

Modulation of glucose homeostasis in rats treated with bacterial lipopolysaccharide: Role of L-arginine counterstrategy

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Abstract

Lipopolysaccharide (LPS), an endotoxin derived from the outer membrane of Gram-negative bacteria, mediates a local or systemic inflammatory response. Its action is carried out through stimulation of Toll-like receptor 4 (TLR4) activity and subsequently, production of interleukin-1 β (IL-1 β), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and interferon-gamma (INF- γ). The purpose of this study was to assess the effect of L-arginine (L-Arg) on endocrine pancreas function in rats treated with LPS. Therefore, rats were weighed, randomly divided into 4 equal groups (20 rats/group) as each group using 4 replications (n=5) and intraperitoneally injected as follow: Control group (saline, 1ml/Kg b.wt for 7 consecutive days), LPS group (LPS, 1 mg/kg b.wt once), L-Arg group (L-Arg, 10 mg/kg b.wt, for 7 days), and LPS+L-Arg group (L-Arg, 10 mg/kg b.wt, for 7 days then once injected with LPS, 1mg/kg). Serum IL-6, glucose and insulin levels were assessed at 6, 12, 24, and 72 hours after LPS or saline injection. Histological examination of the pancreas tissue was also performed. Serum IL-6 elevated significantly 6 and 12 hours after LPS injection and retrieved at 24 and 72 hours. Association of LPS and L-Arg potentiate IL-6 production at 6 and 12 hours higher than that in L-Arg-treated group. Serum glucose levels were declined in single LPS treated rats 6 and 12 hours and recovered at 24 and 72 hours after LPS injection. On contrary, Serum glucose levels were elevated 6 and 12 hours and declined near to control level 24 and 72 h after L-Arg injection. However, insulin levels were slightly elevated 12, 24 and 72 hours after LPS injection and 12 hours after LPS+L-Arg injection the rescued around the normal values at 24 and 72 hours. On contrary, serum insulin level decrease at 6 hours after L-Arg injection then recovered to control level. Histologically, rats treated with LPS showed necrosis, vacuolation in islets of Langerhans with infiltration of inflammatory cells. Pre-treatment of L-Arg prior LPS attenuated the alteration in pancreas tissue architecture. However, Rats treated with L-Arg showed normal pancreas tissue. In conclusion, the results of this study support the hypothesized relationship between L-Arg and modulation of glucose metabolism and suggest that L-Arg may have a dual role in the modulation of glucose homeostasis depending on intensity and stage of inflammation.

Keywords: Insulin, L-Arginine, Lipopolysaccharides, Nitric Oxide, Pancreas.

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Introduction

Glucose hemostasis is a process of a balance between insulin and glucose to maintain the level of glucose in the blood under the control of pancreas that plays a key roles in control the glucose level in blood via its various hormones, glucagon produced from α -cells, and glucose-lowering hormone; insulin produced β -cells (Röder et al., 2016). Glucose hemostasis is achieved by opposing action of glucagon and insulin hormones; in hypoglycemia particularly during sleeping and in between meals, glucagon secreted from α -cells to elevate blood glucose level through hepatic glycogenolysis and hepatic and renal gluconeogenesis. On contrary, insulin secreted from β -cells under stimulation of elevation of exogenous glucose levels (Komatsu et al., 2013). Lipopolysaccharide (LPS) is a bacterial endotoxin which present in the outer layer of the cell wall of Gram-negative bacteria and causes inflammatory responses in the body (Metukuri et al., 2010). LPS causes stimulation of Toll-like receptor 4 (TLR4) in the plasma membrane of immune cells (Winnall et al., 2011). Activation of immune cells produces many inflammatory mediators such as NO, prostaglandin E2 (PGE2), tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) (Kern et al., 1995). Many deleterious changes can be caused in the body such as hypoglycemia and insulin resistance and β -cells dysfunction due to acute inflammation of the pancreas caused by LPS. In addition, the pathway of LPS-induced Pancreatitis achieved through activation of TLR-4 subsequently, stimulation of proinflammatory cytokines as TNF and IL-1 β (Garay-Malpartida et al., 2011).

L-arginine (L-Arg) is one of the most versatile amino acid that can be derived from dietary intake (approximately 4–6 g of arginine per day) (Gad, 2010). L-arginine is a source of many importance molecules of metabolic and clinical importance such as nitric oxide (NO), proline, glutamate and

creatine (Appleton, 2002, Morris, 2006); . Furthermore, it plays a decisive role in regulating different cellular processes in different organs (McConell, 2007). L-Arg modulates the action of NO and could be control the inflammation process and immune response through killing of pathogens and tumor cells, vasodilatation, neurotransmission and inhibition of platelet aggregation (Lee and Cheng, 2008). Nitric Oxide synthesis from L-Arg is catalyzed by nitric oxide synthase enzymes (NOS), which exists in three isoforms: neuronal NOS (nNOS, NOS1), inducible NOS (iNOS, NOS2) and endothelial NOS (eNOS, NOS3). Both NOS1 and NOS3 are constitutive and Ca²⁺-dependent isoforms which catalyze a continuous basal production for NO. However, NOS2 is Ca²⁺-independent isoform which induces NO production as a result of inflammatory stimuli such as bacterial endotoxin and pro-inflammatory cytokines (Förstermann and Sessa, 2012)

The current study is an endeavor to investigate the effect of L-Arg on LPS-induced acute inflammation of pancreas in adult rats and clarify the pathway by which LPS enhances pancreas inflammation and hypoglycemia and the potential role of L-Arg to mitigate pancreas dysfunction. Therefore, the current study is designed to clarify the effect of L-Arg alone or co-administered with LPS on the serum insulin and glucose levels and to evaluate the histological changes of pancreas tissue in experimental model of LPS-induced acute inflammation in adult rats.

Materials and methods

1. Ethical Approval

The Maintenance and treatment of all the animals were performed according to the guidelines of South Valley University's Animal Ethics Committee, Egypt. Animals were cared for in accordance with the Egypt National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

They were examined and adapted to the new environmental conditions for a week before the formal experiment.

2. Chemicals

Lipopolysaccharide (LPS, *Escherichia coli* (*E. coli*) serotype O111:B4) and L-arginine (L-Arg, $\geq 99.5\%$) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). All chemical reagents were of analytical grade and freshly prepared by dissolving in a physiological saline (0.9% sodium chloride (NaCl)) pyrogen-free (sterile) solution. The doses of the chemicals used for each group were calculated according to the previous studies (Arab et al., 2020, Moustafa et al., 2015).

3. Experimental animals

Eighty healthy adult male albino rats (150-200 g) of 8-10 weeks old age were purchased from the private Laboratory Animal House. Rats were moved to the Physiology Department, Faculty of Veterinary Medicine, South Valley University, Qena. They were kept in the sterile plastic cages and provided with standard rat pellet feed and tap water *ad libitum* and conserved on a 12-hour light/12-hour dark cycle at a temperature of $22\pm 3^\circ\text{C}$ and humidity of $55\pm 5\%$. Animals were adapted for one week to the experimental site.

4. Experimental design

After the end of acclimatization period, rats were randomly distributed into four groups (20 rats/group) as each group using 4 replications ($n=5$). The experimental rats were subjected to the following treatment:

Group I (Control): Rats were intraperitoneally (i.p.) injected with physiological sterile saline (0.9% NaCl) at dose 1 ml/kg body weight (BW) once per day for 8 consecutive days.

Group II (LPS-treated): Rats were i.p. injected with a single dose of LPS (1 mg/kg BW) after the end of acclimatization period.

Group III (L-Arg-treated): Rats were i.p. injected with L-Arg (10 mg/kg BW) for 7 consecutive days.

Group IV (LPS + L-Arg-treated): Rats were i.p. injected with L-Arg (10 mg/kg BW) once per day for 7 consecutive days followed by a single dose of LPS (1 mg/kg BW, i.p. in the same day).

5. Clinical signs, mortalities recording and post-mortem examination

Along the experimental period, animals were checked twice a day for uncommon clinical symptoms and mortality rates during the experimental period. In addition, the dead rats were dissected, and all organs were examined for post-mortem changes.

6. Blood samples collection

After the end of experimental time, rats were sedated with diethyl ether at 6, 12, 24 and 72 h after the last injection. Blood samples were collected from the retro-orbital venous plexus in clean dry test tube and centrifuged at 3000 rpm for 15 minutes and sera were collected in Eppendorf tubes then stored at -80°C until the evaluation of biochemical parameters. Rats will be anesthetized with an intraperitoneal injection of sodium thiopental (50 mg/kg bw) then sacrificed at 6, 12, 24 or 72 h after the last injection and their pancreases were harvested for histological examination.

7. Biochemical Estimations

7.1. Evaluation of interleukin-6 (IL-6) levels:

Measurement of serum IL-6 was performed using enzyme-linked immunosorbent assay (ELISA) kit (a specific rat IL-6 ELISA kit) according to manufacturer's instructions (Thermo Scientific, Waltham, MA, USA) catalogue NO. (BMS625). Hormonal assay was done by microplate reader (Infinit 50, Männedorf, Switzerland) at wavelength 450 nm (Arsenijevic et al., 2015).

7.2 Measurement of blood glucose levels

Blood glucose level was determined by colorimetric method (Spectrophotometry) using specific kits according to manufacturer's instructions (Spectrum diagnostics, Obour city, Cairo, Egypt) catalogue NO. (250 001). Blood glucose determination was carried out by spectrophotometer T80 UV/VIS spectrophotometer (PG Instruments, UK) at wavelength 546 nm (Tietz, 1995).

7.3 Serum insulin hormonal assay

Serum insulin levels were measured using enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer's instructions (Cal biotech, El Cajon, CA, USA) Catalogue NO. (IS130D). Hormonal assay was done by microplate reader (Infinite 50, Männedorf, Switzerland) at wavelength 450 nm (Kao et al., 1994). In addition, insulin resistance estimation carried out using homeostasis model assessment method (HOMA-IR) and was calculated using the following formula according to Majid et al. (2017):

$$\frac{\text{Serum glucose (mg/dL)} \times \text{Serum insulin (\mu U/ml)}}{405}$$

8. Histopathological examination

At the end of the experiment, the pancreas from all animals was collected according to standardized necropsy procedures (Kittel et al., 2004). Instantly, the pancreas tissue was fixed in 10% neutral buffered formalin, dehydrated in ethanol (70-100%), cleared in two changes of xylene (one hour each), processed to paraffin impregnation, and was embedded, sectioned at five-microns tissue thickness, and stained with hematoxylin and eosin (Bancroft and Layton, 2013). The slides were examined microscopically, and any histopathological alterations will be reported.

9. Statistical analysis

The data were expressed as mean \pm SEM. Differences between the groups were evaluated statistically through One and Two-way variance test (ANOVA), followed by Tukey's Post-Hoc test for multiple comparison. Significant differences were accepted when $P < 0.05$. The computer program GraphPad (ISI Software, Philadelphia, PA, USA) was used for analysis and data collection.

Results

1. Clinical manifestations

The rats were monitored after acute intraperitoneal injection of LPS, and clinical signs were recorded at 6, 12, 24, and 72 h. The onset of systemic inflammation induced by bacterial endotoxemia including reduced motor activity, diminish of food and water consumption, lethargy, piloerection, hunched posture, hyperthermia and watery or soft feces. In addition, the symptoms of neurotoxicity were observed represented by shivering and tremors of the hind limbs. Furthermore, close eyes with secretion and labored breathing with gasping were also observed indicating pain caused by acute inflammation. All the previous signs were markedly obvious in LPS and LPS+L-Arg injected rats 6 and 12 h after LPS injection however, the rats recovered partially and completely from the signs of acute inflammation after 24 and 72 h of LPS, respectively. On contrary, the rats of control and L-Arg groups appeared normally, moved freely around the whole cage, with normal food and water consumption, their eyes opened free from secretion, and they showed normal, rapid and regular respiratory rate.

2. Post-mortem examination

The dead rats among LPS- and LPS+L-Arg injected groups were dissected at 6, 12, 24 and 72 h after the last injection to figure out the gross changes caused by LPS

toxicity. Postmortem findings revealed darkening and enlargement of liver, spleen and lungs with hemorrhagic foci distributed on the lungs in addition to congestion of the testes. Moreover, characteristic findings concerning to endotoxicity were markedly observed including gaseous and milky fluid in the stomach, intestine and urinary bladder. Controversially, saline- and L-Arg- rats were dissected at the end of the experimental period showed normal organs without any change.

3. Mortality rates

Percent death of the experimental rats were recorded in control and treated groups at 6, 12, 24 and 72 h after the last injection (Fig. 1). In LPS-treated rats, the mortality rate was 5% after 6 h of LPS injection and the percent elevated to be constant at 10% after 12, 24 and 72 h. Similarly, LPS+L-Arg injected rats showed mortality rate 5% after 6 and 12 h of LPS injection with marked increased to 10 and 15% at 24 and 72 h after injection, respectively. However, all rats of control and L-Arg-treated groups survived along the experimental period with mortality rate 0%.

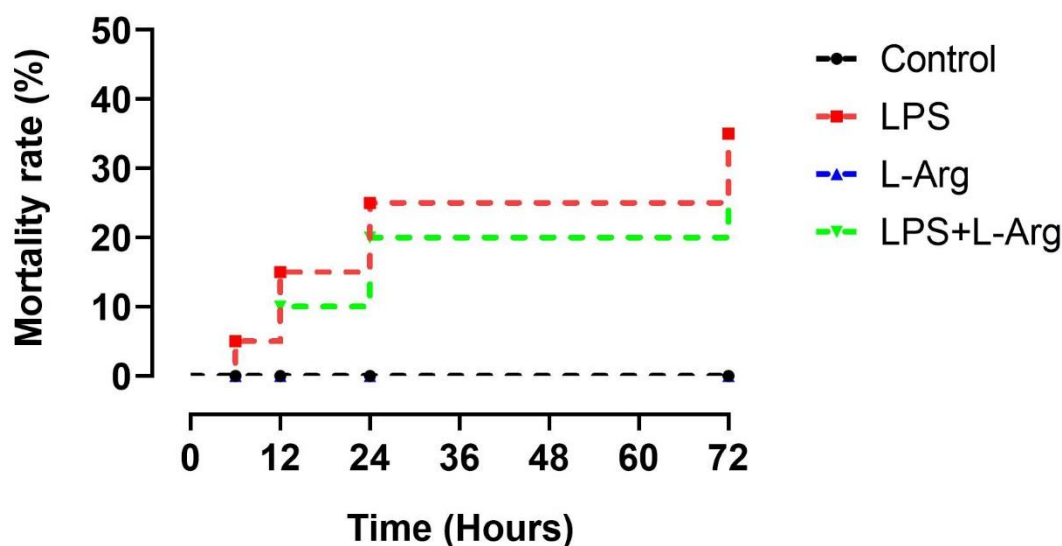


Fig. 1. Mortality rates of experimental rats of different treated groups at 6, 12, 24 and 72 h after the last injection. In: Control, LPS-treated (LPS), L-Arg-treated (L-Arg) and LPS with L-Arg-pretreated (LPS + L-Arg) groups. Values expressed as a percentage (%) of dead rats compared with the initial group (n = 20).

4. Serum interleukin-6 (IL-6) level

Fig. 2 illustrates the serum level of pro-inflammatory IL-6 (pg/mL) in control and treated groups 6, 12, 24 and 72 h after the last injection. LPS injection initiated an obvious significant ($P < 0.05$) elevation of IL-6 serum level (3703.30 ± 364.39 and 223.30 ± 41.66 , respectively) after 6 and 12 h from the last injection compared with control rats (83.26 ± 1.35 and 90.43 ± 4.67 , respectively). However, this elevation frustrated (96.14 ± 4.82 and 96.16 ± 2.15 , respectively) at 24 and 72 h to near the

control values (85.81 ± 3.24 and 89.10 ± 3.28 , respectively). Likewise, L-Arg administration for 7 consecutive days demonstrated no change in serum IL-6 6 h after the last injection (122.87 ± 17.94) compared with control. Surprisingly, serum IL-6 level elevated (107.52 ± 5.03 , 116.40 ± 12.36 and 114.19 ± 8.67 , respectively) significantly ($P < 0.05$) compared with control after 12, 24 and 72 h of the last injection. On contrary, serum IL-6 level decreased significantly ($P < 0.05$) in the same group after 6 and 12 h compared with LPS-treated group. Similarly,

association of LPS and L-Arg injection, raised the serum IL-6 levels (587.77 ± 108.50 and 183.07 ± 27.36 , respectively) after 6 and 12 h significantly ($P < 0.05$) compared with corresponding control and L-Arg groups. Conversely, serum level of IL-6 decreased significantly ($P < 0.05$) 6 h after last injection compared with LPS-treated group. Furthermore, serum level of IL-6 raised (114.56 ± 0.63) significantly ($P < 0.05$) 24 h after the last injection compared with the control and LPS groups

5. Glucose levels in blood

Fig. 3 discloses the serum glucose level (mg/dL) in all experimental rats at 6, 12, 24 and 72 h after the last injection. Single injection of LPS alone or associated with L-Arg lowered serum glucose level non-significantly at 6, 12 and 24 h after last injection (106.03 ± 2.55 , 122.01 ± 20.13 and 157.85 ± 29.47 , respectively for LPS group) and (109.89 ± 10.58 , 118.51 ± 13.97 and 133.16 ± 17.69 , respectively for LPS+L-Arg group) compared with control (138.30 ± 17.63 , 150.27 ± 21.99 and

184.73 ± 48.84 , respectively). However, serum glucose level rescued and showed non-significant elevation in both groups at 72 h (171.34 ± 30.30 and 181.91 ± 42.16 , respectively) compared with control (156.33 ± 15.20). On contrary, injection of L-Arg for 7 successive days revealed non-significant and significant ($P < 0.05$) increase of serum glucose level after 6 and 12 h (181.22 ± 22.60 and 248.99 ± 33.56 , respectively) compared with control. Moreover, serum glucose level increased significantly ($P < 0.05$) after 6 and 12 h (181.22 ± 22.60 and 248.99 ± 33.56 , respectively) compared with LPS. Predictably, serum glucose level recovered at 24 and 72 h after last injection (176.01 ± 40.58 and 157.23 ± 18.40 , respectively) around the control values (184.73 ± 48.84 and 150.27 ± 21.99 , respectively). Meanwhile, the serum glucose level in LPS-L-Arg group decreased (109.89 ± 10.58 and 118.51 ± 13.97) significantly ($P < 0.05$) after 6 and 12 h, respectively of the last injection compared with L-Arg-injected group.

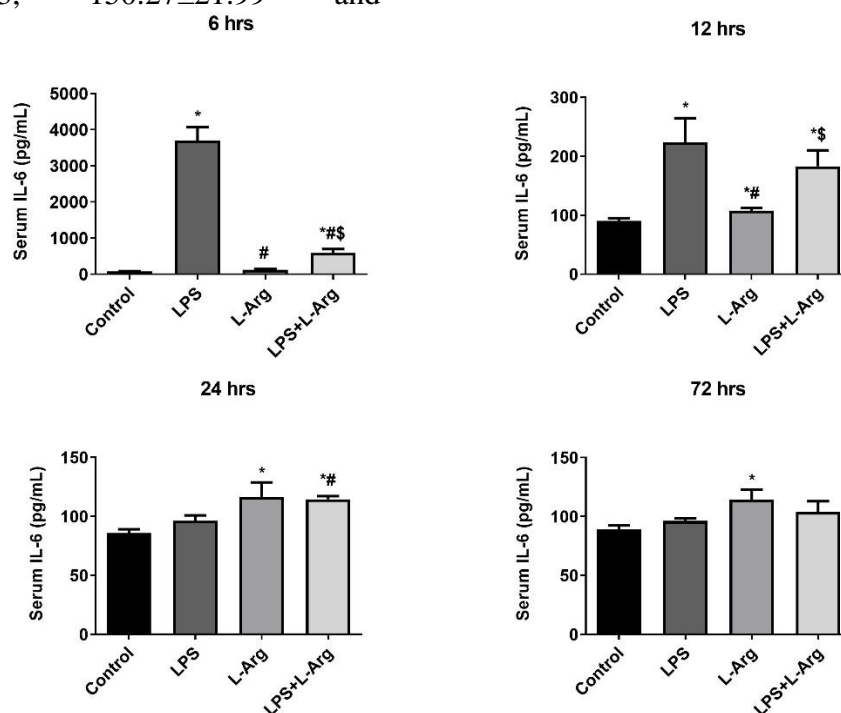


Fig. 2. Serum IL-6 level (Pg/mL) at 6, 12, 24 and 72 h after the last injection in control and treated groups. LPS-treated (LPS), L-Arg-treated (L-Arg) and LPS with L-Arg-pretreated (LPS + L-Arg) groups. Data are expressed as the mean \pm SEM ($n = 3-5$, One-way ANOVA) * $P < 0.05$, vs. Control, # $P < 0.05$, vs. LPS, and \$ $P < 0.05$, vs. L-Arg.

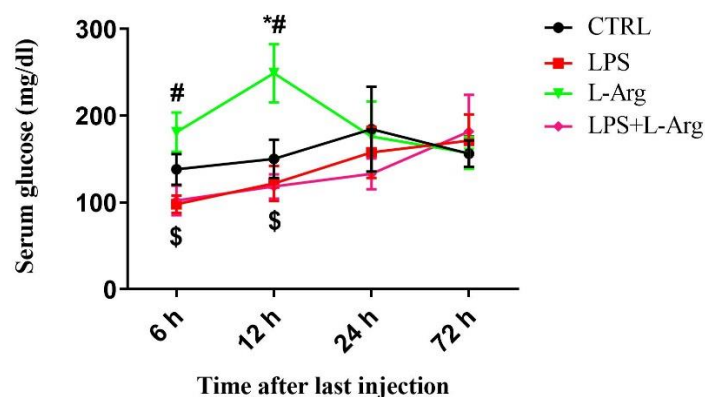


Fig. 3. The serum glucose levels at different time points (6, 12, 24 and 72 h) after the last injection in control and treated groups. LPS-treated (LPS), L-Arg-treated (L-Arg) and LPS with L-Arg-pretreated (LPS + L-Arg) groups. Data are expressed as the mean \pm SEM (n = 3-5, Two-way ANOVA) *P < 0.05, vs. Control, #P < 0.05, vs. LPS, and $^{\$}$ P < 0.05, vs. L-Arg

6. Serum insulin level

Serum insulin level (μ IU/mL) was evaluated in all experimental rats at 6, 12, 24 and 72 h after the last injection as clarified in figure 4. Serum insulin level disclosed non-significant increase after 6 h in all treated groups (LPS, L-Arg and LPS+L-Arg) (80.91 \pm 5.23, 75.83 \pm 16.02 and 80.62 \pm 12.39, respectively) compared with control (67.24 \pm 5.51). In addition, after 12 h, administration of LPS only or associated with L-Arg induced non-significant elevation of serum insulin level (91.60 \pm 10.40 and 98.90 \pm 13.03, respectively) compared with control (71.79 \pm 12.25). However, L-Arg initiated non-significant reduction in serum insulin level (58.46 \pm 10.49) at the same time point compared with control. Constantly, Serum insulin level increased non-significantly in LPS, L-Arg and LPS+L-Arg after 24 h (106.90 \pm 13.08, 87.49 \pm 19.35 and 85.31 \pm 11.00, respectively) and 72 h (103.82 \pm 20.68, 77.80 \pm 19.32 and 82.75 \pm 27.39, respectively) compared with control (76.30 \pm 11.58 and 65.03 \pm 7.87, respectively).

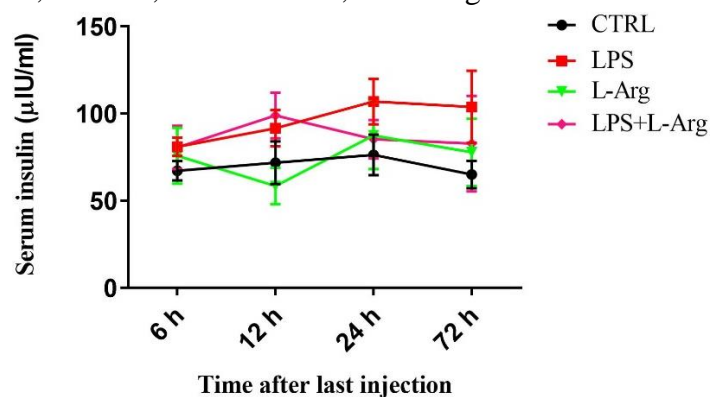


Fig. 4. The serum insulin levels at different time points (6, 12, 24 and 72 h) after the last injection in control and treated groups. LPS-treated (LPS), L-Arg-treated (L-Arg) and LPS with L-Arg-pretreated (LPS + L-Arg) groups. Data are expressed as the mean \pm SEM (n = 3-5, Two-way ANOVA).

Fig. 5 showed HOMA-IR values of the different treated groups. LPS-injected group showed HOMA-IR values close to the control in all time points. However, L-Arg-treated group revealed non-significant elevation of HOMA-IR values compared with control and LPS groups after 6 and 12 h then recovered after 24 and 72 h. Moreover, LPS+L-Arg group disclosed variation in HOMA-IR values in different time points; there was no change compared with control and LPS groups after 6 and 12 h. However, HOMA-IR values declined

then elevated non-significantly after 24 and 72 h, respectively.

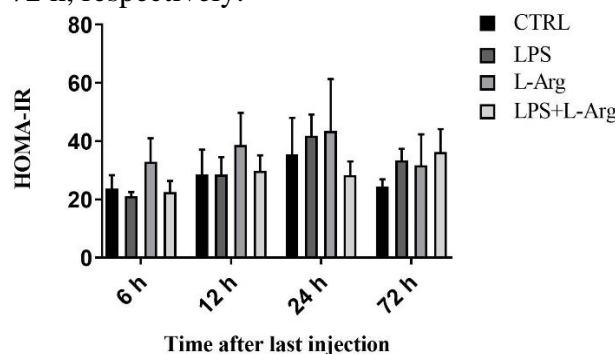


Fig 5. HOMA-IR values in control, LPS, L-Arg and LPS+L-Arg groups. Data are expressed as the mean \pm SEM (n = 3-5, Two-way ANOVA).

7. Histopathological findings

The pancreas tissue samples were collected 6, 12, 24 and 72 h (each, n = 5) after the last injection and immediately processed for histopathological examination under a light microscope. The microscopical examination of pancreas tissue of the control group which injected with saline at different time points showed apparently normal pancreas tissues with normal Langerhans cells. Since the pancreas is divided into lobules separated by connective tissue septa. Lobules are composed largely of grape-like clusters of exocrine cells called acini. Besides, the

endocrine cells termed Langerhans cells which consisted of β -cells, alpha and delta cells as shown (Fig. 6a, 6b, 6c and 6d) for 6, 12, 24 and 72 h, respectively. While the pancreas of the LPS-treated group exhibited severe necrosis and vacuolation of β - cells of the islets of Langerhans with infiltration of inflammatory cells after 6 and 12h after last injection (Fig. 7a & 7b, respectively). Also, edema with perivascular inflammatory cells infiltration was noticed 24 h (Fig. 7c), in addition to dilatation and thickening of wall of the blood vessels after 72 h (Fig. 7d). The pancreas of the L-Arg-treated group showed apparently normal pancreas tissues exhibited by normal pancreas acini at 6 h (Fig. 8a), normal intralobular ducts at 12 h (Fig. 8b), besides few inflammatory cells' infiltration at 24 and 72 h (Fig. 8c & 8d, respectively). LPS+L-Arg-treated group recorded mild pathological changes of the pancreas characterized by mild vacuolation of B-cells of the islets of Langerhans at 6 and 12 h (Fig. 9a & 9b, respectively). Intralobular ducts were appeared minimally dilated. Besides few inflammatory cells' infiltration was detected at 24 h (Fig. 9c). In addition to there was moderate congestion and hemorrhage of the blood vessels at 72 h (Fig. 9d).

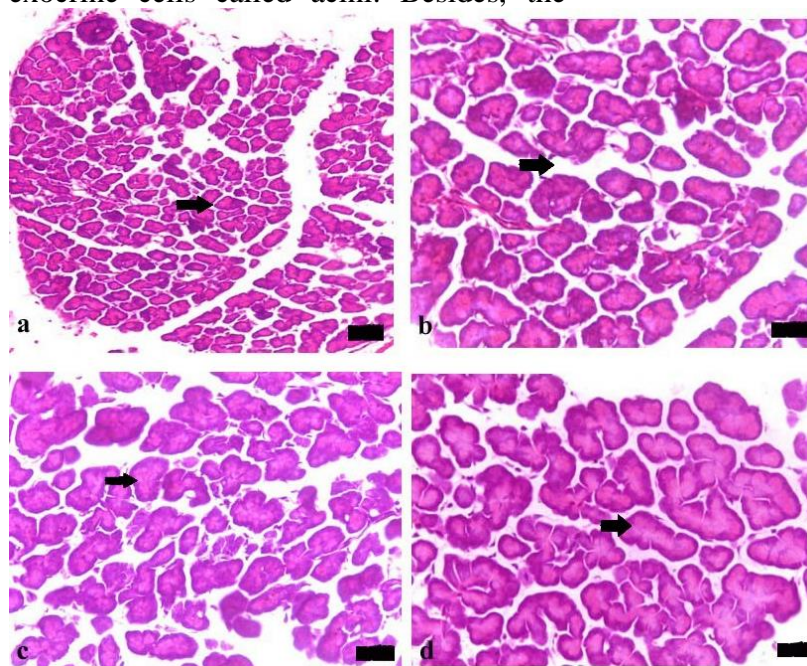


Fig. 6 (a-d):

Photomicrograph of the pancreas of the control group showing normal pancreas tissues with normal Langerhans cells after 6 h (a). High power showing normal intralobular ducts after 12 h (b), in addition to intact pancreas acini after 24 and 72 h (c and d, respectively). (H&E, X 100 & 400).

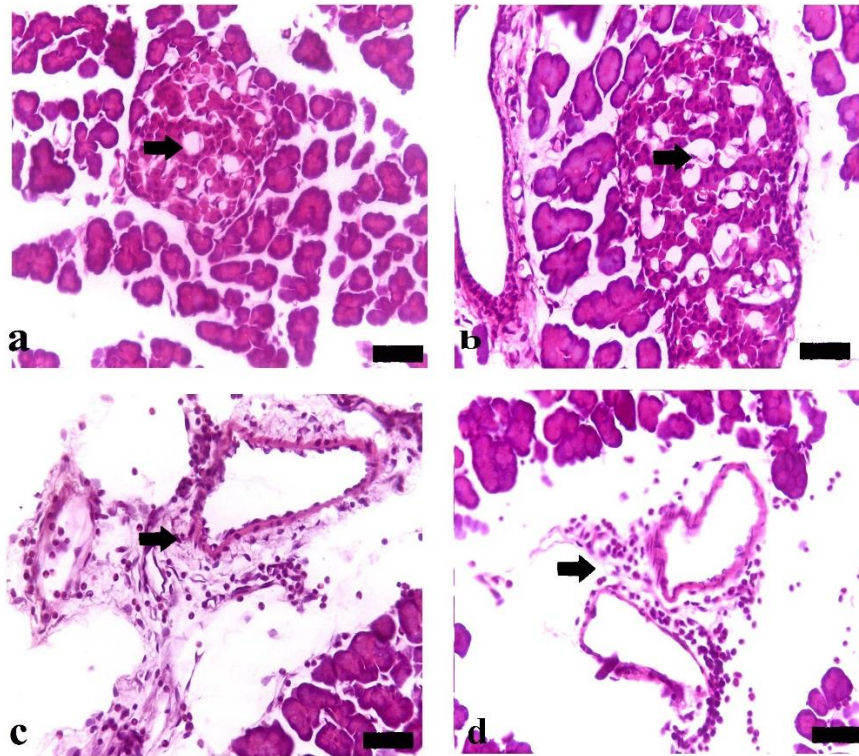


Fig. 7. Photomicrograph of the pancreas of the LPS-treated group showing severe necrosis and vacuolation of β - cells of the islets of Langerhans at 6 and 12 h after LPS injection (a & b, respectively), edema with perivascular inflammatory cells infiltration at 24 h (c), in addition to dilatation and thickening of wall of the blood vessels at 72 h (d). (H&E, X 400).

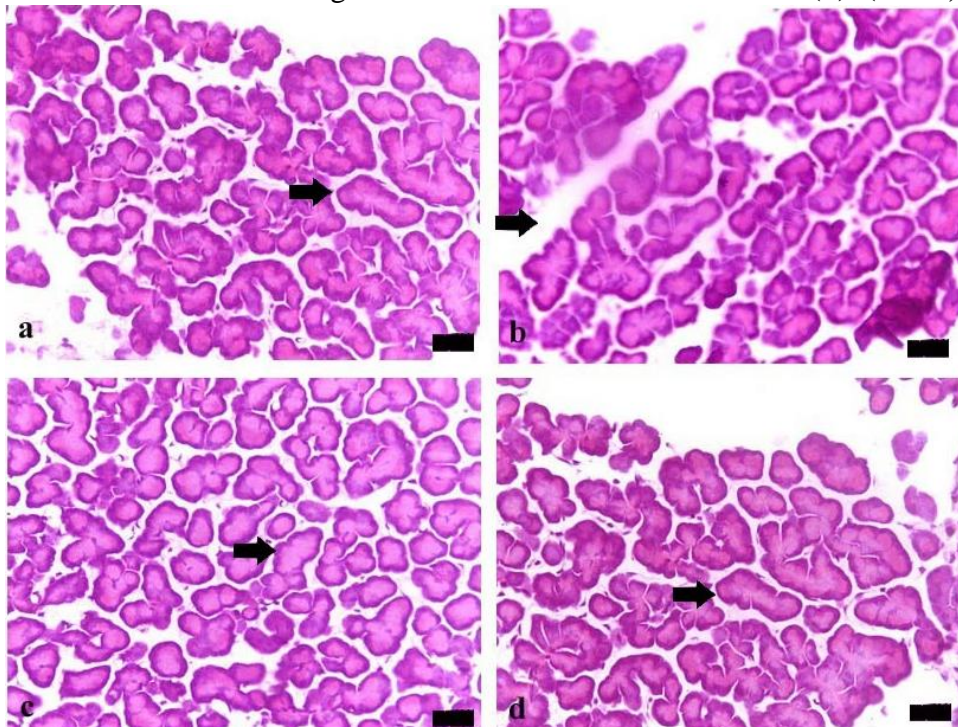


Fig. 8. Photomicrograph of the pancreas of the L-Arg-treated group showing normal pancreas acini at 6 h (a), normal intralobular ducts at 12 h (b), besides few inflammatory cells' infiltration at 24 and 72 h (c & d). (H&E, X 400).

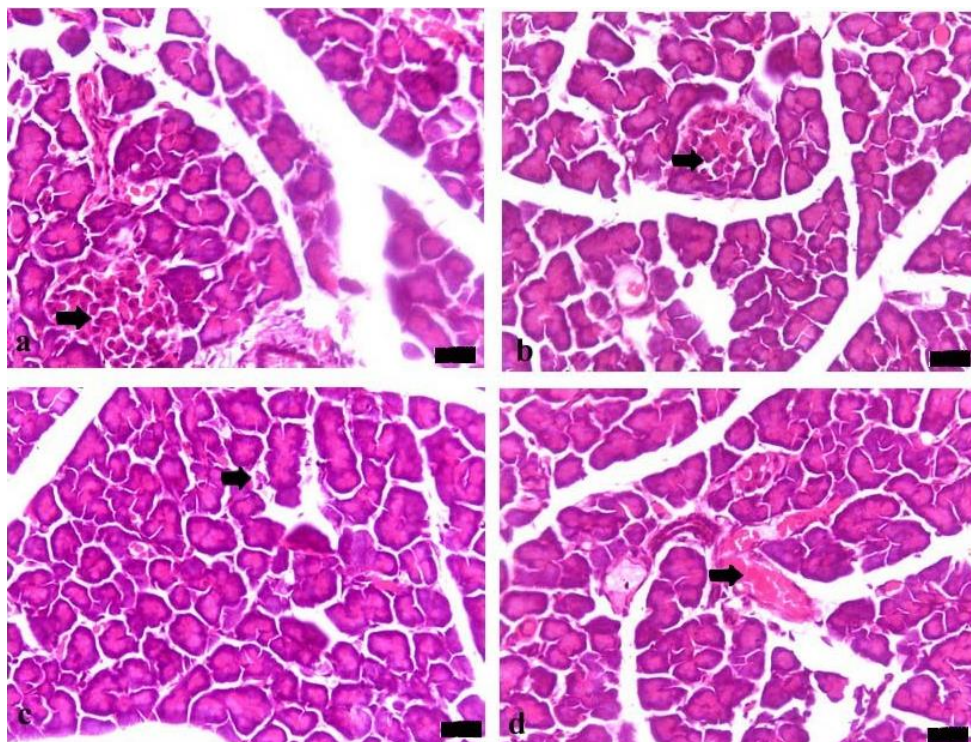


Fig. 9. Photomicrograph of the pancreas of the LPS+L-Arg group showing mild vacuolation of B- cells of the islets of Langerhans at 6 and 12 h (a & b, respectively), few inflammatory cells infiltration at 24 h (c), in addition to moderate congestion and hemorrhage of the blood vessels at 72 h (d). (H&E, X 400).

Discussion

The current study is an endeavor to elucidate the effect of lipopolysaccharide (LPS) alone in form of a single dose (1 mg/kg BW, i.p) or associated with L-arginine (L-Arg) (10 mg/kg BW, i.p.) which is nitric oxide (NO) donor on the general health conditions, pancreas function and blood glucose level in endotoxemia model rats.

Inflammation is a complex biological process takes place in response to deleterious effect on tissue homeostasis induced by biological or chemical stimulus (Wong et al., 2016). Under above mentioned stimuli, the innate immune system initiates a regulatory role to control inflammation-induced adverse effect (Bradford et al., 2015). LPS is a glycolipid located at the outer membrane of gram-negative bacteria and considered the most

important inducer of immune system by activation of Toll-like receptor 4 (TLR4) in the presence of the cluster of differentiation 14 (CD 14) (Yermak et al., 2020). Thus, LPS-induced inflammation opposed by proinflammatory mediators such as IL- β , IL-6, TNF and nitric oxide (NO) (Cherng et al., 2007). Herein, single injection of LPS induced marked elevation of IL-6 6 and 12 h post injection that is supported by aforementioned results. Interestingly, association of LPS and L-Arg exacerbated expression of IL-6 compared with administration of L-Arg alone. L-Arg is NO donor under the regulation of nitric oxide synthase enzymes (NOS), endothelial NOS (eNOS), neural NOS (nNOS) and inducible NOS (iNOS). Inducible NOS is a regulator of NO production from L-Arg under immune system stimulation, besides, NO production under the control of iNOS is thousands compared with that produced by other nitric oxide synthase enzymes.

Therefore, the high concentration of cellular NO is a potential contributor for LPS-induced inflammation (McConnell, 2007). Further, huge amount of NO enhances macrophages to secrete inflammatory mediators and production of reactive oxygen species (ROS) enhancing aggravating the inflammatory process (Ogilvie et al., 2000). On contrary, the injection of L-Arg alone was associated with low level of IL-6 after 6 and 12 h from the last injection compared with that produced after LPS alone or combined with L-Arg. Indeed, NO produced in low concentration as a result of L-Arg administration only; in such case, NO is a potential anti-inflammatory (Tripathi et al., 2007). The previous studies are matching with the current findings as NO plays a pivotal pro- and anti-inflammatory roles depending on its location and concentration. Therefore, the excessive NO production results in tissue damage and alteration of physiological function while, low NO cellular concentration act as anti-inflammatory and prevent tissue damage (Iwata et al., 2020).

The present experimental data reported transient disturbance of glucose homeostasis represented by decrease of serum glucose level 6 and 12 h after single LPS injection. In accordance with Toda et al. (2020), the acute exposure to LPS enhanced a hypoglycemia and insulin resistance. In addition, LPS-induced hypoglycemia is caused by cessation of glucose production via inhibition of hepatic glycogenolysis and gluconeogenesis through nuclear factor Kappa B (NF κ B), TLR4 and MYD88 pathway (Ghosh and Karin, 2002). In fact, the role of the aforementioned pathway in LPS-induced hypoglycemia owing to the inhibition of glucocorticoids and glucagon hormones (Waltner-Law et al., 2000). Besides, LPS induced transient inhibition of glucose producing enzymes; glucose-6-phosphatase (G-6-P) and phosphor-phenylpyruvate

carboxy kinase (Feingold et al., 2012). Furthermore, the proinflammatory cytokines particularly IL-6 that associate LPS administration are a potential contributor in induction of hypoglycemia by depletion of hepatic gluconeogenesis and exhaustion of hepatic glycogen storage (Metzger et al., 2004). Moreover, NO as proinflammatory mediator plays an essential role in hypoglycemia occurred after LPS injection as it inhibits glycogen breakdown and gluconeogenesis in the liver (Anavi et al., 2013). Thus, the current data in accordance with the previously mentioned explanation. Interestingly, co-administration of LPS and L-Arg denoted similar effect after 6 and 12 h or slightly synergized hypoglycemia after 24 h from the last injection compared with LPS only. Co-administration of LPS and L-Arg is associated with massive amount of NO regulated by iNOS, as mentioned before, high concentration of NO has a synergetic effect to LPS producing similar or powerful hypoglycemia.

The cellular stress resulted from inflammation has been confirmed to be the pathological pathway in insulin resistance and type 2 diabetes induction (Wellen and Hotamisligil, 2005). Concordantly, the current experimental data showed elevation of serum insulin level after LPS injection, and this elevation attenuated 24 h after co-administration of LPS and L-Arg. However, serum insulin level dropped transiently 6 h after the last injection of L-Arg injection only which increased the insulin sensitivity and decreased its resistance. Subsequently, L-Arg-NO pathway modulating glucose homeostasis, promoting lipolysis, maintaining hormone levels and ameliorating insulin resistance (Hu et al., 2017). In accordance with Toda et al. (2020), the serum insulin level raised after LPS-induced endotoxemia which ending with insulin resistance. Further, LPS-induced insulin resistance developed under the regulation of IL-10 produced by adipose

tissue macrophage under inflammatory stress. Additionally, TLR4, MYD88 and NF κ B cell signaling pathway is involved in insulin resistance and hyper insulinemia caused by acute exposure to LPS (Raetzsch et al., 2009). Insulin resistance was postulated in a systemic counter-regulatory response to LPS-induced hypoglycemia. Consequently, insulin resistance may be linked to the proinflammatory cytokines production or counter-regulatory hormones such as catecholamines, glucagon and glucocorticoids (Hotamisligil, 1999). As described before, association of LPS and L-Arg resulted in production of high amount of NO under the regulation of iNOS which is considered a potential contributor factor in development of insulin resistance by tyrosine nitration causing insulin desensitization (Pilon et al., 2010).

LPS-induced inflammation extended to pancreas tissue with alteration of pancreas architecture. Acute pancreatitis developed after single LPS injection and pancreas tissue characterized by necrosis, vacuolation and swelling of β -cells of islets of Langerhans with infiltration of inflammatory cells. The current histological findings are matching the results of Sayed and Abdel-Kader (2013) who reported the adverse effect of LPS on pancreas tissue. The former deterioration of pancreas tissue may be attributed to the oxidative stress and ROS production by LPS. ROS is the main cause of the damage of cellular building block including DNA, protein and lipid ending with cell death and apoptosis (Moskalev et al., 2013). Also, it is postulated that vacuolation and swelling and bursting of β -cells of islets of Langerhans is a powerful contributing mechanism in elevation of serum insulin level after inflammatory process. Surprisingly, pre-administration of L-Arg rescued the alteration of pancreas tissue and attenuated the adverse effect of LPS also, the pancreas tissue revealed normal histological structure after L-Arg injection.

L-Arg has ameliorative and protective effect against the oxidative stress through the induction of antioxidant enzymes activity such as glutathione peroxidase and superoxide dismutase (Hosseini et al., 2012, Tripathi et al., 2010). Furthermore, L-Arg has anti-apoptotic properties, increase the level of total thiol and ascorbic acid associated with decrease of lipid peroxidation, carbonyl content, serum cholesterol and pro-oxidant enzyme as well as xanthine oxidase enzymes (Tripathi et al., 2009).

Conclusion

The results of this study support the hypothesized relationship between L-Arg and modulation of glucose metabolism and suggest that L-Arg may have a dual role in the modulation of glucose homeostasis depending on intensity and stage of inflammation. In addition, the microscopic examination of pretreated group with L-arginine (L-Arg) in LPS injected rats help in decrease adverse effects of LPS on pancreas tissue and protected against acute pancreatitis.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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