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L-ARGININE DOWN-REGULATED TNF-ALPHA GENE IN LPS INDUCED INFLAMMATION IN EXPERIMENTAL RATS.

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ABSTRACT

Lipopolysaccharide (LPS) induced the over- expression of a large amount of inflammatory mediators such as TNF-alpha and IFN-gamma which induced the transcription of inducible nitric oxide synthase (iNOS). L-Arginine modulates the action of NO and could be control the inflammation process. This study was conducted to elucidate the importance of L-Arginine as immunomodulator upon intraperitoneal injection of rats with potent inflammatory inducer, LPS and to deduce the exact mechanism by which L-arginine modulate or activate the immunity system either through induction of nitric oxide synthase (NOS) or arginase pathway. To evaluate these objectives, 5 groups, were intraperitoneally injected as follow; Control group), protection group: injected with 10 mg/kg L-Arginine for 7 days then once injected with 4mg/kg LPS, treatment group: injected with LPS followed by L-Arginine, Inhibition group: injected sequentially with LPS, L-Arginine and 10mg/kg NG nitro L-Arginyl methyl ester (L-NAME), and finally induction group injected with LPS only. Cytokines IL-6 and IL-1b levels were estimated by using ELISA while TNF alpha was estimated by qPCR. Furthermore, hepatic, renal functions and oxidative stress parameters were estimated colorimetric. Our results showed that, the level of total plasma nitrite was increased in both treatment and protection group and decreased in the inhibition group. Moreover, the level of pro-inflammatory cytokines (IL-1B, IL-6 and TNF

alpha) were increased in the both induction and inhibition group and decreased in the treatment and protection groups. Altogether, L-Arginine plays an important role as an immunomodulator through NO-iNOS metabolic pathway.

KEYWORDS: L-Arginine, Lipopolysaccharide, Pro-inflammatory cytokines, L-NAME, TNF-alpha, NO.

INTRODUCTION

L-Arginine is a semi-essential amino acid in human and weaning rats (Barbul, 1986) .It has received much attention in the past decades mostly for being the sole precursor for nitric oxide (NO) synthesis; the latter regulates numerous biological functions (Osorio et al, 2000; Boger et al, 2001). Intestinal epithelial membrane arginine transport is essential in maintaining arginine homeostasis as dietary arginine accounts for 80% of arginine production (Yu et al, 1996) .Like all other amino acids, intestinal arginine absorption occurs via discrete amino acid transporter systems (Stevens, 1992). The absorbed arginine is utilized either locally with enterocyte or systemically via systemic circulation in various tissues. Major arginine metabolic pathways in enterocyte include (1) conversion of arginine to NO via nitric oxide synthetase (NOS) and (2) degradation of arginine to urea or ornithine by arginase.

During inflammatory insult, the early phase is characterized by the conversion of L-Arg by NOS to trigger high-output generation of NO in the millimolar range, exerting protective actions in mammalian tissues due to its cytostatic and cytotoxic antimicrobial activities towards pathogens. Nitric oxide has been shown to mediate the expression of tumor necrosis factor-alpha (TNF-α), a well-known cytokine, which seems to induce the accumulation of neutrophils by indirect mechanisms involving the macrophage, and induces the production of other cytokines [Rodriguez-pena et al, 2004; Appelberg R.et al,1992]. Upon reperfusion, TNF-α has been shown to act as a continuous stimulant for polymorphonuclear neutrophil infiltration in the liver, a critical event in ischemia and reperfusion (I/R) injury [Suzuki S et al,1994]. TNF-α has also been suggested to stimulate chemokine synthesis in ischemic tissues, and to activate nuclear factor-κB (NF-κB), a transcription factor involved in the regulation of genes related to the inflammatory response (Frangogiannis NG et al,2007).NO donors administered during the ischemic event appear to suppress TNF-α (L´opez-Neblina F, et al, 1996].

Inflammation is the major and complex reaction of the body against infection upon tissue injury. It consists of recruitment and activation of leukocytes and plasma proteins at the site of infection to eliminate the infectious agent (Kindt et.al. 2004). The infectious microorganisms, after gaining bodily access to the site of injury, cause local inflammation (Lundberg, 2010). The local inflammatory response is later accompanied by a prominent systemic response known as acute phase response {APR} (Male et.al. 2006).

This response is marked by the induction of fever, anorexia, solmonescence, lethargy, increased synthesis of hormones such as adrenocorticotropic hormone {ACTH} and hydrocortisone (Willey et.al. 2008) increased leukocytosis and altered production of large number of proteins in the liver [34]. Those proteins whose levels change during inflammation are termed acute phase proteins {APP}(Zheng et.al. 1995) .Many bacterial components and products such as peptidoglycans, lipoteichoic acid, exotoxins, lipoproteins and glycolipids can initiate the local inflammatory processes. (Paul et al,, 2008).

Lipopolysaccharide (LPS),a cell wall component of Gram-negative bacteria, is a well-known activator of macrophage to initiate Toll-like receptor 4 (TLR4)associated innate immune response (Dziarskietal.,2000) in which processes the production of inflammatory cytokines including necrosis factor-a (TNF-a),interleukin-1b (IL-1b), interleukin-6 (IL-6) and nitric oxide (NO) (VanSnick,1990; Medzhitov and Janeway,1997). Thus, these cytokines are recognized as excellent biomarkers for inflam mation (Yangetal.,2011; Chiangetal.,2012; Huangetal.,2012).LPS acts as the most important patterns recognized by TLR4 during the host defense responses to pathogens (Palsson-McDermott and O'Neill,2004). Upon forming the complex with CD14 ,LPS trigger serial signal transduction events which lead to the activation of NF- kB and MAP kinases such as ERK,JNKandp38.Theactivation of these proteins leads to the production of various inflammatory cytokines including TNF-a, IL-1b andIL-6,which are all essential for the eradication of the infectious microorganisms (Zhangand Dong, 2005) and ,on the other hand, contribute to the pathogenesis of inflammatory diseases(Huo etal.,2012).

LPS can also cause iNOS expression in Kupffer cells and hepatocytes of the liver (Duval et al., 1996; Rockey and Chung, 1996; Roland et al. 1996) Consequently, there is a potential for large amounts of NO to be generated in the liver during sepsis and this could impair hepatic function by directly injuring cells (Darley-Usmar et al. 1995; Szabo et al. 1996; Li and Billiar, 1999).

LPS also triggers the synthesis of reactive oxygen species, such as superoxide in the lung(Demling et al. 1986; Milligan et al. 1988),liver(Bautista and Spitzer, 1990) and kidney (Zurovsky and Gispaan, 1995; Faas et al., 1998). NO and superoxide react spontaneously to form the potent and versatile oxidant peroxynitrite (ONOO2). This highly toxic species reacts with lipids, proteins, DNA, and GSH and leads to their destruction (Stamler et al. 1992; Pryor and Squadrito, 1995) Furthermore, subsequent to the bacterial invasion, many cell types residing in the mucosa or skin may produce molecules important in controlling infections (Kindt et.al. 2004).

Among the important resident host cells are the mast cells, popularly known for their stores of histamine, serotonin (Moshage, 1997) and also for containing preformed TNF-α and various cytokines(Arnett et.al. 2010) such as interlukin-1b {IL-1b} and interlukin-6{IL-6}which have profound behavioral, neuroendocrine and metabolic effects (Rich et.al. 2008). The concentration gradient of various tissue products released activates the vascular system and the cells of inflammation. These responses in turn are associated with production of more cytokines and other inflammatory mediators which diffuse to the extracellular fluid compartment and circulate in the blood (Ceciliani et. al. 2002).

The aim of this study was to know if L-Arginine activates or modulates the immunity system of the rat upon LPS intraperitoneal injection. The second objective was to deduce the exact mechanism of the action of L-arginine on immune system modulation either through the induction of NOS or by arginase pathway.

MATERIAL AND METHODS

Reagents

L-Arginine was purchased from WinLab, UK. L-NAME (NG nitro L-Arginyl methyl ester) and *Salmonella typhimurium* (LPS) were purchased from Sigma-Aldrich-USA. L-Arginine, L-NAME and LPS were dissolved in 0.9% NaCl at concentrations 10mg/kg, 10mg/kg and 4mg/kg body weight, respectively, and stored at 4 °C.

Animals

A total of 30 male Sprague-Dawley rats weighing from 110-134 gm were obtained from the animal house of the Department of Histology, Faculty of Medicine, and University of Alexandria. All rats were housed in cages with free access to a regular diet and tap water for

one week prior to handling. The animals were kept under conventional conditions of temperature and humidity with 12 hours light-dark cycle. All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals, National Institutes of Health.

Experimental protocols

Rats were randomly divided into the following groups;

Group 1: Control group, animals were intraperitoneally (IP) injected with 0.5ml, saline.

Group2: L-Arginine + LPS (protection group): Animals were IP injected with L-Arginine (10mg/kg body weight) for seven days then in the 8th day they were IP injected by LPS (4mg/kg body weight).

Group3: LPS+ L-Arginine (Treatment group): Animals were intraperitoneally injected with LPS (4mg/kg body weight) then after 10 they were reinjected with L-Arginine (10mg/kg body weight). Group4: L- Arginine + LPS+ L-NAME (Inhibition group): Animals were IP injected with L-Arginine (10mg/ kg body weight) then injected with LPS (4mg/kg body weight) and injected with L-NAME (10mg/kg body weight), sequentially. Finally, group 5: LPS only (Induction group): All animals were intraperitoneally injected with LPS (4mg/kg body weight).

After 12 hours from the last injection, animals were decapitated then blood and tissues were collected, serum was isolated from blood and liver homogenate were prepared according to (Oser et al, 1965).

1-Routine Biochemical parameters

Liver Function test: Alanine aminotransferase (ALT) and aspartate aminitransferase (AST) were determined in serum according to the instructions of commercial kits purchased from Diamond (Egypt).

Renal function test: creatinine was determined in serum according to the instructions of commercial kits purchased from Randox (France) while urea level was determined according to the instructions of commercial kits purchased from Biocin (Egypt).

2-Prooxidant/ antioxidant parameters

Lipid peroxidation was determined in the form of malonaldehydehyde according to Tapel et al, (1959) the concentration of serum malonaldehyde calculated by the following equation Concentration of Malonaldehyde (nmol/ml)=At X 0.156

While for tissue homogenate the concentration was calculated by the following equation Concentration of malonaldehyde (nmol/ g wet tissue) = At X 0.156X 10

The reduced glutathione was measured by the method of Paglia et al (1967). The plasma protein level was precipitated by adding 2ml TCA (5%) to 0.5 ml plasma then the mixture was centrifuged at 4000 rpm for 15 min at 4 °C.

One milliliter of supernatant was added to 500ul of Ellman's reagent (0.0198% DTNB in 1% sodium citrate) and 3 ml of 0.2 M of potassium phosphate buffer, pH 8, and incubated for 10 min. The absorbance of yellow color was read against blank at 412 nm.

The superoxide dismutase (SOD) activity was assessed by (Markuland method ,1974). The autooxidation of pyrogallol leads to formation of superoxide that will be decreased in the presence of SOD, The percentage inhibition of pyrogallol was calculated by using this equation;
100- (At /min/ml sample)/ (Ar/min/ml reference) X 100.

The SOD concentration in the samples was computed with standard curve as ng/ml. One unit of SOD activity is defined as amount of the enzyme that inhibits the rate of auto oxidation of pyrogallol by 50%.

3- Inflammatory Parameters

NO was determined in serum and tissue homogenate according to the method of Montogomery et al (1961). Hundred micro liter of plasma was added to one milliliter of sulphanilamide and incubated for 5 min, then one hundred microliter of N-(1-naphthyl) ethylenediamine was added and stand for 20 min at room temperature. The absorbance was against blank at 540 nm. The plasma reduced nitric oxide was measured by the following equation as uM= At / As X concenteration of standard of sodium nitrite.

The Hepatocytes cytokines (IL-1b and IL-6, which purchased from Ray Biotech, USA) were measured by (ELISA) according to the manufacture instructions.

4- TNF alpha expression level

Approximately, 30mg of liver tissue were homogenized in guanidium thiocynate buffer using sterile syringes and needles. RNA isolation was taken place according to Gene jet purification column instructions. Synthesis of cDNA takes place in sterile nuclease free tube, 5ul template RNA (total RNA) added to 4ul of 5X reaction buffer, 0.5ul of RNase inhibitor and 2ul of dNTP mixture, RNA was reversely transcriped by using moloney murine leukemia virus reverse transcriptase (Revert Aid H minus, Fermentas, Canada). The mixture was incubated for 60 min at 42°C for transcription and the reaction stopped at 70°C for 10min.PCR was performed by using 1 U of Taq- Polymerase (Maxima probe, Fermentas, Canada) 0.2uM dNTP and 100pmol of each primer, in total volume 25ul in Rotor gene 6000 corbett thermocycler (Qigagen, Germany) the GADPH primers were sense; TGAAGGTCGGAGTCAACGGATTTT, antisense; CATGTGGGCCATGAGGTCCACCAC while the TNF primers were sense; AT GAGCACAGAAAGCATGATCC and antisense; GAAGATGATCTGAGTGTG. The PCR conditions was denaturation at 95°C for 15sec., annealing at 60°C for 30s and extension at 72°C for 30s for 40cycles.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using primer of Biostatistics (Version 5) software program. Significance of mean ±SD was detected groups by the multiple comparisons Student-Newman- Keuls test as p 0.05.

RESULTS

Routine Biochemical Parameters

LPS injection increased serum ALT and AST activities by 458.8% and 644.4%, respectively ($p \leq 0.05$). The blood levels of creatinine and urea increased in the induction group when compared to the control group by 169.3% and 61.3%, respectively.

The intraperitoneal injection by L-arginine as curative or prophylactic agent showed preventive effect for hepatotoxicity and nephrotoxicity which produced from LPS injection where it resulted in notice reduction in the levels of AST, ALT and creatinine but it elevated the urea level ($p \leq 0.05$).

In the Inhibition group where intraperitoneal injection of L-NAME with LPS and L-Arginine leads to positive increment in the serum level of AST and ALT by 122.8% and 397.4% respectively compared to the control group.

Also the serum level of creatinine and urea where elevated in the inhibition group compared to the control group by 76.15% and 91.6% respectively as shown in figure 1.

The Intraperitoneal injection of the rats by L-Arginine showed a protective effect against LPS hepatotoxicity and nephrotoxicity. In the protection group and treatment groups, L-Arginine decreased AST and ALT by 35.8%, 23.45% and 12.42%, 12.37% respectively ($p<0.05$). The level of creatinine in the protection group was decreased by 7.23% and 8.9%. The level of urea increased in the protection and treatment group by 85.72% and 124.56% respectively as shown in figure 1.

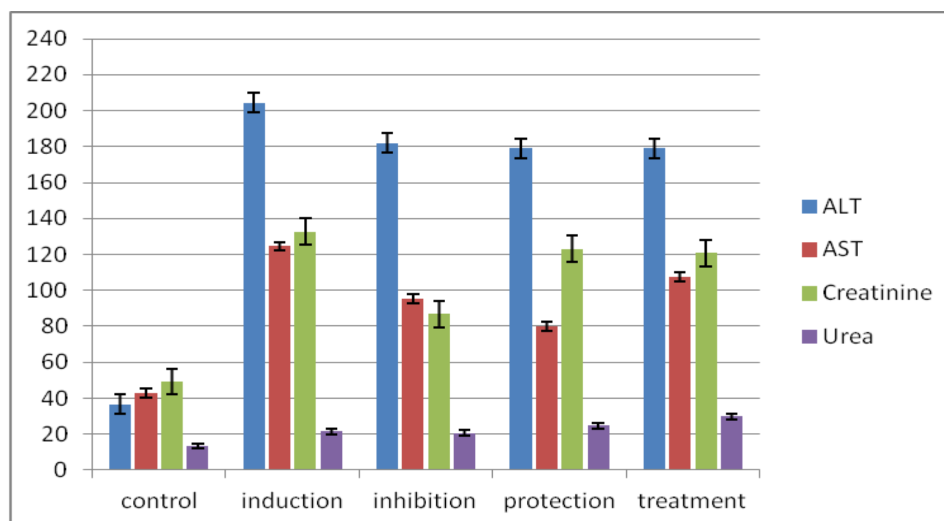

Figure 1: Serum Routine Biochemical analysis in different treated group.

Table 1: Serum and tissue oxidative stress parameters in different animal groups.

Groups	TBARS		GSH		SOD
	serum (nmol/ml)	Liver (nmol/g wet tissue)	Serum (mg/dl)	Liver (mg/g wet tissue)	Serum (ng/ml)
Control	1.24±0.02	1.298±0.2	12.12±3.03	13.37±1.8	16.3±0.47
Induction	3.43±0.03*#	10.8±0.04*#	47.4±6.3*#	47.75±6.44*#	22.5±1.25*#
Inhibition	2.96±0.59*#	4.39±0.042*#	36.3±3.03*#	36.39±3.1*#	22.7±0.39*#
Protection	1.42±0.15*#	1.68±0.29*#	23.2±4.6*#	22.97±4.74*#	19.7±0.3*#
Treatment	1.57±0.042*#	2.21±0.04*#	20.2±4.6*#	25.04±4.73*#	18.1±0.3*#

LPS administration resulted in positive increment in the level of TBARS by176.8% in serum and 732% in the liver tissue which associated with elevation in the level of GSH by 291.1% in serum and 257.1% in the liver tissue compared to the control group.

The level of GSH also was elevated in the inhibition group by 299% in serum and 272% in the liver tissue LPS administration showed an elevation of serum superoxide dismutase

(SOD) by 38.0% ,39.26% compared to the control group in both induction and inhibition groups respectively.

L-Arginine act as anti oxidants and anti-inflammatory agent because it was obviously decreased the level of TBARS and normalized GSH and SOD activity in both serum and liver tissue during protection or treatment.

Serum inflammatory markers (IL-1b, IL-6 and Nitrite Level)

NO was increased progressively in all treated group that that of control one. On one hand, protection with Arginine showed the highest NO level. On the other hand, the treatment with it showed NO level higher than that of induction group, at $p<0.05$, as showed in figure 2.

The level of TNF alpha in the induction group increased by 30.1% compared to the control group. The level of TNF alpha decreased upon injection with L-Arginine before (protection group) or after (treatment group) administration of LPS by 19.06% and 22.07% respectively, compared to the induction group. However, this decrease in levels of TNF alpha was inhibited by the inclusion of L-NAME with L-Arginine in the inhibition group (table 2.).

Consequence over expression of TNF lead to production of another cytokines such as IL 1B and IL 6 which increased markedly in the induction group as shown in figure 2. The protection or treatment with L-Argnine significantly decreased IL 1B by 13.4% and 6.4% respectively, and IL-6 by 66.25% and 65% respectively when compared to the LPS induced group. This decrease in levels of IL-1b and IL-6 was inhibited by the inclusion of L-NAME in the inhibition group compared to both of the treatment and protective groups.

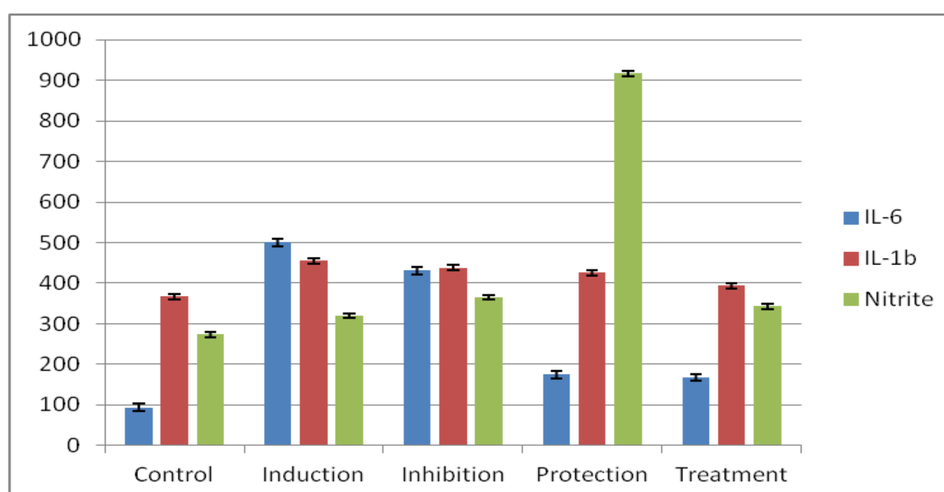

Figure 2: **Serum inflammatory markers in different treated group.**

Table 2: Gene expression of TNF alpha in different animal groups.

Group	(TNF alpha (ug/ml)
Control	10.97±0.028
Induction	14.27±0.014*#
Inhibition	12.54±0.014*#
Protection	11.55±0.025*#
Treatment	11.12±0.033*#

DISCUSSION

Lipopolysaccharide (LPS or endotoxin) a component of Gram-negative bacteria cell walls is associated with tissue injury and sepsis. One of the major features of endotoxic shock is the induction of nitric oxide synthase in the liver (Billar et al.1990). Inducible nitric oxide synthase (iNOS) induced by cytokines and LPS produces nitric oxide (NO) in large amounts (Vos et al,1997). NO is known to be a crucial factor in acute inflammation and sepsis. *In vivo*, NO has protective effects in inflammation and endotoxemia induced hepatic injury (Harbrecht et al,1994). In addition, in response to endotoxin, proinflammatory cytokines including interleukines (IL-6 and IL-1b) and TNFa and anti-inflammatory cytokines such as IL-10 are produced by inflammatory cells. In the liver, LPS activates the resident macrophages, which results in cytokine release (Aono et al,1997). Furthermore, LPS is cleared by the liver, mainly by Kupffer cells.Bacterial LPS (endotoxin) induces extensive damage to a variety of organs, especially tissues rich in cells of the reticuloendothelial system such as liver and spleen (Sugino *et al*, 1987), due to the increased production of reactive oxygen intermediates and a resultant rise in lipid peroxidation (Kono et. al. 2003; Matsuda et. al. 1998). In the liver, Kupffer cells (fixed macrophage) are the major targets of LPS, which are activated by LPS attack and consequently produce excessive amounts of O2•– (Bautista et. al. 1990). Additionally, LPS induces migration of activated polymorphonuclear leukocytes (PMNs) into the liver (Levy et. al. 1967), which constitutes another source of free radicals. Thus, severe oxidative stress is induced in liver by LPS.

This concept was in agreement with the results of the present study, where the level of TBARS increased 176.8% in plasma and 732% in the liver tissue when compared to the control group levels. These results emphasized the previous reports about LPS detrimental effect on liver tissue through the overproduction of ROS. The inhibition group showed a higher TBARS level in both hepatic tissue and serum than that of control one and this might be mainly due to the predominant effect of LPS that evokes the liver damage. The level of GSH (reduced glutathione) was elevated in the induction group by 291.1% in plasma and

257.1% in the liver tissue compared to the control group and this elevation might be due de novo synthesis of GSH from its amino acid constituents that occurs as an adaptive response to oxidative stress (Huang et. al. 1993).

The level of GSH also was elevated in the inhibition group by 299% in plasma and 272% in the liver tissue and this illuminates the effect of LPS that evokes the liver damage in combination with L-NAME. LPS administration showed an elevation of serum superoxide dismutase (SOD) by 38.03% and 39.26% compared to the control group in both induction and inhibition groups respectively, and this shows that production of reactive oxygen species such as O2 -, H2O2, and HO-, occurs at the site of inflammation and contribute to tissue damage. Therefore, enhanced tissue SOD at endotoxemia may be a preventive measure of the host to handle the superoxide anion load after bacterial LPS administration.

LPS injection increased serum ALT and AST activities, compared to the control group. The blood levels of creatinine and urea increased in the induction group compared to the control group, the strong correlation between the elevations of ALT, AST, urea and creatinine levels in blood indicates tissue damage which may predispose to lipid peroxidation associated with the inflammatory response (Park et al. 2000). The present results showed found the level of urea in the induction was elevated by compared to the control group, indicating renal dysfunction.

The present results showed that, LPS injection induced the production of nitrite, IL-1b, IL-6 and TNF alpha in plasma, where the level of nitrite in the induction group was higher than that in the control group by 16.9% in plasma. Also, the level of IL-1b, IL-6 and TNF alpha were increased compared to the control group in plasma by 24.1%, 433.5% and 31% respectively.

L-Arginine has been previously reported to have protective effects in renal dysfunction and oxidative stress (Yang et al., 1998; Kadkhodaee, 2004; Kurus et al. 2005; Secilmia et al. 2005). This was in agreement with our findings, where creatinine plasma levels decreased by 7.23% and 8.9% in the protection and treatment groups, respectively compared to the induction group.

Arginase exists in two isoforms, AI and AII. They differ in cellular sublocalization and in tissue distribution. AI, or liver type, is the cytosolic isoform, whereas AII is the mitochondrial

isoform, located in the kidney, prostate, small intestine, and breast (Jenkinson et al.1996). Several cells can express both isoforms such as murine macrophages (Louis et al.1998) and rat aortic endothelial cells (Buga et al.1996). AI is increased in experimental glomerulonephritis (Kettler et al;1996) or LPS-induced inflammation (Sonoki et al. 1997). This was in agreement with our studies that the level of urea in the protection and treatment increased by 85.72% and 124.56%, respectively compared to the control group.

During infection with LPS, nitric oxide is produced by the action of iNOS, where L-arginine–nitric oxide pathway is activated. Nitric oxide has a protective effect against inflammation and this is in agreement with our findings where, the levels of IL-1b, IL-6 and TNF alpha decreased by 6.39%, 13.39% , 65% in the protection group and by 66.2%, 19.06% and 22.1% in treatment groups, respectively ($p \leq 0.05$). Nitric oxide acts as anti-inflammatory agent and this was obvious in the level of TBARS that was decreased in the protection and treatment groups compared to the induction group in plasma and in the liver homogenates by 58.6%, 54.06% and 84.4%, 79.53%, respectively ($p \leq 0.05$).

L-Arginine serves as a precursor to the synthesis of NO that may have an indirect antioxidant role by scavenging $O2^-$ (Pabla et al,1996), which is the substrate for the SOD enzyme and this in agreement with our studies where, the level of SOD in the protection group and treatment groups were decreased compared to the induction group by 12.4% and 19.5% respectively. This shows that L-Arginine has some protective roles against ROS attack possibly because of its direct chemical property interaction with $O2^-\cdot$, thus suggesting a protective effect for L-Arginine involving antioxidant mechanisms (Lass et al. 2002).

Nitrite (NO2-), acts as a physiologically inert metabolite and biomarker of the endogenous vasodilator NO. During infection and inflammation, iNOS produces high levels of NO. The major source of systemic iNOS-derived NO metabolites during inflammation or sepsis seems to be of parenchyma cells origin rather than from blood cells (Bultinck et al. 2006), and this in agreement with our investigation that the level of nitrite in the protection group and treatment group in plasma were elevated compared to the induction group by 186.9% and 7.2% respectively. iNOS- derived NO was also credited with protective effects in TNF-alpha, LPS-, or sepsis-induced shock models (Cauwels, 2007).

To investigate the mechanism of action of L-arginine, an irreversible competitive inhibitor of NOS was used such as L-NAME was used in combination with LPS in the inhibition group.

Our results showed that the decrease in activity of ALT and AST in plasma in the treatment group by the use of L-Arginine was prevented by the use of L-NAME, indicating that L-arginine effect is likely to be through NO-NOS pathway. Also, the decrease in plasma creatinine levels in the treatment group by using L-arginine was inhibited by the inclusion of L-NAME in the inhibition group, indicating that L-arginine protection against renal dysfunction might be due to increased production of NO through NOS pathway. Likewise, the decreased levels of TBARS and cytokines (IL-1b, IL-6 produced by L-arginine in the treatment group was inhibited by the inclusion of L-NAME in the inhibition group, giving an indication of the mechanism by which L-arginine does its protective effects against inflammation and oxidative stress. Administration of L-Arg. in the treatment and protective groups significantly showed higher levels compared to the induction group .However, the inclusion of L-NAME with L-Arginine in the inhibition group has significantly decreased plasma nitrite levels compared to both of treatment and protection groups. The same pattern was shown for hepatic nitrite levels, indicating that many of the effects produced by L-Arginine are most likely to be through the NO-NOS pathway.

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