VERSITA

Cent. Eur. J. Med. • 4(3) • 2009 • 340-347
DOI: 10.2478/s11536-008-0092-0

Central European Journal of **Medicine**

Effect of *Plasmodium* and *Salmonella* co-infection in a murine model

Research Article

Geeta Shukla[1*], Divya Singh[1], Lalita Sharma[1], Ashwani Koul[2], Praveen Rishi[1]

[1] Department of Microbiology, Panjab University, Chandigarh-160 014, India

[2] Department of Biophysics, Panjab University, Chandigarh-160 014, India

Received 29 August 2008; Accepted 18 November 2008

Abstract: The present study was designed to evaluate the effect of *Plasmodium* and *Salmonella* co-infection in LACA mice. The parasitaemic level, bacterial load, histological alterations and levels of oxidants/antioxidant activity were measured. Co-infected mice had a high parasitaemic level, increased bacterial load, and died earlier than *Plasmodium*-infected mice. Histologically, co-infected mice had more architectural damage in the liver, spleen, kidney, and brain than the control groups. The level of lipid peroxidation was significantly increased and the activities of antioxidative enzymes (superoxide dismutase and catalase) were decreased in all organs of co-infected mice compared to the control groups, indicating depression of the antioxidant defense system. The present study demonstrates more severe histological and biochemical alterations in co-infected mice, highlighting the importance of early diagnosis for selection of appropriate treatments and reducing the likelihood of further complications.

Keywords: *Malaria • Typhoid • Co-infection • Oxidant • Antioxidant*

1. Introduction

Infectious diseases have high degree of morbidity and mortality. Infections caused by parasites, bacteria, and viruses have become a major public health concern, particularly in developing and under-developed countries due to poor sanitation, lack of potable water, and inadequate treatment. The situation is even worse in neonates, children, pregnant women, and immunocompromised individuals. Several studies have reported an enhanced susceptibility of HIV-infected individuals to various infections, including hepatitis, toxoplasmosis, pneumonia, malaria, and salmonellosis [1-3]. Similarly, prior infection with *Helicobacter pylori* infection enhances the susceptibility to infection with *Salmonella typhi* [4].

Malaria and typhoid are endemic in developing countries, including India. These diseases affect millions of children and adults every year [5]. Although they have similar symptoms and epidemiology, little is known about their coexistence. It has been reported that the prevalence of typhoid is higher in areas where drug-resistant malaria is common and that 35% of malaria patients have false-positive Widal tests, whereas 22% of malaria patients have positive Widal test [6-7].

Recently, bacteriemia, especially due to non-typhoidal *Salmonella* infection, has been documented in African children with malaria [8]. Clinically, malaria and salmonellosis have been found to be associated, but there is little data on the incidence of co-infection, and their relative contribution to pathogenesis during co-infection is unknown. Thus, in the current study, we used a murine model to examine the effects of co-infection on the level of parasitemia, bacterial load, and histological alterations related to immune status.

* E-mail: geeta_shukla@pu.ac.in

2. Material and Methods

2.1. Microorganisms

Plasmodium berghei (NICD, National Institute of Communicable Diseases, New Delhi, India) strain NK 65 was maintained in LACA mice by intraperitoneal (i/p) inoculation with parasitized blood. *Salmonella enterica* serovar typhimurium (virulent strain NCTC 74) was obtained from the Central Research Institute, Kasauli, India. The strain was cultured on nutrient agar slants at 37ºC and stored at 4ºC. The strain was examined biochemically and serologically prior to storage and use.

2.2. Animals

Crossbred LACA mice, 8-10 weeks old, weighing 18-25 g were used. The animals were bred in the Central Animal House, Panjab University, Chandigarh, India and were provided standard pellet diet and water *ad libitum*. Animal care, use and disposal were in accordance with the guidelines of our institutional ethical committee.

2.3. Colony formation assay

Salmonella enterica serovar typhimurium (NCTC 74) was grown in nutrient broth for 9 h at 37°C, harvested, washed, and resuspended in phosphate-buffered saline (PBS; 0.1 M, pH 7.2). Viable counts were determined by spreading 10 µl each of 10-fold serial dilutions of the culture on nutrient agar plates. The plates were incubated overnight at 37°C. Following incubation, bacterial colonies were counted, and colony-forming units (CFU) were calculated.

2.4. Animal studies

The animals were divided into the following four groups: group I (n=8), control mice inoculated intraperitoneally (i/p) with normal saline; group II (n=16), *P. berghei*-infected mice, inoculated i/p with 10^5 parasitized red blood cells; group III (n=8), *S. typhimurium*-infected mice, inoculated with 10^4 *S. typhimurium* cells i/p; group IV (n=16), mice infected with both *P. berghei* and *S. typhimurium*, inoculated i/p with both *P. berghei* (10^5) and *S. typhimurium* (10^4) on the same day. Animals in groups I and III were sacrificed 5 days after inoculation. Eight mice in groups II and IV were used to study the pattern of parasitemia, and the remaining 8 were sacrificed at the parasitemia level of 30% to 40%. Animals were sacrificed by cervical dislocation. Parasitemia, histopathology, lipid peroxidation (oxidant) and antioxidant levels [reduced glutathione (GSH), superoxide dismutase (SOD) and catalase] were measured.

2.5. Measurement of parasitemia

Percent parasitemia was monitored on every alternate day by tail vein blood smear, followed by fixation in methanol, staining with Giemsa, and analysis of at least 500 cells.

2.6. Collection of tissue, histopathology, and preparation of tissue homogenates

The liver, spleen, kidney, and brain of all animals were removed aseptically and rinsed in normal saline. Tissues weights were measured, and a portion of the tissue was fixed in 10% buffered formalin, were processed, stained with hematoxylin & eosin, mounted with DPX and examined microscopically. The remaining tissue was homogenized for 30 to 45 s in PBS using a Potter-Elvehjem homogenizer with a Teflon pestle on ice. The protein content in the tissue homogenates was estimated according to the method of Lowery *et al.* [9].

2.7. Assessment of bacterial load

Bacterial load in tissues was assessed by plating 10-fold serial dilutions of tissue homogenates on nutrient agar. Cultures were incubated for 24 h at 37°C. Bacterial load in the blood of mice belonging to groups III and IV was assessed by streaking tail vein blood directly on to MacConkey agar.

2.8. Isolation of PMS

Tissue homogenates were centrifuged at 2500 rpm for 10 min. The pellet was discarded, and the supernatant was further centrifuged at 12,000 rpm for 10 min at 4°C. Supernatants (PMS) were collected and used for the estimation of various oxidants and antioxidants level.

2.9. Analysis of lipid peroxidation

Lipid peroxidation was assessed as described by Wills [10] using the molar extinction coefficient of chromophore ($1.56 \times 10\ M^{-1}cm^{-1}$).

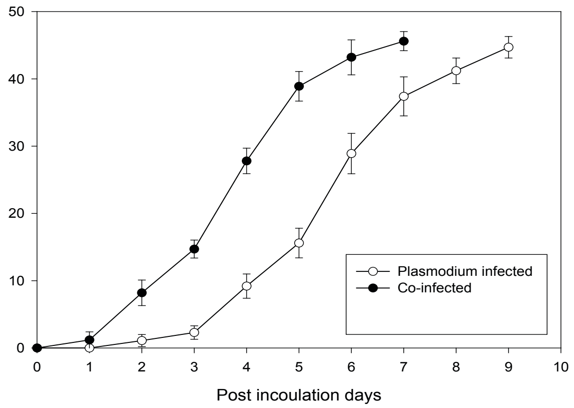
2.10. Measurement of GSH levels

GSH levels in various tissues were assessed as described by Ellman [11], and the results are expressed as nanomoles of GSH per milligram of protein.

2.11. Assay of SOD activity

SOD activity was measured according to the method of Kono [12] and expressed as units SOD per milligram protein, where one unit of activity is defined as the amount of SOD required to inhibit the rate of reduction of nitro blue tetrazolium by 50%.

Figure 1. Parasitaemic level in *Plasmodium*-infected and co-infected mice. Values are means ± standard deviation.



2.12. Assay of catalase activity

Catalase activity was assayed by the method of Calabiborne [13] using the absorption at 240 nm, and the results are expressed in K min^{-1}.

2.13. Statistical analysis

Means were compared using Student's *t*-test with an equal number of observations. The oxidant and antioxidant data was analyzed by two-way analysis of variance with an equal number of observations. Differences were considered significant at $p<0.05$.

3. Results

3.1. Parasitemia

Plasmodium-infected mice showed a gradual increase in parasitemia that began 2 days after inoculation and increased gradually until day 9 post-inoculation, with maximal parasitemia of 44.7%. In contrast, co-infected mice showed a rapid increase in parasitemia beginning 1 day after inoculation, with maximal parasitemia of 46% on after 7 days (Fig. 1). Co-infected mice died earlier than *Plasmodium*-infected mice (day 8 vs. day 10).

3.2. Qualitative and quantitative assessment of bacterial cells

The bacterial load in the blood of *Salmonella*- and co-infected mice 3 days after inoculation is shown in Figures 2 and 3. Co-infected mice had higher bacteremia and mortality than *Salmonella*-infected mice (Table 1 and 2). Interestingly, in the *Salmonella*-infected group, none of the mice had died 5 days post-inoculation, whereas more than 60% of mice in the co-infected group were dead at this time (Table 2). The bacterial load in various organs was significantly higher in the co-infected mice than in the *Salmonella*-infected mice (Table 1). In addition, the weights of all organs were significantly higher in co-infected mice than in the control groups (Table 3).

Figure 2. Bacterial loads in *Salmonella*-infected mice 3 days after inoculation.

Figure 3. Bacterial loads in co-infected mice 3 days after inoculation.

Table 1. Quantitative assessment of bacterial load in the vital organs.

Tissue	*Salmonella-infected*	Co-infected infected
Liver	$9.2X10^5 \pm 0.65$	$9.8X\ 0^5 \pm 0.68^b$
Spleen	$2.8X10^5 \pm .35$	$7.3X10^5 \pm 0.49^a$
Kidney	$1.3X10^4 \pm 0.55$	$7.2X10^4 \pm 0.50^b$
Brain	$9.3X10^3 \pm 0.72$	$1.7X10^4 \pm 0.40^b$

Values represent mean CFU/ml ± standard deviation. $^a p<0.01$, $^b p<0.05$

Table 2. Bacterial load in the blood of *Salmonella* and co-infected mice.

Post inoculation days	*Salmonella* infected (P/M/N/ %)	Co-infected (P/M/N/ %)
1	- / 0 / 8 / 0	- / 0 / 8 / 0
2	- / 0 / 8 / 0	- / 0 / 8 / 0
3	+ / 0 / 8 / 50	+ / 1 / 8 / 87.5
4	+ / 0 / 8 / 100	+ / 2 / 8 / 100
5	+ / 0 / 8 / 100	+ / 2 / 8 / 100

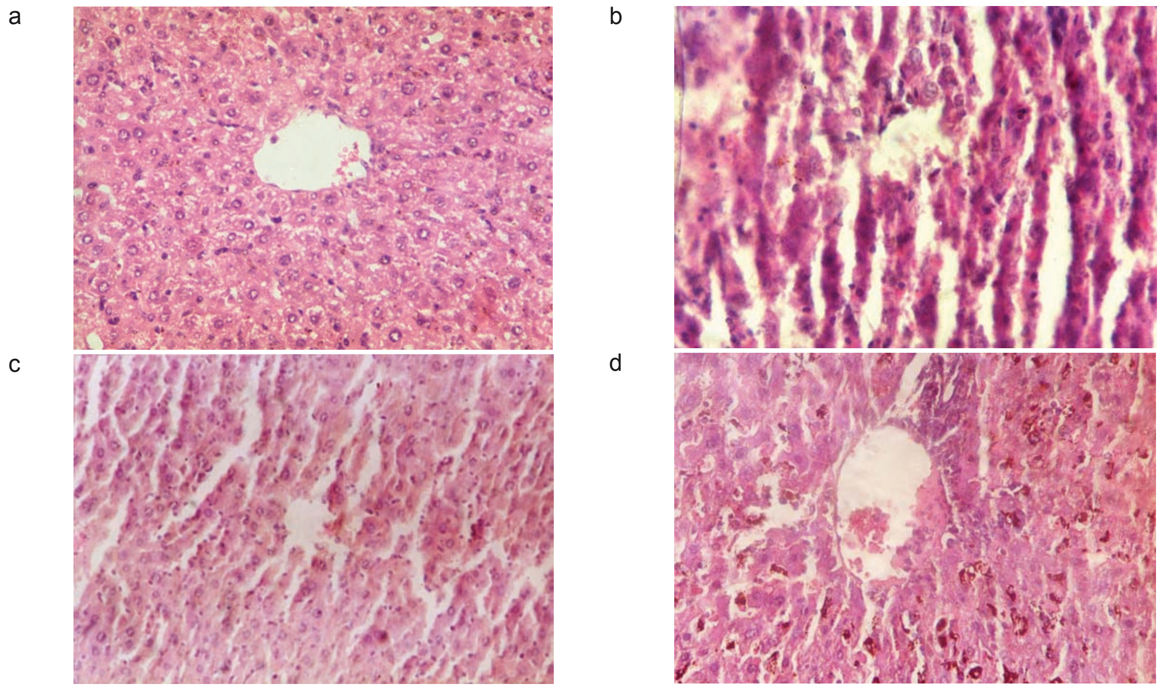
P, presence/absence of organism; M, mortality; N, total number of mice; %, percent of mice showing bacterial colonies.

Table 3. Vital organ weights.

Organ	Control	*Plasmodium-infected*	*Salmonella-infected*	Co-infected
Liver	0.98 ± 0.020	1.79 ± 0.063 [a]	1.24 ± 0.067 [a,b]	1.87 ±0.078 [a,b,c]
Spleen	0.053 ±0.019	0.318 ± 0.036 [a]	0.12 ± 0.031 [a,b]	0.34 ± 0.043 [a,b,c]
Kidney	0.15 ±0.021	0.205 ± 0.013 [a]	0.16 ± 0.023 [a,b]	0.21 ± 0.016 [a,b,c]
Brain	0.352 ± 0.030	0.208 ± 0.021 [a]	0.31 ± 0.039 [a,b]	0.22 ± 0.014 [a,b,c]

Values are means ± standard deviation. [a]Significant ($p<0.05$) difference vs. control group. [b]Significant ($p<0.05$) difference vs. Plasmodium-infected group. [c]Significant ($p<0.05$) difference vs. Salmonella-infected group.

Figure 4. Photomicrograph showing a liver stained with hematoxylin & eosin (magnification, 250x). (a) Control mice. (b) *Plasmodium*-infected mice. (c) *Salmonella*-infected mice. (d) Co-infected mice.



3.3. Histopathology

Histopathologically, the livers of control mice showed hepatic plates radiating outwards from the central vein and towards the periphery of the sinusoids between the plates of hepatocytes (Figure 4a). In contrast, the livers of *Plasmodium*-infected mice showed distortion in the central vein and prominent sinusoid spacing, and the livers of *Salmonella*-infected showed structural disintegration in hepatic plates along with dilated sinusoids and increased intracellular gaps. In addition, severally damaged central veins and mildly distorted hepatic lobules were observed (Figures 4b and 4c). Interestingly, the livers of co-infected mice showed overall distortion of the histoarchitecture, dilated sinusoids, an enlarged nucleus, and poorly formed lobules (Figure 4d).

The spleens of control mice showed a normal splenocytes texture (Figure 5a), whereas the spleens of both *Plasmodium*- and *Salmonella*-infected mice showed inflammation of the splenocytes (Figure 5b), with the *Salmonella*-infected mice having a greater extent of inflammation (Figure 5c). Similarly, co-infected mice had a maximal extent of splenocytes inflammation and altered architecture, along with prominent sinusoids (Figure 5d).

The kidneys of *Plasmodium*-infected mice showed damaged convoluted tubules (Figure 6b), whereas the kidneys of *Salmonella*-infected mice showed damaged glomeruli and Bowman's capsules (Figure 6c). In contrast, co-infected mice had highly inflamed glomeruli and Bowman's capsules (Figure 6d).

The brains of control mice had normal pyramidal cell structures in the cortex region (Figure 7a). The brains of *Plasmodium*-infected mice, in contrast, showed inflammation of the giant pyramidal cells and broadly damaged interstitia (Figure 7b). The giant pyramidal cells were not prominent in *Salmonella*-infected mice (Figure 7c). The brains of co-infected mice had thin interstitia in the cerebral cortex and a damaged cellular architecture (Figure 7d).

Figure 5. Photomicrograph showing a spleen stained with hematoxylin & eosin (magnification, 250x). (a) Control mice. (b) *Plasmodium*-infected mice. (c) *Salmonella*-infected mice. (d) Co-infected mice.

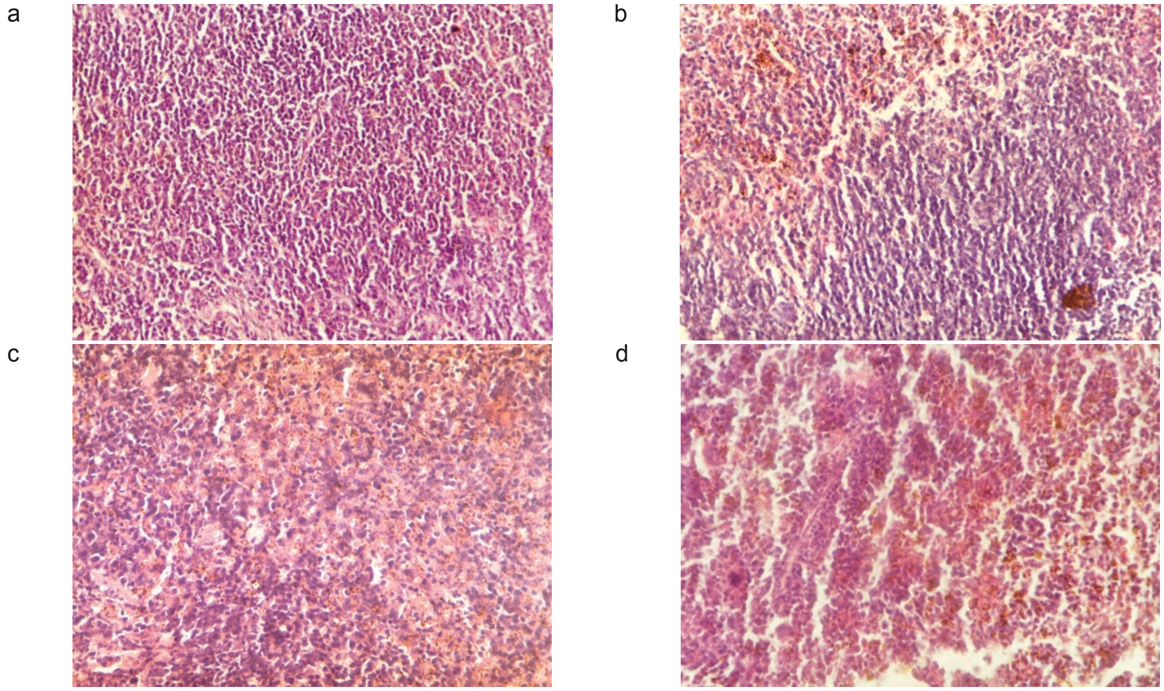


Figure 6. Photomicrograph showing a kidney stained with hematoxylin & eosin (magnification, 250x). (a) Control mice. (b) *Plasmodium*-infected mice. (c) *Salmonella*-infected mice. (d) Co-infected mice.

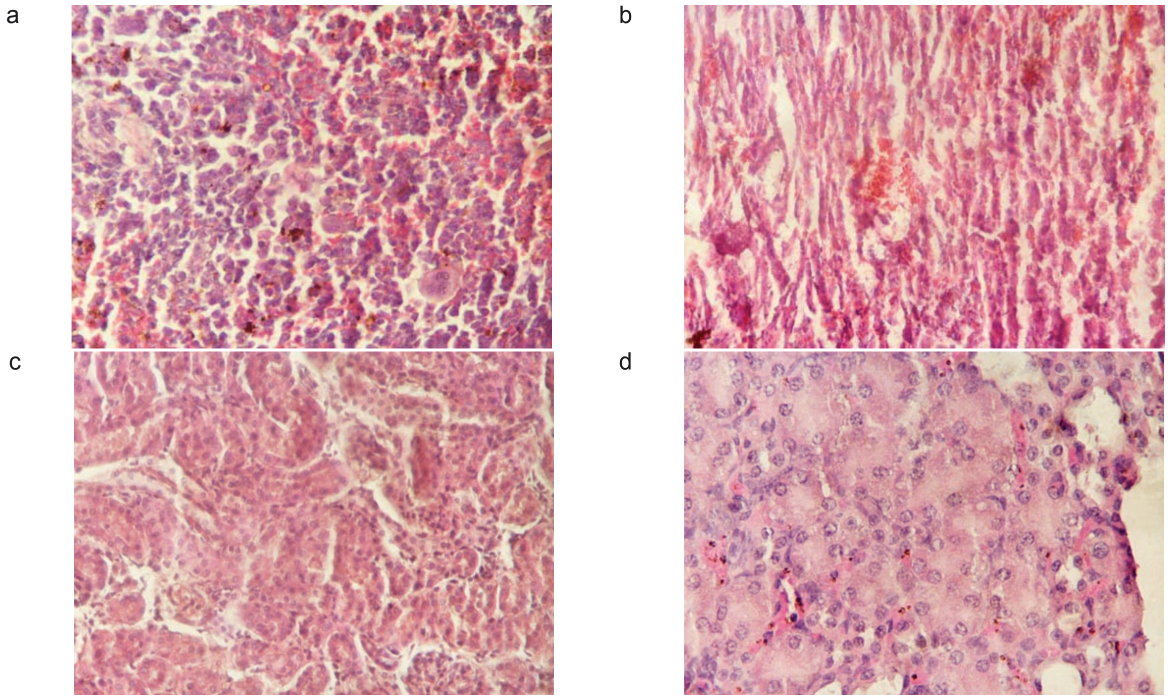


Figure 7. Photomicrograph showing a brain stained with hematoxylin & eosin (magnification, 250x). (a) Control mice. (b) *Plasmodium*-infected mice. (c) *Salmonella*-infected mice. (d) Co-infected mice.

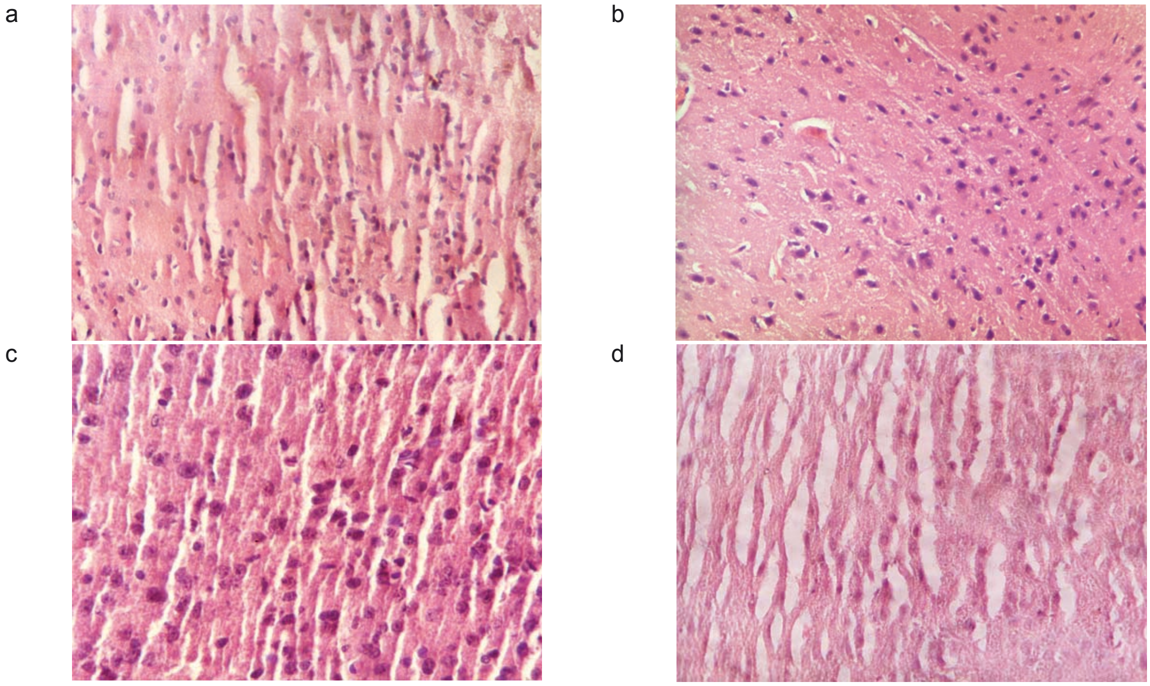


Figure 8. Melanodialdehyde levels. Values are means ± standard deviation. [a]Significant (p<0.05) difference vs. control group. [b]Significant (p<0.05) difference vs. *Plasmodium*-infected group. [c]Significant (p<0.05) difference vs. *Salmonella*-infected group.

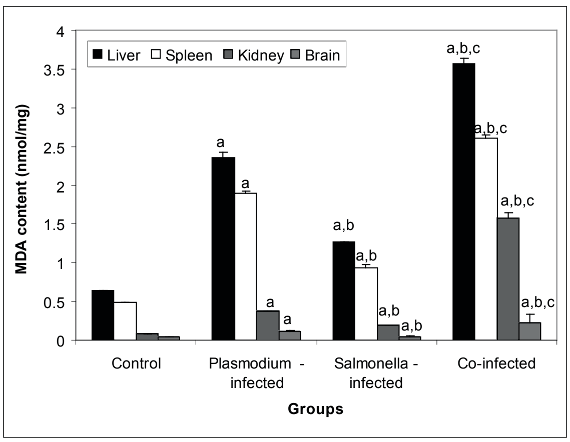
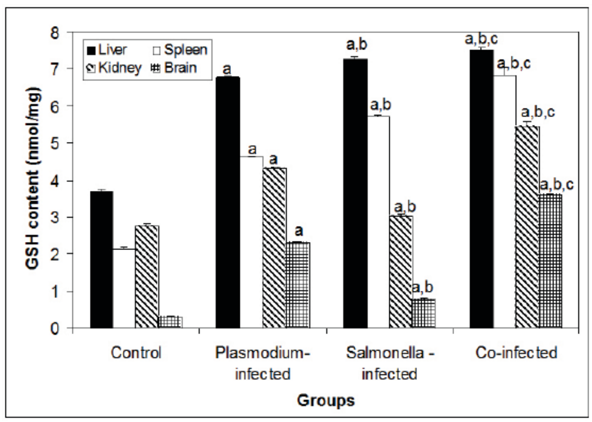


Figure 9. Levels of GSH. Values are means ± standard deviation. [a]Significant (p<0.05) difference vs. control group. [b]Significant (p<0.05) difference vs. *Plasmodium*-infected group. [c]Significant (p<0.05) difference vs. *Salmonella*-infected group.



3.4. Oxidant and antioxidant level

Plasmodium- and *Salmonella*-infected mice had increased levels of MDA in all organs compared to the control group. The level of MDA in co-infected mice, however, was much higher than in the controls (Figure 8). Similarly, the GSH level was significantly higher in all three test groups compared to the controls (Figure 9). SOD and catalase activities in organs, however, were significantly lower in co-, *Salmonella*-, and *Plasmodium*-infected mice compared to control mice (Tables 4 and 5).

4. Discussion

Malaria and *Salmonella* are more prevalent in underdeveloped countries, and their co-existence in the same individual may result in further deterioration of the health status. Thus, we carried out the present study to examine how co-infection affects the host.

Co-infected mice had a gradual increase in parasitemia due to increased reticulocytosis [14]. The infection was more severe in co-infected mice, a finding

Table 4. SOD activity in different groups of mice.

Group	SOD activity (units/mg protein) in Liver			
	Liver	Spleen	Kidney	Brain
Control	279.31±0.32	117.24±0.22	110.07±0.23	127.9±0.47
Plasmodium	147.86±0.18^{a}	82.82±0.19^{a}	66.77±0.18^{a}	67.58±0.21^{a}
Salmonella	186.75±0.21^{a,b}	91.92±0.19^{a,b}	94.14±0.21^{a,b}	98.4±0.51^{a,b}
Co-infected	118.54±0.07^{a,b,c}	64.92±0.14^{a,b,c}	58.32±0.14^{a,b,c}	37.98±0.18^{a,b,c}

Values are means ± standard deviation. ^{a}Significant ($p<0.05$) difference vs. control group. ^{b}Significant ($p<0.05$) difference vs. Plasmodium-infected group. ^{c}Significant ($p<0.05$) difference vs. Salmonella-infected group.

Table 5. Catalase activity in different groups of mice.

Group	Catalase activity ($Kmin^{-1}$) in liver			
	Liver	**Spleen**	**Kidney**	**Brain**
Control	0.38±0.24	0.15±0.21	0.25±0.22	0.15±0.64
Plasmodium	0.19±0.13^{a}	0.08±0.19^{a}	0.21±0.21^{a}	0.05±0.11^{a}
Salmonella	0.23±0.12^{a,b}	0.08±0.18^{a,b}	0.24±0.22^{a,b}	0.11±0.29^{a,b}
Co-infected	0.15±0.09^{a,b,c}	0.06±0.16^{a,b,c}	0.17±0.18^{a,b,c}	0.03±0.09^{a,b,c}

Values are means ± standard deviation. ^{a}Significant ($p<0.05$) difference vs. control group. ^{b}Significant ($p<0.05$) difference vs. Plasmodium-infected group. ^{c}Significant ($p<0.05$) difference vs. Salmonella-infected group.

supported by the observation of an increased bacterial load in the blood and organs. This suggests that co-infection makes the host more vulnerable to infection. These observations are supported by earlier studies showing an elevated bacterial count in the livers of infected mice [15-16].

Lipid per oxidation is a process of oxidative degradation of polyunsaturated fatty acids, and it results in the generation of excess of free radical intermediates, a characteristic of cell injury. The level of lipid peroxidation products, such as MDA, can be used an index of peroxidation. Reactive oxygen species are important mediators of tissue injury during malaria and typhoid [17-19]. The enhanced level of MDA in all of the organs of all three test groups supports that the damage observed was due to enhanced production of oxidants, in agreement with earlier findings [18-20]. In addition, it is well established that malarial parasites invading erythrocytes cause pathological alterations in organs, ranging from hyperplasia and color change (*i.e.*, from pink to dark brown due to accumulation of malarial hemozoin pigment) and increased production of reactive oxygen species due to activation of neutrophils and degradation of hemoglobin [21].

Tissue injury depends upon the balance between the generation of toxic radicals and tissue antioxidant status [19,22]. GSH, an antioxidant, may act either by protecting cells from lipid peroxidation or by protecting protein sulfhydrl groups from irreversible oxidation after oxidant injury. In addition, GSH released by liver parenchymal cells are thought to trap reactive oxygen species generated by Kupffer cells and neutrophils in the vasculature and therefore attenuate liver injury [23]. However, in the present study, the level of GSH was increased despite an increased severity of infection, suggesting that GSH might have a dual role *in vivo*. In contrast, an increased level of GSH during infection by malaria may be an attempt by the host to neutralize oxidative stress generated by the parasite.

The decreased antioxidant enzyme (SOD and catalase) activity observed here may be due to a reduced ability to neutralize superoxide anions and inefficient detoxification of hydrogen peroxidase, which would result in the formation of hydroxyl radicals. The increased production of hydroxyl radicals may, in turn, enhance the peroxidation of lipid membranes as suggested by an increased extent of lipid peroxidation in all three test groups of mice. These findings are supported by our histopathological studies, where, in spite of an increased level of GSH, there was clear damage to all organs, in accordance with earlier studies [20,24].

In summary, the results of present study suggests that both individual infection by *Plasmodium* or *Salmonella* or co-infection by the two microorganisms may cause tissue damage due to the accumulation of free radicals, resulting in decreased levels of antioxidant enzymes. Our results suggest that the manifestations of infection were more pronounced in co-infected mice due to increased oxidative stress and depressed antioxidative response.

References

[1] Gilson R.J., Hawkins A.E., Beecham M.R., Ross E., Waite J., Briggs M., McNally T., Kelly G.E., Tedder R.S., Weller I.V., Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection, AIDS, 1997, 11, 597-606

[2] Xiao L., Owen S.M., Rudolph D.L., Lal R.B., Lal A., Plasmodium falciparum antigen-induced human immunodeficiency virus type-1 replication is mediated through induction of tumor necrosis factor alpha, J. Infect. Dis., 1998, 177, 437-45

[3] Froebel K., Howard W., Schafer J.R., Howie F., Whitworth J., Kaleebu P., Brown A.L., Riley E., Activation by malaria antigens renders mononuclear cells susceptible to HIV infection and re-activates replication of endogenous HIV in cells from HIV-infected adults, Parasit. Immunol., 2000, 26, 213-7

[4] Bhan M.K., Bahl R., Sazawal S., Sinha A., Kumar R., Mahalanabis D., et al., Association between Helicobacter pylori infection and increased risk of typhoid fever. Journal of Infectious Diseases, 2000, 186, 12, 1857-1860

[5] Keong B.C.M., Sulaiman W., Typhoid and malaria Co-infection – An interesting finding in the investigation of a Tropical fever, Malaysian J. Medical Sciences, 2006, 13, 1, 74-75

[6] Frimpong E.H., Feglo P., Essel-Ahun M., Addy P.A.K., Determination of diagnostic widal titers in Kumasi Ghana, West Afr. J. Med., 2000, 19, 34-38

[7] Petit P.L.C., Wamola I.A., Typhoid fever. A review of its impact and diagnostic problems, East Afr. med. J. 1999, 11, 183-188

[8] Bronzan R.N., Taylor T.E., Mwenechanya J., Temb M., Kayira K., Bwanasia L., et al., Bacteremia in Malawian Children with Severe Malaria: Prevalence, Etiology, HIV Co-infection and outcome, Infectious Disease, 2000, 195, 895-904

[9] Lowry O.H., Rosenbrough N.J., Farr A.L. Randell R.J., Protein measurement with folin's phenol reagent, J. Biol Chem., 1951, 193, 265-275

[10] Wills E.D., Mechanism of lipid peroxidation formation in animal tissues, Biochem. J., 1966, 99, 667-676

[11] Ellman G.L., Tissue sulfhydryl groups, Arch. Biochem., 1959, 82, 70-77

[12] Kono Y., Generation of superoxide radical during autooxidation of hydroxylamine and an assay of superoxide dismutase, Arch. Biochem. Biophys., 1978, 186, 189-195

[13] Claiborne A., Handbook of Methods for Oxygen Radical Research, CRC Press, 1985

[14] Zuckerman A., Blood loss and replacement in Plasmodium infection I., Plasmodium berghei in untreated rats of varying age and in adult rats with erythropoetic mechanisms manipulated before inoculation, J. Infect. Diseases, 1957, 100, 172-177

[15] Richter-Dahlfors A., Buchan A.M.J., Finlay B.B., Murine Salmonellosis Studied by Confocal Microscopy: Salmonella resides intracellularly inside macrophages and exerts a cytotoxic effect on phagocytes in vivo, J. Exp. Med., 1997, 186, 569-580

[16] Ninalue N.A., Shnyra A., Hultenby K., Linderberg A.A., Salmonella choleraesuis and Salmonella typhimurium associated with liver cells after intravenous inoculation of rats are localized mainly in kupffer cells and multiply intracellularly, Infect. Immun., 1992, 60, 2758-2768

[17] Clark A., Chaudhri G., Cowden W.B., Some roles of free radicals in malaria, Free Rad Boil Med, 1989, 6, 315-321

[18] Siddiqui N.J., Puri S.K., Dutta G.P., Maheshwari R.K., Pandey V.C., Studies on hepatic oxidative stress and antioxidant defence system during chloroquine/poly ICLC treatment of Plasmodium yoelii nigeriensis infected mice, Molecular and Cellular Biochemistry, 1999, 194, 179-183

[19] Rishi P., Kaur H., Tirkey N., Chopra K., Bharrhan S., Chanana V., Koul A., Are the increases in local tumor necrosis factor and lipid peroxidation observed in pre-starved mice infected with Salmonella typhimurium, markers of increased liver damage, Microbes. Infect., 2006 (In press)

[20] Srivastava P., Puri S.K., Dutta G.P., Pandey V.C., Hepatic superoxide scavenging system during Plasmodium berghei infection and chloroquine treatment, Med. Sci. Res., 1991, 19, 307-308

[21] Kremsner P.G., Greve B., Lell B., Luckner D., Schmid D., Malarial anemia in African children associated with high oxygen radical production, Lancet, 2000, 355, 40-41

[22] Windrow V.R., Winyard P.G., Morris C.J. Blake D.R. Free radicals in inflammation: second messengers and mediators of tissue destruction, Br. Med. Bull., 1993, 49, 506-522

[23] Jaesctike H., Enhanced sinusoidal glutathione efflux during endotoxin induced stress in vivo, Am. J. Physiol., 1992, 263, G60-G68

[24] Mehta A., Singh S., Ganguly N.K., Role of reactive oxygen species in Salmonella typhimurium-induced enterocyte damage, Scand. J. Gastroenterol., 1998, 33, 4, 406-414