

# Manipulation of nitric oxide in an animal model of acute liver injury. The impact on liver and intestinal function

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## Abstract

**Background:** Nitric oxide may have a protective effect on the liver during endotoxemia and chronic inflammation. There is evidence that it maintains liver and intestinal tissue integrity during inflammatory processes. We evaluated the impact of altering nitric oxide release on acute liver injury, the associated gut injury and bacterial translocation, at different time intervals. **Methods:** An acute rat liver injury model induced by D-galactosamine was used. Sprague Dawley rats were divided into four main groups: normal control, acute liver injury control, acute liver injury + *N*-nitro-*L*-arginine methyl ester (L-NAME), acute liver injury + L-NAME + *L*-arginine. Each group was divided into three subgroups according to the different time intervals (6, 12, 24 hours) after the induction of the liver injury. Liver enzymes and bilirubin were evaluated, as well as bacterial translocation, cecal and colonic microflora, and histological study of liver, ileum and cecum. **Results:** Liver enzymes increased significantly at all time intervals in acute liver injury + L-NAME compared to liver injury control groups. Bacterial translocation increased significantly in liver injury + L-NAME groups; at 6 hours to the liver, at 12 hours to the liver and mesenteric lymph nodes (MLNs), and at 24 hours to arterial and portal blood, liver and MLNS. Inhibition of nitric oxide increased significantly the *Enterobacteriaceae* count in cecum compared to normal and liver injury control groups. The G-negative anaerobes increased significantly in the colon compared to the liver injury control group. **Conclusion:** Inhibition of nitric oxide in an acute liver injury model potentiates the liver injury as evidenced by increased appearance of hepatocellular necrosis and elevated liver enzymes and bilirubin. It increases the *Enterobacteriaceae* in both cecum and colon and G-negative anaerobes in the colon. It also increases bacterial translocation to extra-intestinal sites. The increased bacterial translocation could be one of the mechanisms potentiating liver injury and nitric oxide may be pathophysiologically involved. Further studies are required to confirm this hypothesis.

**Key Words:** Acute liver injury, Arginine, Bacterial translocation, Intestinal microflora, L-NAME, Nitric oxide.

## Introduction

Nitric oxide (NO) is synthesized from *L*-arginine by the enzyme nitric oxide synthase (NOS). Two types of NOS have been identified: one is constitutive and responsible for the physiological transduction purposes and the other is induced by cytokines and releases NO as part of the immunological response [1]. Macrophages [2,3], Kupffer cells [4] and hepatocytes [5] possess the inducible NOS. Kupffer cells activated by septic stimuli or other inflammatory conditions respond by forming NO themselves and by inducing its formation in neighbouring hepatocytes [6]. Moreover, endotoxin can induce NOS in hepatocytes [7].

The role of endogenous NO on gut function has been previously studied. Stark ME, et al, have shown that increase gastric mucosal levels of NO by augmenting endogenous NO production or providing an exogenous source of NO are effective in the prevention of acute gastric mucosal injury in high-risk setting [8]. The inhibition of NOS facilitates mucosal injury [9] and pre-treatment with an inhibitor of NO synthesis enhances endotoxin-induced small intestinal damage and plasma leakage [10]. The endogenous NO is also important in the prevention of hypoxia-induced intestinal injury [11]. Decreased production and/or increased NO inactivation would likely enhance intestinal permeability [12].

One of the roles of NO production in the liver [13] and intestine [14] is to maintain tissue integrity

during inflammatory processes. NO is important in mucosal defence in that it influences virtually every component of mucosal defence [15]. It has been suggested that NO has a protective effect on the liver during endotoxemia and chronic inflammation [16], as evidenced by reduced hepatocellular necrosis and decreased release of intracellular liver enzymes when NO production is maintained [17]. The protective effect of NO is also evidenced by the appearance of hepatocellular necrosis in animals in which NO production has been blocked [16]. In a model of acetaminophen hepatotoxicity, inhibition of cytokine-mediated NO production potentiated the injury [17] and exogenous delivery of NO significantly decreased the acetaminophen-induced hepatotoxicity [18]. The results of some studies support previous reports of a protective effect of NO on the liver in endotoxemia and suggest that NO may up-regulate hepatic protein synthesis *in vivo* [19].

We have previously shown that bacterial translocation occurs in D-galactosamine acute liver injury [20, 21]. Bacterial infection and sepsis are recognized as serious complications of acute liver failure with gram-negative enteric bacteria being the most frequent causative organisms [22, 23]. The exact mechanisms of bacterial translocation in acute liver injury are unclear. We decided to test the hypothesis that NO inhibition may potentiate liver injury, bacterial translocation,

and that changes in bacterial translocation may influence the degree of liver injury.

The aim of this experiment was to study the role of NO in an acute liver injury model induced by D-galactosamine and to examine the impact of bacterial translocation, intestinal mucosal changes, endotoxemia, and cecal and colonic microflora changes in response to NO manipulation and the extent of liver injury.

### Materials and methods

**Experimental design:** Male Sprague - Dawley rats (BK Universal AB, Sollentuna, Sweden), with a weight range of 220-300 g were used and divided into four main groups (24 rats in each main group, 8 rats in each subgroup): normal control group, acute liver injury control group, acute liver injury + N-nitro-L-arginine methyl ester (L-NAME) group and acute liver injury + L-NAME + L-arginine group. Each group was divided into three subgroups according to the time interval after the liver injury (6, 12, 24 hours). The inhibitor of NOS which we used in our experiment is NG-nitro-L-arginine methyl ester (L-NAME), an inhibitor of both the constitutive and inducible form of NOS. It has been used in many experiments studying the effects of NO on the liver. All animals received normal rat chow (R3, Lactamin AB, and Stockholm, Sweden) and water ad libitum throughout the experiment and were kept at 12 hours light/dark cycle and 22°C room temperature. The Animal Ethics Committee of Lund University approved the experimental protocol. Acute liver injury was induced by intraperitoneal injection of D-galactosamine (Sigma Chemicals Co., St. Louis, MO, USA) 1.1 g/Kg body wt., [24]. L-NAME (Sigma) 100 mg / kg B.W. and L-arginine (Sigma) 300 mg / kg B.W. were injected intraperitoneally half an hour before the liver injury when indicated. Samples were collected (6, 12, 24 hours) following liver injury. Under ether anaesthesia a laparotomy was performed through a midline incision under aseptic technique. Portal blood was collected for bacteriological tests and measurement of endotoxin levels, aortic blood for bacteriological and liver enzyme tests. Samples from the caudate lobe of the liver, mesenteric lymph nodes (MLNs), and cecal and colonic contents were obtained for bacteriological counts. Samples from liver, distal small intestine and cecum were taken for histological examination.

**Bacterial translocation:** Blood samples were immediately placed in EDTA containing sterile tubes. Tissue samples were placed in 5 ml of sterile transport medium [25]. Samples were placed in ultrasonic bath (Millipore, Sundbyberg, Sweden) for 5 minutes and swirled on Chiltern

(Terma-Glas, Gothenberg, Sweden) for 2 minutes. Total aerobic plate count was made by placing 1.0 ml of the sample on Brain Heart Infusion agar BHI (Oxoid, Hampshire, England) and incubated at 37°C for 3 days. Total anaerobic plate count was made by placing the samples on BHI and incubating under anaerobic condition (Gas Pack System, Gas Pack, Becton Dickenson Microbiology Systems, Cockeysville, MD, USA) at 37°C for 3 days. After 3 days the number of colonies formed on each plate were counted and corrected for the weight of the original tissue. Tissue samples were expressed per gram of tissue while blood samples were expressed per ml of blood.

**Intestinal microflora:** Samples taken from cecal and colonic content were immediately placed in 5 ml of sterile transport medium and transferred into ultrasonic bath and swirled on Chiltern as above. After a conventional dilution procedure, viable counts were obtained from brain heart infusion agar BHI (Difco, Detroit, MI) that was incubated aerobically and anaerobically at 37°C for 72 hours (aerobic and anaerobic bacterial count, respectively). From Rogosa (Oxoid, Hampshire, England) and BHI agar containing gram-negative anaerobic supplement (Oxoid, Hampshire, England) incubated anaerobically at 37°C for 72 hours (lactobacilli and gram-negative anaerobic bacterial counts respectively) and from violet red-bile-glucose agar VRBG (Oxoid, Hampshire, England) incubated aerobically at 37°C for 24 hours (Enterobacteriaceae counts)

**Liver enzymes and bilirubin:** After centrifugation of the blood (1000xg, 10 min.) serum bilirubin (Bil), alkaline phosphatase (ALP), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels in aortic blood were measured according to the recommendations of the Committee on the Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology [26].

**Histological study:** Samples from the left lobe of the liver, distal small intestine and cecum were placed in 4% phosphate buffered formaldehyde. Paraffin-embedded samples were sliced and studied under light microscopy after staining with haematoxylin and eosin. At least 3 slides were studied from each specimen in a blinded fashion.

**Serum endotoxin:** Four ml of portal blood were placed in endotoxin free, special sampling tubes containing heparin (Endo Tube, Chromogenix, Mölndal, Sweden) and centrifuged at 3000 g for 15 min. at 4°C. The endotoxin level in the separated plasma was measured by Limulus Amoebocyte Lysate (LAL) using Chromogenix Coatest (Chromogenix, Mölndal, Sweden) [27].

The detection limit of endotoxin in our method was 0.25 units (1.2 EU = 100 pg). Because of the high levels of bilirubin in some of the samples, plasma blank containing 200  $\mu$ L of 20% acetic acid was used as appropriate.

### Statistics

Values are presented as Mean  $\pm$  SEM. Differences between all groups were evaluated by one way ANOVA test followed by all pairwise multiple comparison Student Newman-Keuls method. The incidence of bacterial translocation was evaluated using Fisher's exact Test. Probability levels less than 0.05 were considered significant ( $p < 0.05$ ).

Table 1 Liver function tests at different time points after induction of liver injury

Time	Groups	ALP (uKat/L)	Bil (umol/L)	ASAT (uKat/L)	ALAT (uKat/L)
6 hrs	NC	6.8 $\pm$ 1.1	5.7 $\pm$ 1.3	2.2 $\pm$ 0.4	1.5 $\pm$ 0.4
	A	8.8 $\pm$ 0.7	7.9 $\pm$ 1.6	8.9 $\pm$ 0.9	8.3 $\pm$ 1.1
	B	14 $\pm$ 1.7*	12.6 $\pm$ 1*	15 $\pm$ 1.3*	12 $\pm$ 0.8*
	C	8.7 $\pm$ 0.6 $^\circ$	10 $\pm$ 0.7 $^\circ$	11 $\pm$ 1.4 $^\circ$	9 $\pm$ 0.6 $^\circ$
12 hrs	A	10.8 $\pm$ 0.3	10.9 $\pm$ 0.5	9.8 $\pm$ 0.6	6.7 $\pm$ 0.7
	B	12.1 $\pm$ 0.7	11.2 $\pm$ 0.9	13.9 $\pm$ 1.3	9.8 $\pm$ 0.8
	C	9.8 $\pm$ 0.9	9.2 $\pm$ 0.7	9.3 $\pm$ 1.4 $^\circ$	7 $\pm$ 1 $^\circ$
24 hrs	A	16.1 $\pm$ 1.4	15.6 $\pm$ 1.9	34.6 $\pm$ 4.6	20 $\pm$ 2.9
	B	20.5 $\pm$ 1.2*	30 $\pm$ 3.5*	72.8 $\pm$ 12*	49 $\pm$ 11*
	C	14 $\pm$ 0.8 $^\circ$	21.1 $\pm$ 2 $^\circ$	44.5 $\pm$ 4 $^\circ$	27 $\pm$ 2.8 $^\circ$

A: Acute liver injury control group, B: Acute liver injury + L-NAME groups, C: Acute liver injury + L-NAME + L-arginine groups, NC: normal control. ALP: alkaline phosphatase, Bil: bilirubin, ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase. \* denotes  $P < 0.05$  compared to group A,  $^\circ$  denotes  $P < 0.05$  compared to group B (All pairwise multiple comparisons, Newman-Keuls method).

Table 2 Incidence of Bacterial translocation in experimental groups at different time intervals

Time	Groups	PB	AB	Liver	MLNs
6 hrs	NC	0/8	0/8	0/8	0/8
	A	5/8*	3/8	7/8**	7/8
	B	5/8*	5/8*	8/8***	8/8***
	C	5/8*	2/8	7/8***	4/8
12 hrs	A	5/8*	5/8*	8/8***	8/8***
	B	7/8**	5/8	8/8***	8/8***
	C	4/8	4/8	8/8***	7/8**
24 hrs	A	7/8**	8/8***	8/8***	8/8***
	B	8/8***	8/8***	8/8***	8/8***
	C	4/8	5/8*	6/8**	7/8**

A: Acute liver injury control group, B: Acute liver injury + L-NAME groups, C: Acute liver injury + L-NAME + L-arginine groups, NC: normal control. PB: portal blood, AB: arterial blood, MLNs: mesenteric lymph nodes. \* denotes  $p < 0.05$ , \*\* denotes  $p < 0.01$ , \*\*\* denotes  $p < 0.001$  compared to normal control.

Table 3 Incidence of Bacterial translocation in experimental groups at different time intervals (CFU/g tissue of ml blood)

Time	Group	PB	AB	Liver	MLNs
6 hrs	A	20 $\pm$ 7	6 $\pm$ 3	275 $\pm$ 75	335 $\pm$ 115
	B	19 $\pm$ 9	25 $\pm$ 11	920 $\pm$ 305*	735 $\pm$ 310
	C	15 $\pm$ 6	13 $\pm$ 10	145 $\pm$ 55 $^\circ$	325 $\pm$ 220
12 hrs	A	12 $\pm$ 8	80 $\pm$ 37	255 $\pm$ 80	115 $\pm$ 20
	B	26 $\pm$ 9	135 $\pm$ 84	1865 $\pm$ 740*	680 $\pm$ 215*
	C	10 $\pm$ 4	19 $\pm$ 9	170 $\pm$ 65 $^\circ$	140 $\pm$ 45 $^\circ$
24 hrs	A	19 $\pm$ 10	44 $\pm$ 16	4120 $\pm$ 1495	3770 $\pm$ 1225
	B	83 $\pm$ 21*	138 $\pm$ 36*	8185 $\pm$ 1550*	9070 $\pm$ 2010*
	C	13 $\pm$ 7 $^\circ$	9 $\pm$ 3 $^\circ$	1550 $\pm$ 780 $^\circ$	3135 $\pm$ 1280 $^\circ$

A: Acute liver injury control group, B: Acute liver injury + L-NAME groups, C: Acute liver injury + L-NAME + L-arginine groups, NC: normal control. PB: portal blood, AB: arterial blood, MLNs: mesenteric lymph nodes. \* $P < 0.05$  compared to group A,  $^\circ P < 0.05$  compared to group B (All pairwise multiple comparisons, Newman-Keuls method).

Table 4 Cecal and colonic microflora count (Log CFU/g content)

Groups	Total aerobic	Total anaerobic	G-ve anaerobic	Enterobacteriaceae	lactobacilli
<b>cecaum</b>					
NC	8 $\pm$ 0.1	8.2 $\pm$ 0.1	8.3 $\pm$ 0.1	6.5 $\pm$ 0.3	7.9 $\pm$ 0.1
A	8.1 $\pm$ 0.1	8.3 $\pm$ 0.1	8.1 $\pm$ 0.1	6.5 $\pm$ 0.3	8.1 $\pm$ 0.1*
B	8.1 $\pm$ 0.1	8.3 $\pm$ 0.1	8.2 $\pm$ 0.1	7.6 $\pm$ 0.2* $^\circ$	8.1 $\pm$ 0.1*
<b>colon</b>					
NC	8.2 $\pm$ 0.1	8.6 $\pm$ 0.1	8.6 $\pm$ 0.1	4.1 $\pm$ 1.7	8.4 $\pm$ 0.1
A	8.2 $\pm$ 0.1	8.6 $\pm$ 0.1	8.5 $\pm$ 0.1	7.2 $\pm$ 0.2*	8.4 $\pm$ 0.1
B	8.2 $\pm$ 0.1	8.6 $\pm$ 0.1	8.8 $\pm$ 0.1 $^\circ$	7.9 $\pm$ 0.3*	8.6 $\pm$ 0.1* $^\circ$

A: Acute liver injury control group, B: Acute liver injury + L-NAME groups, \* denotes  $P < 0.05$  compared to group NC,  $^\circ$  denotes  $P < 0.05$  compared to group A (All pairwise multiple comparisons, Newman-Keuls method).

### Results

There was no mortality at any time point in any of the experimental groups. In the liver injury + L-NAME groups, the animals appeared sicker with retarded locomotion and piloerection than the acute liver injury control group.

**Liver enzymes and bilirubin:** Six hours after induction of the liver injury, there was a significant increase of the liver enzymes (ALP, ASAT, and ALAT) and bilirubin levels in the liver injury + L-NAME group compared to the liver injury control group. In the liver injury + L-NAME + L-arginine group the liver enzymes decreased significantly compared to liver injury + L-NAME group (Table

1). Twelve hours after the liver injury, the liver enzymes increased significantly in the liver injury + L-NAME group compared to the liver injury control group (Table 1). At 24 hours there was a significant elevation of liver enzymes and bilirubin in the liver injury + L-NAME group compared to the liver injury control group, which had been decreased significantly after addition of arginine (Table 1).

**Bacterial translocation:** The incidence of bacterial translocation, at all time intervals, increased in all the experimental groups compared to normal control (Table 2). Six hours following liver injury the number of translocated bacteria differed in the experimental groups. Bacterial translocation to the liver increased in the liver injury + L-NAME group compared to the liver injury control group, and this effect was reversed by the addition of arginine. The bacterial translocation after 12 hours showed the same finding to the liver and MLNs, while after 24 hours the same effect was seen in the portal and arterial blood, liver and MLNs (Table 3).

**Intestinal microflora:** In the cecum the Enterobacteriaceae count increased significantly in the liver injury + L-NAME group compared to normal control and liver injury control groups. The Lactobacillus count increased significantly in both: liver injury and liver injury + L-NAME groups compared to normal control. In the colon the G<sup>-ve</sup> anaerobic count increased significantly in the liver injury + L-NAME group compared to the liver injury control group. While the Enterobacteriaceae count increased significantly in the liver injury and liver injury + L-NAME groups compared to normal control. The Lactobacillus count increased significantly in the liver injury + L-NAME group compared to the liver injury and normal control groups (Table 4).

**Histological study:** The liver showed more hepatocellular necrosis and inflammatory cell infiltrations, at all time intervals, in the acute liver injury + L-NAME groups compared to the liver injury control groups (Figures 1, 2, 3). Samples of the distal small intestine and cecum were normal and showed no histopathological changes on light microscopy in any of the experimental groups (data not shown).

**Serum endotoxin levels:** In all the experimental groups the endotoxin levels were below detectable levels (<0.25 U).

## Discussion

The results of this study showed that inhibition of NO synthesis by administration of L-NAME significantly increased hepatocellular damage as indicated by increased release of liver enzymes.

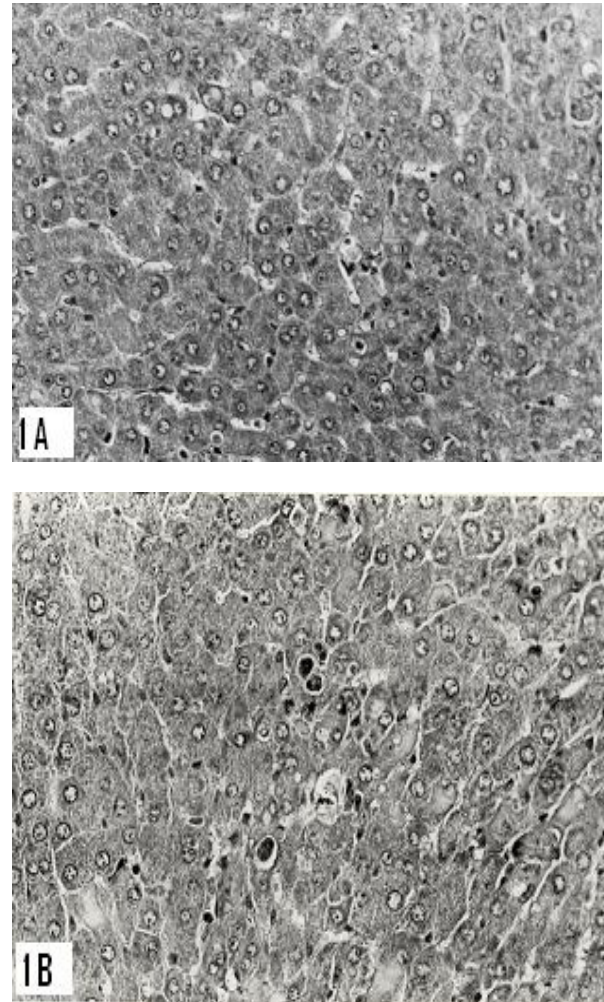


Figure 1: Histological appearance of the liver 6 hours after the injury (Hematoxylin-Eosin, x100). The liver injury control group (Fig. 1A) showing hepatocellular necrosis and inflammatory cell infiltration. The liver injury + L-NAME group (Fig. 1B) showing more hepatocellular necrosis and inflammatory cell infiltration compared to the liver injury control group.

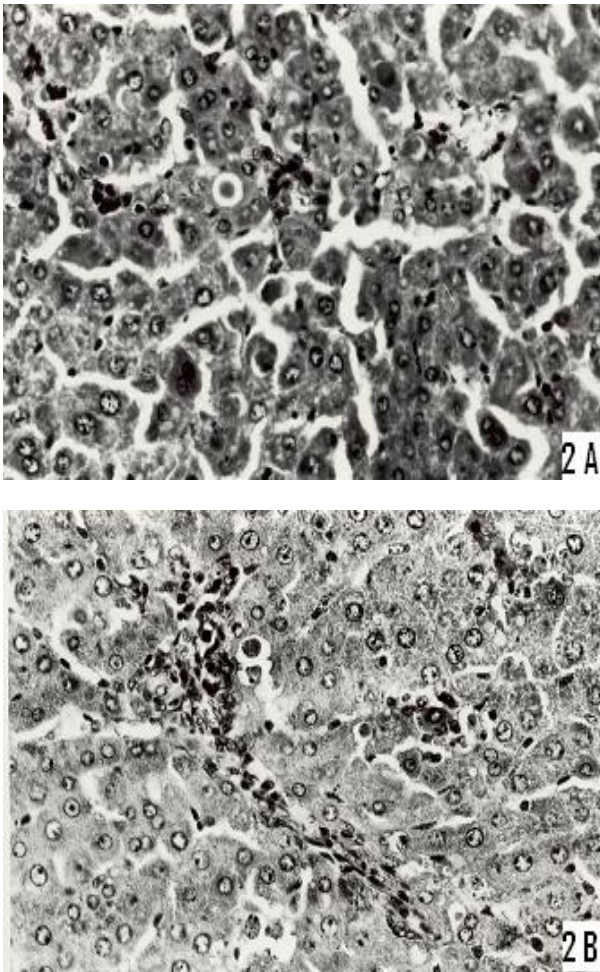


Figure 2: Histological appearance of the liver 12 hours after the injury (Hematoxylin-Eosin, x100). The liver injury control group (Fig. 2A) showing hepatocellular necrosis and inflammatory cell infiltration. The liver injury + L-NAME group (Fig. 2B) showing more hepatocellular necrosis and inflammatory cell infiltration compared to the liver injury control group.

This effect was partially reversed by simultaneous administration of L-arginine. This suggests a possible role for NO in protecting the liver in this acute liver injury model. When NO production was inhibited at all time intervals studied, there was more hepatocellular necrosis and inflammatory cell infiltration compared to the acute liver injury control groups. The protective effect of NO on liver function is in accordance with other studies. Kuo et al [17] showed that maintenance of NO production during endotoxemia and chronic inflammation reduced hepatocellular necrosis and decreased release of intracellular liver enzymes, while blocking the production increased the hepatocellular necrosis.

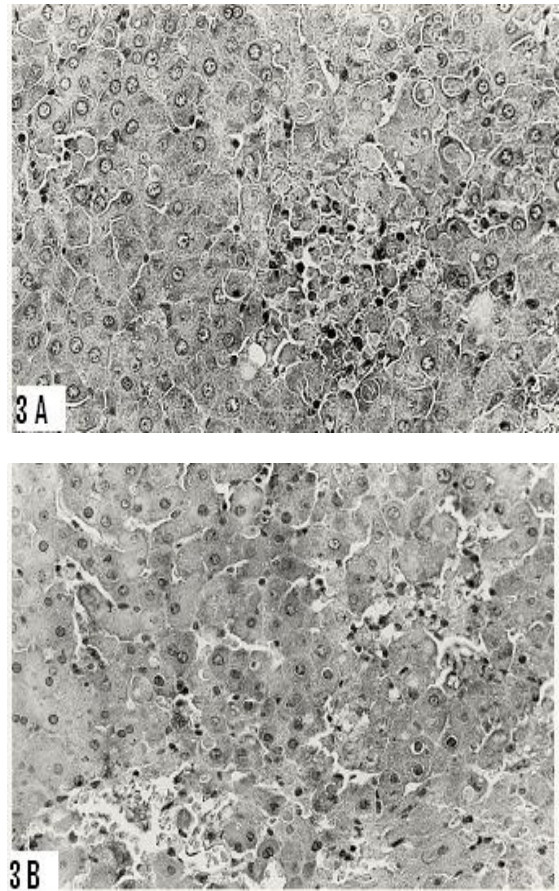


Figure 3: Histological appearance of the liver 24 hours after the injury (Hematoxylin-Eosin, x100). The liver injury control group (Fig. 3A) showing hepatocellular necrosis and inflammatory cell infiltration. The liver injury + L-NAME group (Fig. 3B) showing more hepatocellular necrosis and inflammatory cell infiltration.

In another study, aspartate aminotransferase levels were elevated in endotoxemic rats pretreated with NO inhibitor [28]. In addition, a liver-selective NO donor has been used to almost completely inhibit liver damage in the LPS-galactosamine model [29]. It has been shown that inhibitors of inducible NOS are more effective than an NO donor in reducing carbon-tetrachloride induced liver injury [30].

The liver plays a central role in haemostasis, and acute liver failure results in a very complex coagulopathy [31]. Hepatic NO from Kupffer cells, endothelial cells and hepatocytes may be involved in modulating the local circulation by preventing platelet aggregation in hepatic sinusoids and contributing to increased blood flow [32]. The hemodynamic of the hepatic microcirculation is known to depend on the activity of smooth muscle cells around the arterioles and pre-capillary sphincters [33]. The increased hepatic damage

from inhibition of NO synthesis is mediated in part by superoxide and hydroxyl radicals [16, 34]. It could also be due to increased oxygen free radical availability following removal of NO as a superoxide scavenger [16]. Inhibition of NO formation by L-NAME stimulated the release of superoxide anion by the liver [35] and NO has been shown to inhibit superoxide anion generation by neutrophils [36]. We have shown that NO inhibition increases the hepatocellular damage and the release of the liver enzymes and bilirubin. The mechanisms of the protective effect of NO on the liver may be due to inhibition of one or several of the beneficial effects of NO. These include regulation of both arterial tone and blood flow [37,38], inhibited platelet aggregation [39] and reduced adherence [40], and scavenging of superoxide and other oxygen-derived free radicals [41].

The gut is a major reservoir of bacteria, and under normal conditions a series of local and systemic protective mechanisms prevent passage of these potentially pathogenic bacteria beyond the intestinal lumen. Severe physiologic alterations may affect these mechanisms, favouring translocation of enteric bacteria across the intestinal mucosa [42]. The gut plays an important role in the infectious and septic complications observed in clinical conditions [43]. NO is important in mucosal defence in that it influences virtually every component of mucosal defence and can influence gastrointestinal mucosal integrity through actions on many cells [15]. In this study we found that inhibition of NO increased the incidence and the number of the translocated bacteria, at all time intervals, to the extraintestinal sites. Other studies showed that inhibition of NO with L-NAME causes a rapid increase in mucosal permeability to <sup>51</sup>Cr-EDTA [12]. NO inhibition also activates mast cells in the mucosa and consequently increases epithelial permeability [44]. Synthesis of NO from L-arginine has an important role in maintaining the integrity of the intestinal mucosa following acute challenge with LPS and it has been suggested that endogenous NO has an important acute protective role in the intestinal microvasculature against blood-borne toxins and tissue destructive mediators [10]. The reported increased intestinal permeability by NO inhibition could explain the increased incidence and number of translocating bacteria. It is plausible that increased bacterial translocation due to the inhibition of NO was one of the factors potentiating the liver injury and failure in this liver injury model. Most bacterial infections in critically ill or immunocompromised patients are caused by the patients own microflora and many patients

dying of sepsis or multiple system organ failure have enteric bacteraemia for which no septic focus is identified, indicating that these infections may have originated from the gut [45]. NO is a potent mediator of antimicrobial defence mechanism [46] and the reactive nitrogen intermediates contribute to the ability of activated rodent macrophages to inhibit a wide variety of intracellular and extra cellular pathogens [47]. The inhibition of NO production was associated with impaired chemotaxis in polymorphonuclear leukocytes [48]. The increased incidence of bacterial translocation in our study could also be due to inhibition of NO antimicrobial effects. NO has multiple immunoregulatory and antimicrobial functions and it either kills or inhibits the replication of a wide variety of pathogens including viruses, bacteria, parasites, and fungi. The effect of inhaled NO on infections in humans is just beginning to be evaluated [49].

The inhibition of NO in our study increased significantly the Enterobacteriaceae count in both cecum and colon and the G-negative anaerobes in the colon. It has been previously shown that NO inhibits a wide variety of intracellular and extracellular pathogens [47]. It seems that NO inhibition disturbed the intestinal microflora by increasing the bacterial count especially the potentially pathogenic organisms. An increased translocation of these bacteria may add more damage to the liver and reduce capacity for the clearance of translocated bacteria, with increased risk of sepsis, septic syndrome and multiple system organ failure. We did not observe any endotoxin levels in the experimental groups; variable amounts of bilirubin in the samples could have masked the presence of small amount of endotoxins in the chromogenic assay and make its detection difficult. Similar to our finding, Al-Tuwaijri et al [50] did not detect any systemic endotoxin level after induction of liver injury by D-galactosamine, as well as normal control animals. We have shown previously that lactulose, with its known anti-endotoxin activity, significantly improved the liver cell damage in the D-galactosamine acute liver injury model [51]. This could indicate a role for endotoxin in the liver injury. In our study we have used the D-galactosamine model without exogenous administration of LPS. There are evidences that implicate gut-derived endotoxin in the pathogenesis of D-galactosamine induced hepatic injury [52]. If the colon is removed before challenge with D-galactosamine, hepatic injury is greatly reduced [53]. It has been shown that severely disturbed gut flora in rats with D-galactosamine-induced acute liver failure plays an

important role in the elevation of endotoxin level in the portal vein [54]. High portal level of LPS could lead to a pronounced secretion of pro-inflammatory mediators by Kupffer cell and ultimately to endotoxin-induced liver injury [54,55]. Some other researchers have used D-galactosamine + exogenous LPS which produce a more severe injury and the role of nitric oxide] in that model need to be studied.

NO protection on the liver could be due to increased blood flow, inhibition of platelet aggregation and adhesion, inhibition of intra-hepatic thrombosis and scavenging of superoxide and other oxygen free radicals. NO could also affect bacterial translocation by maintaining the intestinal mucosal integrity and through its antimicrobial activity against a wide variety of intracellular and extra-cellular pathogens. We therefore conclude that inhibition of NO in an acute liver injury model potentiates the liver injury as evidenced by increased appearance of hepatocellular necrosis and elevated liver enzymes and bilirubin. It increased the Enterobacteriaceae in both cecum and colon and G-negative anaerobes in the colon and increased bacterial translocation to extra-intestinal sites. The increased bacterial translocation could be one of the mechanisms to account for the potentiation of liver injury.

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