Research In Pharmaceutical Biotechnology Vol. 3(10), pp. 135-141, December 2011
Available online at http://www.academicjournals.org/rpb
DOI: 10.5897/RPB11.005
ISSN 2141-2324 ©2011 Academic Journals

Full Length Research Paper

Histopathological examination of formulated drugs against typhoid

S. S. Haque*, A. Sharan and U. Kumar

Department of Clinical Biochemistry, Indira Gandhi Institute of Medical Sciences Patna-14, India.

Accepted 30 November, 2011

Typhoid fever (TF) is a systemic bacterial infection caused by *Salmonella typhi*, a facultative and gram negative rods. The infection is usually acquired through the ingestion of contaminated water or food. Almost 80% of cases and deaths occur in Asia. The attack rate as high as 1100 cases per 100000 populations have been documented in developing countries. In typhoid fever, various organs can be involved leading to a wide range of presentation, from uncomplicated to a complicated one involving multiple organs. Histopathological derangements are common in typhoid fever, whereas, hepatic dysfunction has been reported variably from less than 1% to as high as 26%. Nitric oxide (NO•) is synthesized in endothelial cells, from the terminal guanidine nitrogen atom of L-Arginine by means of NO-synthase (NOS). NO• may regulate hepatic metabolism directly by causing alterations in hepatocellular metabolism and function, or indirectly as a result of its vasodilator properties. In this study we evaluated the liver tissues by carrying out a histological examination. We found that extensive tissue with cords of hepatocytes consisting of neutrophils and macrophages, had granulomatous lesions and mild necrosis before and after treatment with the formulated drugs that is L-arginine and ciprofloxacin.

Key words: Histopathology of liver tissue, typhoid, nitric oxide.

INTRODUCTION

Typhoid fever is an important health concern in developing countries (including those in Central and South America, Asia, Indian Subcontinent, Africa and the Caribbean), where hand washing and other such hygienic practices may be less frequent, and where water may be contaminated with sewage. Thus, travelers to these countries are most at risk. In addition, carriers or those who have recovered from typhoid fever may still shed the bacteria. For developed countries such as the United States, the chances of *S. typhi* transmission are very low because of high hygienic standards. *S. typhi* targets intestinal epithelial cells (enterocytes), causing the inflammation of other cells in the intestinal Payer's patches and subsequently the mesenteric lymph nodes, spleen and bone marrow associated with typhoid fever (Ohl et al., 2001). The mechanism by which *S. typhi* attaches to host cell is common among bacteria, which utilizes long, hair-like filaments known as fimbriae that are coated with receptor specific adhesins that recognize and bind to specific types of sites on the surface of target cells (Baulmer et al., 1996). Until 1980s, chloramphenicol was the undisputed first-line drug for the treatment of typhoid. Since 1970, multiple resistant *Salmonella* have caused extensive outbreaks in many developing countries. Third generation cephalosporin and fluoroquinoles have been found effective in treatment of these cases (Manchanda et al., 2006). However, isolates of *S. typhi* with reduced susceptibility to flouroquinoles (as indicated in the laboratory by resistance to nalidixic acid) have now appeared in the Indian subcontinent and other regions. These nalidixic acid resistant but ciprofloxacin sensitive strains have increased MIC (minimum inhibitory concentration) for ciprofloxacin although they are still within the current NCLLS (National Committee for Clinical Laboratory Standards) range for susceptibility (0.125 to 0.5 mg/ml) (Rowe et al., 1995; Brown et al., 1996; Jesudason et al., 1996; Chitnis et al., 1999; Kapil et al., 2002).

Nitric oxide is a highly reactive gas that participates in many biochemical reactions. During the last few years

*Corresponding author. E-mail: sshaq2002@yahoo.co.in.

Table 1. Treatments schedule.

Groups	Treatments
1	Negative control (normal saline)
2	Positive control (*S. typhimurium*(0.6 x LD_{50})+ saline
3	*S. typhimurium* (0.6 × LD_{50}) + Ciprofloxacin (400 mg per kg b. wt)
4	*S. typhimurium* (0.6 × LD_{50}) + Arginine (1000 mg per Kg b.wt)
5	*S. typhimurium* (0.6 × LD_{50})+Arginine (500 mg per kg b. wt) + Ciprofloxacin (200 mg per kg b. wt)
6	*S. typhimurium* (0.6 × LD_{50}) + Arginine (250 mg per kg b. wt) + ciprofloxacin (200 mg per kg b. wt)

the role of NO in health and diseases has been further understood (Nathan, 1997; Fang, 1997). The role of NO in biological system was first reported by Gruetter et al. (1979), he bathed the isolated pre-contracted strips of coronary artery in Krebs' bicarbonate buffer bubbled with NO gas and marked relaxation response in pre-contracted strips of coronary artery was observed. NO is synthesized within cells by an enzyme NO synthase (NOS). NOS produce NO from arginine with the aid of molecular oxygen and NADPH. NO diffuses freely across cell membranes. Since there are so many other molecules, with which it can interact, it is quickly consumed close to where it is synthesized. Key discoveries in 1987 included reports that arginine is the precursor for mammalian nitrite/nitrate synthesis (Hibbs et al., 1987a) and that NO is an endothelium derived relaxing factor (Palmer et al., 1987; Ignarro et al., 1987b). Ciprofloxacin, ofloxacin, perfloxacin and fleroxacin are common fluoroquinolones proved to be effective against typhoid. In children, the first two are only used in our country and there is no evidence of superiority of any particular fluroquinolones. Many studies have suggested that patients in Indian subcontinent or with the history of travel to the Indian subcontinent should receive ciprofloxacin as first line therapy (Rowe et al., 1995; Gulati et al., 1992). Physiological arginine concentrations may be limiting for cellular NO synthesis as plasma concentrations of arginine are typically found in the range of 80 to 110 µM. It has been reported that NO plays a protective role in the liver in septic and inflammatory conditions (Harbrecht et al., 1992, 1994).

The aim of this work was to evaluate histopathogical results to establish the therapeutic effect of L-arginine and ciprofloxacin against typhoid.

MATERIALS AND METHODS

Dose and dosage

Animals

Swiss albino mice (25 to 30 g), 6 to 8 weeks old were obtained from the central animal house of Hamdard University, New Delhi, India. The animals were kept in Poly-propylene cages in an air-conditioned room at 22/25°C and maintained on a standard laboratory feed (Amrut Laboratory, rat and mice feed, Navmaharashtra Chakan Oil Mills Ltd, Pune) and water *ad libitum*. Animals were allowed to acclimatize for one week before the experiments under controlled light/dark cycle (14/10 h). The studies were conducted according to ethical guidelines of the "Committee for the purpose of control and supervision of experiments on animals (CPCSEA)" on the use of animals for scientific research.

Bacteria

In this experiment only, *Salmonella typhimurium* (wild) was used. The standard strain of this pathogen was obtained from the National Salmonella Phage Typing Centre, Lady Harding Medical College, New Delhi, India. This bacterial strain was further confirmed by the Department of Microbiology, Majeedia Hospital, New Delhi, India. The drug was administered orally and *S. typhimurium* intraperitoneally. Animals were divided into six groups. Each group comprised of six animals. The treatments followed the schedule indicated in Table 1.

Histopathological studies

Animals were sacrificed by cervical dislocation. The liver tissues were immediately removed and fixed for the histopathological studies. The steps involved were as follows:

Fixation and processing

The tissues were fixed immediately after dissection in 10% phosphate buffered formaldehyde solution, pH of 7. The tissues were cut into 2 to 4 mm thick sections to ensure that the fixative readily penetrated throughout the tissues. Processing involved dehydration and clearing of tissues as well as their infiltration with paraffin. The tissue block was passed through the series of steps as per the processing schedule, allowing 1 h at each stage. They were dehydrated through graded solutions of alcohol ending in two changes of absolute alcohol for 2 h each. They were cleared 2 changes of xylene, infiltrated in 2 changes of paraffin wax for 2 h each using the automatic tissue processor. During the process of embedding, the tissue blocks were oriented in such a way that sections were cut in a desired plane of tissues. Two L-shaped metal moulds were laid on metal plate so as to enclose a square or a rectangular space. It was then partially filled with melted paraffin and allowed to cool until reasonably firm. The set block of paraffin with tissue was removed from moulds and then trimmed to suitable sizes and fixed on a metal object holder. The block was further trimmed and kept at 0°C.

Section cutting

The sections of 4 to 6 µm thickness were cut on albuminized slides.

Table 2. Staining (hematoxylin and eosin).

Reagent	Amount used
Harris hematoxylin stain	5 g
Ammonium/potassium alum	100 g
Hematoxylin crystal	5 g
Alcohol (100%)	50 ml
Distilled water	1000 ml

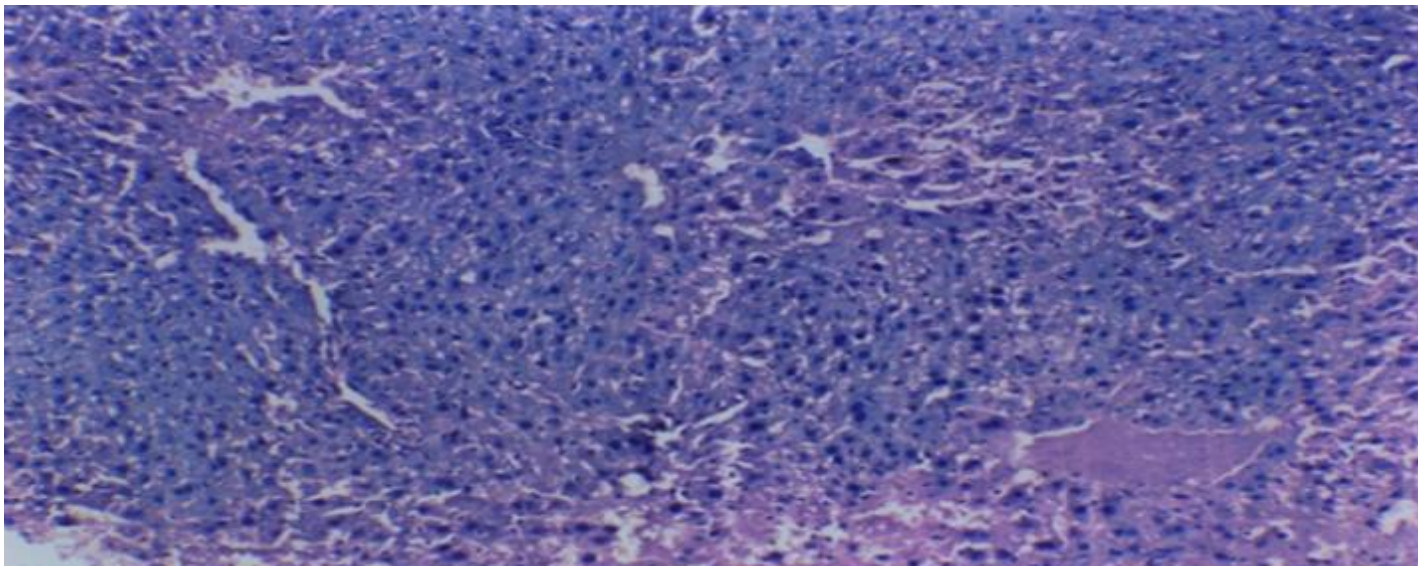

Figure 1a. Histopathological changes in liver. Normal liver section of control mice. Hepatocytes are arranged in columns. No pathological lesions were observed.

The sections were drained with water and dried on a hot plate at about 50°C for 30 min before staining (Table 2). The hematoxylin crystals were dissolved in alcohol and alum in water by the aid of heat. It was then heated to simmer until it became dark purple. The solution was removed from heat immediately and was plunged into basin of cold water until it cooled. Finally, 2 to 4 ml of glacial acetic acid was added (which increases the precision of nuclear stain). The stain was filtered before each use.

Eosin phloxine stain

Eosin (1.0 g) was dissolved in 100 ml of distilled water and 10 ml. 1% phloxine solutions were added. Finally, 780 ml of 95% alcohol and 4 ml of glacial acetic acid was added to it. It was filtered before use.

Procedure

Sections measuring approximately 0.2 x 0.2 cm were taken from the liver of mice. They were dehydrated through graded solutions of alcohol ending in two changes of absolute alcohol for 2 h each. They were cleared in 2 changes of xylene, infiltrated in 2 changes of paraffin wax for 2 h each using the automatic tissue processor and embedded in molten paraffin wax (DPX). Finally the slides were observed for histopathological changes.

RESULTS

Histopathological examination of liver

Histopathological examination of uninfected mice liver showed cords of hepatocytes arranged in columns with central veins and portal triads (Figure 1a). Infection with 0.6 x LD_{50} of *S. typhimurium* showed multiple lesions of nodular microabsces or granulomatous lesions that were composed of degenerated hepatocytes with variable degree of central necrosis surrounded by the sheets of neutrophils and macrophages, and severe necrosis were observed (Figure 1b). In contrast, liver sections of L-arginine treated mice showed tissue with cords of hepatocytes consisting of neutrophils and macrophages, showing granulomatous lesions and mild necrosis were seen (Figure 1c). In ciprofloxacin treated mice infected with 0.6 x LD_{50} of *S. typhimurium,* no significant pathological changes in liver were observed. Sections showed that the liver tissue was comprised of cords of hepatocytes showing mild fatty changes and extensive focal neutrophilic infiltrate consisting of granuloma. No area of necrosis was seen (Figure 1d). Similarly,

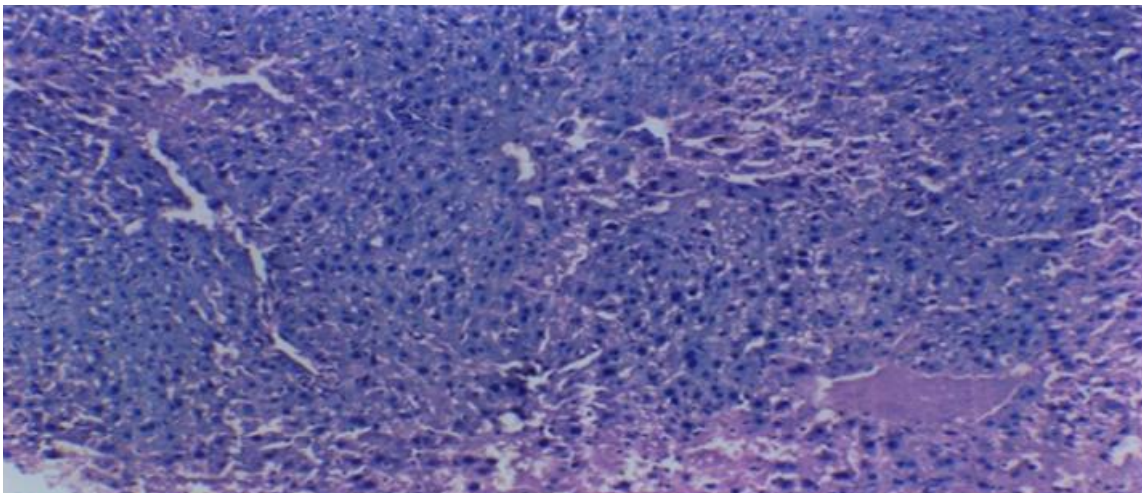

Figure 1b. Histopathological changes in liver. Normal liver section of control mice. Hepatocytes are arranged in columns. No pathological lesions were observed.

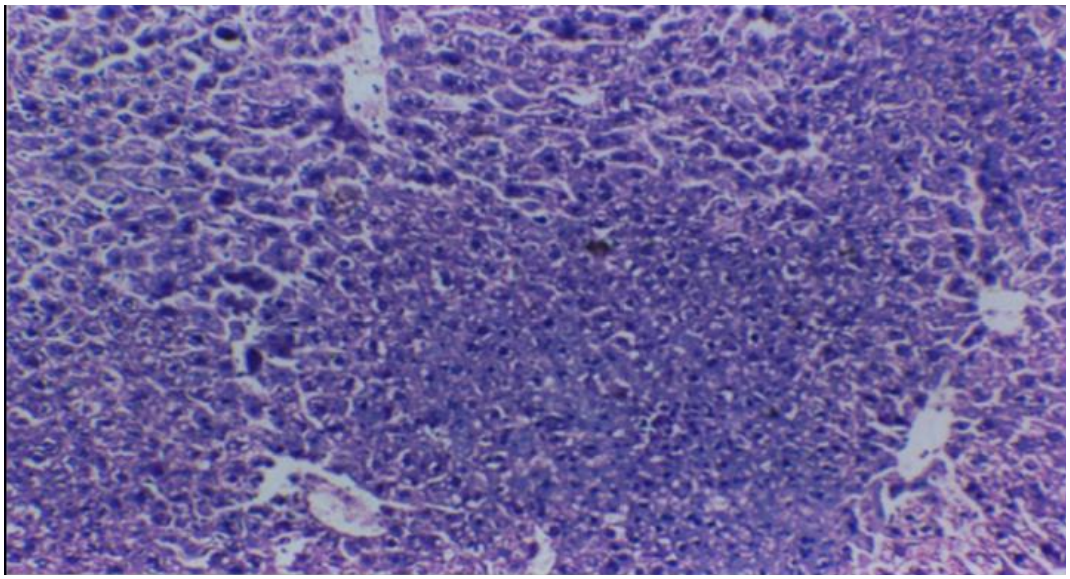

Figure 1c. Effect of L-arginine on *S. typhimurium* infected liver damage. Mice were treated with L-arginine (1000 mg per kg b. wt) after infection with challenge dose of 0.6 x LD_{50} *S. typhimurium*. At day 14, liver section were examined histopathologically which revealed that cords of hepatocytes showing focal granulomatous collection of neutrophils and macrophages (G) and there is mild sign of necrosis (N).

treatment with drugs in combination that is 1/2 Arginine + 1/2 Ciprofloxacin showed less significant pathological changes in the liver, and maximum decreases in the level of granulamatous lesion and necrosis (Figure 1e).

DISCUSSIONS

Histopathological examination of the liver

Histopathological examination of paraffin sections prepared from normal mice showed normal architecture of hepatocytes and infection with 0.6 x LD_{50} of *S. typhimurium* showed multiple lesions of nodular microabscess or granulomatous lesions thatwere composed of degenerated hepatocytes with variable degree of central necrosis surrounded by the sheets of neutrophils and macrophages, and severe necrosis were observed (Figures 1a and b). In *S. typhimurium*, infected mice treated with L-Arg, and sacrificed on day 14, the liver tissue was found to have extensive cords of hepatocytes consisting of neutrophils and macrophages,

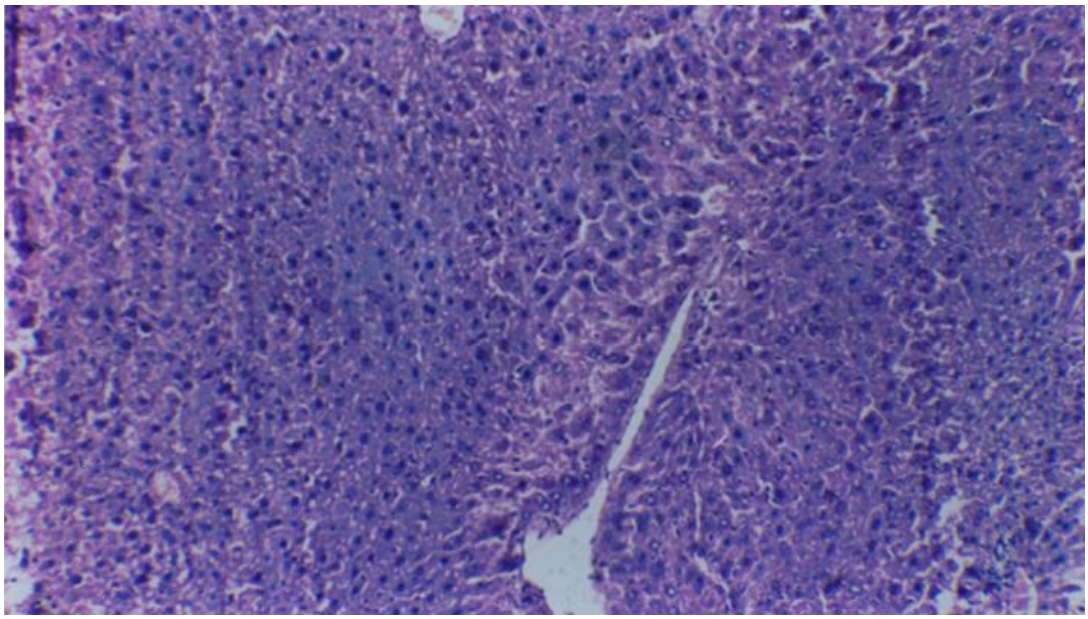

Figure 1d. Effect of ciprofloxacin on *S. typhimurium* induced liver damage. Mice were treated with ciprofloxacin (400 mg per kg b. wt) after infection with challenge dose of 0.6 x LD_{50} *S. typhimurium*. At day 14, liver section were examined histopathologically which revealed that cords of hepat ocytes showing focal granulomatous collection of neutrophils and macrophages (G) and there is no sign of necrosis (N).

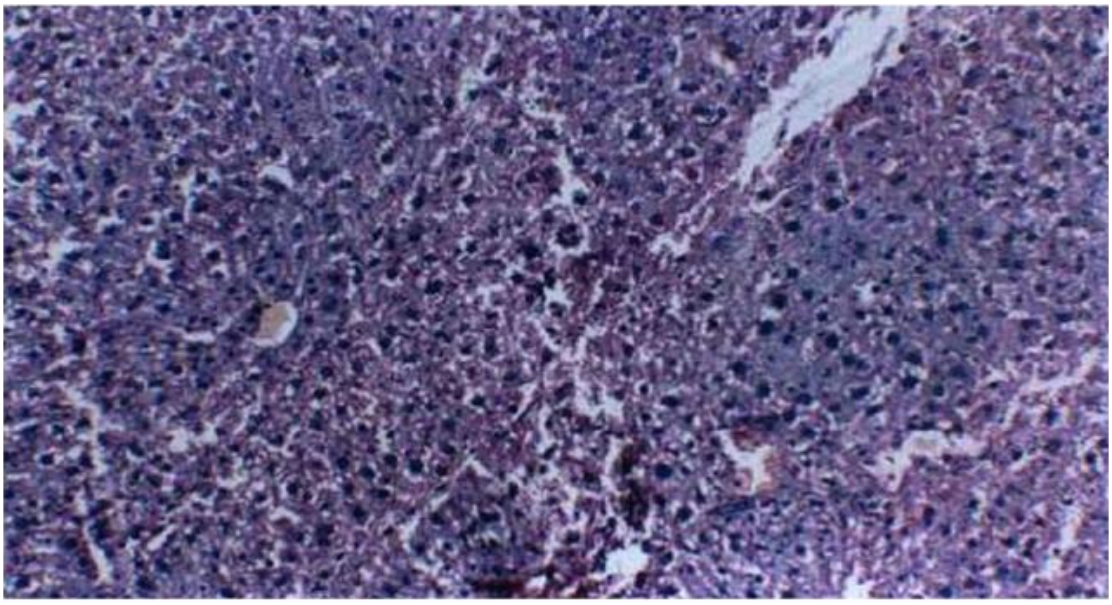

Figure 1e. Effect of combination of drugs on *S. typhimurium* induced liver damage. Mice were treated with B+1/2 Arg+1/2 Cip after infection with challenge dose of 0.6 x LD_{50} *S. typhimurium*. At day 14, liver section were examined histopathologically which revealed that cords of hepatocytes showing focal granulomatous collection of neutrophils and macrophages (G) and there is less sign of necrosis (N).

showing granulomatous lesions and mild necrosis (Figures 1c and d). In ciprofloxacin treated mice, no significant pathological changes in liver were observed.

Conclusion

This study confirms that formulated drugs have significant

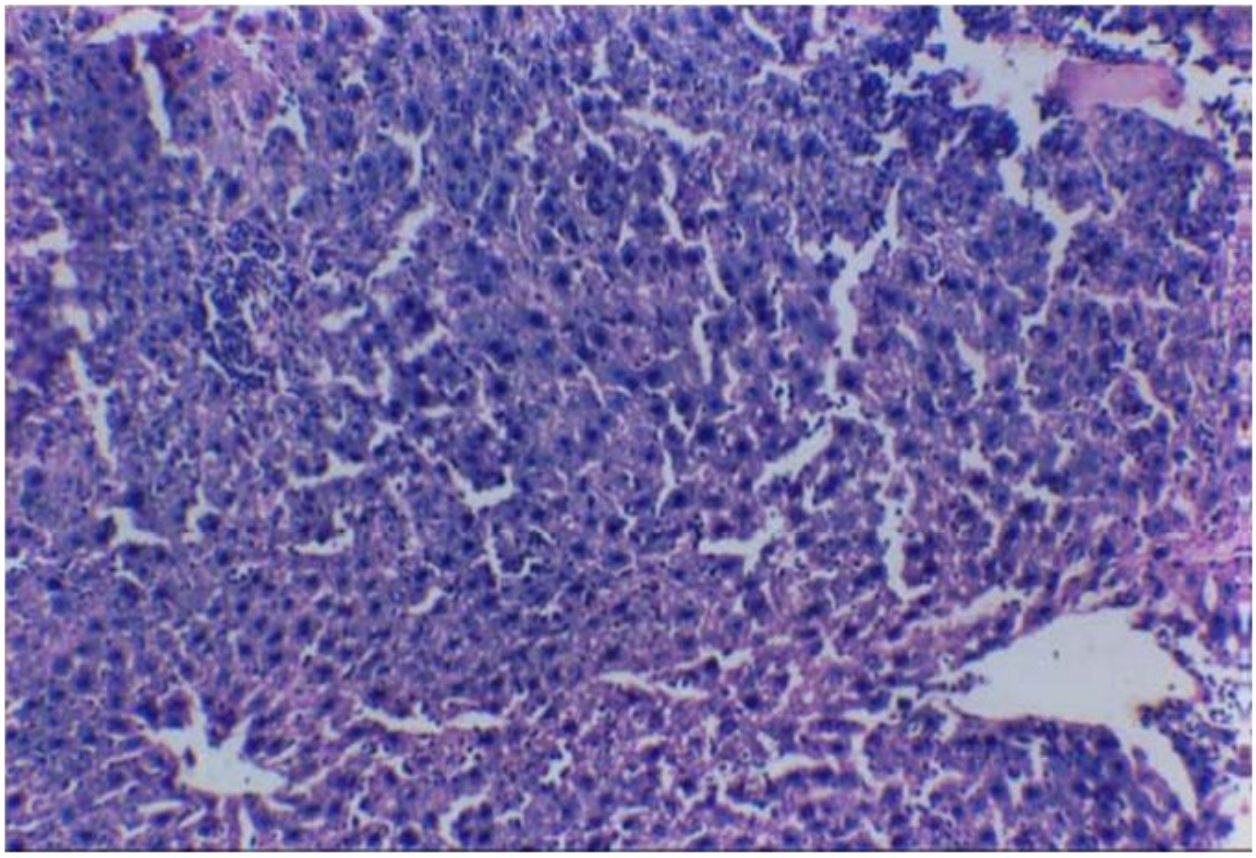

Figure 1f. Effect of combination of drugs on *S. typhimurium* induced liver damage. Mice were treated with B+1/4Arg+1/2Cip after infection with challenge dose of 0.6 x LD_{50} *S. typhimurium*. At day 14, liver section were examined histopathologically which revealed that cords of hepatocytes showing focal granulomatous collection of neutrophils and macrophages (G) and there is mild sign of necrosis (N).

role on typhoid and we have corroborated these findings with normal histopathological observations. Our results are consistent with the earlier reports (Umezawa et al., 1997; MacFarlane et al., 1999; Mastroeni et al., 2000). It was found that when the mice were treated with combination, this combination (B+1/2 Arg+1/2 Cip) caused less granuloma or no necrosis with other combination of drugs (Figures 1e and f).

REFERENCES

Baumler AJ, Tsolis RM, Heffron F (1996). The lpf fimbrial operon mediates adhesion of *Salmonella typhimurium* to murine Peyer's patches. Proc. Natl. Acad. Sci. US A. 9: 279-283.

Brown JG, Brooks BW, Blais BW, Yamazaki H (1996). Application of cloth-based enzyme immunoassay for the characterization of monoclonal antibodies to Salmonella lipopolysaccharide antigens. Immunol Invest. 25(4): 369-381.

Chitnis V, Chitnis D, Verma S, Hemvani N (1999). Multidrug-resistant *Salmonella typhi* in India. Lancet. 7: 354(9177): 514-515.
endotoxemia promotes intrahepatic thrombosis and an oxygen radical-mediated hepatic injury. J. Leukocyte Biol. 52: 390-394.

Fang FC (1997). Perspectives series, host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity. J. Clin. Invest. 99: 2818-2825.

Gruetter CA, Barry BK, McNamara DB, Gruetter DY, Kadowitz PJ, Ignarro LJ (1979). Relaxation of bovine coronary artery and activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside and a carcinogenic nitrosoamine. J. Cyclic Nucleotide Protein Phosphor. Res. 5: 211-214.

Gulati S, Marwaha RK, Singhi S, Ayyagari A, Kumar L (1992). Third generation cephalosporins in multi-drug resistant typhoid fever. Indian Pediatr. 29(4): 513-516.

Harbrecht BG, Billair TR, Stadler J, Demetris AJ, Ochoa J, Curran RD , Simmons RL (1992). Inhibition of nitric oxide synthesis during

Harbrecht BG, Stadler J, Demetris AJ, Simmons RL, Billiar TR (1994). Nitric oxide and prostaglandins interact to prevent hepatic damage during murine endotoxemia. A. J.

Hibbs JB Jr, Taintor RR, Vavrin Z. (1987a). Macrophage cytotoxicity, Role for L-arginine deiminase and imino nitrogen oxidation to nitrite. Sci. 235: 473-476

Ignarro LJ, Byrns RE, Buga GM, Wood KS (1987b). Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. Circ Res. 61: 866-879.

Jesudason MV, John R, John TJ (1996). The concurrent prevalence of chloramphenicol-sensitive and multi-drug resistant *Salmonella typhi* in Vellore, S. India Epidemiol. Infect. 116: 225-227.

MacFarlane AS, Schwacha MG, Eisenstein TK (1999). *In vivo* blockage of nitric oxide with aminoguanidine inhibits immunosuppression induced by an attenuated strain of *Salmonella typhimurium*, potentiates *Salmonella* infection, and inhibits macrophage and polymorphonuclear leukocyte influx into the spleen. Infect. Immun. 67: 891-898.

Manchanda V, Bhalla P (2006). Emergence of non-ceftriaxone-

susceptible Neisseria meningitidis in India. *J. Clin Microbiol*. 44(11):4290-1. Epub 2006 Sep 27.
Nathan C (1997). Perspectives series: nitric oxide and nitric oxide synthases. J. Clin. Invest. 100: 2417-2423.
Ohl ME, Miller SI (2001). Salmonella: a model for bacterial pathogenesis. Annu. Rev. Med. 52: 259-74.
Rowe B, Ward LR, Threlfall EJ (1995). Ciprofloxacin-resistant Salmonella typhi in the UK. Lancet. Sci. 11:346 (8985): 1302. 235: 473-476.
Umezawa K, Akaike T, Fujii S, Suga M, Setoguchi K, Ozawa A, Maedia H (1997). Induction of nitric oxide synthesis and xanthine oxidase and their roles in the antimicrobial mechanism against *Salmonella typhimurium* infection in mice. Infect. Immun. 65, 2932-2940.