011) and modulation of neuronal function during stress and anxiety (Gulati and Ray, 2014). In this study, chronic restraint stress (CRS) was used to induce oxidative stress and the antioxidant effect of L-Arginine was evaluated in serum and brain tissue. Moreover, the role of NOS in the mediation of L-Arg action is assessed through the utilization of L-N^G –nitro-L-arginine methyl ester (L-NAME), a potent common inhibitor of NOS.

Materials and methods

Ethical approval:

All experimental procedures in the present study were performed and approved in accordance with the Ethical Research Committee of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt (approval No.: 2021-15). The protocols of the animal experiment have

performed under the guidelines provided by the Egypt National Institutes of Health for the Care and Use of Laboratory Animals.

Animals

The study involved 30 adult male albino BALB/c mice weighing 35 to 40 g. Mice were bought from the animal house at the Faculty of Medicine, Sohag University, Sohag, Egypt. Animals were kept in plastic cages with 12h dark /12h light cycle at room temperature with *ad libitum* access to food and water. The animals were examined and adapted to the new environment for two weeks before the formal experiment.

Drugs:

L-Arg ($\geq$ 99.5%) and (L-NAME) were purchased from Sigma-Aldrich (St Louis, MO, USA). All chemicals were prepared daily before injection. L-Arg was dissolved with sterile endotoxin-free phosphate-buffered saline (PBS) and injected intraperitoneally (I.P) at a dose of 500 mg/kg b.wt. L-NAME was dissolved in PBS and injected intraperitoneally at a dose of 30 mg/kg b.wt. All drugs were administered in a volume of 0.5 ml/mouse.

Experimental design:

Mice were randomly assigned into five groups (n = 6 mice): The first group received a daily I.P injection of sterile PBS at a dose of 0.5ml/mouse for 10 consecutive days (control group). The second group was injected with PBS at a dose of 0.5ml/mouse followed by application of CRS through immobilization in perforated plastic tubes (2h/day for 10 consecutive days) (CRS group) (Hossain *et al.*, 2017). The third group was injected daily with L-Arg at a dose of 500 mg/kg b. wt dissolved in PBS 30 minutes before exposure to CRS for 10 consecutive days (CRS+L-Arg group). The fourth group was daily injected with L-Arg at a dose of 500 mg/kg b. for 10 consecutive days (L-Arg group) (Pal *et al.*, 2020). The fifth group was injected with L-NAME at a dose of 30 mg/kg b.wt 30 minutes before

L-Arg injection (Safaripour *et al.*, 2018) followed by exposure to CRS (2h/day for 10 consecutive days) (CRS + L-Arg+ L-NAME group).

Application of chronic restraint stress:

CRS was applied two hours daily for 10 consecutive days as mentioned before Hossain et al., (2017) with minor modifications. Mice were immobilized in a cylindrical transparent tube (2.5 cm diameter, 12 cm long, with holes along the sidewall of the tube and in front of the head for ventilation) so the mice could not turn or move. Food and water were not allowed during the stress period. To avoid the habituation of mice to CRS it was applied at different daily time points. Mice were returned immediately to their cages at the end of restraint stress and allowed free access to food and water.

Body and brain weight recording:

Body weights were measured before euthanasia and the body weight gain ratio was calculated as a percentage of the initial body weight as follows:

$$\frac{w1 - w0}{w0} \; X \; 100$$

Where w1 is the final weight and w0 is the initial weight. At the end of the experiment, Animals have been sacrificed, brains were weighted, and the brain weight/body weight ratio was calculated.

Serum and tissue collection:

On one day after the last injection, mice were sedated with diethyl ether and blood samples were collected from the heart via cardiac puncture using plain tubes. Blood was allowed to clot and then centrifuged at 3000 rpm for 15 minutes at room temperature for separation of serum. Sera were collected into Eppendorf tubes and kept at -80° C. The brain was excised, has been weighed and preserved at 10%

formalin for further histopathological examination.

Estimation of total antioxidant capacity and catalase activity:

Total antioxidant capacity (T-AOC) and catalase activity were measured in sera samples using a colorimetric assay kit according to the manufacturer's protocol (Bio diagnostic, Egypt).

Histopathological examination:

All mice were sacrificed one day after the last injection. The brains were rapidly removed, fixed in 10% formalin, processed through the conventional paraffin embedding technique, sectioned at approximately 5 μm thick, and stained with Hematoxylin and Eosin (H&E; Bancroft and Gamble, 2002). Sections were observed under a light microscope equipped with a camera.

Statistical analysis:

Data are expressed as the mean ± standard error of mean (SEM). Differences between groups were examined for statistical significance using the one-way analysis of variance (ANOVA) followed by Tukey's Post Hoc test. Mean differences with P-value less than 0.05 are considered statistically significant.

Result

Body weight gain ratio:

Body weight gain/initial weight ratios were measured 10 days after initial treatment in different groups (Fig.1). There were no significant changes between CRS (1.68 ± 1.13) and control (4.74 ± 1.71) groups.

However, all L-Arg groups showed a significant reduction in body weights when compared to control; including CRS+L-Arg (-1.99 + 1.39 $P< 0.01$), L-Arg (-0.12 + 0.31, $P< 0.05$), and CRS + LArg + L-NAME (-0.06 + 0.01, $P<0.05$).

Brain/body weight ratio:

The brain/body weight ratio showed no significant differences between animals in the different experimental groups (Fig. 2).
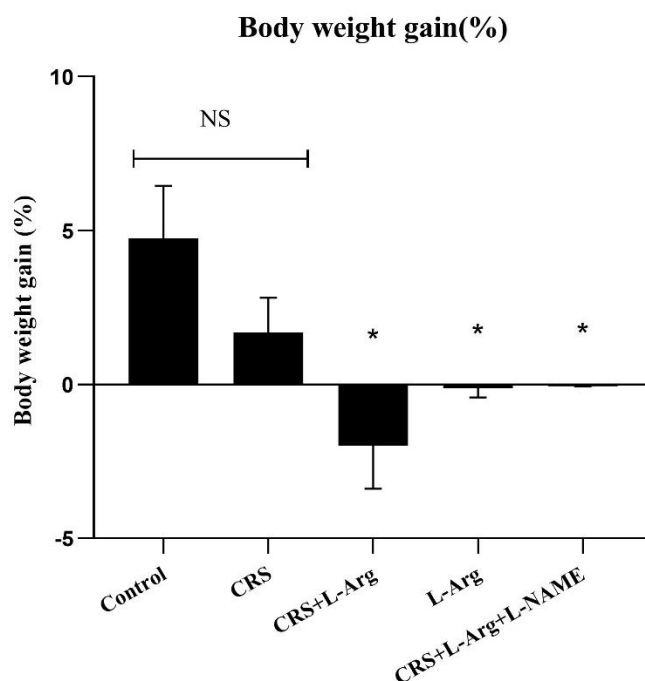
Body weight gain(%)

Fig. 1. Body weight gain ratio on the last day of injection in control, CRS, CRS+L-Arg, L-Arg, and CRS + L-Arg + L-NAME groups. Data are presented as mean ± SEM (n= 5-6). *$P<0.05$, vs. control group, NS = not significant $P>0.05$.

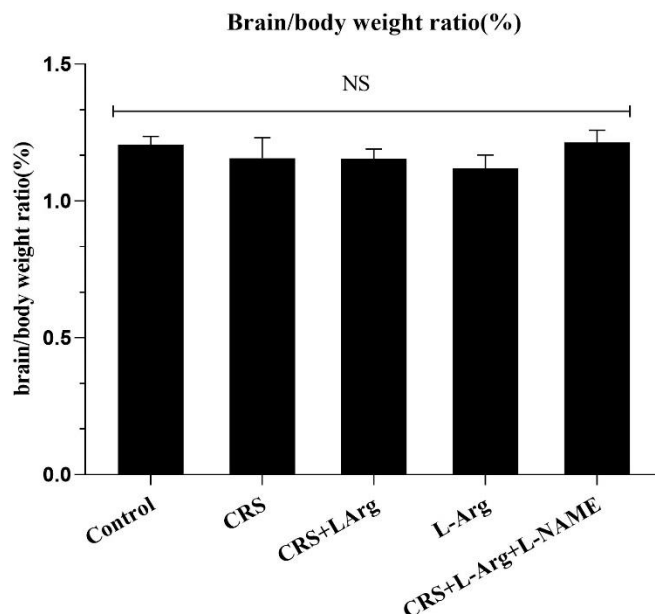**Brain/body weight ratio(%)**

Fig. 2. Brain/body weight ratios in control and treated groups the day after the last injection. Data are presented as mean ± SEM (n=6), NS = not significant P > 0.05.

Influence of L-Arg, and L-NAME on serum T-AOC:

Colorimetric assessment of the T-AOC was done in sera samples 10 days after the initial treatment. CRS significantly suppressed total antioxidant levels (0.57 ± 0.022, $P < 0.001$) when compared to the non-stressed control group (0.97 ± 0.072). Meanwhile, L-Arg + CRS (0.72 ± 0.042) ameliorated CRS-induced reduction of T-AOC when compared to CRS group ($P < 0.05$). However, T-AOC in L-Arg + CRS group was still significant when compared to the control ($P < 0.05$). L-Arg alone showed a significant increase in T-AOC (0.96 ± 0.024, $P < 0.05$) when compared with the CRS group. On the other hand, L-NAME drastically declined total antioxidant levels (0.26 ± 0.05, $P < 0.001$) compared to CRS+L-Arg and control groups which indicate a crucial role for NOS and NO in the regulation of the L-Arg antioxidant effect (Fig. 3).
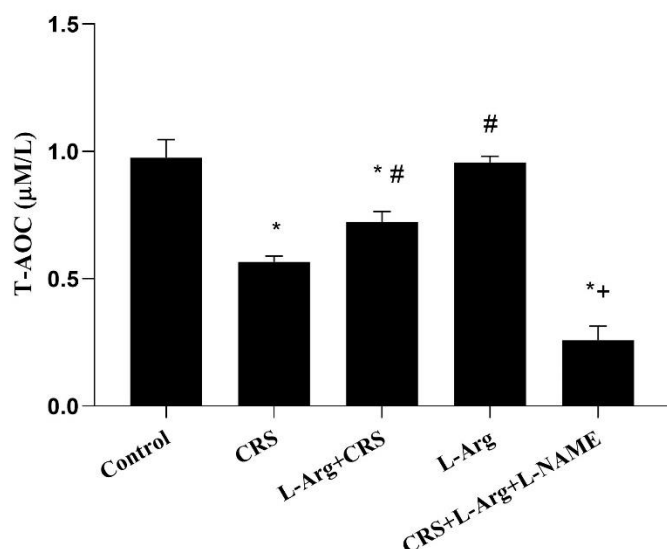
Total antioxidant capacity

Fig. 3. Colorimetric determination of Total Antioxidant capacity (T-AOC) in serum (mM/L) the day after the last injection. Data were expressed as mean ± SEM (n = 6). *P<0.05 vs control, #P<0.05 vs. CRS and +P<0.001, vs. CRS+L-Arg.

Effect of different treatment groups on catalase activity:

Catalase activity was significantly reduced in the CRS group (274.03 ± 25.14, $P < 0.05$) when compared to the control group (447.85 ± 52.70). The activity of catalase in the L-Arg group (456.71 ± 14.86) was not statistically significant when compared to the control group. However, pretreatment of stressed animals with L-Arg (CRS + L-Arg) (344.92 ± 15.18) showed non-significant increase in catalase when compared to the CRS group. In addition, CRS + L-Arg + L-NAME (327.35 ± 72.35) showed no significant differences when compared to the CRS + L-Arg group **(Fig. 4).**
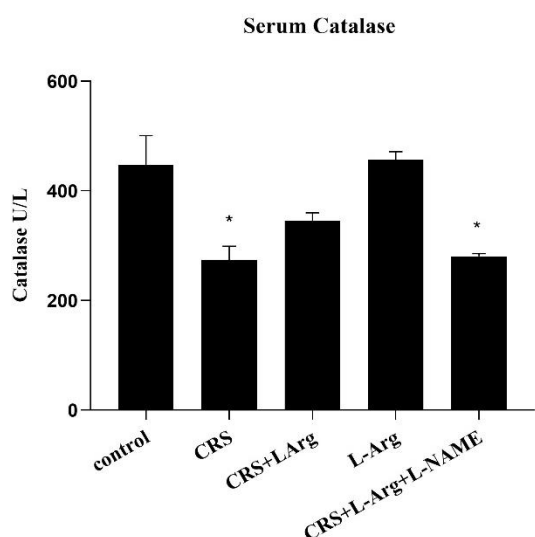
Serum Catalase

Fig. 4. Colorimetric measurement of serum catalase activity (U/L) on the last day of injection in different treatment groups. Data presented as mean ± SEM (n = 6). *$P<0.05$ vs control group.

Influence of L-Arg and L-NAME on the histopathology of stressed mice brains:

The control group that received sterile PBS showed normal nerve cells with mild perineuronal vacuolation (Fig. 5A). The brain of the CRS group showed focal microgliosis, perineuronal vacuolation, and edema in the Virchow-Robin space (Fig. 5B). CRS+L-Arg group displayed normal neurons with mild dilation in the Virchow-Robin space (Fig. 5C). The L-arginine group showed normal neurons, in addition to mild demyelination (Fig. 5D). Mild demyelination with mild neuronal death was detected in CRS+L-Arg + L-NAME (Fig. 5E).
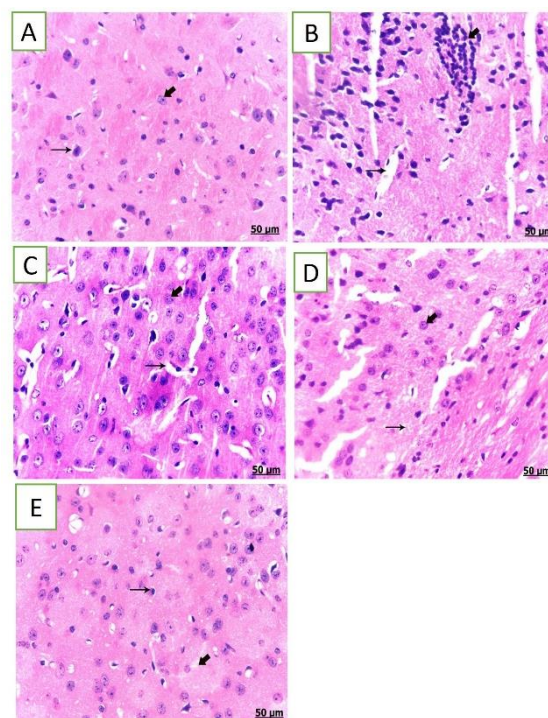

Fig. 5 (A-E). Histopathological micrographs of brain tissue in different treatment groups. (A) The control group showed normal nerve cells (thick arrow) with mild perineuronal vacuolation (thin arrow). (B) The brain of the CRS group showed focal microgliosis (thick arrow), perineuronal vacuolation, and edema in the Virchow-Robin space (thin arrow). (C) CRS+L-Arg group showed normal neurons (thick arrow) with mild dilation in Virchow-Robin space (thin arrow). (D) The brain of the L-Arg group showed normal neurons (thick arrow), in addition to mild demyelination (thin arrow). (E) L-Arg + L-NAME+ chronic resistant stress group showing mild demyelination (thick arrow) with mild neuronal death (thin arrow). (H&E., X 400).

Discussion

The present study investigated the *in vivo* effect of L-Arg administration on brain tissue and serum total antioxidant capacity as well as catalase activity of adult male mice subjected to CRS and the potential role of L-Arg and NOS, the key enzyme of NO production, on CRS-induced oxidative stress in mice. Chronic stress is caused by persistent exposure to one or more types of stressors for days to months and is related to physical, behavioral, and neuropsychiatric symptoms (Lin and Scott, 2009). The cause of illness progression is determined by the levels of antioxidant and oxidant balance. If this balance shifts in favor of the oxidants, oxidative stress may result. Antioxidant levels are critical for free radical counterbalancing.

The results showed that L-Arg groups including CRS+L-Arg, L-Arg, and CRS + L-Arg + L-NAME caused a significant reduction in body weight. However, CRS did not show a significant reduction when compared to the control group. These findings were supported by previous literature showing that L-Arg can reduce body weight, and adipose tissue (McKnight *et al.*, 2010) in addition to the ability of L-Arg to reduce insulin resistance and enhance lipolysis (Thams and Capito, 1999; Wu *et al.*, 2012). Chronic restraint stress significantly decreased total antioxidant levels in serum which was parallel to the findings achieved before by Samarghandian *et al.* (2017) and Pal *et al.* (2020) that clarifies the role of repetitive restraint stress in the reduction of total antioxidant capacity, SOD, catalase as well as GSH levels and the enhancement of MDA levels. However, the antioxidant effect of L-Arg is not always supported as L-Arg can show a decrease in catalase activity in animals without oxidative stress (Delwing *et al.*, 2002). The main mechanism of restraint stress adverse effect was due to restraint stress-induced inflammation which causes oxidative stress and the production of more free radicals (Matough *et al.*, 2012). Additionally, free radicals may damage and kill cells by destroying different cell components such as nucleic acids, proteins, enzymes, and cell membrane lipids which justify the brain pathological finding in the current study (Pandya et al., 2013). Here, we found that L-Arg could not alter CRS-down-regulated catalase activity which might be a result of an L-Arg dose-dependent effect.

It was found that pretreatment with L-Arg (500 mg/kg b.wt.) reversed changes in total antioxidant capacity in mice subjected to CRS whereas pretreatment with L-NAME (30mg/kg) reversed the ameliorative impact of L-Arg on total antioxidant capacity. These findings indicate that the NOS inhibitor, L-NAME, aggravates CRS-induced oxidative stress. Consequently, L-Arg antioxidant effect is mediated by NOS, the enzyme responsible for NO production from L-Arg. Therefore, it is concluded that L-NAME blocked the desirable role of NO which reacts with mitochondrial enzymes of respiratory chains to regulate cellular breathing and allows antioxidant function (Brüne *et al.*, 1997).

The histopathological changes in brain tissue of the CRS group in the present study showed focal microgliosis, perineuronal vacuolation, and edema in the Virchow-Robin space. these results come in agreement with Kumar and Pandey (2013), who found that the brain is more susceptible to stress due to high levels of intracellular mediators that are involved in the stress response. CRS+L-Arg group displayed normal neurons with mild dilation in the Virchow-Robin space. The L-arginine group showed normal neurons, in addition

to mild demyelination. Mild demyelination with mild neuronal death was detected in CRS+L-Arg + L-NAME. Improving the antioxidant system may be useful in combating excessive ROS production (Samarghandian et al., 2015). So that, the availability of L-Arg is a critical factor to suppress oxidative stress, inducing an endogenous antioxidant response (Liang et al., 2018) reducing inflammatory reactions (Tan et al., 2014).

Conclusion

Exposure to CRS induced a reduction in T-AOC and catalase activity. L-Arg pretreatment rescued the antioxidant activity and reduced oxidative stress in a mechanism that depends on NOS and NO production. However, the antioxidant effect of L-Arg is not mediated by catalase activity.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

References

Albaugh VL, Stewart MK, Barbul A (2017). Cellular and Physiological Effects of Arginine in Seniors, in Nutrition and Functional Foods for Healthy Aging; 317–336.

Bancroft JD, Gamble M (2001). Theory and practice of histological techniques. 5th ED. London: Churchill Livingstone; 800.

Brüne B, Götz C, Meßmer UK, Sandau K, Hirvonen MR, Lapetina EG (1997). Superoxide formation and macrophage resistance to nitric oxide-mediated apoptosis. J. Biol. Chem., 272(11): 7253–7258.

Debats IBJG, Wolfs TGAM, Gotoh T, Cleutjens JPM, Peutz-Kootstra CJ, van der Hulst RRWJ (2009). Role of arginine in superficial wound healing in man. Nitric Oxide - Biol. Chem., 21(3): 175–183.

Delwing, Débora, Delwing, Daniela, Dutra-Filho CS, Wannmacher CMD, Wajner M, Wyse ATS, (2002). Arginine administration reduces catalase activity in midbrain of rats. Neuroreport, 13(10): 1301–1304.

El-Missiry MA, Othman AI, Amer MA (2004). L-Arginine ameliorates oxidative stress in alloxan-induced experimental diabetes mellitus. J. Appl. Toxicol., 24(2): 93–97.

Forbes SC, Harber V, Bell GJ (2014). Oral L-arginine before resistance exercise blunts growth hormone in strength trained males. Int. J. Sport Nutr. Exerc., Metab. 24(2): 236–244.

Förstermann U, Sessa WC (2012). Nitric oxide synthases: Regulation and function. Eur. Heart J., 33(7): 829–837.

Fujiwara T, Kanazawa S, Ichibori R, Tanigawa T, Magome T, Shingaki K, Miyata S, Tohyama M, Hosokawa K (2014). L-Arginine stimulates fibroblast proliferation through the GPRC6A-ERK1/2 and PI3K/Akt pathway. PLoS One, 9(3): e92168.

Gulati K, Ray A (2014). Differential neuromodulatory role of NO in anxiety and seizures: An experimental study. Nitric Oxide - Biol. Chem., 43: 55–61.

Hardingham G E, Fukunaga Y, Bading H (2002). Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nature Neuroscience, 5(5): 405–414.

Hossain MS, Abe Y, Ali F, Youssef M, Honsho M, Fujiki Y, Katafuchi T (2017). Reduction of Ether-Type Glycerophospholipids, Plasmalogens, by NF-κB Signal Leading to Microglial Activation. J. Neurosci., 37(15):4074-4092.

Kim YK, Won E (2017). The influence of stress on neuroinflammation and alterations in brain structure and function in major depressive disorder. Behav. Brain Res., 329: 6–11.

Kumar S, Pandey AK (2013). Chemistry and Biological Activities of Flavonoids: An Overview Shashank. Scientific World Journal., 2013:16.

Liang M, Wang Z, Li H, Cai L, Pan J, He H, Wu Q, Tang Y, Ma J, Yang L (2018). L-Arginine induces antioxidant response to prevent oxidative stress via stimulation of glutathione synthesis and activation of Nrf2 pathway. Food Chem. Toxicol., 115:315–328.

Lin GG, Scott JG (2009). Neural Regulation of Endocrine and Autonomic Stress Responses. Nat. Rev. Neurosci., 10: 397–409.

Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J (2012). The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ. Med. J., 12(1): 556–569.

McKnight R, Satterfield MC, Jobgen, Wenjuan S, Smith, Stephen B, Spencer, Thomas E, Meininger, Cynthia J, McNeal, Catherine J, Wu, Guoyao (2010). Beneficial effects of L-arginine on reducing obesity: Potential mechanisms and important implications for human health. Amino Acids. Amino Acids, 39(2): 349–357.

Musazzi L, Milanese M, Farisello P, Zappettini S, Tardito D, Barbiero VS, Bonifacino T, Mallei A, Baldelli P, Racagni G, Raiteri M, Benfenati F, Bonanno G, Popoli M (2010). Acute stress increases depolarization-evoked glutamate release in the rat prefrontal/frontal cortex: The dampening action of antidepressants. PLoS ONE. Public Library of Science, 5(1) :e8566

Niraula A, Wang Y, Godbout JP, Sheridan JF (2018). Corticosterone production during repeated social defeat causes monocyte mobilization from the bone marrow, glucocorticoid resistance, and neurovascular adhesion molecule expression. Journal of Neuroscience, 38(9): 2328–2340.

Pal G, Behl T, Rohil V, Khandelwal M, Gupta G, Jena J (2020a). Evaluation of oxidative stress and its modulation by L-Arginine and L-Ascorbic acid in repetitive restraint stress model in Wistar rats. Obes. Med., 17: 100172.

Pandya CD, Howell KR, Pillai A (2013). Antioxidants as potential therapeutics for neuropsychiatric disorders. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 46: 214–223.

Paul V, Ekambaram P (2011). Involvement of nitric oxide in learning & memory processes. Indian J. Med. Res., 133(5): 471–478.

Pervin M, Unno K, Konishi T, Nakamura Y

(2021). L-arginine exerts excellent anti-stress effects on stress-induced shortened lifespan, cognitive decline and depression. Int. J. Mol. Sci., 22(2):1–14.

Safaripour S, Nemati Y, Parvardeh S, Ghafghazi S, Fouladzadeh A, Moghimi M (2018). Role of l-arginine/SNAP/NO/cGMP/KATP channel signalling pathway in antinociceptive effect of α-terpineol in mice. J. Pharm. Pharmacol., 70(4):507–515.

Samarghandian S, Azimi-Nezhad M, Afshari R, Farkhondeh T, Karimnezhad F (2015). Effects of Buprenorphine on Balance of Oxidant/Antioxidant System in the Different Ages of Male Rat Liver. J. Biochem. Mol. Toxicol., 29(6): 249–253.

Samarghandian S, Azimi-Nezhad M, Borji A, Farkhondeh T (2016). Crocus sativus L. (saffron) extract reduces the extent of oxidative stress and proinflammatory state in aged rat kidney. Prog. Nutr., 18(3): 299–310.

Samarghandian S, Azimi-Nezhad M, Borji A, Samini M, Farkhondeh T (2017). Protective effects of carnosol against oxidative stress induced brain damage by chronic stress in rats. BMC Complement. Altern. Med., 17(1):1–7.

Spiga F, Walker JJ, Terry JR, Lightman SL (2014). HPA Axis-Rhythms. Compr Physiol 4(3): 1273–1298.

Stamper CE, Hassell JE, Kapitz AJ, Renner KJ, Orchinik M, Lowry CA (2017). Activation of 5-HT1A receptors in the rat dorsomedial hypothalamus inhibits stress-induced activation of the hypothalamic–pituitary–adrenal axis. Stress, 20(2): 223–230.

Tan J, Liu S, Guo Y, Applegate TJ, Eicher SD (2014). Dietary L-arginine supplementation attenuates lipopolysaccharide-induced inflammatory response in broiler chickens. Br. J. Nutr., 111(8): 1394–404.

Ter Horst DM, Schene AH, Figueroa CA, Assies J, Lok A, Bockting CLH, Ruhé HG, Mocking, RJT. (2019). Cortisol, dehydroepiandrosterone sulfate, fatty acids, and their relation in recurrent depression. Psychoneuroendocrinology, 100: 203–212.

Thams P, Capito K (1999). L-arginine stimulation of glucose-induced insulin secretion through membrane depolarization and independent of nitric oxide. Eur. J. Endocrinol., 140(1): 87–93.

Wu Z, Satterfield MC, Bazer FW, Wu G (2012). Regulation of brown adipose tissue development and white fat reduction by L-arginine. Curr. Opin. Clin. Nutr. Metab. Care, 15(6): 529–538.