Addis Ababa University

Faculty of Life Science

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Biomedical Science Stream

Experimental Evaluation of Schistosomiasis-Malaria Co-infection In Mouse Model System

By: Wondwossen Sisay

A Thesis submitted to the School of Graduate Studies, Addis Ababa University, in partial Fulfillment of the Requirements of the Degree of Master of Science in Biology (Biomedical Science)

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ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

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A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in (Biomedical Sciences)

Approved by Examining Board:

Prof. Beyene Petros	(Advisor)	02 | 04 | 2012
Prof. Berhanu Erko	(Examiner)	02 | 04 | 2012
Dr. Mekuria Lakew	(Examiner)	02 | 04 | 12
Dr. Fassil Assefa	(Chairman)	02 | 04 | 12

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Abbreviations

CCL	CC Chemokine ligand
CD	Cluster of differentiation
CDC	Center for Disease Control
CR1	Complement receptor 1
g/dl	Gram per deciliter
GPIs	Glycosylphosphatidylinositols
Hb	Hemoglobin
ICAM-1	Intercellular adhesion molecule-1
IFN_{γ}	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
MCP	Membrane cofactor protein
MDC	Macrophage-derived Chemokines
MHC	Major Histocompatibility complex
MIP	Macrophage inflammatory protein
NKC	Natural killer complex
pRBC	Parasitized red blood cell
p/cm	Pixel per centimeter
RBC	Red blood cell
SCID	Severe combined immunodeficient

TARC	Thymus and activation regulated chemokine
TGFβ	Tumor growth factor beta
TH	T-helper cell
TNF-α	Tumor necrosis factor alpha
TRAP	Thrombospondin-related adhesive protein
VCAM-1	Vascular cell adhesion molecule-1
WHO	World Health Organization

Abstract

Mixed parasitic infections are common in many parts of the world. *Plasmodium falciparum* and *Schistosoma mansoni* are co-endemic parasitic infections with broad global distribution. However, little is known about how concurrent infections affect the immunity to and/or pathogenesis of each other. Thus, this study was undertaken to examine the parasitological, histopathological and hematological conditions that occur in *Schistosoma mansoni-Plasmodium berghei* co-infected mice. Mice infected with *S. mansoni* cercariae were divided into two groups, which were super-infected at weeks 4 and 7 post-infection with *P. berghei*. Giemsa stained blood specimens, hematoxyline and eosin stained liver and brain tissues were used for the analysis of parasitaemia, liver granuloma and cerebral sequestration of malaria parasites, respectively. In addition to these, hemoglobin level, percentage weight loss, eggs per gram of liver and survivability of *P. berghei* infected mice were used as parameters for the comparison of co-infected and mono-infected groups. Sampling of hemoglobin and weight measurements were done at days 3, 5, 7, 9 and 11 post-*P. berghei* infection. Sampling of blood for malaria parasitaemia was done at days 5, 7, 9 and 11 post-*P. berghei* infection. Independent *t*-test analysis of the results showed that co-infection of mice with *P. berghei*, 7 weeks post-*S. mansoni* infection resulted in significant ($P<0.05$) increase in malaria parasitaemia, severe loss of hemoglobin and weight, lower number of schistosome eggs per gram of liver, and decrease in granuloma size and number. In addition, reduction of malaria parasite sequestration in the brain with resultant increase in the life span of the infected mice was associated with co-infection. However, there was insignificant difference during co-infection of mice with *P. berghei* 4 weeks post-*S. mansoni* infection. The overall interplay between these two infections may be explained immunologically, with malaria infection down-regulating delayed hypersensitivity reaction, which would lead to reduction in granuloma formation around schistosome eggs. Similarly, chronic schistosome infection may modulate host immunopathological responses that would lead to cerebral sequestration. Taken together, the study showed schistosome and malaria co-infections to profoundly affect each other. These findings can have implications in treatment and in the efficacy of vaccines against schistosomiasis and malaria in co-endemic localities.

Key words: Co-infection, *S. mansoni*, *P. berghei*, Parasitaemia, Hemoglobin, Granuloma, Cerebral sequestration

1. Introduction

1.1. Malaria: The Disease

1.1.1. General characteristics

Malaria is the most serious tropical disease of humankind and the cause of much death and morbidity in areas where it is endemic. Globally, the annual disease burden in endemic countries is estimated as 225 million clinical cases and 781,000 deaths with the greatest impact in sub-Saharan Africa (WHO, 2010). The disease is caused by a protozoan parasite from the genus *Plasmodium*, transmitted through the bite of the female *Anopheles* mosquitoes. Four species of *Plasmodium* are known to cause disease in humans, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium falciparum*, the latter being the most virulent and the major cause of mortality. *Plasmodium knowlesi* is now established as the fifth *Plasmodium* species to cause malaria in humans (Figtree *et al*., 2010).

Most of the clinical signs of this disease are caused by the parasite at stages in which it multiplies asexually in red blood cells. The typical clinical symptoms of malaria are fever, nausea and headache, sometimes accompanied by diarrhea and vomiting. If the condition remains untreated, life threatening complications, commonly termed severe malaria, may follow in *P. falciparum* infections. Severe malaria includes any one or more of the following manifestations: cerebral malaria, severe anaemia, renal failure, pulmonary edema, hypoglycemia, circulatory collapse, spontaneous bleeding, repeated generalized convulsions, acidosis and haemoglobinuria (WHO, 1990). Deaths in children are mainly due to severe anaemia or cerebral malaria often in association with hypoglycemia (Marsh *et al*., 1996). The incidence of severe malaria is believed to be increasing, most probably because of widespread parasite resistance to the commonly available anti-malarial drugs. However, only a small proportion of infected children develop severe complications; in non-immune individuals these can cause severe and life-threatening disease. The reasons for these differences are not fully understood, but, it is likely that host genetics, co-infection with other diseases, immunity, and social and geographic factors may play a role (Fig 1).

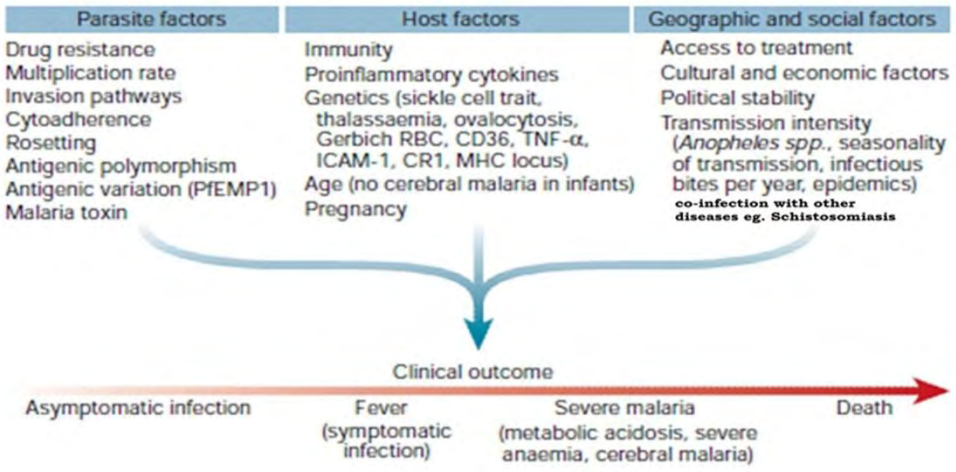

Figure 1. Factors for the clinical outcome of malaria diseases (Modified from Miller *et al*., 2002).

Many attempts have been made by using several *Plasmodium* antigens both in model systems and in humans to develop a malaria vaccine. However, the results, although encouraging, are far from satisfactory. One of the difficulties hindering the design of a successful vaccine against *Plasmodium* parasites is our current incomplete knowledge of protective immunity and how it can be induced. Moreover, the pathogenesis of two of the most severe complications of *P. falciparum* malaria, cerebral malaria and severe malarial anemia both appear to involve dysregulation of the immune system (Miller *et al*., 2002). Therefore, a greater appreciation of the mechanisms of protective immunity on the one hand and of immunopathology on the other would provide crucial clues as to how manipulation of the immune system may best be achieved in order to reach the goal of better vaccines.

1.1.2. Biology and Life cycle

The *Plasmodium* spp. life cycle (Fig. 2) has an asexually reproducing generation, which reproduces by multiple binary fission, and alternates with a generation that reproduces by forming sex cells that fuse with one another and produce individuals with new combinations of traits (Marcus, 2009).

The cycle begins when mosquitoes inject sporozoites into the subcutaneous tissue of the vertebrate host, and less-frequently, directly into the bloodstream. From there, sporozoites travel to the liver, where they invade and replicate in the hepatocytes. Following schizogony, thousands of daughter merozoites are released into the bloodstream and enter red blood cells (RBCs).

The parasites are carried around the circulation within RBCs, but as they grow, they express adherent ligands such as *P. falciparum* erythrocyte membrane protein 1 that enable the maturing parasite to bind receptors expressed by endothelial cells that line the blood vessels in the deep vascular beds of organs such as the brain, lungs and placenta. The parasitized RBCs (pRBCs) rupture and release more daughter merozoites, thereby perpetuating and promoting the blood-stage cycle. Some merozoites differentiate into gametocytes, which, when taken up by another feeding mosquito, complete the sexual phase of the life cycle in the insect.

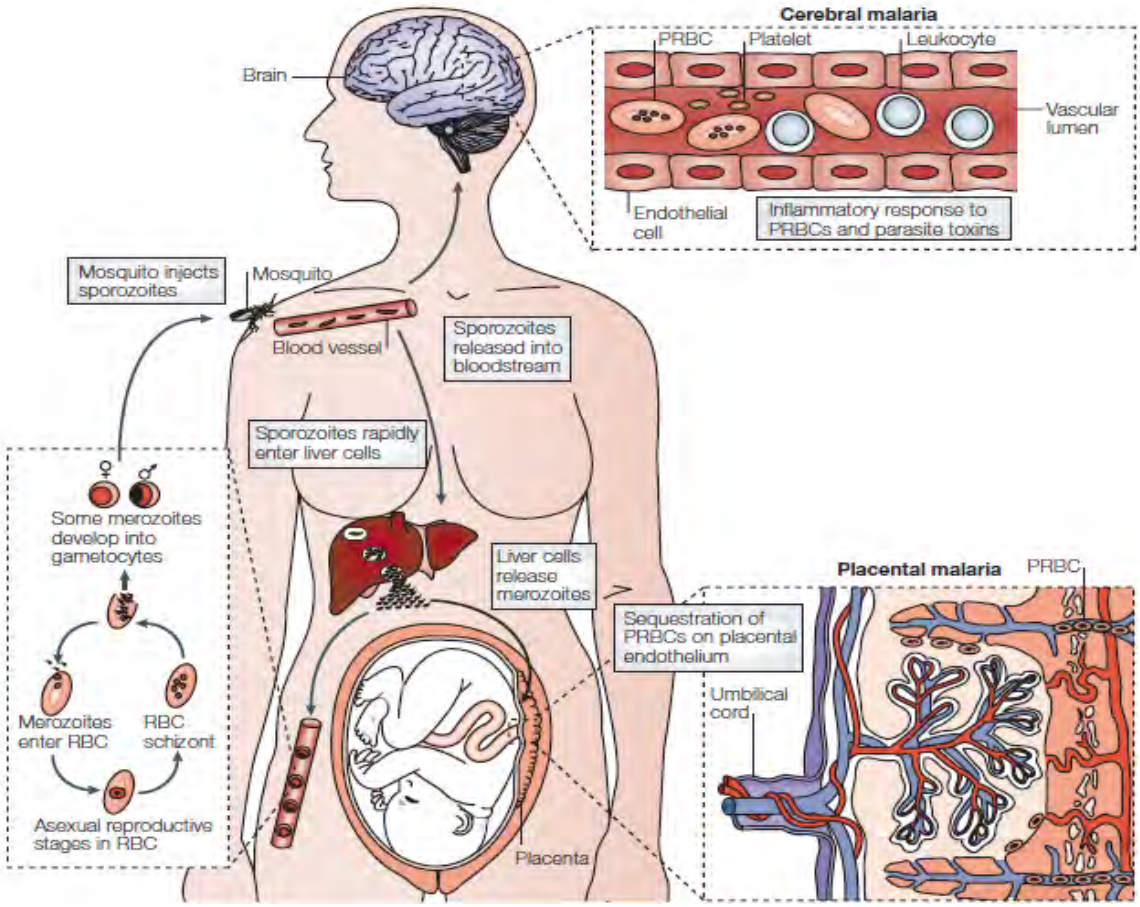

Figure 2. The life cycle of Falciparum malaria (Source: Schofield and Grau, 2005).

A key feature in the biology of *P. falciparum* is its ability to cause infected red blood cells (RBCs) to adhere to the linings of small blood vessels (Miller *et al*., 2002). Such sequestered parasites cause considerable obstruction to tissue perfusion. In addition, in severe malaria, there may be marked reduction in the deformability of uninfected RBCs (Dondorp *et al.*, 2000), which would lead to pRBC accumulation and clogging of the vascular vessels.

1.1.3. Immunopathogenesis of malaria

The early interaction of the parasite with the host immune apparatus is important in determining the nature of subsequent acquired immune response and the pathology associated with the complications of severe malaria such as cerebral malaria, severe anemia, and hypoglycemia. Thus, the manner in which the malaria parasite activates the innate immune system and the cytokines and chemokines induced will all influence the magnitude of the inflammatory response and the types of T and B cell responses elicited. An understanding of these processes might enable us to determine the level at which host responses contribute to malarial disease, or might allow us to dissect out protective from pathological processes, and thus lead to some immunologically based intervention strategies.

Despite the fact that much of the disease results from inflammatory and immune response of the host, host defenses are vital in limiting the infection. Immunity to malaria develops slowly and protection against the parasite occurs later than protection against disease symptoms (Plebanski and VS Hill, 2000). Because of the different location of the parasite and the different antigens expressed at the liver and blood stages, the relevant immune responses and their specificity and regulation will not be same for the liver and blood stages of infection. A thorough understanding of the mechanisms and antigens recognized at both these stages, and the differentiation of immunity to disease and infection, will be important for the construction of an effective vaccine.

The host response to malaria can clearly result in pathology. Glycosylphosphatidylinositols (GPIs) of the parasite, which anchor a range of *Plasmodium* molecules to cell surfaces, are considered likely candidates to induce host inflammatory responses, fever, and other pathology. Antibodies to these GPIs may ameliorate the severity of disease and thus could potentially be used therapeutically.

1.1.3.1. Cerebral Malaria

Cerebral malaria is one of the most serious complications of *P. falciparum* infection and is defined as an acute, diffuse and symmetric encephalopathy (Dorovini-Zis *et al*., 2011). Cerebral involvement occurs in approximately 1% of infected individuals and carries a 15% to 20% case-fatality rate, resulting in three-fourths of 1 to 2 million deaths/year (WHO, 2000). Young children in sub-Saharan Africa account for 90% of cerebral malaria-associated deaths (Dorovini-Zis *et al*., 2011).

The pathogenesis of human cerebral malaria remains unclear. However, the simplified explanation for the mechanism leading to cerebral malaria include: the blockage of cerebral blood vessels by parasitized cells, deposition of immune complexes in brain capillaries, reduced humoral or cell-mediated immune responses, action of endotoxin, and action of tumor necrosis factor (Hunt *et al*., 2006). Among these, the blockage of cerebral blood vessels has been considered to be the major factor in the pathogenesis of cerebral malaria. One possible cause of death associated with cerebral malaria is an imbalance in the production of neurotoxic and neuroprotective factors brought about by parasite-triggered cerebral inflammation. Thus, immune mechanisms that lead to parasite destruction and which are associated with effector cells may induce a range of pathological effects (Clark, 1987). It has been widely believed that the TNF-α secreted during malaria infection contributes to the immune response against the parasite but later during infection may serve as a key mediator of tissue damage (Stevenson *et al*., 1995). The best characterized animal model of cerebral malaria is *P. berghei* ANKA (PbA) infection in CBA or C57BL/6 mice, now used in many laboratories internationally. After parasite inoculation the mice show progressive behavioral, histopathological and immunological changes culminating in coma and death. This model has striking similarities to the human disease (de Souza and Riley, 2002; Hunt and Grau, 2003).

1.2. Schistosomiasis

1.2.1. General characteristics and life cycle

Schistosomiasis is a parasitic disease caused by blood flukes of the genus *Schistosoma.* An estimated 700 million people are at risk of infection in 76 countries, considered endemic, as their agricultural work, domestic chores, and recreational activities expose them to infested water (Ross *et al*., 2007). After malaria, schistosomiasis is the second most devastating tropical disease in the world (Ross *et al*., 2007). The most important species that infect humans are *S. japonicum*, *S. mansoni and S. haematobium*. Adult schistosomes live in mammalian or human host and use freshwater snails as intermediate hosts. The schistosomes develop into adults in the blood vessels surrounding the urinary or intestinal tracts. Adults release eggs which can circulate and become lodged in the veins and other organs causing painful inflammation and chronic illness.

Unlike other trematode infections, schistosome*s* have separate sexes (dioecious). Infection occurs through contact with fresh water that contains infective cercariae released from an intermediate host snail (Fig 3). The cercariae penetrate intact human skin and transforms into the migrating schistosomulum larva, which migrates through the bloodstream to the hepatic portal system to complete the parasites life cycle. Penetration of the skin by the cercariae (usually from species unable to develop in man, particularly cercariae of species of avian schistosome*s*) may result in a form of dermatitis, cercarial dermatitis, though this is not as important in terms of pathology as egg induced pathology. Male and female worms differentiate pair and migrate into the small venules draining the intestine (*S. mansoni, S. japonicum*) or the bladder (*S. haematobium*). The female worm produces 300 to 3000 eggs each day. Eggs pass into the lumen of the intestine or bladder and, if deposited in fresh water, hatch to release ciliated miracidia that infect the snail host. However, many eggs also lodge in the definitive host's liver and intestine or bladder, where they cause the pathology associated with schistosomiasis. In the chronic form of the disease, eggs trapped in the liver elicit the development of a cellular, granulomatous reaction which, with its ensuing fibrosis, gives rise to the most serious disease symptoms of infection.

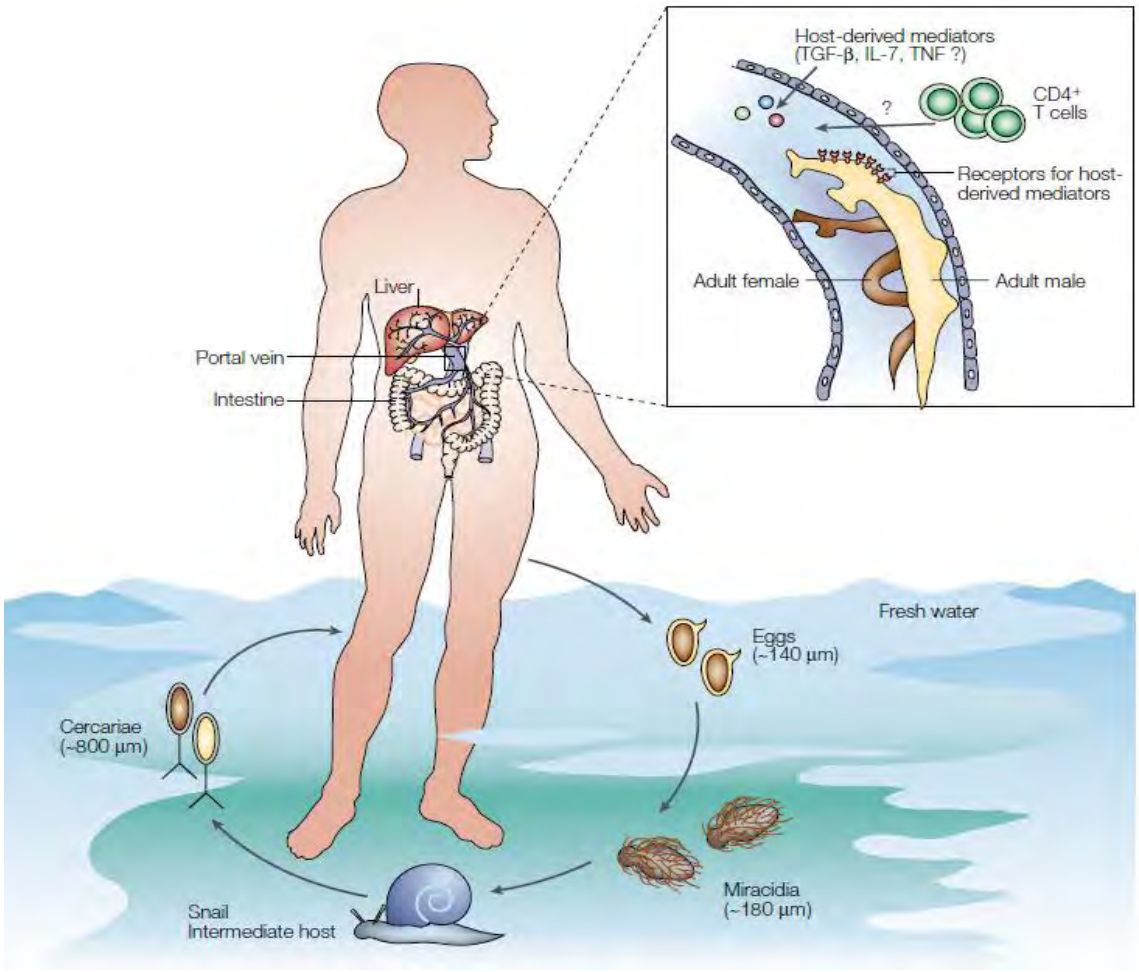

Figure **3**. Life cycle of *S. mansoni*. (Source: Pearce and MacDonald, 2002).

Patterns of schistosomiasis infection are shaped by two factors: host exposure and host immunity. Both factors demonstrate marked heterogeneity within any given population (Butterworth, 1994). The reasons for this heterogeneity are variable and may involve nutritional, genetic, concurrent pathology (malaria, hepatitis, etc), intensity and duration of infection and socio-cultural factors (Abath *et al*., 2006). As a behavior related disease, the risk of infection with schistosomiasis is associated with age, sex, and occupation of individuals (Gryseels, 1991). The conditions responsible for the evolution to the severe forms of the disease are not completely clear although the parasite burden seems to be a major determinant (Sleigh *et al*., 1986).

1.2.2. Transmission

Transmission of *S. mansoni* relies on contamination of water by excrement, adequate environments for appropriate aquatic snail intermediate hosts, and skin exposure to contaminated water. Any contact with contaminated water such as bathing, washing clothes, collecting water for cooking, getting a drink, fishing, sailing, farming canal irrigated lands, and brick making could put one at risk of infection. As little as one exposure to cercariae-containing water per year is sufficient to maintain transmission (King and Dangerfield-Cha, 2008). Social, cultural, behavioral and economic factors interact with local environmental and ecological factors to produce extraordinary variation in the epidemiology of schistosomiasis with respect to prevalence and intensity of infection (Hibbs *et al*., 2011).

Geographic distribution of the disease depends on the distribution of intermediate snail host and the opportunity to infect humans and snails (Lima *et al*., 1987). Infection occurs throughout much of tropical and subtropical areas of the world (Fig. 4). Infection is predominant in endemic countries in school age children, fishermen, farmers, irrigation workers and others using infested water for their domestic and/or recreational purposes. Epidemiologic studies in modern populations typically find a higher prevalence of schistosomiasis among males than females (Abdel-Wahab *et al*., 2000; El-Khoby *et al*., 2000). This is likely the result of a gendered division of labor involving water contact.

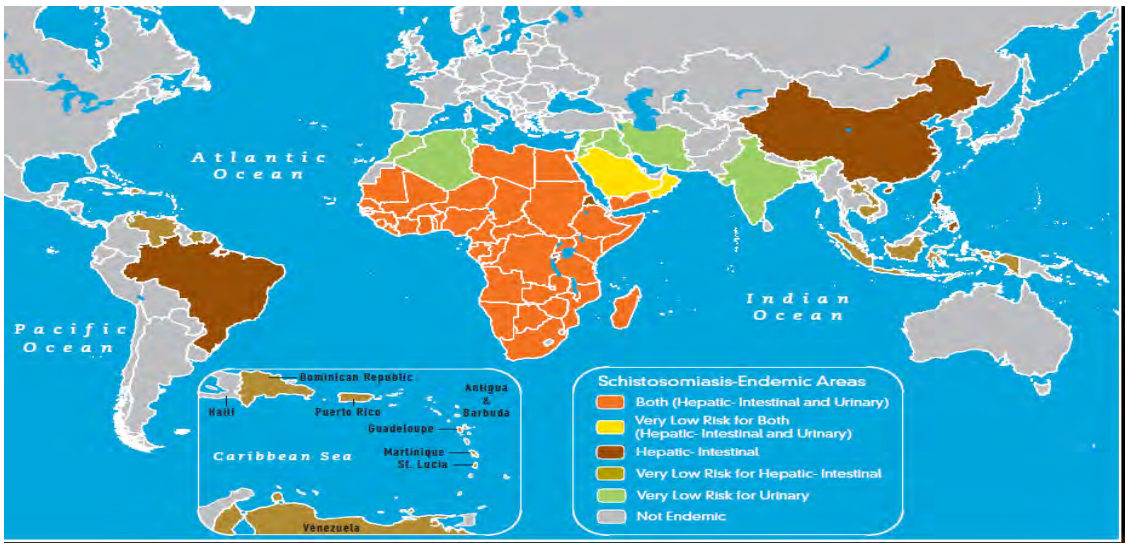

Figure 4. Global distribution of schistosomiasis (CDC, 2011).

1.2.3. Immunopathology

1.2.3.1. Development of the immune response

In the course of an infection, the immune response progresses through at least three phases. In the first 3–5 weeks, during which the host is exposed to migrating immature parasites, the dominant response is T helper 1 (TH1)-like. As the parasites mature, mate and begin to produce eggs at weeks 5–6, the response alters markedly; the TH1 component decreases and this is associated with the emergence of a strong TH2 response. This response is induced primarily by egg antigens.

During the chronic phase of infection (infections are long lived and worms continue to produce eggs~300 per day in the case of each *S. mansoni* female), the TH2 response is modulated and granulomas that form around newly deposited eggs are smaller than at earlier times during infection. From work in the mouse, there now seems to be a correlation between the inability to form granulomas, or the development and persistence of a highly pro-inflammatory TH1-like response beyond the acute phase, and the development of hepatotoxic liver disease (Fallon, 2000). By contrast, TH2-cell-mediated granulomas seem to protect hepatocytes, but allow the development of fibrosis (Cheever *et al*., 2000). Although it is clear that severe fibrosis occurs in human schistosomiasis, there is debate over the existence of the hepatotoxic form of disease (Fallon, 2000).

1.2.3.2. Immune-related pathologies

Schistosomiasis causes a range of morbidities, the development of which seems to be influenced to a large extent by the nature of the induced immune response and its effects on granuloma formation and associated pathologies in target organs (Cheever *et al*., 2000). Two main clinical conditions are recognized in *S. mansoni*-infected individuals: Acute schistosomiasis and chronic schistosomiasis. Acute schistosomiasis is characterized by cercarial dermatitis and Katayama syndrome (Caldas *et al*., 2008). Cercarial dermatitis is an IgE-mediated hypersensitivity response directed against penetrating cercariae (Caldas *et al*., 2008), occurs infrequently among endemic populations but is common among visitors and migrants and after primary infections. Katayama

syndrome is an immune-complex mediated hypersensitivity reaction against migrating schistosomula and early egg deposition (Ross *et al*., 2007).

Chronic disease the most serious form and life-threatening hepatosplenic disease, which is usually accompanied by severe hepatic and periportal fibrosis, portal hypertension and portosystemic shunting of venous blood (Dunn and Pearce, 1999). Although TH2 responses seem to have a crucial role in modulating potentially life-threatening disease during the initial stages of schistosomiasis, prolonged TH2 responses contribute to the development of hepatic fibrosis and chronic morbidity (Cheever *et al*., 2000).

1.2.3.2.1. The Granuloma

Granuloma is a delayed type hypersensitivity reaction against schistosome egg antigens. The CD4+ T-cell response that is induced by egg antigens orchestrates the development of granulomatous lesions, which are composed of collagen fibers and cells, including macrophages, eosinophils and CD4+ T cells around the individual eggs (Dunn and Pearce, 1999) (Fig 5) . As the eggs die, the granulomas resolve, leaving fibrotic plaques. Severe consequence of infection with *S. mansoni* is the result of an increase in portal blood pressure as the liver becomes fibrotic, congested and harder to perfuse. Under these conditions, the diameter of the portal vein increases and the wall of the portal vein become fibrotic. Associated with these changes is the development of ascites (the accumulation of serous fluid in the peritoneal cavity) and portal–systemic venous shunts (new blood vessels that bypass the liver), which can rupture, leading to life-threatening bleeding. Although, hepatic granulomas have essential host-protective role it become pathogenic by precipitate fibrosis, which obstructs blood flow, increases portal blood pressure, and ultimately promotes development of portal-systemic venous shunts (Gryseels *et al*., 2006).

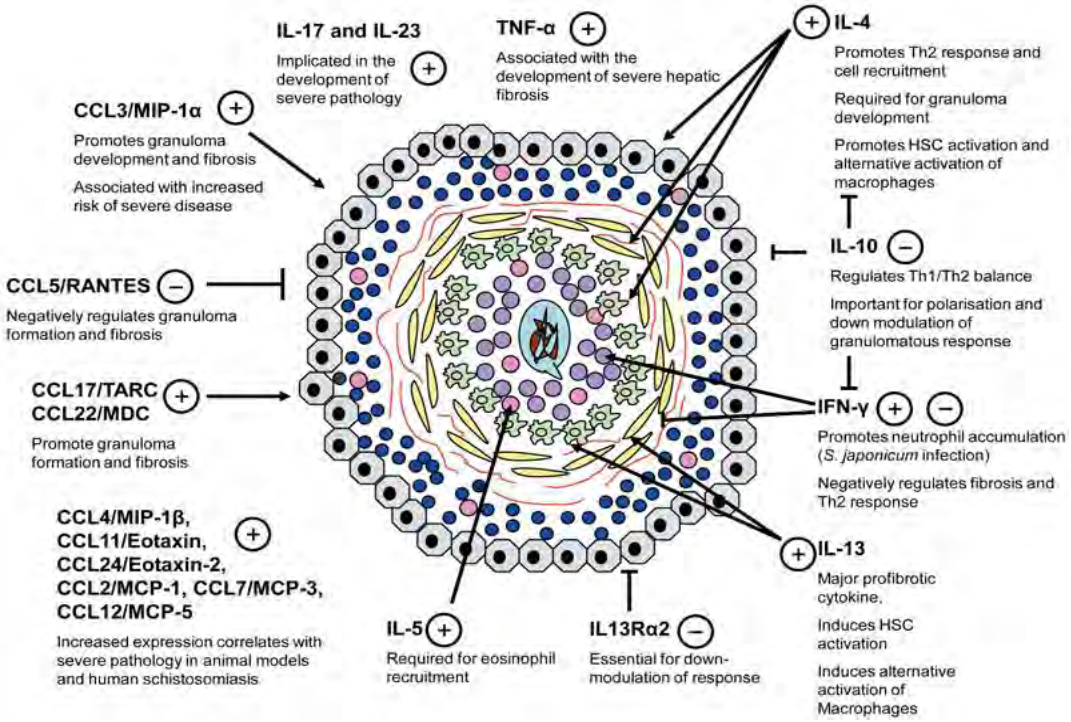

Figure 5. Major components of the granulomatous response to Schistosome eggs in the host liver and the main cytokines and chemokines that regulate this response. (Source: Burke *et al*., 2009).

1.3. Schistosomiasis-Malaria Co-infection

Concomitant parasitic infections are common events in different regions of the world. Malaria and schistosomiasis are among the parasitic infections that shares common transmission areas in various tropical regions, especially on the African continent. Hence concomitant infection with these two parasites is more of „„the rule rather than the exception" in human populations living in these areas (Mwangi *et al*., 2006). Co-infection by these two parasites may have an important influence on the regulation of immune response associated with the development of these infections and their respective morbidity (Diallo, T. O., 2004). It has increasingly been speculated that helminth infections may alter susceptibility to clinical malaria, and there is now increasing interest in investigating the consequences of co-infection, with studies yielding contrasting results. Such knowledge is of importance for the rational design and optimization of vaccination protocols and treatment programs (Helmby *et al*., 1998).

Most recent experimental studies of co-infection reported that mice with ova producing *S. mansoni* infection have increased malaria parasitaemia with *Plasmodium* infection (Laranjeiras *et al*., 2008, Waknine-Grinberg *et al*., 2010 and Bucher *et al*., 2011) delayed parasite clearance after chloroquine treatment of *P. berghei* (Legesse *et al*., 2004) compared with those *P. berghei* infection alone although, earlier studies (Long *et al*., 1981; Lwin *et al*., 1982 and Rahman, 1990) reported the decreased malaria parasitaemia in co-infected mice. *S. mansoni* infection has been shown to reduce the incidence of murine cerebral malaria (Waknine-Grinberg *et al*., 2010 and Bucher *et al*., 2011). On the other hand, malaria conferred reduction in *S. mansoni* morbidity, as demonstrated by reduced worm counts, granuloma sizes and high schistosome-specific IgG levels in the co-infected mice (Kanyugo *et al*., 2009).

Most studies that examined naturally occurring co-infection in humans indicated that co-infection with schistosome and malaria organisms has an effect on the host, both in terms of pathology and in terms of immunological response (Abruzzi and Fried, 2011). The direction of this response seems to depend on the species of schistosome and the worm burden, host age and malaria parasitaemia. Studies that examined the effects of co-infection on the host found that co-infected hosts had lower malaria parasitaemia (, Lyke *et al*., 2005 and Arinola, 2005), lower hemoglobin level and high prevalence of anemia (Midzi *et al*., 2010 and Okafor and Elenwo,

2007) and higher level of IL-10 (Courtin *et al*., 2011 and Wilson *et al*., 2009). In contrast to these studies, co-infection also resulted in higher hemoglobin level (Nmorsi *et al*., 2009) and higher overall prevalence of malaria parasite with greater incidence and densities of gametocytes than *P. falciparum* single infected children (Sangweme *et al*., 2010). Though the malaria species was not identified, decreased splenomegaly was also observed in co-infected humans (Friis *et al*., 2000). Overall, the studies indicated that co-infection with *S. haematobium* probably mediated the incidence and severity of infection with *P. falciparum* (Nmorsi, *et al*., 2009), possibly in an age-dependent manner (Lyke *et al*., 2006).

There are also human studies that examined the effects of *S. mansoni* co-infection with *P. falciparum*. Most studies with *S. mansoni* found a detrimental effect of the coinfection on the host, with increased malaria attacks (Faye *et al*., 2008) or increased host hepatomegaly and splenomegaly (Wilson *et al*., 2009 and Mwatha *et al*., 2003). An age effect was also observed, in which children under the age of five had higher levels of parasitaemia than older children (Faye *et al*., 2008). Increased egg load of *S. mansoni* (Sokhna *et al*., 2004) and production of more anti-schistosome IgE and IgG_3 (Mutapi *et al*., 2000) was also associated with co-infection. Small sample size, disparate enrollment ages, and retrospective analysis limit interpretation of these results. The argument and evidence is plausible, but not conclusive, and there remains a need to conduct longitudinal studies to conclusively substantiate and to investigate the associations further. Thus, this study was performed to examine the parasitological, histopathological and hematological conditions that occur in mice, during schistosomiasis-malaria co-infection.

Hypothesis

Pre-existing (chronic) *S. mansoni* infection would reduce cerebral sequestration of malaria parasite (cerebral malaria). On the other hand malaria infection might not have an effect on severity of *S. mansoni* infection.

2. Objectives

2.1. General objective

- To investigate the interplay between the *S. mansoni* and *P. berghei* parasites during concurrent infections.

2.2. Specific objectives

- To assess the effect of pre-existing (acute and chronic) *S. mansoni* infection on malaria parasitaemia and cerebral sequestration of malaria parasite.
- To assess the effect of *P. berghei* infection on the severity of pre-existing *S. mansoni* infection by examining number and size of schistosome induced granuloma and the number of *S. mansoni* eggs in the liver of mice.
- To examine the effect of co-infection on hemoglobin level, weight gain and survivability of mice.

3. Materials and Methods

3.1. Experimental setup

The study was carried out in Swiss albino mice, an experimental model for both malaria and schistosomiasis. The study entailed an infection and analysis experiments on the parasitological, hematological and pathological responses, weight loss and survivability of mice.

At the beginning of the study, 20 mice were infected with 100±10 *S. mansoni* cercariae by tail immersion method. Four weeks post-*S. mansoni* infection 6 mice from schistosome infected mice and 5 control mice were infected with 0.2 ml of blood containing 10^6 *P. berghei* parasitized red blood cells intraperitoneally. Seven weeks after infection with *S. mansoni* 7 mice from schistosome infected group and 5 control mice were also infected with the same dose of *P. berghei* and same method of infection. Additional control group consisted of mice infected with only *S. mansoni* was used for each co-infection schedule.

Parasitaemia was monitored at day 5, 7, 9 and 11 post-*P. berghei* infection by thin blood smears prepared from tail blood and blood samples were also taken on day 3, 5, 7, 9 and 11 post-*P. berghei* infection for determination of hemoglobin level using HemoCue machine. On day 3, 5, 7, 9 and 11 post-*P. berghei* infection the weight of mice were measured using a digital weight balance for the analysis of weight lost. In addition number of eggs of *S. mansoni* parasite per gram of mice liver was evaluated for co-infected and mono-infected mice.

Histopathology of brain tissue for examination of cerebral malaria and liver tissue for analysis of liver granuloma were examined using frozen sectioning method. Cerebral sequestration observed in brain pathology of *P. berghei* infected dead mice and a sign of coma before death were used as an indicator of cerebral malaria. Survivability of mice post-*P. berghei* infection was also assessed. Mice that died at parasitaemia of up to 15% with cerebral sequestration were considered to have died of cerebral malaria. But mice which did not die from cerebral malaria died from severe malarial anemia and high parasitaemia.

3.2. Parasites, hosts and infections

S. mansoni cercariae shaded from *Biomphalaria pfeifferi* was used throughout this work as schistosome parasite. Cercarial shading was done from infected snails collected form Burka (Bishangari, South central Ethiopia) and Kemise (Northern Ethiopia) by exposing them to florescent illumination for approximately one and half hours by putting individual snail on a shedding chamber filled with dechlorinated water.

After one and half hours of illumination, the chambers were checked for the presence and species of the cercariae using a dissecting microscope at 40X magnification to discriminate *S. mansoni* cercariae from bird and other animal schistosome*s*. Cercariae from different shedding chambers were pooled in to test tubes to allow mixing of sexes to avoid the possibility of single infection. The total number of cercariae was determined from counts made in1ml iodine killed cercariae.

The pooled cercariae-containing solution was mixed by hand shaking to create a uniform cercarial suspension. Then 100±10 cercariae were used to infect each mouse using tail immersion method through two hours of exposure. Finally infected mice were transferred into the animal house for a standard care and keeping. Starting from the fourth week post-infection, mice were checked for the presence of *S. mansoni* eggs in their feces using Kato Katz method (WHO, 2004).

The *P. berghei* ANKA strain malaria parasite was used in the study. Parasitized blood diluted in normal saline was used to infect mice intra-peritoneally. The parasite was maintained by serial passage of blood from infected mice to the non-infected ones on weekly basis. A blood sample taken from donor mouse with the growing parasitaemia of 30-40% was diluted with normal saline, so that each 0.2 ml of blood contained 10^6 infected erythrocytes were used to infect each mouse.

Swiss Albino mice aged three-five weeks were used in all experiments. The mice were bred at the animal house of Addis Ababa University and caged in groups and fed on commercial pellets and water provided *ad libitum.* They were kept under a natural light-dark cycle of 12/12 hours, at an ambient temperature of 25^{o}C.

3.3. Preparation of Giemsa stained thin blood films

Thin blood films from tail blood were made on standard microscope slides, and air-dried before fixing the films in methanol for 5 minutes. They were stained with fresh Giemsa solution (10% v/v in distilled water). The stained blood films were observed under a standard light microscope using the x100 objective lens with immersion oil. Infected and uninfected erythrocytes in different fields of view were identified and counted. pRBC were counted microscopically in at least five microscopic fields, each approximately 300 cells (Bucher *et al*., 2011). Parasitaemia was quantified as percentage using:

$$\% \text{ Parasitaemia} = \frac{\text{parasitized RBCs}}{Total\ RBCs\ counted}\ X\ 100$$

3.4. Egg Count in Tissue

The eggs in the liver of schistosome infected mice were counted according to Lewis (1998). The liver of the co-infected and schistosome only infected mice were dissected out. Then 1gm liver tissue was taken and put in to a bottle containing 10ml 5%KOH, which digests out the tissue and releases the eggs, and incubated at 37^{o}C for 16-24hrs. The solution was mixed thoroughly and the egg count was done by taking 1ml of the solution. Then egg per gram of liver was extrapolated for 10ml.

3.5. Determination of the Percentage of Mouse's Body Weight

The control and experimental mice were weighed using digital balance and percentage weight loss of mice during the experiment was calculated according to the following equation:

$$\frac{\text{Average body weight of normal mouse} - \text{The body weight of infected mouse}}{\text{Average body weight of normal mouse}}\ \text{x}\ 100$$

3.6. Measurement of hemoglobin level

Hb concentration was measured using a portable HemoCue® 201 photometer (Angelholm, Sweden). Micro-cuvet was filled with a drop of tail snip blood from the mouse and then placed into the HemoCue machine, which read out the Hb level in g/dl.

3.7. Histopathological examination of tissues

Liver tissues (from co-infected and *S. mansoni* mono-infected mice) and brain tissues (from co-infected and *P. berghei* mono-infected mice) were taken and covered with mounting media, then frozen in the microtone under -26^{0}C (brain tissue) -27 ^{0}C (liver tissue) for 20 minute. A section of 7µm (for liver tissue) and 6µm (for brain tissue) thickness were cut using a rotary microtome. Then the sections were transferred into frozen microscopic glass slides and stained with haematoxylin and counter-stained with eosin.

Slides were examined under 100X and 400X light microscope magnification and a picture was taken using a computer connected to microscope. Liver granuloma count was calculated as the number of granulomas in 5 successive fields using the low power 100X (Riad *et al*., 2007). Granuloma measurements were done only for solitary granulomas containing a single egg in their center by using Adobe Photoshop CS3 version 11 software and measured at picture resolution of 28.346 p/cm and dimension of 225.78 x 203.2 mm. The mean diameter of every granuloma was obtained by measuring the length and width of each granuloma with a centrally placed schistosome egg. The averages of the length and width were taken as the granuloma size (Farah *et al*., 2000*)*. Five granulomas were measured for each mouse of the two Schistosome-infected groups: the co-infected and *S. mansoni* controls.

Statistical analysis

Difference in the parameters (parasitaemia, eggs per gram of liver, liver granuloma size and number and hemoglobin levels) measured for the different test groups were tested for significance by the independent t- test using SPSS 17 computer software. Differences in between groups were considered significant when *p*- value was less than 0.05. Percentage of weight loss and survived mice were also used as comparing parameters for the different groups.

4. Results

4.1. Malaria parasitaemia

During co-infection of mice with *P. berghei* four weeks post-*S. mansoni* infection, malaria parasitaemia develop on day 5 in both single and co-infected group. On day five after *P. berghei* infection the *Plasmodium* mono-infected group developed a parasitaemia of 4.4%± 0.48% when compared to 3.9%±0.29% of the co-infected group (p=0.36). The parasitaemia in co-infected and *Plasmodium* mono-infected group at day 7 were 16 .9% ± 0.45% and 16.1% ± 1.97%, respectively (p=0.64). The parasitaemia peaked at day 9 for both groups with 38.3% ± 1.14 of the co-infected mice and 37.13% ± 1.25% of mono-infected groups (p=0.53) (Fig.6). At day 11after *P. berghei* infection the parasitaemia declined in both groups and became 32.5% ± 1.13% for co-infected and 33.6% for malaria only group.

Infection of mice with *P. berghei* seven weeks post-*S. mansoni* infection resulted in faster development and increased malaria parasitaemia than *P. berghei* mono-infected mice that wasn‟t seen during four weeks post-*S. mansoni*. The co-infected mice had parasitaemia of 12.8% ± 0.31% on day 5, compared to 3.6% ± 0.34% in the *P. berghei*-mono-infected group (p<0.01). At day 7 the co-infected group developed peak parasitaemia of 47.9% ± 0.92%, which is significantly higher than the malaria only group, with parasitaemia of 20.64%±0.64%. The malaria only group developed a peak parasitaemia of 39.8%±1.51% at day 9 after infection which was lower than the co-infected group parasitaemia (45.7 %) (Fig.6).

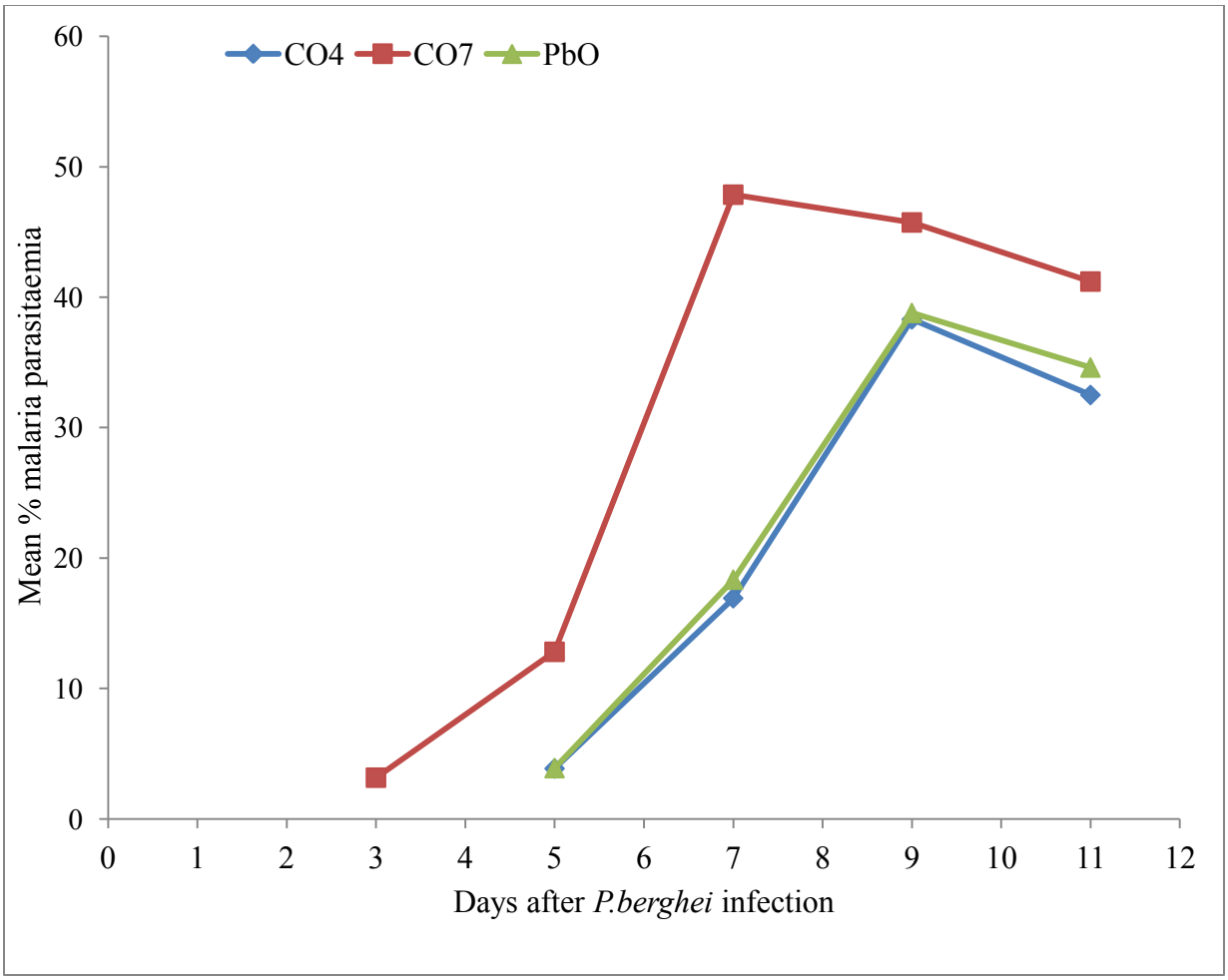

Fig 6. Percentage *P. berghei* parasitaemia in mice co-infected with *P. berghei* 4 weeks and 7 weeks post-*S. mansoni* infection and in mice infected with *P. berghei* only. (CO4: 4th week co-infected, CO7: 7th week co-infected, PbO: *P. berghei* mono-infected).

4.2. Hemoglobin level

The group of mice infected with *Schistosoma* parasite for 4 weeks had Hb level of 16.7g/dl±0.27g/dl which was insignificantly different from normal uninfected mice with Hb level of 17.3g/dl±0.36g/dl before *P. berghei* infection (Fig 7). On day 3 after *P. berghei* infection, the Hb level become lower (15.6g/dl±0.84g/dl) in co-infected group as compared with malaria only group (17g/dl±0.32g/dl, P=0.17) and schistosome only group (16.6g/dl±0.46 g/dl, P=0.44). The schistosome only group has insignificantly lower Hb level than malaria only group. Until day 7 the Hb level in the three groups was not significantly different. On day seven the *P. berghei* mono-infected group and the co-infected group had similar Hb value (14g/dl). The Hb

level on day 9 becomes 13.1g/dl ±0.26g/dl for co-infected and 12.8 g/dl ±0.28g/dl for *P. berghei* mono-infected group which was significantly lower when compared with the Hb level of schistosome mono-infected group (16.1 g/dl ±0.19 g/dl). But the Hb level between co-infected and *P. berghei* mono-infected was not significantly different.

For the group of mice co-infected with *P. berghei* 7 weeks after *S. mansoni* infection, the baseline Hb level before *Plasmodium* infection, was significantly different between seven weeks schistosome infected groups (14 g/dl ±0.6 g/dl) and uninfected healthy mice (17.7 g/dl ±0.3 g/dl,) (Fig. 7). On day 3 after *P. berghei* infection the co-infected group scored the lowest level of Hb even if it was not significantly different from the schistosome mono-infected (13.2 g/dl ±0.4 g/dl Vs 14.2 g/dl ±0.3 g/dl) but it was significantly lower than *P. berghei* mono-infected group (16.9 g/dl ±0.4 g/dl; $P<0.01$). The co-infected mice had significantly lower Hb level on day 5 when compared with schistosome mono-infected mice (12.4g/dl±0.4g/dl vs 13.9g/dl±0.1g/dl), but not significant when compared with *P. berghei* mono-infected mice. On day 9 the Hb level in the co-infected mice declined (9.5g/dl±0.54 g/dl) and significantly lower than *P. berghei* mono-infected mice (12.9g/dl ±0.49g/dl). Until day 11 the schistosome and *P. berghei* mono-infected mice did not have significantly different Hb. But on day 11 the *P. berghei* mono-infected mice scored significantly lower Hb count (11.5g/dl) than schistosome mono-infected mice (12.8g/dl±0.17g/dl) but still higher than the co-infected mice which had Hb level of 7.4g/dl±0.67g/dl.

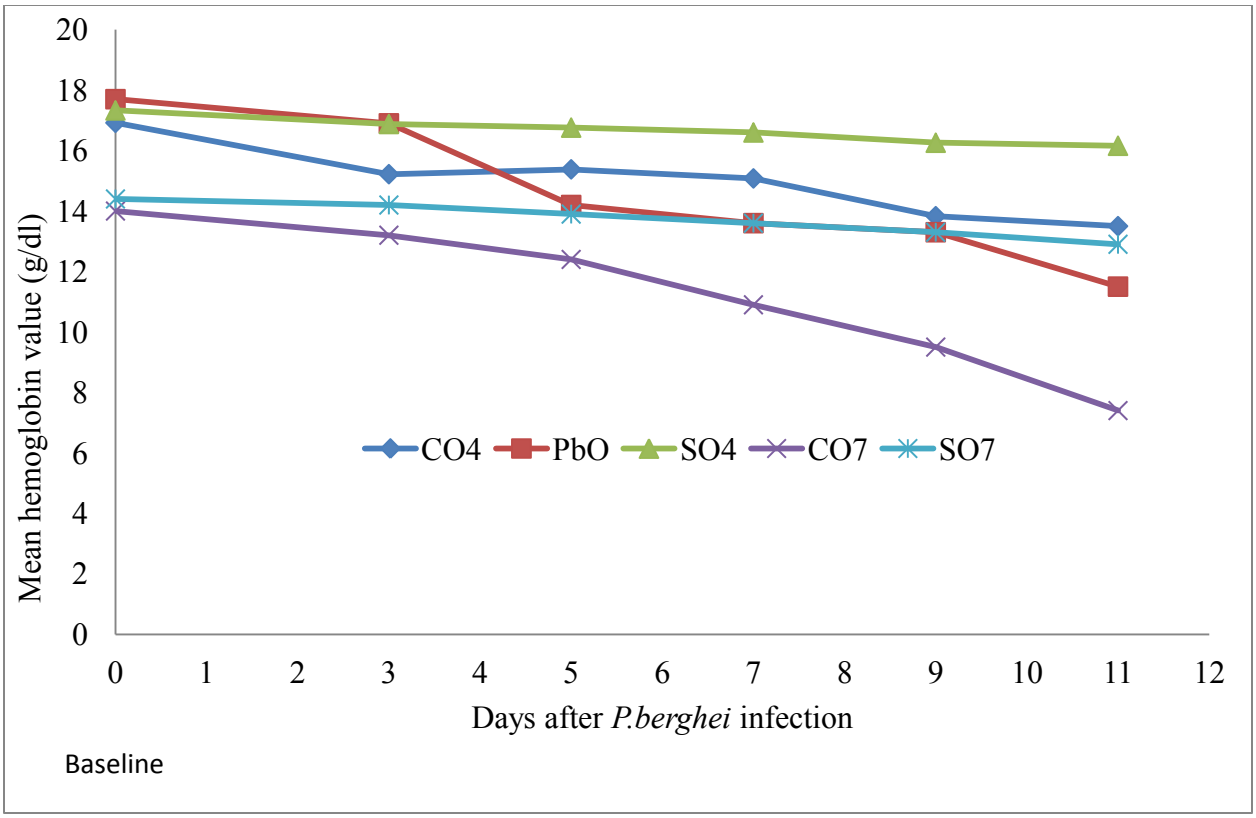

Figure 7. Mean Hb value for, mice co-infected with *P. berghei* 4 weeks and 7 weeks post-*S. mansoni* infection, *P. berghei* mono-infected and schistosome mono-infected mice. **(**CO4: 4th week co-infected, CO7: 7th week co-infected, PbO: *P. berghei* mono-infected, SO4: *S. mansoni* mono-infected for 4 week, SO7: *S. mansoni* mono-infected for 7 week**).**

Although the mean hemoglobin value in the *P. berghei* mono-infected and schistosome mono-infected mice looks similar during the 7th week co-infection schedule, the percentage loss of Hb was higher in the *P. berghei* mono-infected mice next to the co-infected group as indicated on Fig 8.

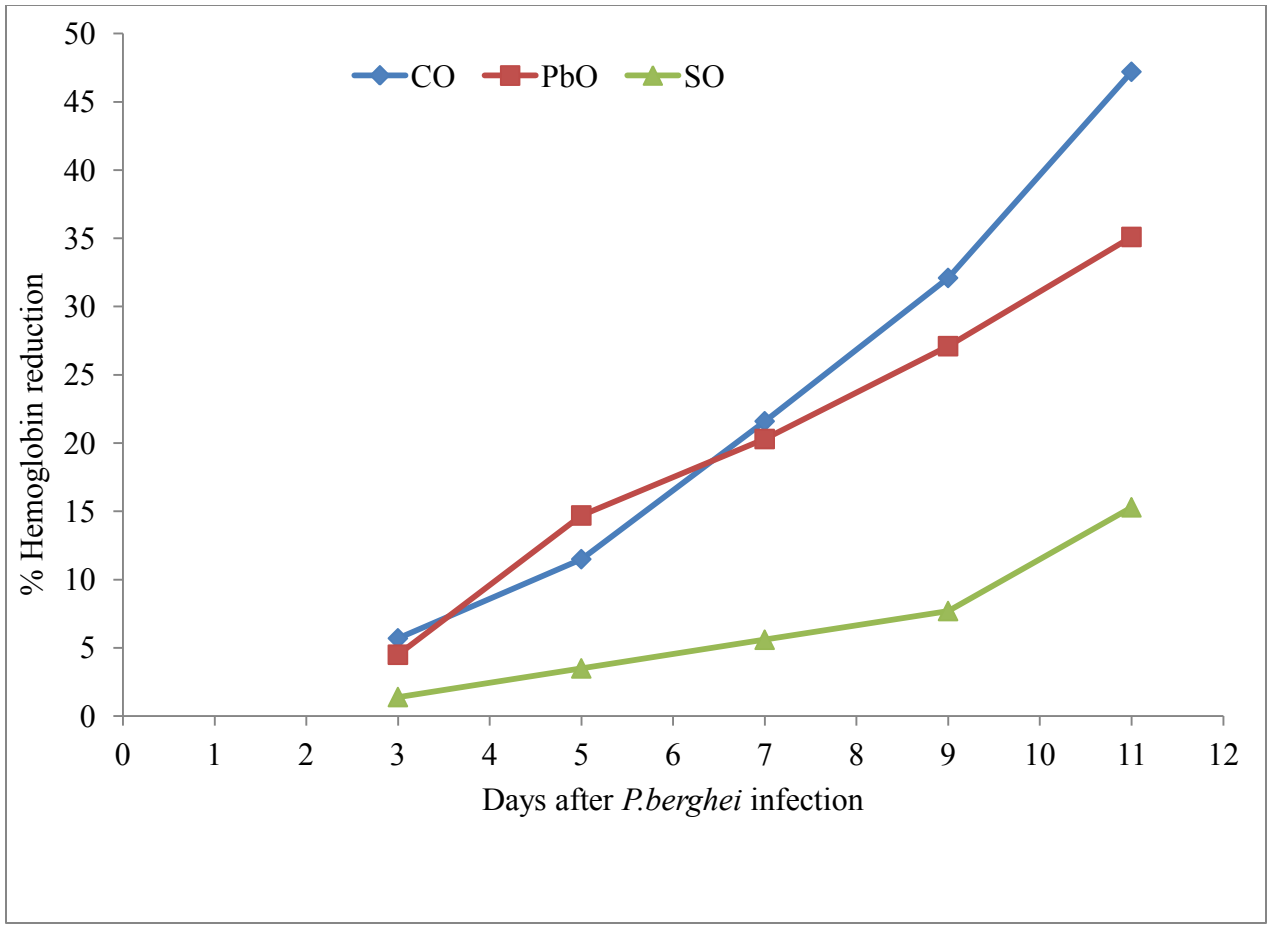

Figure 8. Percentage reduction of Hb in *S. mansoni-P. berghei* co-infected and Schistosome/*Plasmodium* mono-infected mice during 7th week infection schedule. **(**CO: co-infected, PbO: *P. berghei* only infected, SO: *S. mansoni* only infected).

4.3. Weight loss

During co-infection of mice with *P. berghei* 4 weeks post-*S. mansoni* infection, significant difference in weight loss was not observed. At the beginning of the experiment, before *P. berghei* infection, the co-infected mice had baseline weight of 35.6g and the mice that are infected with *P. berghei* alone had weight of 33.9g. After co-infection the percentage weight loss in co-infected and mono-infected group increase proportionally (Fig.9). But the most severe weight loss was observed on day 11 post-*P. berghei* infection. The co-infected group lost 2.04%, 6.04%, 8.54% and 12.62% of their weight on day interval of 3, 5, 7 and 9 post-*P. berghei* infection, respectively. Similarly, the *P. berghei* mono-infected group lost 1.17%, 4.13%, 7.57%

and 11.47% of their weight on the same day intervals as the co-infected. Although, the *S. mansoni* mono-infected group lost some weight, it was significantly lower when compared with the malaria and co-infected groups.

In the *S. mansoni-P. berghei* co-infected mice the weight loss was synergistically caused in patent *S. mansoni* infections as shown in figure 9, the highest amount of percent weight loss was recorded in the co-infected groups- 2.71% on day 3, 7.4% on day 5, 12.4% on day 7, 15.5% on day 9 and 23.1% on day 11 from their baseline weight. Furthermore, the percentage weight loss in *P. berghei* mono-infected group (1.44% on day 3, 6.47% on day 5, 6.38% on day 7, 11.4% on day 9 and 14.8% on day 11 from their baseline weight), was lower than the co-infected but still higher than the schistosome mono-infected with an average weight loss of 9.99% on day 11.

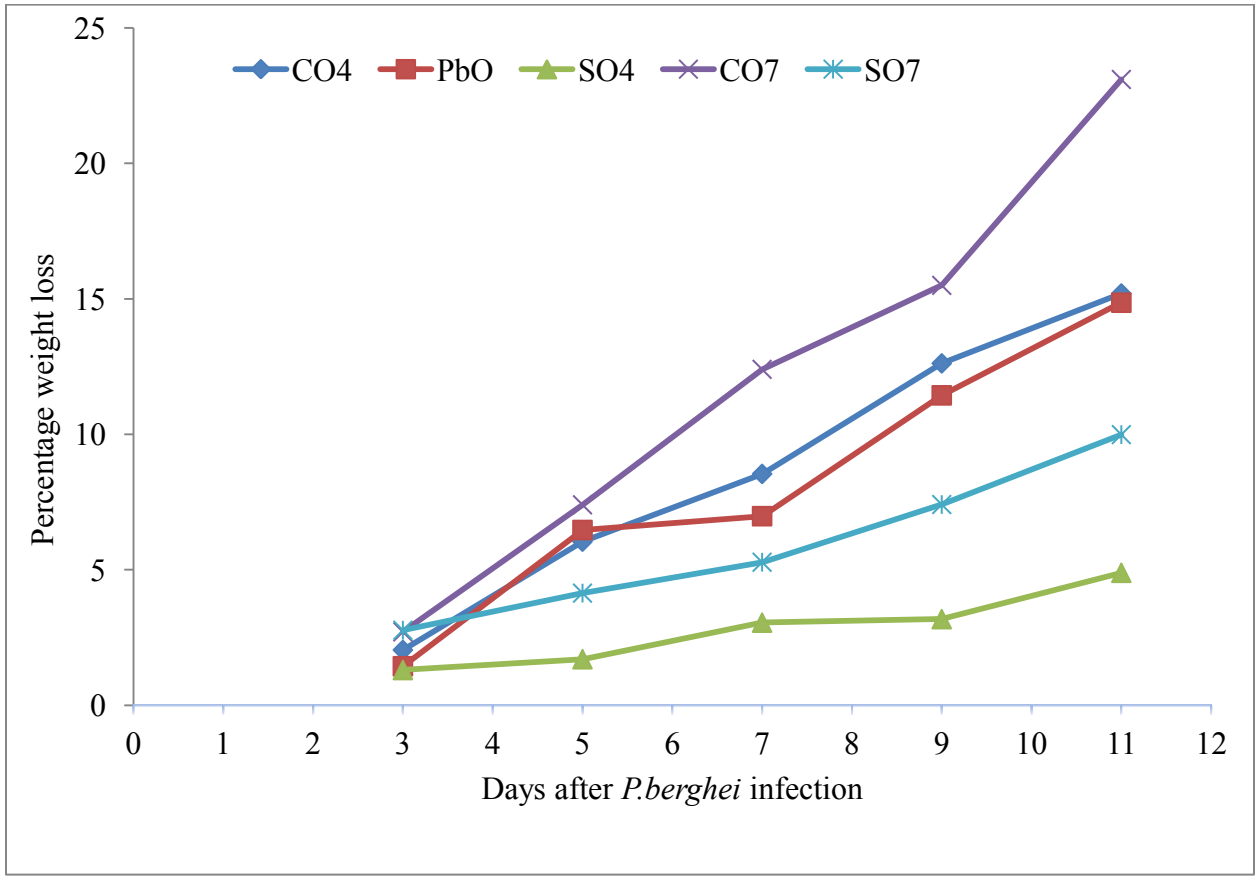

Figure 9. Percentage weight loss in 4 and 7 weeks post-*S. mansoni* infected mice after co-infection with *P. berghei* and in *Plasmodium* and schistosome mono-infected group. (CO4: 4th week co-infected, CO7: 7th week co-infected, PbO: *P. berghei* mono-infected, SO4: *S. mansoni* mono-infected for 4 weeks and SO7: *S. mansoni* mono-infected for 7 weeks).

4.4. Eggs per gram of liver

The mean egg load in hepatic tissues of co-infected and schistosome mono-infected groups revealed a significantly lower number of *S. mansoni* eggs in the liver of the co-infected mice when compared with the corresponding schistosome mono-infected control group (Fig. 10), (8860±456.1 eggs /gram of liver for co-infected and 11272±487.2 eggs/gram of liver for mono-infected group).

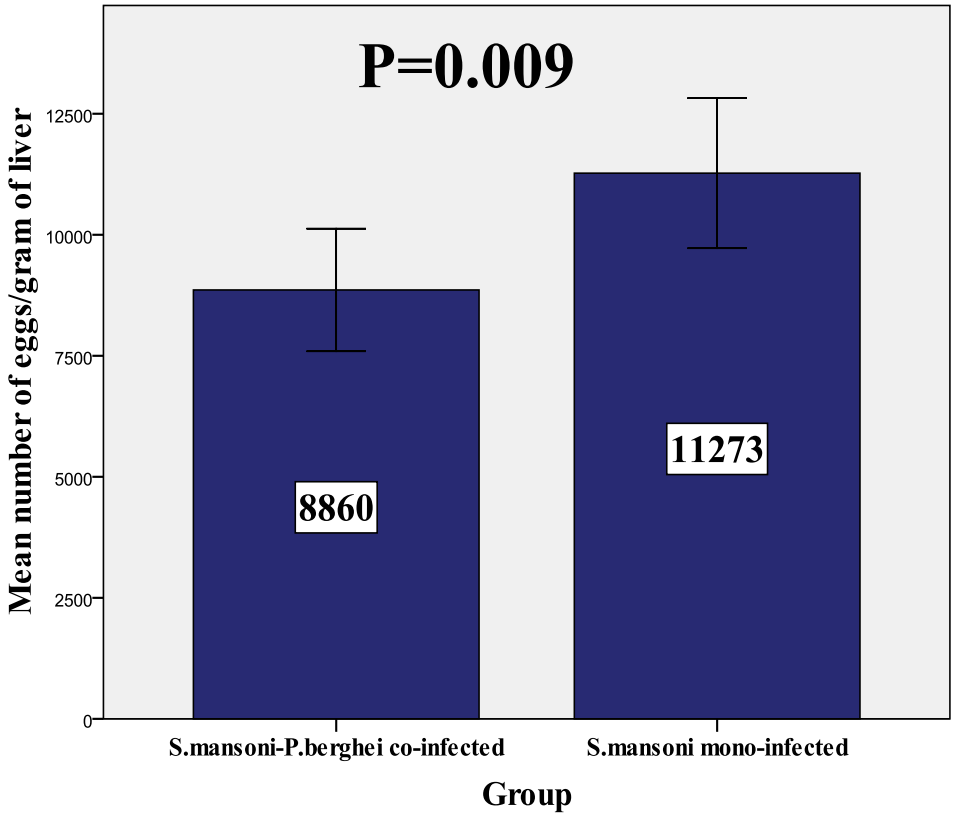

Figure 10. Mean eggs per gram of liver in *S. mansoni* mono-infected and *S. mansoni-P. berghei* co-infected mice**.** (Error bars: 95% CI).

4.5. Histopathology

4.5.1. Liver granuloma

Comparison of the mean diameter and number of the schistosome granulomas in the liver of *S. mansoni-P. berghei* co-infected and *S. mansoni* mono-infected mice showed that mice co-infected with *P. berghei* and *S. mansoni* had smaller granuloma (75.1mm±1.7mm) as compared to the *S. mansoni* mono-infected mice, which had significantly larger granulomas (105.8mm ± 3.1mm) (Fig. 11A).

In *S. mansoni-P. berghei* co-infected mice, a significant reduction in the number of hepatic granuloma was observed (4.2 ±0.3/5 successive fields of microscope) when compared to the corresponding *S. mansoni* mono-infected (10 ± 0.5/5 successive fields of microscope) mice (Fig. 11B).

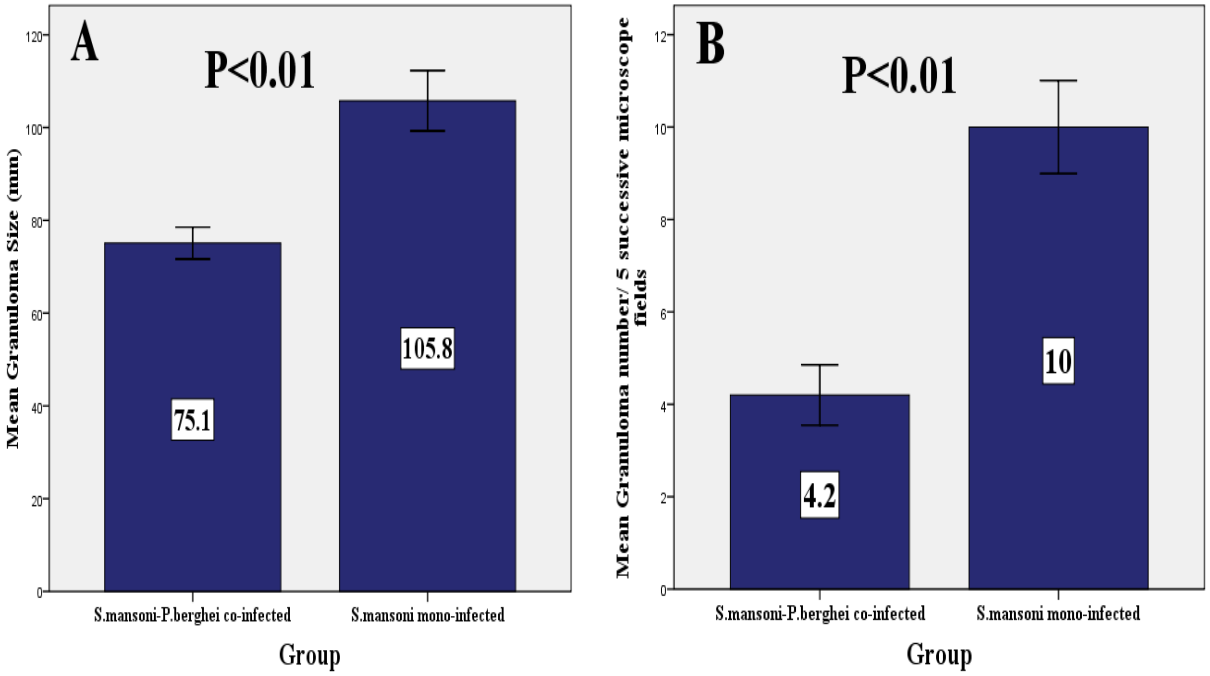

Figure 11. Mean granuloma size (A) and number (B) of *S. mansoni-P. berghei* co-infected and *S. mansoni* mono-infected mice. (Error bars: 95% CI).

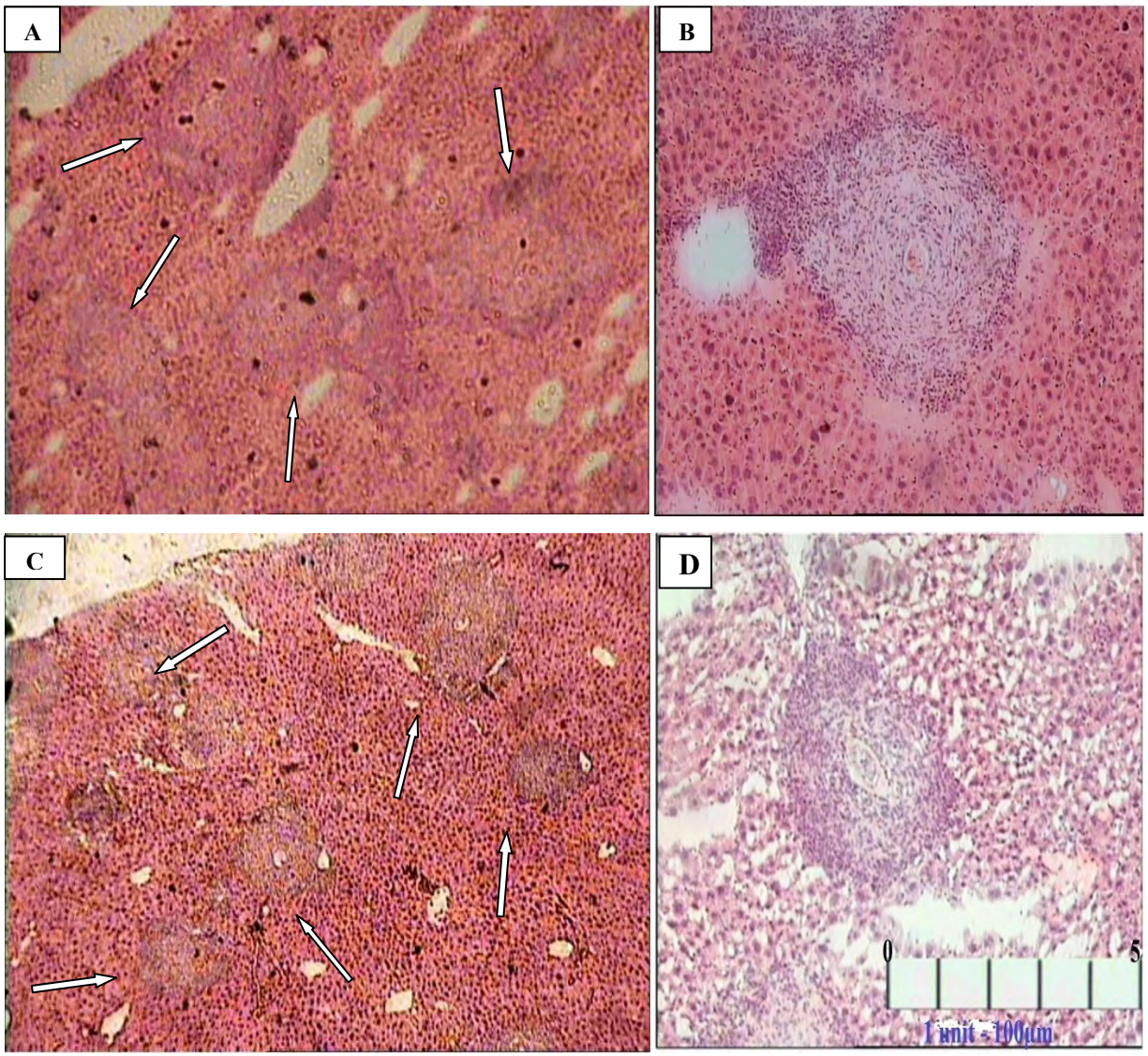

Figure 12. Photomicrograph of liver granuloma: I. Liver granulomas from mice infected with *S. mansoni* only: (A: 40X and B: 100X). II. Liver granuloma from mice co-infected with *S. mansoni* and *P. berghei* (C: 40X and D: 100X). Arrow represents single granuloma. (1 unit of the bar represents 100 µm).

4.5.2. Brain Histopathology

To assess the effect of patent schistosomiasis on *P. berghei*-induced brain pathology, brain sections of *P. berghei* mono-infected and *S. mansoni–P. berghei* co-infected mice were examined (Fig. 13). Two different infection schedules were examined in order to determine the effect of *S. mansoni* infection on cerebral sequestration of malaria parasites. In mice co-infected four weeks post-*S. mansoni* infection, no significant effect was seen on cerebral sequestration. In contrast, when mice were co-infected with *P. berghei* seven weeks after cercariae infection, a reductive effect of the pre-existing *S. mansoni* infection was seen: co-infected mice displayed lower rates of cerebral sequestration: 20% vs. 80% in *P. berghei* mono-infected mice (Table 1).

Table 1. The effect of acute and chronic *S. mansoni* infection on cerebral sequestration of malaria parasites in mice infected with *P. berghei*.

		S. mansoni – P. berghei co-infection			
		4 weeks post-*S. mansoni* infection (n=5)		7 weeks post-*S. mansoni* infection (n=5)	
P. berghei cerebral sequestration		CO	PbO	CO	PbO
	Positive	4 (80%)	5(100%)	1(20%)	4(80%)
	Negative	1 (20%)	0	4(80%)	1(20%)
Total		5	5	5	5

(CO: co-infected, PbO: *P. berghei* only infected, SO: *S. mansoni* only infected).

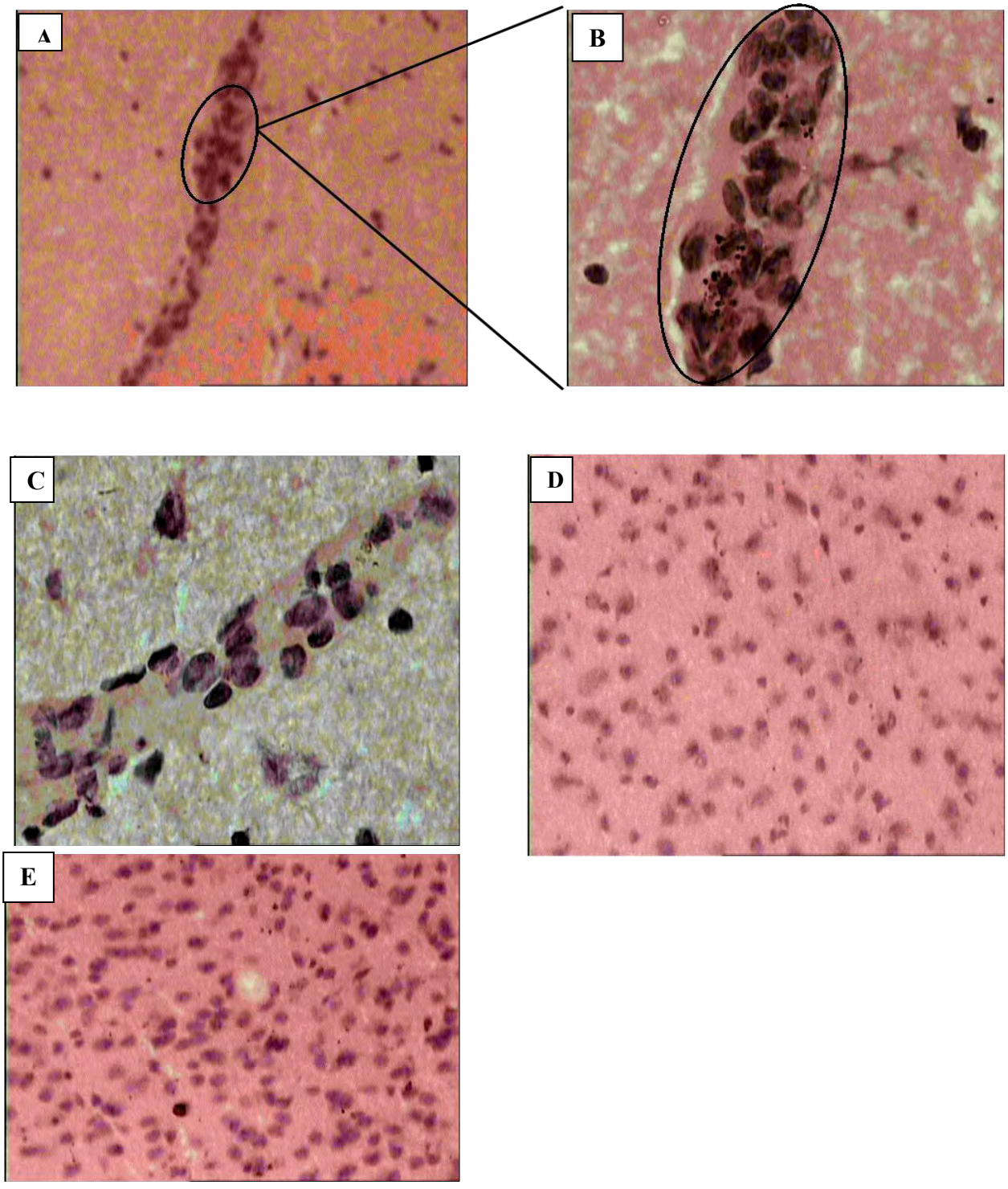

Figure 13. Histopathology of brain sections of: A) (400X) and B) (1000X) of *P. berghei*-mono-infected, showing sequestration of pRBC; (C) mice co-infected with *P. berghei* 4 weeks after *S. mansoni* infection (1000x) showing cerebral sequestration; (D) Brain section of a 7 weeks *S. mansoni–P. berghei*-co-infected mouse showing a (clear) uninfected brain tissue (400x), and (E) brain section of uninfected healthy mice (400X).

4.6. Survivability

During co-infection with *P. berghei* four weeks after *S. mansoni* infection, mice started to die on day 4 after *P. berghei* inoculation with all infected dying by day 12 post-infection. On the other hand, the *P. berghei* mono-infected mice started to die on day 6 post-infection, culminating in all death on day 10. However, all mice in schistosome mono-infected group survived over the entire experiment (Fig. 14).

In chronic *S. mansoni* infection, that is, seven weeks after *S. mansoni* infection, all the co-infected mice survived until day 11 started to die then after. 80% of the *P. berghei* mono-infected mice survived until day 4 and only 20% survived until day 10, whereas, 100% of the co-infected mice still survived until them. On day 11 all *P. berghei* mono-infected died whereas 60 % of the co-infected mice survived until day 14 post-*P. berghei* infection. The schistosome mono-infected mice survived all along and were sacrificed for histopathological assessment.

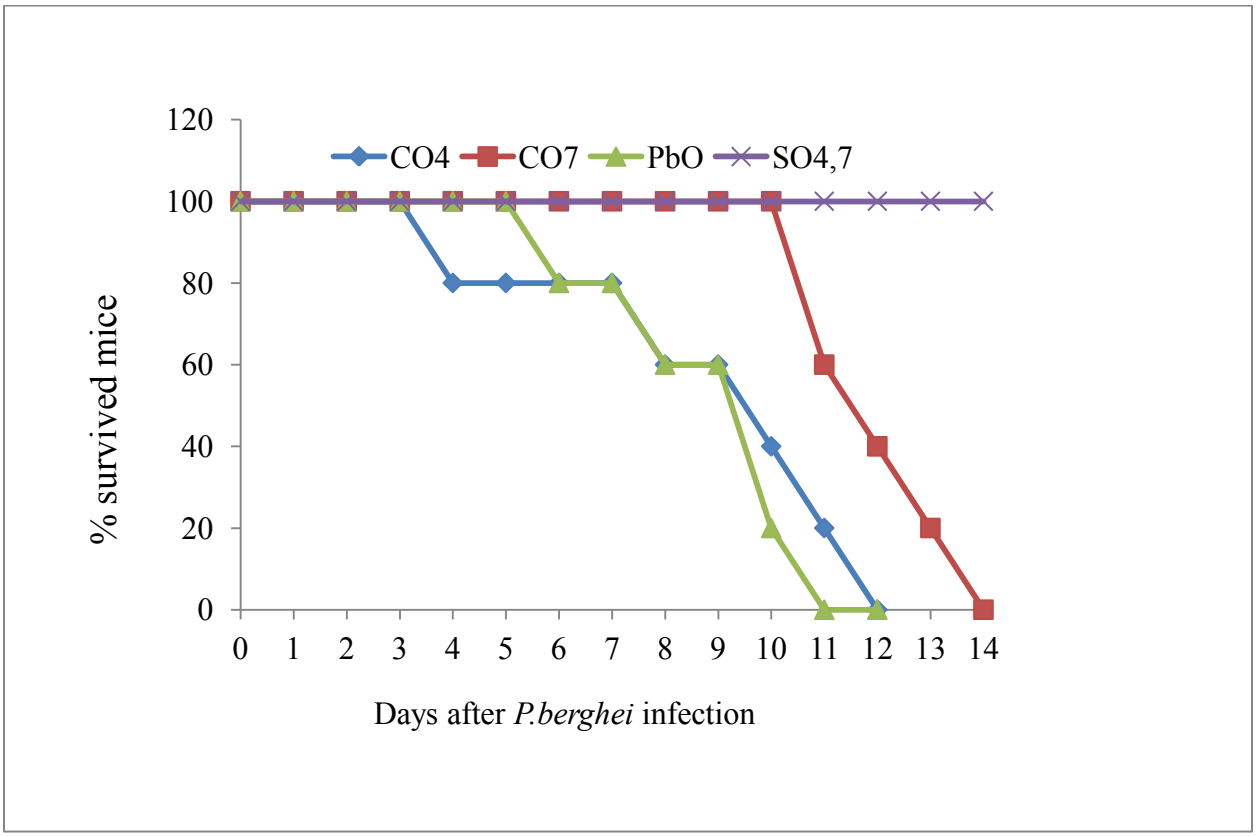

Figure 14. Length of survival in mice infected with *P. berghei* 4 weeks and 7 weeks post-*S. mansoni* infection and in schistosome/*Plasmodium* mono-infected mice. (CO4: 4th week co-infected, CO7: 7th week co-infected, PbO: *P. berghei* mono-infected, SO4**,** **7**: *S. mansoni* mono-infected for 4 and7 weeks).

5. Discussion

Findings of the present study showed mice co-infection with *P. berghei* 7 weeks post-*S. mansoni* infection to favor rapid *P. berghei* development and increased *P. berghei* parasitaemia than co-infection of mice with *P. berghei* 4 weeks post-*S. mansoni* infection. A number of previous studies also have demonstrated higher malaria parasitaemia in mice co-infected with various species/strains of rodent malaria and worms than those infected with the malaria parasite alone. For instance, Bucher *et al*. (2011) had reported increased malaria parasitaemia during early stages of malaria in chronic *S. mansoni*-*P. berghei* co-infected than mono-infected mice. Also, earlier studies by Yoshida *et al.* (2000), Legesse *et al.* (2004), and Kanyugo *et al.* (2009) had demonstrated elevated malaria parasitaemia in chronic *S. mansoni*-*P. berghei* co-infected mice than in mice infected with *P. berghei* alone. Therefore, the rapid *P. berghei* development seen in 7 weeks post-*S. mansoni* infection appears to indicate that chronic *S. mansoni* infection predisposes the host to more severe infection than during acute *S. mansoni* infection.

However, earlier works that reported no increased malaria parasitaemia in mice co-infected with *S. mansoni* and malaria parasite was observed (Lewinsohn, 1975 and Lwin *et al.*, 1982). This discrepancy in the findings may have been caused by difference in the *P. berghei* strains used that may be different in virulence and these reporting findings may possibly based on acute schistosomiasis infection. The elevated malaria parasitaemia seen in *S. mansoni*-*P. berghei* co-infected mice compared to *P. berghei*- mono-infected mice could perhaps be explained by a schistosome parasite induced Th2-mediated immune inhibition of the *P. berghei*-induced Th1 response. This might be possible because, resistant mice have Th1 type protective immune response against blood-stage *P. berghei* infection (Taylor-Robinson, *et al*., 1993). There has been much speculation about possible immunological modulation against severe malaria in schistosome-malaria co-infection, as chronic schistosomiasis is known induce a Th2 immune response (Muturi *et al*., 2008). A most likely explanation for the increased malaria parasitaemia seen in the co-infected mice is the *S. mansoni*-induced suppression of macrophage activation, probably through IL-10 and possibly also IL-4 and/or TGF-β (Oswald *et al*., 1992) that would lead to inability of the macrophages to kill parasitized red blood cells.

According to Mutapi *et al*. (2000), analysis of specific anti-schistosome egg responses in children co-infected with schistosomiasis and malaria shows that malaria positive children produce significantly more anti-schistosome IgE, which mediates immune cells, like macrophages, eosinophils and platelets that kill the parasite through antibody dependent cellular cytotoxic mechanism (Klion and Nutman, 2002). Worm killing would result in decreased worm load leading to the lower number of eggs per gram of liver detected in the co-infected mice in the present study. Further immune mediated damage to the schistosome parasite has also been demonstrated to be through antigen-specific IgG activation in co-infected mice (Kanyugo *et al.,* 2009). Similar findings exist from *Leishmania major* schistosome co-infection where by reduction in *S. mansoni* worm counts were reported (Yole *et al*., 2007).

The present finding confirmed earlier demonstration that hepatic granulomas in murine schistosomiasis are suppressed by the presence of concomitant malaria infection that suppressed the hosts‟ immune response to schistosome eggs (Van Horn *et al*., 1972 cited in Abdel-Wahab *et al*., 1974). Similarly, a study by Kanyugo *et al.* (2009) has demonstrated the decrease in size and number of granuloma in *S. mansoni* and *P. berghei* co-infected mice. Similar reports from other pathogen co-infection also exist such as that of Furze *et al.* (2005) who noted that co-infection of mice with influenza virus during the early phase of trichinosis to have resulted in a reduced inflammatory infiltrate in the lungs. This may be explained by the fact that malaria has a direct influence on an immunologic reaction of the delayed hypersensitivity type. The findings of Helmby *et al*. (1998) that showed the lower levels of IL-4 and IL-5, the major cytokines that up regulate granuloma, in *S. mansoni-P.chabaudi* co-infected mice would support the proposed immunological induction by the malaria parasite leading to decrease in the size and number of granuloma due to schistosome eggs.

Studies investigating the impact of helminths on the pathogenesis of cerebral malaria have conflicting reports. Some studies have reported chronic schistosome infection to protect (Bucher *et al*., 2011) and reduce (Waknine-Grinberg *et al*., 2010) brain pathology in murine model of cerebral malaria during concurrent infection. Likewise, the present study had demonstrated chronic *S. mansoni* co-infection to result in reduction of malaria parasite sequestration in the brain. However, the reduction of cerebral sequestration was dependent on the timing of *S. mansoni* infection. This is in agreement with observation of Christensen *et al*. (1988) who

reported that the outcomes of experimental co-infections with protozoan and trematode parasites to be dependent on the parasite species as well as the relative timing of infection. The time-dependence reduction of cerebral sequestration associated with schistosome parasite co-infection with malaria may have been caused by the protective cytokines and chemokines secreted during the chronic phase of schistosomiasis. The infection of mice with *S. mansoni* for seven weeks resulted in the marked reduction of *P. berghei* cerebral sequestration in *S. mansoni* chronically infected mice and the lack of effect of *S. mansoni* acute infection on cerebral sequestration of *P. berghei* coincides with absence of schistosome parasite egg production four weeks after cercarial infection. This suggests that each stage of schistosomal infection may affect the development of cerebral sequestration of malaria parasite in a different manner. The preexisting chronic *S. mansoni* infection, which causes a Th2 shift, can be expected to have translated into an improved disease profile in *P. berghei* infected mice. The immunological basis for this can be extrapolated from the fact that during the prepatent period of *S. mansoni* infection (the first 4-5 weeks following exposure to cercariae) the immune response is primarily Th1 (hypersensitivity), and an egg antigen-specific Th2 response (an immunomodulatory) is seen by seven weeks post-infection (Kaplan *et al*., 1998). This shift in immune profile may have resulted in an overall modulation of balance in cytokines, chemokines and adhesion molecules leading to reduction in cerebral sequestration of malaria parasite.

The basis for the protection appears to be modulation of the Th1/inflammatory immune response which is associated with cerebral sequestration of malaria parasite (Hartgers *et al*., 2008). The chronic schistosome infection, which commonly generates Th2 anti-inflammatory responses, would dampen the inflammatory damages (Maizels *et al*., 2004). Therefore, an estimate of several factors (e.g. a sum of Th1 vs. Th2 cytokines) might better predict and correlate with the clinical situation in cerebral malaria. Thus, during cerebral malaria, IFN_{γ} and TNFα have been shown to play an important role in up-regulation of cellular adhesion molecules (CAM) on brain endothelial cells (Weiser *et al*., 2007). This process leads to the sequestration of leucocytes in microvessels and is required for the development of murine cerebral malaria (Favre *et al*., 1999). However, the elevated level of IL-10, the anti-inflammatory Th2 cytokine, in the co-infected mice (Bucher *et al*., 2011) seems to result in down regulation of the inflammatory cytokines leading to reduction of cerebral sequestration of leucocytes. Niikura *et al*. (2010) have also shown the role of IL-10 in protection of experimental cerebral malaria. On the other hand,

according to de Souza and Helmby (2008), concurrent chronic gastro-intestinal nematode infection does not alter the development of experimental cerebral malaria, which suggests that nematode and schistosome parasites trigger distinct reactions inherent to their own life cycle and anatomic location within the host. This is apparent in spite of the fact that both helminth parasites have in common the induction of a host-protective Th2- type immune response (Gause *et al*., 2003).

The time-dependent effect of schistosomiasis during co-infection with *P. berghei* was also seen on weight loss, hemoglobin level and survivability of the mice. However, an earlier study by Lewinsohn (1975) had reported lack of effect of chronic schistosomiasis-malaria co-infection on anemia. This report is contradicted by a recent work (Bucher *et al*., 2011) that showed the most severe weight and hemoglobin loss in mice concurrently infected with *S. mansoni* and *P. berghei*. Similarly, Waknine-Grinberg *et al*. (2010) had shown the highest loss of weight in *S. mansoni* and *P. berghei* co-infection. The findings of the present study, whereby chronic schistosome co-infection resulted in high loss of weight and hemoglobin is in agreement with these recent reports. The fact that this effect could only be seen during the chronic period of infection suggests a synergetic effect of chronic *S. mansoni* and *P. berghei* acute infection on the pathogenesis of anemia. The distinct mechanisms of anemia induction in schistosomiasis, which includes extra-corporeal loss of iron, splenomegaly leading to red blood cell sequestration and autoimmune hemolysis (Friedman *et al*., 2005) and that of malaria, which includes rupture of pRBC, haemolysis and phagocytosis of infected and uninfected RBC (Chang and Stevenson, 2004) seems to synergize to exacerbate anemia severity.

The post-infection survivability of mice concurrently infected with *S. mansoni* and *P. berghei* is similar to the enhanced survival of rats concurrently infected with the protozoan, *Trypanosoma brucei*, and the nematode, *Strongyloides ratti* (Onah *et al.,* 2004). In addition to this, Waknine-Grinberg *et al*. (2010) had reported that a concomitant patent *S. mansoni* infection in ICR HSD mice led to an increased survival in addition to the reduction of *P. berghei*-induced cerebral malaria. Although Bucher *et al*. (2011) reported that chronic co-infection with *S. mansoni* does not influence the survival of *P. berghei* infected mice, the data obtained in the present study showed that chronic *S. mansoni-P. berghei* co-infected mice survived an average of 3 days longer than those mice with *P. berghei* only.

The observed disparity in experimental outcomes from different studies may have resulted from differences in *Plasmodium* species/strain used in the study, the patency of *S. mansoni* during co-infection and differences in the dose of *S. mansoni* cercariae used to infect the mice. This explanation is supported by the findings of Waknine-Grinberg *et al*. (2010) who showed the reduction of cerebral malaria during concomitant *S. mansoni* and *P. berghei* infection to be dependent on infection schedule and infecting cercariae number. This report is in agreement with the findings of the present study whereby infection schedule was shown to have an effect on cerebral sequestration of malaria parasite.

6. Conclusions

- The patency of *S. mansoni* infection is the major factor in disease outcome during co-infection with *P. berghei* as all the effect of co-infection was apparent during chronic phase of infection.
- Co-infection of mice with *P. berghei* during chronic *S. mansoni* infection resulted in rapid *P. berghei* development and high malaria parasitaemia, severe hemoglobin and weight loss; accompanied with enhanced survivability of the mice and reduced sequestration of malaria parasite in the brain.
- Reduction of cerebral sequestration was dependent on chronicity of *S.mansoni* infection.
- Co-infection of mice with *P. berghei* and *S. mansoni* resulted in reduced severity of *S. mansoni* infection as indicated by reduction of eggs deposited in the liver and granuloma.

7. Recommendations

- Appropriately planned and controlled field studies and further laboratory experiments on non-human primate models will be required for elucidation of the importance of heterologous interactions in concurrent parasite infection in schistosomiasis and malaria.
- Since the presence or absence of a pre-existing infection may partly explain why certain individuals develop cerebral malaria while others exhibit minor symptoms only, the presence of multiple parasitic infections in patients from endemic areas should therefore be carefully noted in clinical trials and in the development of standard treatment protocols for malaria infection.

8. References

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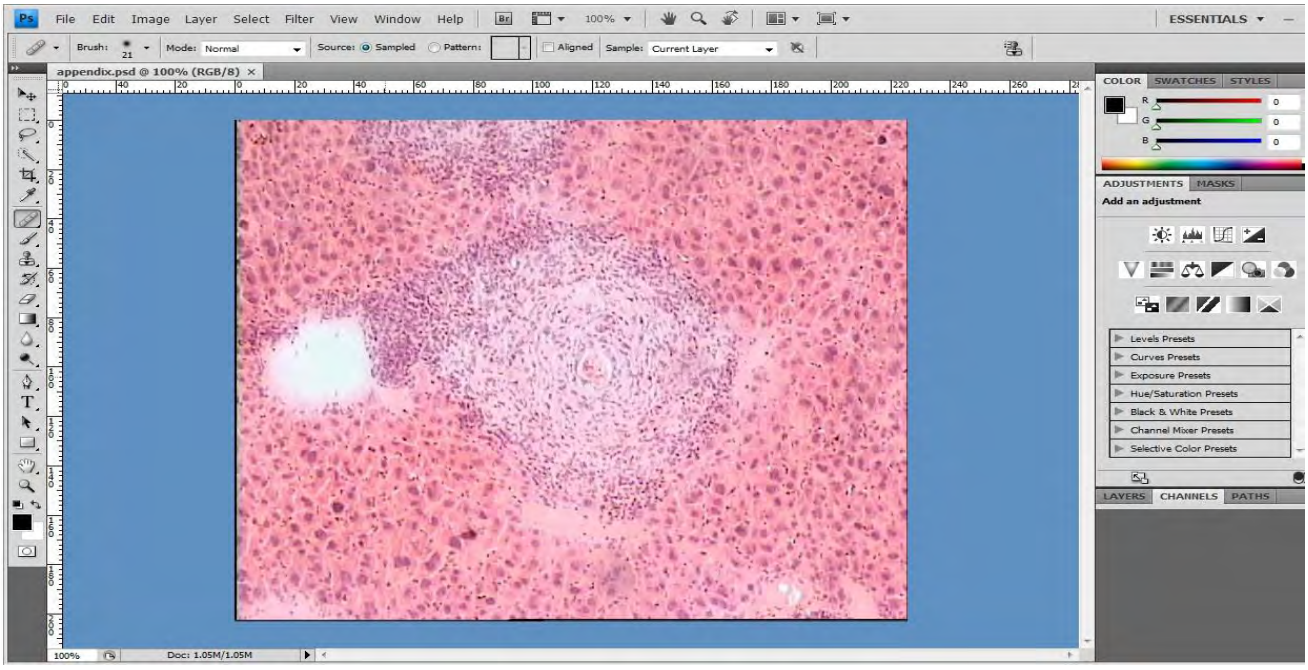
Appendix I: Picture of instrument and software used during the study

A. Method of capturing histopathological pictures from microscopic slide by microscope connected computer

B. Method of granuloma measurement using Adobe Photoshop



(Picture resolution: 28.346 p/cm and dimension: 225.78 x 203.2 mm)