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L-ARGININE AGGRAVATES LIPOPOLYSACCHARIDE-INDUCED ANXIETY-LIKE BEHAVIORS AND INTERLEUKIN-6 LEVELS IN MALE ALBINO RATS

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ABSTRACT

In rodents, lipopolysaccharide (LPS, a product of Gram-negative bacteria) induces septic shock and depression. Animal studies based on the relationship of nitrergic system and the pathogenesis of such neuropsychiatric disorders is still unclear. This study was designed to find out whether the L-arginine (L-Arg)/nitric oxide (NO) pathway and the proinflammatory cytokine interleukin-6 (IL-6) have a role in anxiety and behavioral responses to systemic LPS administration in rats. The open field test (OFT) was chosen for assessment of anxiety-like behaviors and L-Arg, a NO precursor, was used to evaluate the role of nitrergic system in LPS-induced anxiogenesis. The animals were identified, weighed and randomly divided into four groups (five in each): Control group (saline, 1ml/kg b.wt), LPS group (LPS alone, 1 mg/kg b.wt), L-Arg group (L-Arg alone, 10 mg/kg b.wt for 7 consecutive days), and LPS+L-Arg group (L-Arg, 10 mg/kg b.wt for 7 consecutive days then once injected with LPS, 1mg/kg). All drugs were administered intraperitoneally (i.p.). Behavioral tests were performed 3 h after saline/ LPS injection using LE8811 Actimeter Pan LAB device. Serum IL-6 levels were measured 6 h after saline/LPS injection with test kits by enzyme-linked immunosorbent assay (ELISA) method. The results showed that acute systemic administration of LPS induced a significant increase anxiety-like behaviors as indicated by reduced frequency of central square entries and less time spent in the central region of the open field. Additionally, LPS increased serum IL-6 levels in rats. Pretreatment of rats with L-Arg aggravated the anxiogenic effects of LPS, as well as induced an increment in serum IL-6 levels. Interestingly, systemic administration of L-Arg alone caused mild anxiety-like behaviors in rats with significantly increase in serum IL-6 levels. These findings lead to the conclusion that L-Arg increases the severity of LPS-induced anxiogenesis, most likely by inducing IL-6 production in the present experimental model.

Key words*: *L-arginine, endotoxin, anxiety, interleukin-6, rats

INTRODUCTION

Anxiety is a frequent negative emotional state characterized by feelings of worry and accompanied by specific behavioral manifestations (Nuss, 2015). Hall (1934) originally described the open field test (OFT) for the study of emotionality in rats. The procedure consists of subjecting an animal to a novel environment from which escape is prevented by surrounding walls (Doukkali *et al.,* 2016). During the host response to infection, several changes in behavior occur, some of which are mediated by the central nervous system (CNS) (Dantzer *et al.,* 2008). In an effort to identify neurobiological mechanisms linking inflammatory system to neurobehavioural changes, LPS has been used to experimentally activate the innate immune system in rodents (Belzung, 1999).

Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria (Schwechheimer and Kuehn, 2015), playing a central role as potent endotoxins in the pathogenesis of septic shock (Kirsten *et al.,* 2013). LPS is considered a potent cytokine-inducer (Teeling *et al.,* 2010). Moreover, the peripheral and central administrations of LPS consistently depress general activity and social interactions in rats (Hansen *et al.,* 1998). LPS-induced behavioral changes can be divided into transient changes such as decreases in locomotor activity and food intake, and persistent changes such as depressive-like behavior and exploratory behavior deficit (Haba *et al.,* 2012).

Lipopolysaccharide (LPS) is a ligand for toll-like receptor 4 (TLR4) and initiates signaling cascades that lead to an increase in the secretion of

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proinflammatory cytokines such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor-alpha (TNF-α) by macrophages and other cells (Bluthé *et al.,* 2000). IL-6 acts as an endogenous pyrogen (Gruol and Nelson, 1997). Administration of IL-6 either peripherally or into the CNS reproduces some of the symptoms of sickness behavior, i.e., fever (Kluger *et al.,* 1995), anorexia (Plata–Salaman, 1999), and activation of the hypothalamic-pituitary-adrenal (HPA) system (Turnbull and Rivier, 1999), Schöbitz *et al.* (1995) demonstrated that centrally injected IL-6 increased body temperature and suppressed locomotor activity and food intake, whereas Bluthé *et al.* (2000) showed that endogenous IL-6 both at the periphery and in the brain participates in the development of sickness behavior.

L-arginine (L-Arg) is a common catalyzing substrate of nitric oxide synthase (NOS) and arginase (Wang *et al.,* 2011). Arginase has two isoforms: arginase I (Arg I) and Arg II. Arg I generally express in the liver and also exist in extrahepatic tissues, whereas Arg II is found in several extrahepatic tissues (Kenyon *et al.,* 2008). Arg I is increased in experimental glomerulonephritis (Kettler *et al.,* 1996) or LPS-induced inflammation (Sonoki *et al.,* 1997). Arginase, which converts L-Arg to urea and ornithine and therefore shares a common substrate with NOS, has been shown to deplete plasma L-Arg levels *in vivo* to levels that do not affect basal blood pressure (Griffith *et al.,* 1992), and in early studies on tumor inhibition was shown to have no significant toxicity. L-Arg depletion by arginase reduces NO synthesis as detected by electron paramagnetic resonance spectroscopy in a model of septic shock (Bune *et al.,* 1995).

The discovery of NO as a neurotransmitter in the brain raised the issue of its role in the function of the CNS (Knowles *et al.,* 1989). NO generated enzymatically from L-Arg by the three different is of or ms of NOS including; neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS) (Wood and Garthwaite, 1994). NO has been implicated in the regulation of various behavioral, cognitive, and emotional processes, including anxiety (Talarek *et al.,* 2017). In physiological concentrations, NO plays a neuroprotective role in the nervous system (Nahrevanian, 2009) whereas, it promotes apoptosis and cell death in high concentrations (Brown and Borutaite, 1999). It has been suggested that overproductions of NO take place due to activation of nNOS by the stimuli such as endotoxins and cytokines (Paul and Ekambaram, 2011).

The aim of the present study was to further evaluate the effects of LPS and/or L-Arg on locomotion, anxiety and exploratory activity in the open field, as well as investigating the role of IL-6 in mediating these LPS-induced behavioral changes in a rat model.

MATERIALS AND METHODS

Ethical Approval

Animal handling and experimentation were performed in line with approved Institutional Animal Care and Use Committee (IACUC#: 12-214) protocols at the South Valley University (Qena, Egypt) and complied with the ethical standards established by the Egyptian animal welfare laws and policies and followed the national authority (Ministry of Higher Education and Scientific Research, Egypt) guidelines for the detention, use and the ethical treatment of laboratory animals. Also, all animal protocols were approved by the Animal Use Subcommittee and by the Research and Ethical Review Committee of the Faculty of Veterinary Medicine, South Valley University.

Experimental animals

Adult male Wistar albino rats were originally obtained from Animal Care Facility at the Faculty of Medicine, Assiut University (Assiut, Egypt) and subsequently maintained in the Animal Care Facility at the Faculty of Veterinary Medicine, South Valley University. Animals (5 per cage) were kept in the laboratory at 22±2°C with 60% relative humidity under a 12 h light/dark cycle for 2 weeks prior to experimentation. Tap water and food pellets were always freely available.

Drugs and Chemicals

Lipopolysaccharide (LPS) isolated from *Escherichia coli* (cat# L2630, serotype 0111:B4) was purchased from Sigma Aldrich (St. Louis, MO, USA) and injections were prepared daily from 0.34 mg/ml stock solutions on the morning of injections. LPS was dissolved in sterile, endotoxin-free 0.9% saline and injected intraperitoneally at a dose of 1 mg/kg body weight (b.wt). This dose of LPS acutely induces a transient sickness behavior response followed by the development of a distinct depressive-like behavioral phenotype in the forced swim and tail suspension tests (O'Connor *et al.,* 2009). L-Arg (cat# 11009) was purchased from Sigma Aldrich (St. Louis, MO, USA) and prepared fresh daily on the morning of injections. L-Arg was diluted to 3.4mg/ml with sterile, endotoxin-free 0.9% saline and injected intraperitoneally at a dose of 10 mg/kg b.wt. All drugs were administered intraperitoneally in a volume of 0.1 ml/100 g b.wt three hours prior to testing with the exception of L-Arg which was administered 7 days prior to testing day.

Experimental Design

Twenty adult male Wistar albino rats (12 weeks old and 200-250g weight) were used in these studies. The protocol used in the present study is the same as described previously (Moustafa *et al.*, 2015). The animals were randomly divided into four experimental groups (five rats per group), and selected for intraperitoneal administration of saline/LPS or L-Arg as follows:

Group I (Control): rats were received 0.9% w/v NaCl solution (saline) (1 ml/kg b.wt).

Group II (LPS): rats were treated with LPS (1 mg/kg b.wt).

Group III (L-Arg): rats were pretreated with L-Arg (10 mg/kg b.wt), once daily for 7 consecutive days, before saline injection.

Group IV (LPS+L-Arg): LPS-treated rats were pretreated with L-Arg (10 mg/kg b.wt), once daily for 7 consecutive days, before LPS injection.

Behavioral Testing

All rats were monitored daily by research staff beginning two weeks prior to the experiment. Before testing, rats were handled once daily for one week to acclimate them to gentle manipulation. Any environmental or physical stress was avoided in order to habituate the rats to manipulation for behavioral testing. The apparatus used in behavioral testing was thoroughly cleaned after each test session with a lightly wet cloth and then dry it with a dry cloth.

Open-Field Locomotor Activity to Test Activity and Anxiety

In the 8th day, three hours after injection of the saline/LPS, all rats were assessed for locomotor activity in a novel open field environment using an infrared LE8811 Actimeter system and measured using Acti Track software (Panlab, Barcelona, Spain) (Mantha *et al.*, 2013). The anxiety-like phenotype of an individual rat's behavior was assessed by determining the amount of time during the test that was spent exploring the central area of the chamber or hugging the perimeter chamber walls (thigmotaxis) (Simon *et al.*, 1994). As figure 1A illustrates, the open field chamber consisted of a 45 cm (width) x 45 cm (depth) arena of black plexiglass enclosed with four clear acrylic walls (35 cm in height), as well as an infrared frame that produced a 16 × 16 grid of intersecting beams used to track the movement of each rat. Infrared beam-break data were used to calculate locomotor activity. Movement data were also analyzed by dividing the arena into an outer, 11.25 cm-wide periphery zone and an inner, 22.5 × 22.5 cm central zone to allow for the evaluation of thigmotaxis (Fig. 1B). Testing was conducted during the light phase between 07:30 h and 12:30 h, and the apparatus was placed in a room homogeneously illuminated at 100 Lx. Each rat was placed in the same corner of the arena when beginning the trial, and was allowed to freely explore for 5 minutes. The following behaviors were analyzed: locomotion & distance traveled in each zone, time spent moving & latency to enter the inner and center zones, number of entries into zones, and resting time in each zone. At the end of the testing, the rat was removed and returned to its home cage.

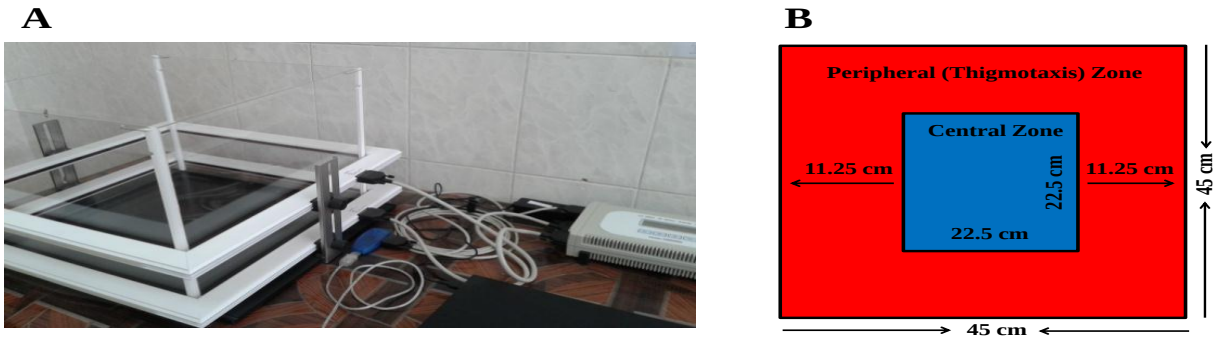

Figure 1: Apparatus used to assess anxiety-like traits. A. An open field arena apparatus. Lower frame is used to track horizontal movements (locomotion), while the upper frame tracks vertical movements (rearing). **B.** Schematic of zones in open field arena with separation of the peripheral (Thigmotaxis) zone (red) and the central zone (blue) to be used for analysis of thigmotaxis.

Serum Collection

Six hours after LPS challenge, the rats were sacrificed to collect serum. Blood was collected through cardiac puncture and centrifuged at 2,000 x g for 20 min to obtain the serum. Sera were separated and collected using dry Pasteur pipette. Serum samples were labeled and stored at -70°C to determine proinflammatory cytokine IL-6 levels as previously described (Teeling *et al.*, 2010).

Determination of serum proinflammatory cytokine IL-6 levels

Levels of proinflammatory cytokine IL-6 in the serum were measured by sandwich enzyme-linked immunosorbent assay (ELISA) using rat-specific IL-6 colorimetric kit (Thermo Scientific, Rockford, IL, USA). ELISA was performed according to the manufacturer's instructions and as previously described (Teeling *et al.*, 2010). Absorbance at 450 nm was measured using the Tecan Infinite M1000 Pro microplate reader with Magellan data analysis software (Tecan Systems, Inc). All samples and standards were measured in duplicate. Values are expressed in concentration (pg/mL).

Statistical Analysis

The results are presented as mean± standard errors of the mean (SEM); *n* represents the number of animals in each group. Data analyses were performed with Origin 6.0 (Origin Lab Corp., Northampton, MA, USA) software for Windows. Group means for all measures were compared using unpaired Student's *t* test (for two group comparisons) to verify significant differences between groups. Differences were considered statistically significant when a minimum value of *P* less than 0.05.

RESULTS

Effects of L-Arg pretreatment on the Locomotor Activity in the LPS-treated rats

Intraperitoneal injection of LPS (1 mg/kg) reduced the central and peripheral locomotor activity (3.50±0.50 and 215.20±127.81, respectively), but this did not reach significance when compared with the control (saline) group (58.60±19.82 and 303.40±204.18, respectively) and the L-Arg group (22.20±4.98 and 502.00±67.06, respectively) in a novel open field (Fig. 2A,B).

Intraperitoneal injection of L-Arg (10 mg/kg) was associated with an increase of the central and peripheral locomotor activity (22.20±4.98 and 502.00±67.06, respectively), but statistically not significant, compared to the LPS injected group (3.50±0.50 and 215.20±127.81, respectively), in this behavioral experimental model in rats (Fig. 2A,B).

Intraperitoneal injection of L-Arg (10 mg/kg) 7 days before intraperitoneal injection of 1 mg/kg LPS to rats almost completely abolished central locomotor activity (Fig. 2A) and significantly reduced the peripheral locomotor activity (105.20±44.36) compared with those injected with L-Arg alone (Fig. 2B).

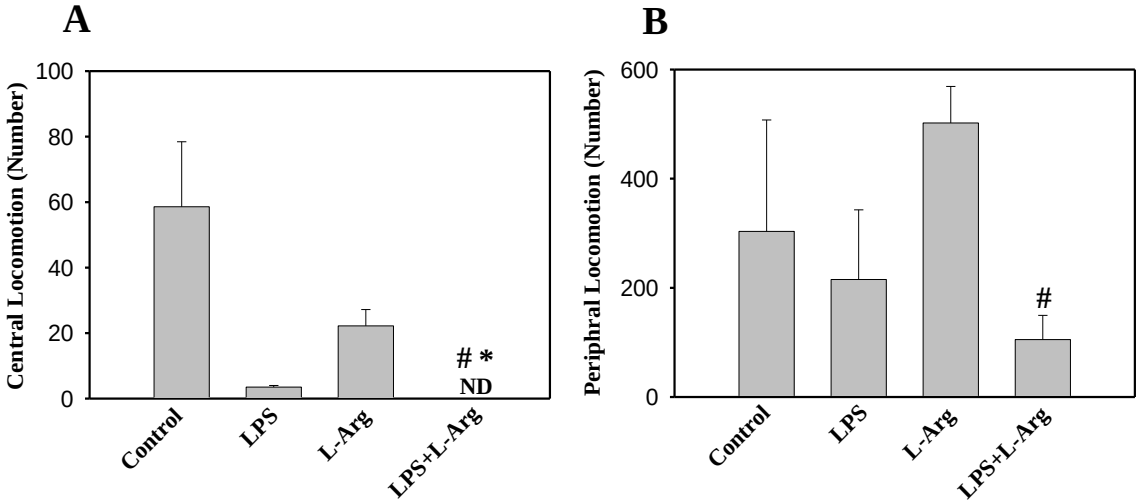

Figure 2: The rats' locomotor activities in a novel open field environment. Mean (±SEM) infrared beam breaks of control and different treated rats 3 h after saline/LPS injection over a 5 min period (*n*= 5 rats per group) in the center (**A**) and periphery (**B**) zones of the open-field chamber. ND, non detectable. *, $p < 0.05$; compared to the control (saline) group. #, $p < 0.05$; compared to the L-Arg group.

Effects of L-Arg pretreatment on the distance travelled in the LPS-treated rats

LPS reduced the traveled distance in central zone (6.22±3.96), but this did not reach significance when compared with the control group (91.82±42.93), while the reduction in the moved distance reach significance when compared with the L-Arg group (59.88±14.33) in the open field (Fig. 3A). There

were no significant differences between LPS (296.10±195.66), L-Arg (837.86±164.31), and the control (535.60±361.24) groups when the peripheral-zone traveled distance was considered (Fig. 3B).

Also, the use of L-Arg reduced the traveled distance in central zone (59.88±14.33), but this did not reach significance when compared with the control group (91.82±42.93) in this experimental behavioral model in rats (Fig. 3A).

Administration of L-Arg, for 7 days before LPS injection, markedly diminished traveled distance in central zone (Fig. 3A) and significantly reduced the traveled distance in peripheral zone (128.46±73.50) compared with those injected with L-Arg alone (Fig. 3B).

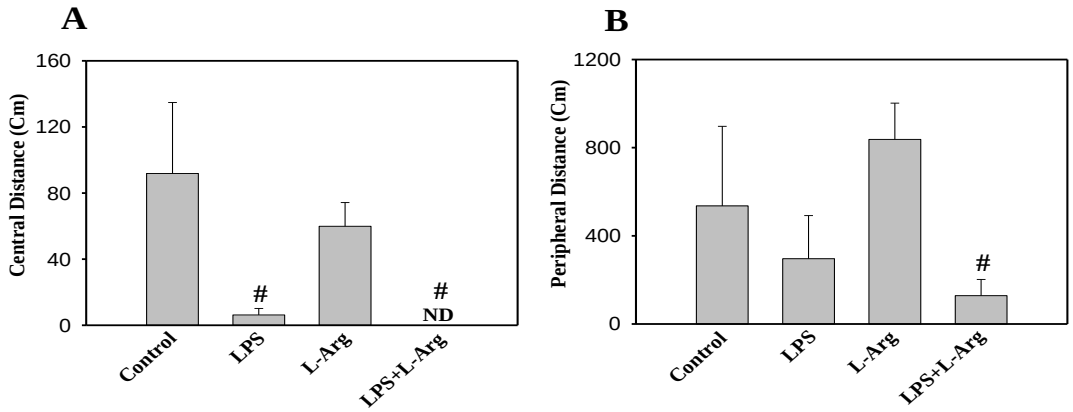

Figure 3: Distance travelled (Cm) by rats in a novel open field environment. Mean (±SEM) infrared beam breaks of control and different treated rats 3 h after saline/LPS injection over a 5 min period (n = 5 rats per group) in the center (**A**) and periphery (**B**) zones of the open-field chamber. ND, nondetectable. #, $p < 0.05$; compared to the L-Arg group.

Effects of L-Arg pretreatment on the Permanence Time in the LPS-treated rats

Administration of LPS markedly reduced the time spent in the central zone (0.20±0.13) compared with the control group (8.64±3.51) and the L-Arg group (2.36±0.66) in a novel open field (Fig. 4A). The animals of LPS group spent more time in the peripheral zone (299.80±0.13) compared to the control (291.36±3.51) and L-Arg (297.64±0.66) groups (Fig. 4B).

The treatment with L-Argalone was associated with a statistically non-significant ($p<0.06$) decrease of the time spent in the central zone (2.36±0.66), comparing with the control group (8.64±3.51), during the session of experimentation (Fig. 4A). There were no significant differences between L-Arg (297.64±0.66) and control (291.36±3.51) groups in the time spent in the peripheral zone (Fig. 4B).

Administration of L-Arg, for 7 days before LPS injection, markedly diminished time spent in the central zone (Fig. 4A) and significantly increased the time spent in the peripheral zone (300.00±0.00) compared with those injected with saline or L-Arg alone (Fig. 3B).

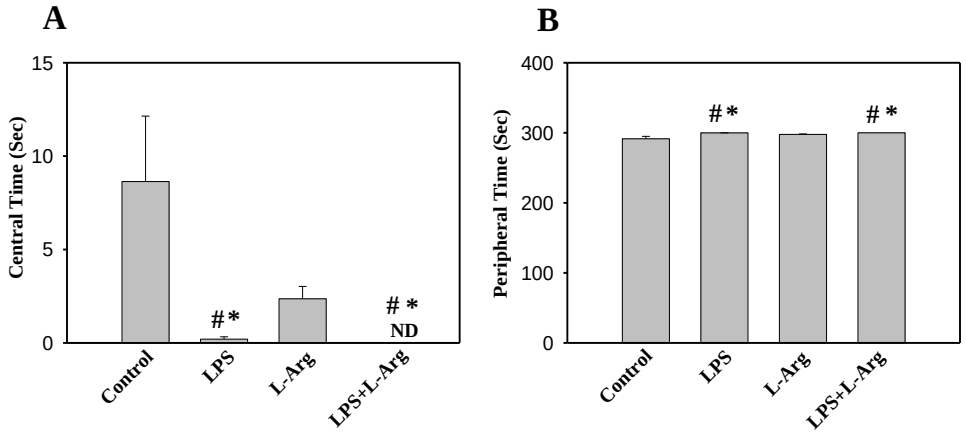

Figure 4: Time of Permanence in the different zones of the arena. Mean (±SEM) time in seconds spent by control and different treated rats 3 h after saline/LPS injection over a 5 min period (n = 5 rats per group) in the center (**A**) and periphery (**B**) zones of the open-field chamber. ND, nondetectable. *, $p < 0.05$; compared to the control group. #, $p < 0.05$; compared to the L-Arg group.

Effects of L-Arg pretreatment on the latency of first visiting a zonein the LPS-treated rats

Intraperitoneal injection of LPS markedly increased the latency to first enter the central and peripheral zones (178.00±13.60 and 461.50±7.50, respectively) compared with the control group (27.60±2.10 and 59.52±16.41, respectively) and the L-Arg group (49.56±17.43 and 77.76±13.03, respectively) in a novel open field (Fig. 5A,B).

Intraperitoneal injection of L-Arg did not determined considerable modifications in the latency to first enter the central and peripheral zones (49.56±17.43 and 77.76±13.03, respectively) comparing with the control group (27.60±2.10 and 59.52±16.41, respectively), in the experiment (Fig. 5A,B).

Intraperitoneal injection of L-Arg (10 mg/kg) 7 days before intraperitoneal injection of 1 mg/kg LPS to rats almost completely abolished entering into the central zone (Fig. 2A) and significantly reduced the latency to first enter the peripheral zone (25.92±11.31) compared with those injected with L-Arg alone (Fig. 5B).

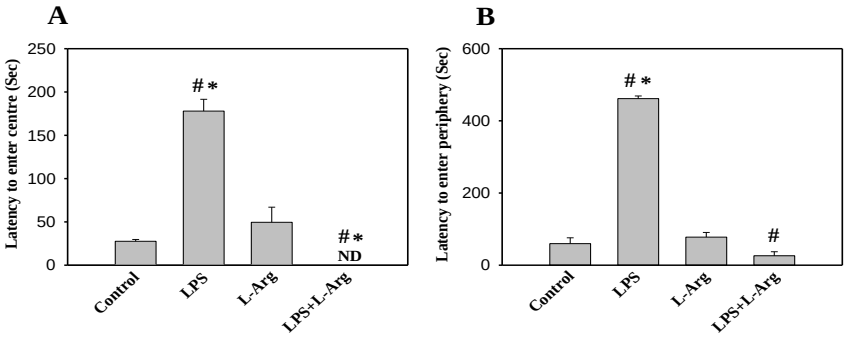

Figure 5: Duration (Sec) to first entry into the different zones of the arena. Mean (±SEM) latency in seconds to first entry by control and different treated rats 3 h after saline/LPS injection over a 5 min period (*n* = 5 rats per group) into the center (**A**) and periphery (**B**) zones of the open-field chamber. ND, nondetectable. *, $p < 0.05$; compared to the control group. #, $p < 0.05$; compared to the L-Arg group.

Effects of L-Arg pretreatment on the total and central entries in the LPS-treated rats

LPS reduced the number of entries into the center zone (0.40±0.21), but this did not reach significance when compared with the control group (11.20±5.34), while the reduction in the number of entries into the center zone reach significance when compared with the L-Arg group (5.20±1.16) in the open field (Fig. 6B). The total number of entries by the animals of LPS group (8.00±4.94) was significantly lower than that of control group (65.20±15.53) and L-Arg group (56.80±8.26) (Fig. 6A).

Intraperitoneal injection of L-Arg did not determine considerable modifications in the total number of entries (56.80±8.26) comparing with the control group (65.20±15.53), in the experiment (Fig. 6A). The treatment with L-Arg alone was associated with a statistically non-significant ($p<0.06$) decrease of the number of entries into the center zone (5.20±1.16), comparing with the control group (11.20±5.34), during the session of experimentation (Fig. 4A).

Intraperitoneal injection of L-Arg (10 mg/kg) 7 days before intraperitoneal injection of 1 mg/kg LPS to rats almost completely abolished entries into the center zone (Fig. 6B) and significantly reduced the total number of entries (15.60±9.44) compared with those injected with L-Arg alone (Fig. 6A).

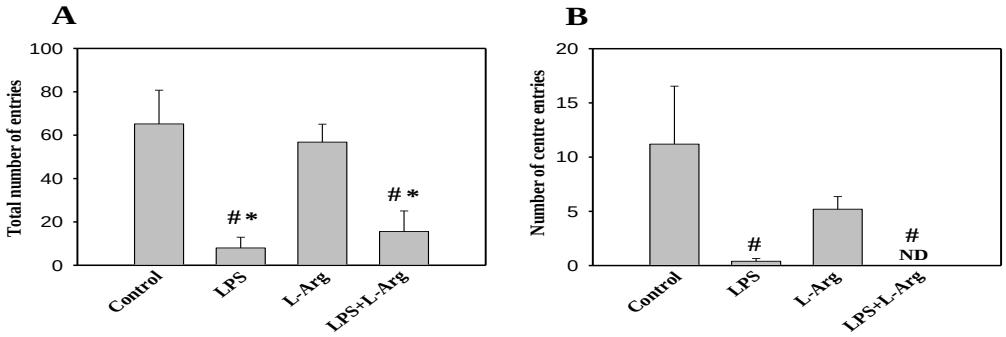

Figure 6: Numbers of entries into the different zones of the arena. Mean (±SEM) of the number of total entries by control and different treated rats 3 h after saline/LPS injection over a 5 min period (*n* = 5 rats per group) into the whole arena (**A**) and central square (**B**) of the open-field chamber. ND, nondetectable. *, $p < 0.05$; compared to the control group. #, $p < 0.05$; compared to the L-Arg group.

Effects of L-Arg pretreatment on rest time (total & in periphery) in the LPS-treated rats

Intraperitoneal injection of LPS increased the total resting time (256.64±23.13) compared with the control group (133.52±27.65) and the L-Arg group (163.40±12.93) in a novel open field (Fig. 7A). There were no significant differences between LPS (199.92±54.66), L-Arg (163.40±12.93), and the control (132.40±27.91) groups when the time spent in resting at peripheral-zone was considered (Fig. 7B).

Intraperitoneal injection of L-Arg did not determine considerable modifications in the total resting time (163.40±12.93) and in the time spent in resting at peripheral-zone (163.40±12.93) comparing with the control group (133.52±27.65 and 132.40±27.91, respectively), in the experiment (Fig. 7A,B).

Administration of L-Arg, for 7 days before LPS injection, significantly increased both total resting time (256.20±15.82) (Fig. 7A) and the time spent in resting at peripheral-zone (256.20±15.82) compared with those injected with saline or L-Arg alone (Fig. 3B).

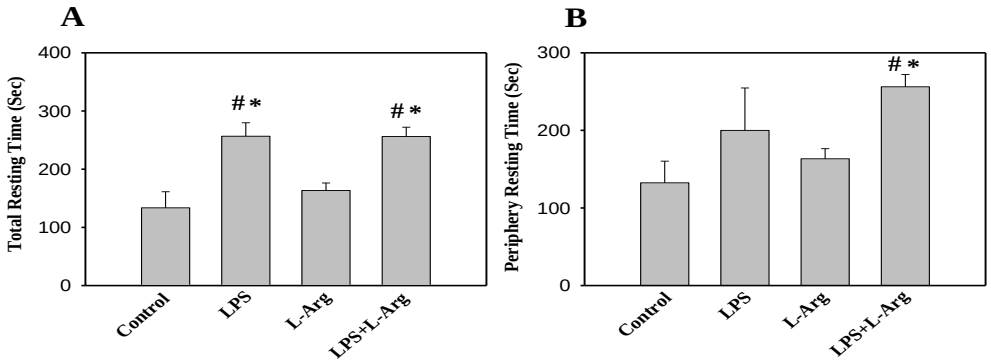

Figure 7: Resting time in the different zones of the arena. Mean (±SEM) resting time in seconds of the control and different treated rats 3 h after saline/LPS injection over a 5 min period (n = 5 rats per group) in the whole arena (**A**) and peripheral square (**B**) of the open-field chamber. *, $p < 0.05$; compared to the control group. #, $p < 0.05$; compared to the L-Arg group.

Effects of L-Arg pretreatment on serum IL-6 levels in the LPS-treated rats

Serum levels of the proinflammatory cytokine IL-6 were measured by sandwich ELISA technique and are illustrated in Figure 8. The time point chosen for this analysis (6 h after saline/LPS injection) correspond to the peak and relevant time course of circulating cytokine levels in this study model. Acute systemic LPS administration resulted in a significant increase in levels of IL-6 in serum, reaching 4279.56±465.21 pg/ml at 6 h after administration, compared to the control (saline-administered rats) group (4.66±0.87) and L-Arg group (35.74±5.44). The animals treated with L-Arg alone showed a significant ($p<0.05$) increase of the proinflammatory cytokine IL-6 compared with those injected with saline alone. However, treatment with L-Arg in LPS-treated rats (LPS+L-Arg group) did induce a significant increase in serum levels of the proinflammatory cytokine IL-6 (5252.63±1691.66) compared with those injected with saline or L-Arg alone.

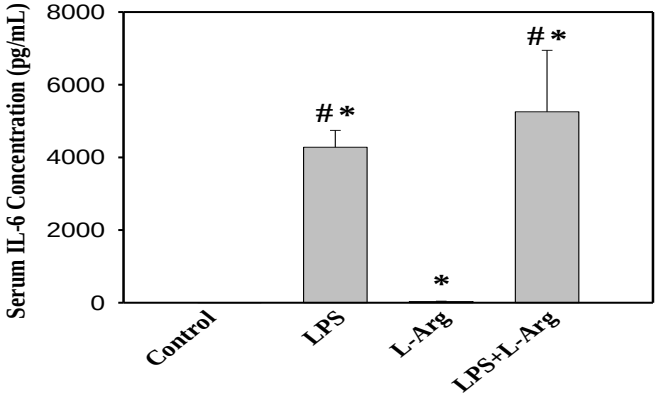

Figure8. Serum concentrations of IL-6 (pg/ml) after 6 h of saline/LPS injection. Data are expressed as means ± SEM of 5 animals per group as determined by sandwich ELISA technique. *, $p < 0.05$; compared to the control group. #, $p < 0.05$; compared to the L-Arg group.

DISCUSSION

Several studies indicate a relationship between anxiety and the immune system (Michopoulos *et al.*, 2017). Multiple immune pathways contribute to the regulation of the HPA system and other neurobiological mechanisms that modulate the behavioral responses to different stressors (Haroon *et al.*, 2012). The peripheral or central administration of LPS, the principal element of bacterial endotoxin, is frequently used to study inflammation-mediated behavioral and biochemical changes in rodents (Pintado *et al.*, 2012). In the current study, the hypothesis that peripheral immune activation leads to neuroinflammation and anxiety-like behaviors in rats were investigated. Moreover, the effects of L-Arg, a NO precursor, on LPS-induced anxiety-like behaviors were also investigated using the open field test.

The open field test is used primarily to examine motor function by means of measuring spontaneous activity in a novel open field. The animal is placed in the open fieldarena and the movements of the animal are monitored by automated computer programs (Hrnkova *et al.*, 2007). General movement, number of entries, preference for particular sections, and/or resting time can all be calculated to examine behavior and anxiety (Hart *et al.*, 2010). The open fieldarena is divided into distinct zones (inner and outer) to measure thigmotaxis (Kas *et al.*, 2008). Decreased tendency to explore the inner area of a novel arena was used as an indicator of increased thigmotaxis as an anxiety-like behavior (Lynch *et al.*, 2011).

The present data indicate that a single intraperitoneal dose of LPS into rats induced anxiogenic-like effects with locomotor impairment. The anxiogenic-like effect of LPS was indicated by a significant reduction in the time spent in the center of the apparatus and frequency of central square entries. It has been proposed that intraperitoneal injection of LPS in rats induced a robust sickness behavior and mild anxiogenic-like effects in rats (Kirsten *et al.*, 2015). In agreement with the present results, there is a report showing that acute systemic LPS administration produced slight sickness behavior in rats using the open field test (Embark, 2017). In another study, Martin *et al.* (2013) reported that LPS-induced sickness and anxiety-like behaviors were dose-and time-dependant. Furthermore, it has been reported that proinflammatory cytokines are important ininflammation-related mood disorders (Teeling *et al.*, 2010), and this in agreement with our findings that LPS caused a strong increase in proinflammatory cytokine IL-6 in the serum 6 h after injection.

The present data showed that administration of the NO precursor L-Arg alone into the rats produced mild anxiogenic effects with slight locomotor impairment. This result is in agreement with other reports showing an increase in motor activity following administration of the L-Arg (Embark, 2017). However, most reports have documented an anxiolytic rather than anxiogenic effect for L-Arg (Akar *et al.*, 2014). These results were surprise for us, as we suspected that L-Arg has anxiolytic effect.

However, surprisingly, in the present study pretreatment with L-Arg in LPS-treated rats induced a robust anxiogenic-like effect with locomotor impairment. There are no reports showing the effect of L-Arg on LPS-induced anxiety-like behaviors in animal studies. So, the present data seems to be the first to report that pretreatment with L-Arg in LPS-treated rats accentuates endotoxin-induced anxiety-like behaviors compared with those treated with saline and/or LPS alone. Furthermore, L-Arg pretreatment alone or with LPS caused a strong increase in IL-6 levels in the serum in rats. Also, these results were surprise for us, as we suspected that LPS-induced NO production as a protective agent from L-Arg, NO precursor, via NOS induction (Paul and Ekambaram, 2011).

CONCLUSIONS

The results of this study confirmed the findings of our previous research, suggesting that the NO precursor L-Arg aggravated LPS-induced neurobehavioral and neurochemical alterations in male albino rats. Taken together, these results indicated that LPS and/or L-Arg have an anxiogenic-like effect in the present rodent model. Further experiments are clearly required to determine precisely the specific neurobiological mechanisms underlying these behavioral changes in LPS-induced systemic inflammation in a rat model.

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DISCLOSURES

The author declares that there are no conflicts of interest regarding publication of this article.

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إل-آرجينين يفاقم سلوكيات القلق المستحث بواسطة عديدات السكر الدهنية ومستويات انترلوكين ٦ في ذكور الفئران البيضاء

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في القوارض، عديدات السكر الدهنية (LPS، وهو منتج من البكتيريا سالبة الجرام) تحدث صدمة إنتانية واكتئاب. الدراسات على الحيوانات على أساس العلاقة بين النظام النيتريرجك والتسبب في مثل هذه الاضطرابات العصبية والنفسية لا تزال غير واضحة. وقد صممت هذه الدراسة لمعرفة ما إذا كان مسار إل-آرجينين (L-Arg)/ أكسيد النيتريك (NO) والانترلوكين ٦ (IL-6) لهم دور في القلق والردود السلوكية على إعطاء عديدات السكر الدهنية في الفئران. وقد تم اختيار الاختبار الميداني المفتوحة لتقييم السلوكيات المتعلقة بالقلق وإل-آرجينين، سَلائِفُ أكسيد النيتريك، لتقييم دور نظام النيتريرجك في احداث سلوكيات القلق التي يسببها عديدات السكر الدهنية. تم ترقيم الحيوانات، وزنها وقسمت عشوائيا إلى أربع مجموعات (خمسة في كل مجموعة): مجموعة التحكم (محلول ملحي، ١ ملي/كغ)، مجموعة عديدات السكر الدهنية (1 ملغ/كغ)، مجموعة إل-آرجينين (إل-آرجينين ، ١٠ ملغ/كغ، لمدة ٧ أيام)، مجموعة عديدات السكر الدهنية مع إل-آرجينين (إل-آرجينين، ١٠ ملغ/كغ، لمدة ٧ أيام ثم يحقن مرة واحدة مع عديدات السكر الدهنية، 1 ملغ/كغ). تم حقن جميع الأدوية داخل الغشاء البريتوني. وقد أجريت الاختبارات السلوكية ٣ ساعات بعد حقن محلول ملحي/عديدات السكر الدهنية باستخدام جهاز (LE8811 Actimeter). تم قياس مستويات انترلوكين ٦ في مصل الدم ساعات بعد الحقن بالمحلول الملحي/ عديدات السكر الدهنية بواسطة تحليل الإليزا (ELISA). وأظهرت النتائج أن حقن عديدات السكر الدهنية يسبب زيادة في سلوكيات القلق بدرجة كبيرة كما يتضح من انخفاض وتيرة الدخول الي المربع المركزي وقلة الوقت المنقضي في المنطقة الوسطى للجهاز. بالإضافة إلى ذلك، عديدات السكر الدهنية تؤدي الي زيادة في مستويات انترلوكين ٦ في مصل الفئران. معالجة الفئران بإل-آرجينين يؤدي الي تفاقم آثار عديدات السكر الدهنية المؤدية الي سلوكيات القلق، وكما انه يسبب زيادة في مستويات انترلوكين ٦ في مصل الفئران. ومن المثير للاهتمام، حقن إل-آرجينين منفرداً يسبب سلوكيات القلق بشكل اقل في الفئران مع زيادة كبيرة في مستويات انترلوكين ٦. هذه النتائج تؤدي إلى استنتاج مفاده أن إل-آرجينين يزيد من شدة احداث سلوكيات القلق المستحث بواسطة عديدات السكر الدهنية، على الأرجح عن طريق تحفيز إنتاج انترلوكين ٦ في النموذج التجريبي الحالي.