OUTCOME OF CHRONIC SCHISTOSOMIASIS IN REGULATION OF MALARIA DISEASE SEVERITY AND PATHOLOGICAL EVENTS IN A MOUSE MODEL

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DECLARATION

This thesis is my original work, and as far as I am aware, it has not been presented for the award of any degree in any University.

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DEDICATION

To the Almighty God for the strength and commitment he gave me throughout this thesis.

To Muganda's family for the spiritual, financial, and emotional strength they offered me.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BSA	Bovine Serum Albumin
CDC	Centre for Disease Control
EDTA	Ethylene Diamine Tris Acetic acid
ELISA	Enzyme-linked Immuno-sorbent Assay
GSH	Reduced glutathione
IFN- γ	Interferon gamma
IgG	Immunoglobulin gamma
IL- 10	Interleukin- 10
IPR	Institute of Primate Research
IPSE	Induced protein of Schistosomes eggs
*Pb*A	*Plasmodium berghei* ANKA
*Pf*EMP1	*Plasmodium falciparum* erythrocyte membrane protein 1
pH	Hydrogen potential
PTEX	Plasmodium translocon of exported proteins
RBC	Red blood cells

ROS	Reactive oxygen species
Sm	*Schistosoma mansoni*
TNF- α	Tumor Necrosis Factor- alpha
WHO	World Health Organization
WMR	World Malaria Report
WSR	World Schistosomiasis Report
WT	Wild type

ABSTRACT

Malaria is a severe infection caused by the *Plasmodium* parasite. It causes high mortality and morbidity, especially in the malaria endemic region. Schistosomiasis is caused by blood flukes and is the second leading parasitic infection after malaria in morbidity and mortality rate. These two infections are co-endemic in many regions. Both parasites have definitive and vector/intermediate hosts and each utilizes the host protein differently. The objective of this study was to determine the outcome of chronic *S. mansoni* infection in the regulation of *P. berghei* ANKA associated disease severity and pathological events in a mouse model. Mice were infected with 200 *Schistosoma mansoni* cercaria. After chronic *S. mansoni* infection had established, mice in co-infection group were inoculated with 10000 *Pb*A infected red blood cells. Parasitemia and physiological parameters were monitored on a two-day interval, to track the infection levels. Furthermore, hematological parameters, and inflammatory markers were quantified at the end of the study and analyzed at a $p < 0.05$. Co-infection of *S. mansoni* and *Pb*A enhanced survival of mice which was independent to parasitemia. Chronic *S. mansoni* infection resulted in hepatosplenomegaly which contributed to an increase in the body weight and introduction of malaria in the host reduced both organ enlargement and body weight. Liver functionality was disrupted by measurement of ALP enzyme which is a marker of liver damage. Schistosomiasis reduced host metabolites such as protein, lipids as schistosomes cannot synthesis their own *de novo.* Co-infection restored disruptive effect of *S. mansoni* in the host. Moreover, co-infection of *S. mansoni* and *Pb*A dampened *Pb*A induced elevated levels of TNF-α and IFN-γ cytokines, associated with inflammation. Co-infection with *S. mansoni* and *Pb*A enhanced anti-inflammatory cytokine IL-10, hence reduced inflammation. There was evident oxidative stress in the host, liver and brain of mice infected with *S. mansoni* or *Pb*A alone. However, coinfection of *S. mansoni* and *Pb*A ameliorated oxidative stress. Standard histopathological analysis revealed that when mice were infected with *S. mansoni* or *Pb*A alone they had pronounced organ damage, which was assuaged by co-infection with both parasites. Overall, this study demonstrates that chronic *S. mansoni* infection is critical in regulation of *Pb*A infection associated severity & pathological events in a mouse model**.** It is recommended to check the differential gene expression during singular infection and in co-infections.

CHAPTER 1

INTRODUCTION

1.1 Background information

Malaria is a life-threatening disease common in many tropical and subtropical areas. It is caused by the protozoan *Plasmodium* that is transmitted to people via bites of infected female *Anopheles* mosquitoes (WHO, 2021). *Plasmodium falciparum, P. vivax, P. malariae,* and *P. ovale* are the causative agent for human malaria. Humans occasionally become infected with *Plasmodium* species that normally infect animals, such as *P. knowlesi.* Nevertheless, there is no known report of human-mosquito-human transmission of such "zoonotic" form of malaria (WMR, 2017). In 2020, 241 million cases were estimated in 87 countries and the estimated number of fatality owing to malaria stood at 621,000 (WHO, 2021). In 2017, the incidence of malaria in Kenya was at 8.53% in a study conducted worldwide (WHO, 2018), and an incidence of 57% reported in 2019 worldwide (WHO, 2020)**.** Overall, the burden of malaria continues to worsen globally as a devastating hindrance to the economy of developing countries and the world at large.

Schistosomiasis commonly known as bilharzia or snail fever, is an acute and chronic infection caused by blood flukes also called schistosomes. It is a neglected tropical disease affecting almost 240 million people globally (WSR, 2018; WHO, 2019). The species of schistosomes that are known to cause schistosomiasis include; *Schistosoma mansoni (Sm), S. mekongi, S. intercalatum, S. guineensis, S. japonicum,* and *S. haematobium*. Schistosomiasis represents a major public health problem globally (WHO, 2018). Out of the 240 million estimated cases of schistosomiasis in the whole world, almost 90% occurred in sub-Saharan Africa (WHO, 2019). Schistosomiasis mostly affects poor and rural communities, especially those that engage in agriculture and fishing. Mostly

women and children that do domestic chores in infested water and inadequate hygiene are at risk and vulnerable to this infection (French *et al*., 2018). However, the entire community in the endemic regions is vulnerable to the infection. The Center for Disease Control (CDC) considers schistosomiasis a "Neglected Tropical Disease" and is ranked as the second most devastating parasitic disease after malaria (French *et al*., 2020). During schistosomiasis, it has been established that once the blood flukes have been established in the mesenteric veins, the female adult, start laying eggs that pass to be secreted in feces. Some eggs get trapped in the liver causing inflammation and elicit immunological responses (Marr *et al*., 2012).

Despite worldwide initiatives, emerging drug resistance, and insufficient knowledge about the exact mechanism behind malaria pathogenesis has provided unprecedented challenges in the management and eradicating the diseases (Acharya *et al*., 2007). Malaria is also known to affect blood coagulation, hence the fatal hemorrhage complications (WHO, 2018). Previous studies have demonstrated that parasite-induced alteration of the host protein have a great impact on diagnosis and prognosis in light infections associated with malaria (Acharya *et al*., 2007). Such findings from these studies provide a better understanding of the disease pathogenesis, host-parasite interaction, and host immune response.

Occurrence of malaria or schistosomiasis, being caused by blood parasites, result in anemia mostly due to high parasite burden (Marr *et al*., 2012; Niikura *et al*., 2011; Butler *et al*. 2012). In most developing countries, mortality resulting from bilharzia among other helminthic infections has been grossly underestimated (WHO, 2019). Schistosomiasis causes loss of 10.4 million Disability Adjusted Life Years (DALYs). However, it causes lower mortality than the morbidity it is usually

associated with. These include and not limited to hepatosplenomegaly, hematochezia, lasting pains, diarrhea (WHO, 2018). Women and children that do domestic chores in infested water and inadequate hygiene are at risk and vulnerable to infection (French *et al*., 2020). In 2019, malaria caused up to 67% mortality in children under the age of five (WHO, 2020). In addition, an estimate of 822000 children were born with low birthweights in 33 countries in the case of malaria infection during pregnancy (WHO, 2020). Co-infection of schistosomiasis and malaria in children, influences the development of acquired immunity associated with the resistance or the pathology linked to schistosomiasis (Diallo *et al*., 2010). In a co-infection study involving *P. berghei* and *Trypanosoma brucei* in murine models, increased severity of both infections was observed (Ademola *et al*., 2016). From this perspective, co-infection of malaria and schistosomiasis can result into higher morbidity and mortality.

1.2 Problem statement

S. mansoni infection results in disability adjusted life years, anemia, hepatosplenomegaly or death whereas malaria results in anemia, coma, neurological syndromes as well as death. Schistosomiasis infection occurs in various endemic regions in Kenya namely in the Coastal region, Central region, Eastern region and along Lake Victoria region. Meanwhile, malaria is also prevalent in the Lake Victoria Region basin, Central region and Coastal region. From this epidemiological distribution, both infections share common geographical regions, hence increased likelihood of an individual in these regions harboring the two infections concurrently. Both infections have defined immune responses, physiological, hematological and pathological parameters when they occur separately in the host (Diallo *et al*., 2010). The detailed severity process due to co-infection of *P. berghei* and *S. mansoni* remains scanty with regards to metabolites utilization, protective or accelerated phenotype; in immune response modulation and whether there would be a compromised or

enhanced immunity in co-infection between *P. berghei* and *S. mansoni* infections. Therefore, there is need to understand metabolites utilization, whether there will be a compromised or enhanced immunity.

1.3 Justification

The study on the immunological responses and other associated parameters during co-infection of schistosomiasis and malaria is critical in understanding how these infections are modulated in an individual. Individual occurrence of malaria or schistosomiasis, is associated with inflammation, oxidative stress, & chronic pathology. This study helps to advance knowledge on immunological response, pathology, and metabolic changes during co-infection and disease progression. Additionally, it generates critical information to understand pathological, inflammation, immunological events associated with co-infection. The findings from this study provides critical information in developing strategies for the management of schistosomiasis and malaria co-infection as a tool towards overall reduction of morbidity and mortality in the two infections. Finding from this study generate information which will be exploited in critical management of co-infection such as policy making.

1.4 Null hypothesis

Chronic *S. mansoni* infection has no effect on malaria disease severity and pathological events in a mouse model

1.5 Research Questions

1. What are the effects of chronic *S. mansoni* infection on selected physiological and hematological parameters during *Pb*A infection?
2. How does the effect of chronic *S. mansoni* infection immune regulation and metabolic changes during *Pb*A infection?
3. What are the effects of chronic *S. mansoni* infection on *Pb*A *co*-infection driven oxidative stress and tissue inflammation?

1.6 General objectives

To determine the outcome of chronic *S. mansoni* infection in the regulation of *P. berghei* ANKA associated disease severity and pathological events in a murine model

1.6.1 Specific objectives

1. To determine the effects of chronic *S. mansoni* infection on selected physiological and hematological parameters during *Pb*A infection
2. To determine the effect of chronic *S. mansoni* infection on immune regulation & metabolic changes during *Pb*A infection
3. To evaluate the effects of chronic *S. mansoni* infection on *Pb*A- driven oxidative stress and tissue inflammation

CHAPTER 2

LITERATURE REVIEW

2.1 Malaria and schistosomiasis prevalence

Parasitic infections are said to be the major cause of morbidity and mortality mostly in developing countries. Concomitant parasite infections in humans are common. For example, malaria and schistosomiasis as well as visceral leishmaniasis and malaria (Yves *et al.,* 2014). Epidemiological studies conducted on malaria and bilharzia indicate that co-infections are common in people living in co-endemic areas. A study conducted in lake Victoria basin and its environs, indicated that there was a high prevalence of malaria as well as substantial level of intestinal schistosomiasis (Kinung'hi *et al*., 2017) as shown in Figure 2.1. It was not a surprise that the heightened prevalence of anemia was observed in the study villages and significantly correlated with high co-infection levels of *Plasmodium falciparum* and *Schistosoma mansoni* (Kinung'hi *et al*., 2017).

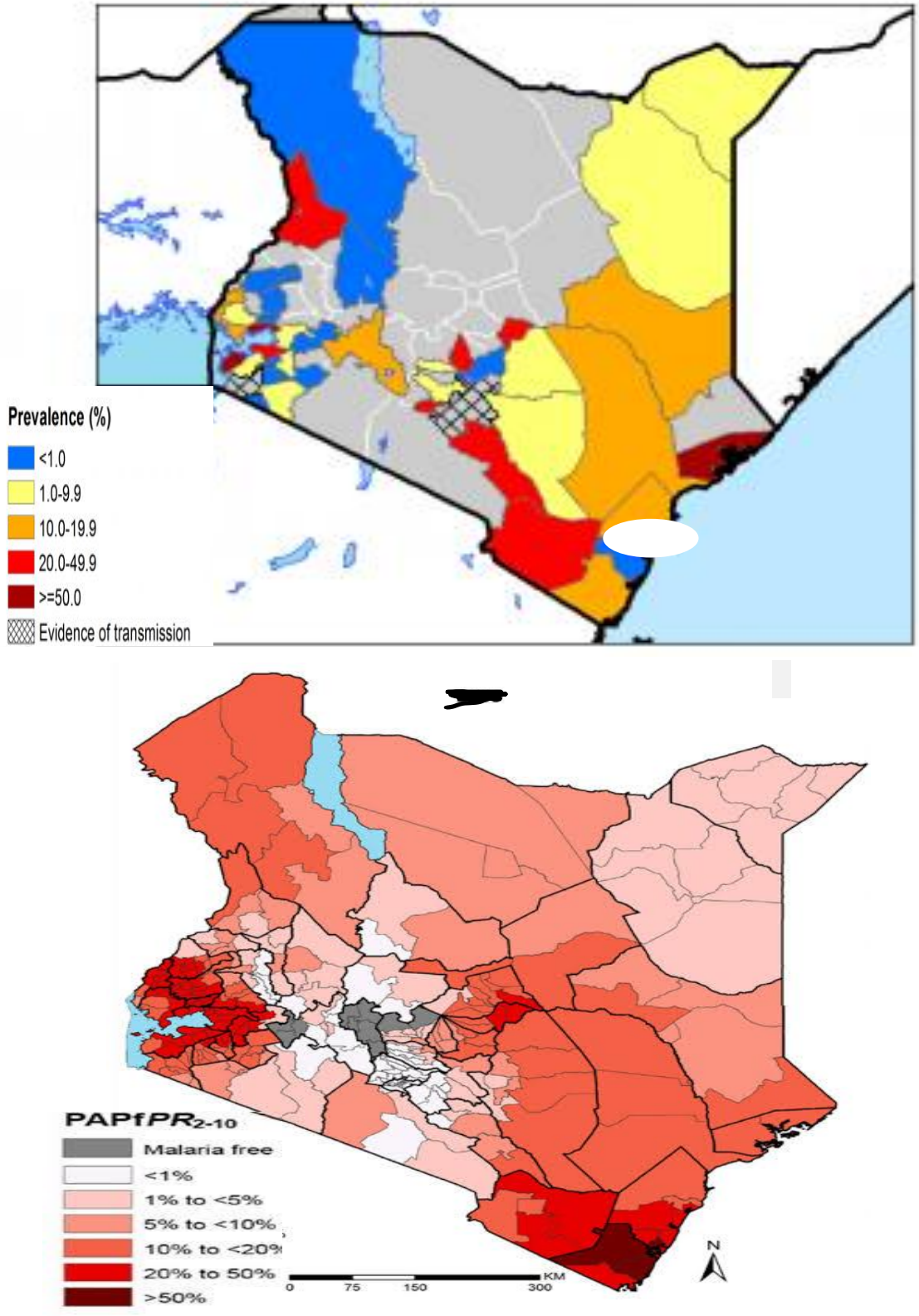

Figure 2.1: Endemic distribution region for schistosomiasis in Kenya
(Top; researchgate.net, date downloaded: 02/08/2021) and malaria (down; Ghilardi *et al.*, 2020))

2.2 Plasmodium life cycle and biology

Human malaria is transmitted via the bite of an infected female anopheles mosquito that harbors the *Plasmodium* parasites in form of sporozoites. Once the mosquito inoculates the sporozoites into the host; then the sporozoites move to the host liver where they develop into merozoites in the hepatocytes after asexual reproduction. The hepatocytes rupture and merozoites are released into the bloodstream and infect erythrocytes (Marti *et al*., 2013). The merozoites enter the host erythrocytes forming ring stages that asexually reproduce forming schizonts and the cell ruptures to release new merozoites that invade new red blood cells. Some merozoites develop into microgamete and macrogametes (Elsworth *et al*., 2014). When a female anopheles mosquito blood feeds from an infected person, it takes up the gametocytes in the meal. A zygote is formed in the gut which later forms an oocyst. Inside the oocyst, thousands of active sporozoites develop. Eventually, the oocyst bursts, releasing sporozoites into the body cavity. The sporozoites travel to the mosquito's salivary glands and are injected into another host (Marti *et al*., 2013; Elsworth *et al*., 2014; CDC, 2019).

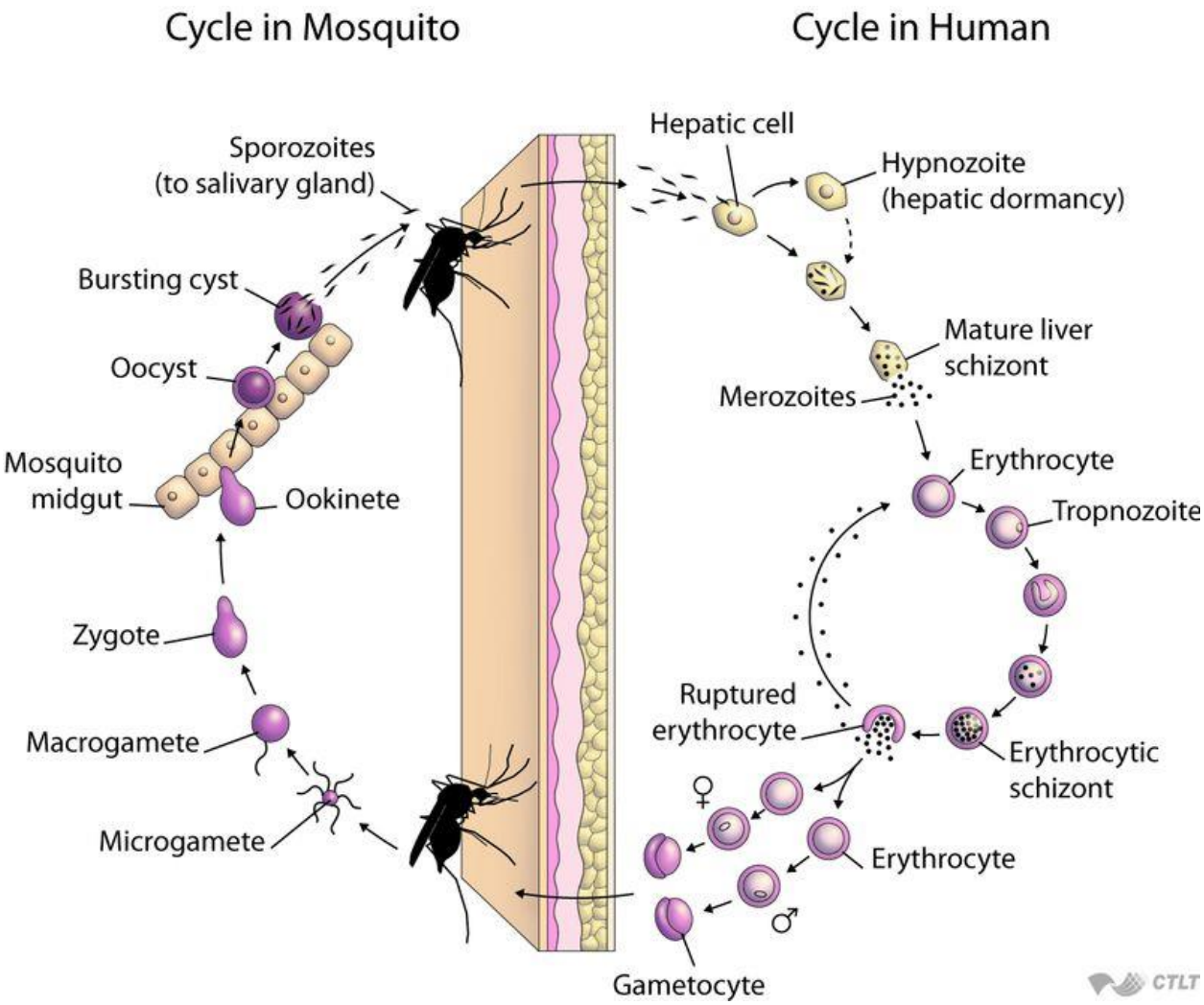

Figure 2.2: Plasmodium species life cycle
(Malaria site, date downloaded: 02/08/2021)

The terminally differentiated red blood cells lack many cell organelles and associated processes like synthesis of proteins and trafficking. *Plasmodium* species such as *P. falciparum* export proteins that impose changes to host red blood cells such as permeability, rigidity, and cytoadherence characteristics (Maier *et al*., 2009; Prajapati *et al*., 2014). In the mechanism of translation, proteins are exported in host red blood cells and navigate into the endoplasmic reticulum. In intracellular parasites, parasitophorous vacuoles are generated during the invasion

and the protein gets to them via the secretory system and parasite plasma membrane (Marti *et al*., 2013). Some of the exported protein enter the host red blood cells' cytosol with the help of parasitophorous vacuolar membrane (PVM) in the erythrocytes cytosol (Prajapati *et al*., 2014).

2.3 Schistosomes biology and life-cycle

During schistosomes' life cycle, it is known that both definitive and intermediate hosts such as humans and snail respectively are involved. Infection begins when swimming cercaria with the help of elastase enzyme, penetrates the host skin causing cercarial dermatitis. (CDC, 2019). As indicated in Figure 2.3, the tail sheds off and schistosomula emerges and enters the lungs then finally in the hepatic veins where they develop into an adult. *S. mansoni* develops in the vein draining the intestines (WHO, 2018). Upon maturation, these dioecious parasite mate, and the female produces eggs into the host liver and intestines. The eggs mature and exit the host back via fecal sample. When the eggs get into freshwater, they hatch into miracidium. The miracidium swims and gets into *Biomphalaria* snails for *S. mansoni*. In the snail, the miracidia develop into sporocyst which develop into cercaria to cause reinfection when it penetrates a host's skin (CDC, 2019; WHO, 2018).

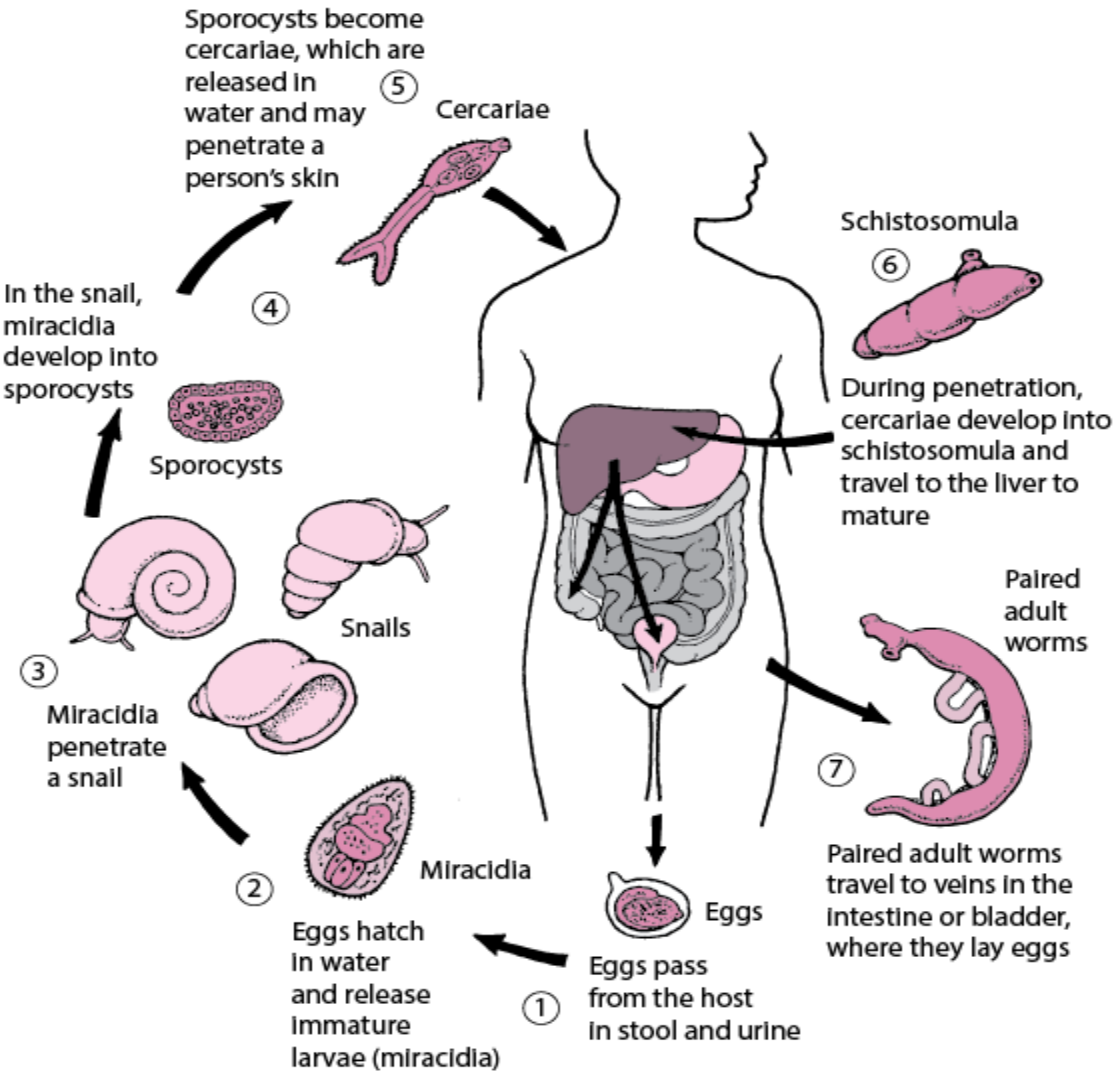

Figure 2.3: Schistosoma species life cycle (MSD Manual, 2020)

Parasite-human protein interaction occurs in four out of the six parasite life stages in *S. mansoni* (French *et al*., 2018). Avalanche body of evidence has shown that such interactions are found in cercaria and schistosomula protein expressed in the skin, schistosomula protein expressed in the lung, liver, immune cell, and erythrocytes. Also, adult *S. mansoni* proteins are expressed in the liver, immune cells, and erythrocytes as eggs protein are expressed in the liver, immune cells, and erythrocytes (Bear *et al*., 2018).

2.4 Biochemical interactions in schistosomiasis or malaria infections

Eight weeks post-infection with *S. mansoni*, there are unique changes in protein expression in livers of infected mice. These changes involve a decrease in protein relating to amino acid metabolism, the citric acid cycle, fatty acid cycle, and urea cycle which are essential in life. In contrast, protein relating to stress and structural components such as peroxiredoxin and actin are increased (Harvie *et al*., 2007).

Occurrence of an antigen in a host elicits immunological responses to or by the host immunity. Schistosomes' eggs antigen elicits immunological responses as the antigens trigger a granulomatous reaction in the host (WHO, 2018).

Reduced glutathione (GSH) is an antioxidant that quenches free radicals and protects the host tissues from damages that can be caused by the free reactive species (Vega-rodríguez *et al*., 2015; Marr *et al*., 2012). In a study conducted on serum antioxidants in Nigeria, occurrence of malaria is known to lower the levels of reduced glutathione in human (Abubakar *et al*., 2016). Notably, a study conducted in India, indicates that parasitic clearance leads to a decrease in reactive oxygen species (ROS) generation by the parasite (Tyagi *et al*., 2017). This could lead to normalization of levels of reduced glutathione antioxidant in the host as there would be no free radicles to counter. Host-parasite association in schistosomiasis leads to the production of free radicals (Egal, 2006). These biochemical changes mostly occur in the host liver, brain, kidney, and spleen antioxidants as a means to scavenge and eradicate these radicals.

Cerebral malaria (CM) is when the blood brain barrier (BBB) has been compromised and mononuclear cells cross to the brain. Cerebral malaria histology is known by presence of

microhemorrhages and subarachnoid bleeding (Lackner *et al*., 2006). On a close interaction with schistosomiasis, Wheater *et al*., (1979) , stated that brain tissues can be affected 8 days post infection when the cycle is past schistosomula stage. At the severe stage of these infection, the host metabolites cells are compromised.

Severe anemia in the host is brought by ingestion of erythrocytes by schistosomes and phagocytosis of infected red cell in malaria (Niikura *et al*., 2011; Marr *et al*., 2012; Leticia *et al*., 2014). Hematuria and lower levels of hemoglobin is one the morbidity caused by schistosomiasis (WHO, 2018). Severe malaria infections is well known to cause anemia by depreciating hemoglobin and hematocrit levels (Al-salahy *et al.*, 2016). Leukocytes, especially eosinophil, are considered a marker for helminths infection as they are altered and/ or elevated (Castro *et al*., 2018). In a hematological profile of patients with malaria, platelets level decrease with an increase in the parasite level (Al-salahy *et al*., 2016; Sakzabre *et al*., 2020).

In malaria, the levels of Alkaline phosphatase (ALP) is reported to increases (Auta, 2018). The increase of serum liver enzymes is directly linked to the higher intensity of malaria parasite (Houmsou, 2018). However, in schistosomiasis, abnormalities as a result of liver fibrosis and portal hypertension (Menezes *et al.*, 2013), leads to deprived levels of liver functionality enzymes such as Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) (Letícia *et al*., 2018).

Schistosomes do not produce their cholesterol and long chain fatty acids *de novo* hence they depend on, and acquire the same from host via the tegument (Marr *et al*., 2012). Malaria patients are known to have higher bilirubin levels both direct and total compared to uninfected ones (Al-salahy *et al*., 2016; Houmsou, 2018). Furthermore, patients with hepatosplenic schistosomiasis

recorded an elevated bilirubin levels (Menezes *et al.*, 2013). There is marginal interaction of these infections known to either elevate or decrease these essential biochemical parameters. There would be more interesting results in this co-infection study to rule out which of these two infections would influence the parameters to their favor.

2.5 Co-infection studies of schistosomiasis and malaria

Several studies conducted on animal models shows immunological, histological, and biological responses induced by a parasite were modified, either positively or negatively, in the presence of another parasite (Tangpukdee *et al*., 2009). Other studies have shown the existing relationship between immune responses elicited by both parasites, although the nature is not yet fully elaborated and is under investigation (Nacher *et al*., 2015). Pierrot *et al*. (2006) observed that *Schistosoma mansoni* LRR gene (SmLRR) expressed in different stages of *S. mansoni* contains antibody binding sites that cross-react with both *P. berghei* and *P. falciparum*. In human infections, the cross-reactive responses seem to be predominantly IgG3 isotype responses to malaria and IgG4 response to schistosomiasis. An association was found between mild *S. haematobium* infection but not moderate or heavy egg counts, and parasitemia of *P. falciparum* (Yves *et al*., 2014).

Other studies conducted to check the effect of schistosomiasis on malaria indicated that schistosomiasis may be associated with an increased malaria risk (Bakare & Nwozo, 2016). The interaction between malaria and schistosomiasis was seen to have the capacity to reduce the effectiveness of malaria treatment for controlling transmission (Bakare & Nwozo, 2016).

In a study of *T. brucei* and *Plasmodium berghei* co-infected in mouse model, it was highlighted that parasitemia in mice was increased and they had least survival time when compared with singly

infected mice. In the same study, anemia and hypoglycemia were severe in co-infection mice (Ademola *et al*., 2016). Similarly, a study conducted in Mount Cameroon showed that pregnant women with malaria and *S. haematobium* infection developed severe anemia compared with those with this either of the infections (Anchang-kimbi *et al.*, 2017).

The schistosomes' protective effect against malaria has been observed in *S. mansoni* and *P. yoelli* co-infection. It was observed that *S. mansoni* reduced malaria parasite level in liver. This reduction led to reduction in blood stage levels and concurrently gametocyte levels (Moriyasu *et al*., 2018). Hence *S. mansoni* was observed as a reducing agent in transmission of malaria both to/from mice and from/to mosquitoes respectively (Moriyasu *et al*., 2018).

2.6 Molecular pathogenesis of malaria and schistosomiasis

2.6.1 Malaria

Plasmodium falciparum is known for its modification of the parasitized erythrocytes. Creation of an adhesive phenotype, that inhibits the parasite from circulating for almost half of its asexual life cycle, is a unique time to intracellular parasites like *Plasmodium* species. The infected red blood cells can adhere to platelets, endothelium or uninfected red blood cells (Smith *et al*., 2013). The parasite cytoadhering state is known to be accomplished through the *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP1), an outcome of *var* gene transcription (Smith *et al*., 2013). Several factors that add variation to the host- parasite interaction are not limited to co-infections, delayed treatment, human genetic variations (Milner, 2017). Pathogenesis of malaria can either be complicated or uncomplicated. Sequestration of the parasite in tissues with elevation of cytokines, toxins and, lack of timely therapy results in fatality. For the other cases, disease severity is due to

an imbalance in the interaction of parasite and host (Milner, 2017). An example of the immune response in malaria is when an infected/ parasitized red blood cell activates dendritic cell to maturity. Once a dendritic cell's pattern recognition receptors (PRR) recognize a malaria pathogen associated molecular pattern (PAMP), the antigen is phagocytosed and the dendritic cell is activated. This upregulates the expression of Major histocompatibility complex (MHC) class II molecules which binds and activates CD4 T helper cells to release pro-inflammatory cytokines (TNF-α, IFN-γ). These cytokines activate macrophages to phagocytes infected red blood cells (iRBC) and in the process releases nitric oxide and reactive oxygen species as shown in the Figure 2.4 (Good, 2001), which in return induces oxidative stress hence need to check antioxidant levels that might quench these effects.

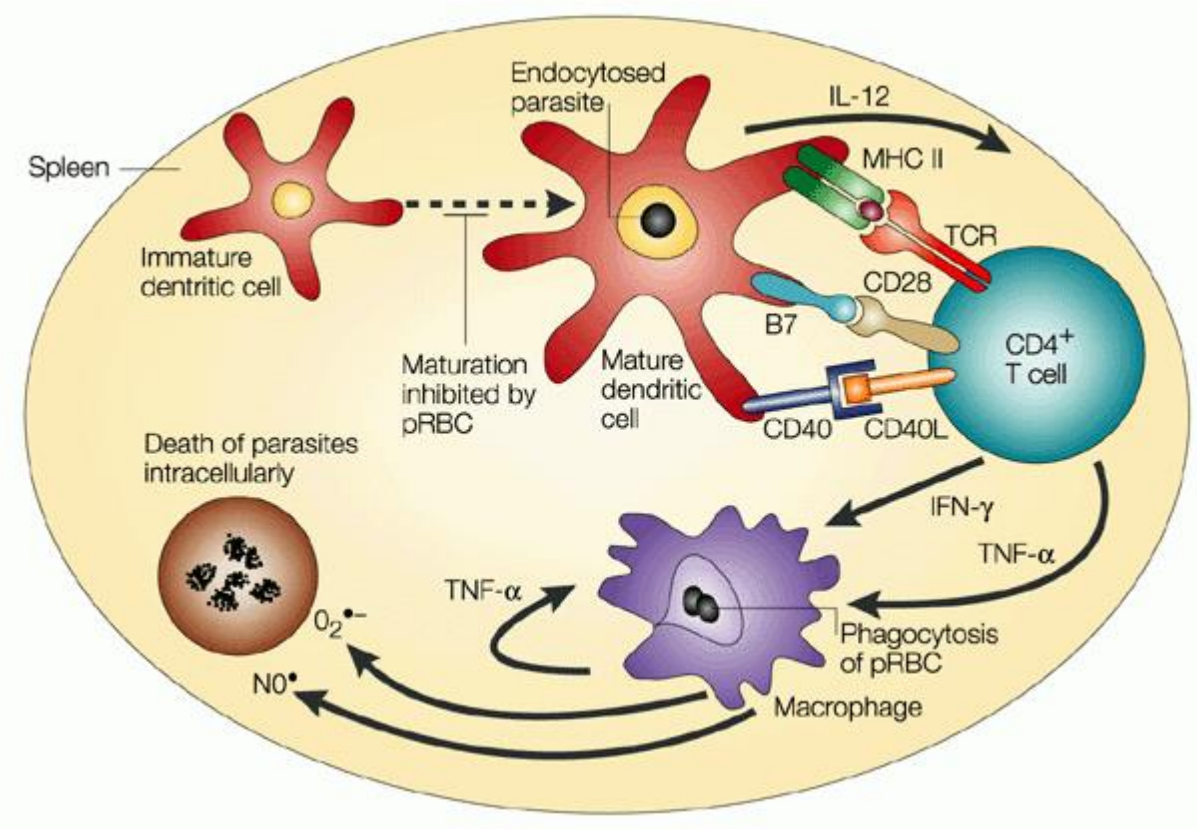

Figure 2.4: T- helper cell 1 (TH1) immune response to intracellular parasites (Good, 2001).

2.6.2 Schistosomiasis

In the early stages of schistosomiasis, the liver, spleen and, intestinal walls lack granuloma as immature eggs contain an inert eggshell to immune responses. Once the eggs are mature, they secrete granuloma inducing antigen through the egg shell rapidly recruiting macrophages to form granuloma. The granuloma protects egg from host immunity and promotes translocation into intestinal lumen for mature eggs to be shed in the feces (Takaki *et al*., 2020). The initial inflammation that occurs in schistosomiasis is thought to be as a result of the passing eggs, with a spine, through the multilayered epithelium (Abdulla *et al*., 2011). This inflammation results in more production of pro-inflammatory cytokines such as gamma interferon, tumor necrosis factor alpha. The protein that is abundantly secreted by the blood flukes eggs is Induced protein of schistosomes eggs (IPSE) for *S. haematobium* and Interleukin 4 for *S. mansoni* (Abdulla *et al*., 2011). Overproduction of these cytokines and movement of the eggs with hooks results into tissue damage of the host. As highlighted by Wiedemann *et al.*, (2020) in Figure 2.5, helminthes utilizes T- helper cell 2 (Th2) immune response after activation of dendritic cells. Th2 cell releases cytokines that activates eosinophil, mast cells, B cells, goblet cell among others. Mast cells and basophils secrete inflammatory mediators to counter the parasitic infection.

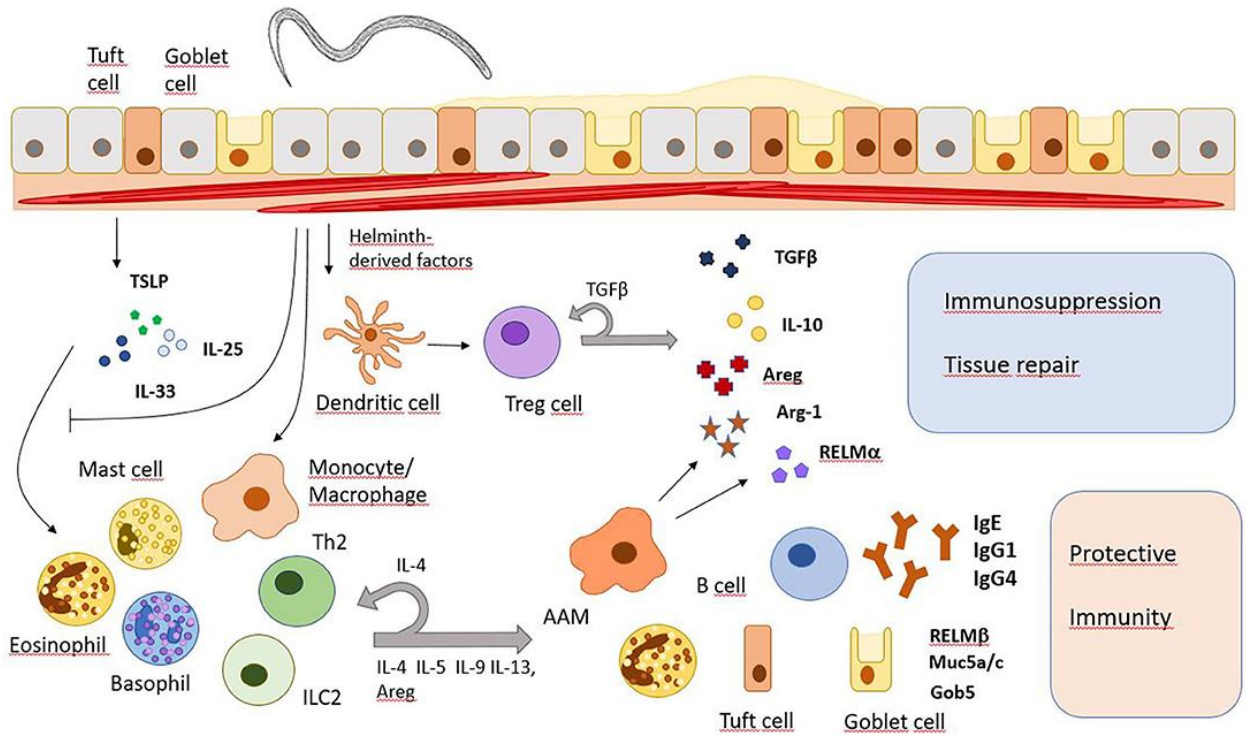

Figure 2.5: T helper cell 2 (TH2) immune response to extracellular parasites (Wiedemann *et al.*, 2020).

2.7 Protein metabolites variation during *S. mansoni* and *Pb*A co-infection

In individual malaria and schistosomiasis infections, the *Plasmodium* species interconverts amino acids as schistosomes takes up amino acids for their use hence the molecular weights changes. Also, in malaria, the proteins transported pass vacuole membrane through plasmodium translocon of exported proteins (PTEX). This PTEX is a molecular machine that unfolds and cleaves hundreds of malaria proteins and transports them across the vacuole membrane into the RBC (Ho *et al*., 2018). Identification of potential disease-related markers and, understanding host immune response can be achieved through analysis of human serum proteomes as most serum protein exhibit rapid changes in quantity and their expression pattern as they respond to infections and also shows association with progresses in disease (Ray *et al*., 2012). In schistosomiasis, female

schistosomes ingest many red blood cells presumably in response to demand for amino acid and proteins used for egg formation and cause release of other proteins into the host (Marr *et al*, 2012). Hence, there is need to check how the protein are expressed with regards to their molecular weights.

Protein such as plasma cytokines and chemokine are important in immune response and immunopathology. Given their importance, it was hypothesized that they could be affected by malaria transmission intensities (Aguilar *et al*., 2019). Both the humoral and cellular arms of the adaptive immune system are essential in eliminating of malaria from the host, and both depend on lymphocytes (Fresno, 2002). In other studies on Schistosomiasis, *Schistosoma* infection intensity is significantly associated with *Schistosoma* antigen-induced cytokine profiles and the variation in cytokine responses in a population (Meurs *et al*., 2014).

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Study site, experimental animals and ethical statement

This study was carried out at the Technical University of Kenya in the School of Biological and Life Sciences (SBLS). Six weeks old Female adult BALB/c mice (weighing between 20-30g) and *Plasmodium berghei* ANKA (*Pb*A) were obtained from Institute of Primate Research (IPR), Karen Nairobi. The mice were housed in cages at the Animal House in the Technical University of Kenya. The mice were fed on mice chow. Wood chippings were used as bedding material. Distilled drinking water was provided *ad libitum*. Laboratory experiments were carried out in the Postgraduate Research laboratory in SBLS. Before the experiment, all mice were treated with 0.02ml of Ivermectin injected subcutaneously and left to acclimatize for two weeks. 72 mice were marked using picric acid to give them numbers for easy identification. Experimental mice were randomly selected and then divided into seven groups. *Biomphalaria pfeifferi* snails infected with *S. mansoni* were obtained from Kenya Medical Research Institute (KEMRI) Kisumu.

3.2 Experimental design

The study involved a completely randomized design. Seventy-two female BALB/c mice were assigned randomly into seven groups containing eight or twelve mice. Survival analysis was performed on three groups of eight mice each. Survival 1: *S. mansoni* infected groups of mice, survival 2: *S. mansoni* - *Pb*A co-infected groups of mice and survival 3: *Pb*A infected group of mice. For experimental groups, four groups were used, each group had twelve mice each. The groups were divided into Group 1: naive control mice, Group 2: *S. mansoni* infected mice, Group 3: *S. mansoni*- *Pb*A co-infected mice and Group 4: *Pb*A infected mice as indicated in Figure 3.1.

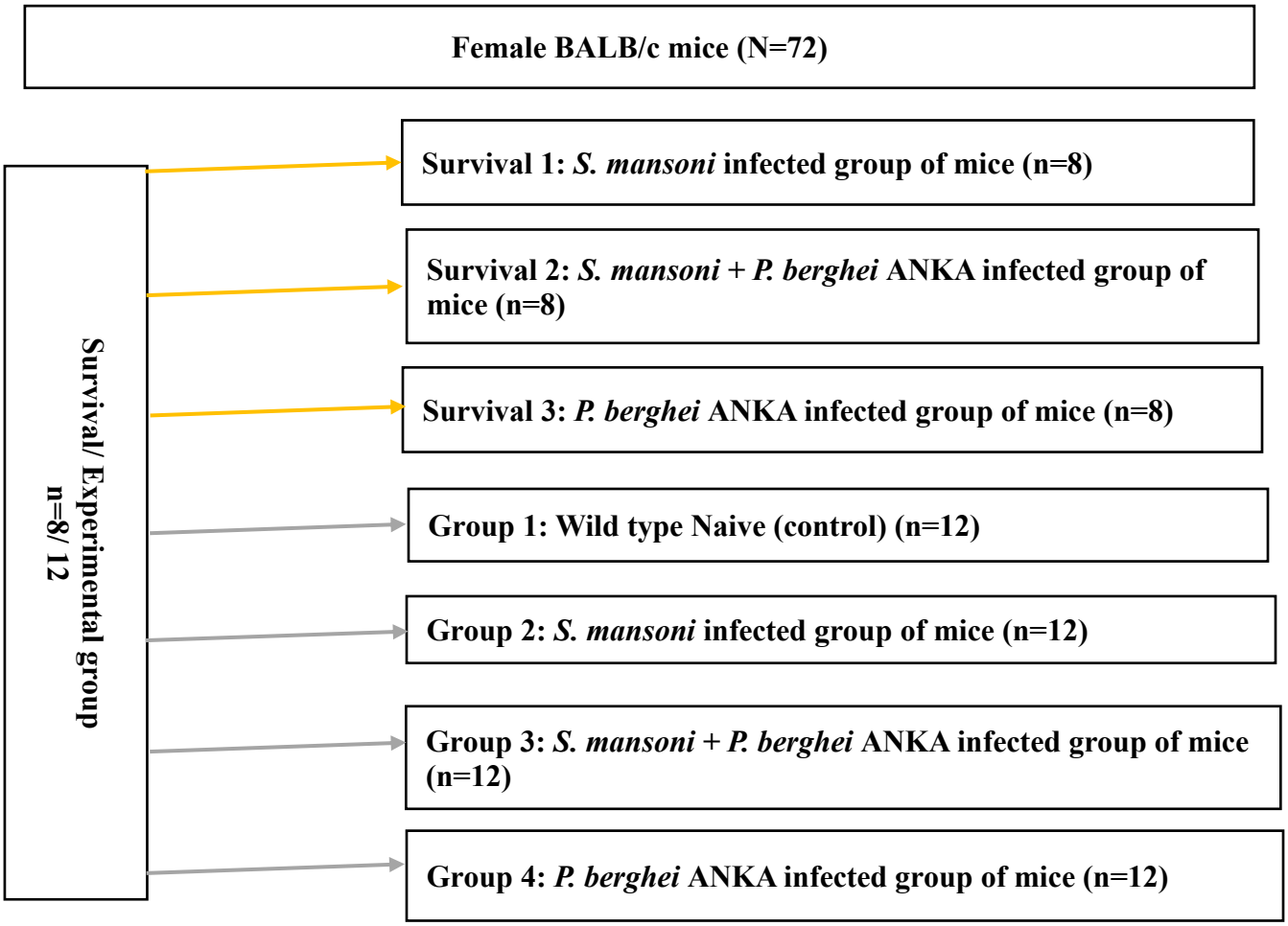

Fig 3.1: Experimental design: Showing the number of mice used and group distribution.

3.3 Malacological studies

3.3.1 Handling of snails

Infected *Biomphalaria pfeifferi* snails were placed in an aquarium containing de-chlorinated water. The water was aerated with an aerator. The snails were fed with clean green dried lettuce. Dried oyster shells were placed as substratum and the water was changed twice a week.

3.3.2 Shedding and quantifying cercaria from snails

Snails were rinsed gently in de-chlorinated water to remove mucus which would cause shed cercariae to clump. The snails were placed in 250ml beaker with about 100ml of de-chlorinated water. The beaker was covered with transparent glass to prevent snails from crawling out and allow light to pass through. The beaker was illuminated with 100 watts electric lamp but taking care not to overheat the beaker. Cercarial suspension was poured in a clean beaker. The cercariae suspension was mixed and an aliquot of 50ul placed on a clean petri dish. The petri dish was placed on a dissecting microscope and cercariae observed at X10 objective. The number of cercariae per 50ul was counted after staining with Lugol's iodine. Volume containing 200 cercariae was calculated.

3.3.3 Infection procedures

3.3.3.1. Schistosoma mansoni

Mice were anaesthetized with intraperitoneal ketamine injection of 0.02ml ketamine per 30-g mouse. The mice were shaved in their lower abdomens. The mice were placed on an infection rack and a ring placed on the shaved area. Approximately 200 *S. mansoni* cercariae were placed in the ring for 30 minutes.

3.3.3.2. Plasmodium berghei ANKA (*Pb*A)

Malaria parasites, transgenic *Plasmodium berghei* ANKA (*Pb*A) stabilates had been stored in 10% glycerol at -196°C liquid nitrogen; in small capillary tubes of 2ml each. They were brought in a cryovial and left to thaw before inoculating the parasite. Viability of the parasites in the capillary tube were checked by placing a drop of its contents on a slide. They were stained with giemsa and viewed under a microscope. EDTA saline glucose (ESG) buffer was used to dilute the parasites. Viability was checked again, before injecting 0.2ml of the diluted parasite into each donor mouse. On the ninth day of infection, parasitemia was checked by taking a drop of blood from the tail and examining under a microscope. The number of parasitized red blood cells (pRBC) was quantified. No. of pRBC= Average parasitemia x 10^6 RBC/ml.

Serial dilution was done so as to get a solution of 25.0 x 10^4 pRBC/ml. To each study mouse, 0.2 ml of this dilution was injected intraperitoneally so that each mouse gets 5.0 x 10^4 pRBC. For co-infection group, *P. berghei* was inoculated only after *S. mansoni* infection had reached the chronic stage with and indicative *S. mansoni* eggs from the liver at week 7 post infection.

3.4 Parasitological studies

3.4.1 *P. berghei* ANKA parasitemia determination

Pre-cleaned slides were labeled with mice number and date. Mice infected with *Pb*A were placed in a restrainer and the tail disinfected using a cotton wool soaked in 70% alcohol. Using a sterile blade, the tip of the mouse tail was cut and the tail squeezed gently to obtain a drop of blood. A drop of blood was added to the center of a completely clean grease-free microscope slide. The

edge of another slide (pusher slide) was brought in contact with the drop of blood and allowed the drop to bank evenly behind the slide. The angle between the two slides was kept approximately 45˚. The blood pusher was pushed away from the blood drop in a smooth, quick motion; the smear covered about half the slide. The smears were allowed to air dry with the slide in a horizontal position. The slides were fixed in methanol for one minute then stained with 20% giemsa stain for 20 minutes. The stained slides were briefly rinsed with running water. The slides were allowed to air dry in a vertical position. Parasitemia was determined by observing under microscope using oil emulsion (×100) and the following formulae was used to determine the percentage of Parasitemia;

$$\text{Parasitemia} = \frac{Total\ \ infected\ erythrocyte}{Total\ number\ of\ erythrocytes\ within\ the\ field} * 100$$

3.4.2 *Schistosoma mansoni* confirmation

Six weeks post-schistosomiasis infection, two mice, for each group were sacrificed and, perfused using phosphate buffered saline (PBS). Mesenteric vein was nipped and PBS injected into the heart of prilumbal surgically sectioned mice. Effluent containing adult worms was collected in a beaker and left to settle. The worms were observed at X20 magnification and images taken. Livers were observed for granuloma and images were taken. The livers were surgically removed and placed in petri dishes containing normal saline. The livers were gently crushed between glass plates and then homogenized using a blender. The homogenates were centrifuged at 1000 revolution per minute (RPM) for 3 minutes. Washing was done three times using normal saline discarding the supernatant. After the third wash, normal saline was added to the pellet and suspension filtered using a sieve. The filtrates were observed for eggs under X10 objective and images taken.

3.4.3 Survival analysis

The two survival groups mice; survival 1 (Wild type *S. mansoni* + *Pb*A infected) and survival 2 (Wild type *Pb*A infected) were utilized for this experiment. Survival experiments employed use of 8 mice per group. The mice were monitored by checking the physiological parameters explained in section 3.5 below. The time and the day at which each mouse succumbed was recorded in excel sheet and then analyzed with Log-rank (Mantel-Cox) Test.

3.5 Physiological parameters of *Plasmodium berghei* ANKA and *Schistosoma mansoni* on mice

3.5.1 Rapid murine and coma behavior scale (RMCBS)

RMCBS comprised of ten parameters: groom, gait, balance, motor performance, body position, touch escape, pinna reflex, limb strength, toe pinch and aggression. Each parameter usually has a maximum score of 2, hence mice were scored from 0-2 for each parameter separately, with 0 correlating to the lowest function while 2 being the highest. During the first 90 seconds of assessment, the mice were placed in the top left corner of an observation box (length, 31.8 cm; width, 19.8 cm; height, 10.5 cm) with a grid floor and the mouse assessed for hygiene related behaviour, gait, body position, exploratory behaviour, and balance. In the subsequent 90 seconds, the mouse was assessed for reflexes, limb strength, and self-preservation actions. These were done after every other two days post co-infection until the termination of the experiment.

3.5.2 Determination of total body weight

The body weight of each mouse was measured on a two-day interval basis since the co-infection day using an analytical weighing balance (Mettler PM34, DoltaRange) up to day of sacrificing.

3.6 Euthanizing the mice and organ collection

The mice were sacrificed on the 7^{th} days post malaria infection. Prilumbal surgical sectioning of mice was performed to draw blood via cardiac puncture after the mice were anesthetized with 0.02ml ketamine per 30-gram mouse. Blood was placed in labelled vials. The blood samples for hematological assay were collected in heparinized tubes. Spleen, brain, kidney, heart, lung and liver were collected surgically and assessed. The organs were weighed as stated in section 3.6.2 below. The organs were assigned into two groups. Some organs stored in a freezer for reduced glutathione experiment and other organs were stored in 4% formalin for histopathology.

3.6.1 Preparation of sera

Vials containing blood were left at room temperature for 3 hours to clot then centrifuged for 6 minutes at 8,000 RPM and stored at 4℃ for immunological and serological assays. (Centurion Scientific Ltd K240R, UK).

3.6.2 Relative organ weights determination

The weight of the organs was measured using an analytical balance (Mettler PM34, DoltaRange). The liver and the spleen were assessed for hepatosplenomegaly by checking for inflammation and

comparing the relative organ weights (ROW) of the naive control mice and the infected group of mice.

$$\text{ROW} = \frac{Organ\ weight}{Weight\ of\ mice\ at\ euthanasia} * 100$$

3.7 Determination of full hemogram, Liver and Kidney function tests

3.7.1 Full hemogram

The blood samples were collected into EDTA tubes for full hemogram analysis using automated Benchman Coulter counter (Benchman, Indianapolis, USA, serial no. 66384). Parameters analyzed here include; red blood cells, hemoglobin, hematocrit, white blood cells (WBC), WBC differential count, and platelets. The results were examined and compared with the normal levels in blood of naive control mice.

3.7.2 Liver and Kidney function tests

Aliquots of the prepared sera were analyzed using automated analyzer (COBAS Intra- 400 plus analyzer, Roche, Basel, Switzerland, serial no. 400,402). Liver functionality enzymes such as alkaline phosphatase, Alanine aminotransferase, and Aspartate aminotransferase as well as kidney function test such as urea level were analyzed. The results were examined and compared with the normal levels in naive sera.

3.8 Determination of cytokines

Cytokines were measured by ELISA kit according to manufacturer's instruction.

3.8.1 Reagent preparation

Coating buffer (1X) was prepared by making a 1:10 dilution of 10X PBS in deionized water. Capture antibodies (Anti-human IFN Gamma, Anti-human TNF alpha and Anti-human IL-10) was diluted 250 times in 1X coating buffer in the ratio 1:250. ELISPOT was diluted 5 times in deionized water in the ratio 1:5. Standards which were human interferon (IFN) gamma, human tumor necrosis factor (TNF) alpha and human interleukin- 10 (IL-10) standard were reconstituted with 750µL of ELISPOT diluent (1X). They were mixed well prior to dilutions. Detection antibodies (Anti-human IFN Gamma, Anti-human TNF alpha and Anti-human IL-10) were diluted 250 times in ELISPOT diluent (1X) in the ratio 1:250. The enzymes Avidin- HRP (horse radish peroxidase) and Streptavidin- HRP were diluted 100 times in 1X ELISPOT diluent.

3.8.2 ELISA procedure

Corning costar 9018 ELISA plate was coated with 100µL/well of capture antibody in coating buffer. The plate was sealed and incubated overnight at 4˚C in a refrigerator. The wells were aspirated and washed 3 times with 300µL wash buffer. During each wash, one minute time was allowed for soaking hence increasing the effectiveness of the wash. The plate was blotted on adsorbent paper to remove any residual buffer. The wells were blocked with 100µL of ELISPOT diluent (1X) and incubated at room temperature for one hour. The plates were aspirated and washed once with wash buffer. Two-fold serial dilutions of human IFN gamma, human TNF alpha and IL-10 standard were performed to make the standard curve for a total of 8 points. 50µL of samples

were added to the appropriate wells. 50µL of the ELISPOT diluent (1X) was added to the blank well. The plate was sealed and incubated at room temperature for 2 hours for maximum sensitivity. The wells were aspirated and washed 3 times with 300µL wash buffer. The plate was blotted on adsorbent paper to remove any residual buffer. 50µL of diluted detection antibody was added to each well. The plate was sealed and incubated at room temperature for 1 hour for maximum sensitivity. The wells were aspirated and washed 3 times with 300µL wash buffer. The plate was blotted on adsorbent paper to remove any residual buffer. 50µL of diluted avidin-HRP was added to each well of tumor necrosis factor and interleukin-10 and streptavidin- HRP was added to each well of interferon gamma respectively. The plate was sealed and incubated at room temperature for 30 minutes for maximum sensitivity. The wells were aspirated and washed 4 times with 300µL wash buffer. During each wash, one minute time was allowed to soak hence increasing the effectiveness of the wash. The plate was blotted on adsorbent paper to remove any residual buffer. 50µL of 1X TMB solution was added to each well. The plate was sealed and incubated at room temperature for 15 minutes for maximum sensitivity. 50µL of stop solution (2N sulfuric acid) was added to each well. The plate was observed for a characteristic color change from blue to yellow; for qualitative test and read using ELISA optical reader (Multiscan ex-355, Thermo electron corporation, Waltham, Massachusetts, USA) for quantitative reading at 450nm.

3.9 Determination of reduced glutathione (GSH) levels.

The reduced glutathione levels for collected organs were measured. GSH was determined by employing the method of Griffith (1980) with slight modification. Organ GSH from naive mice was measured and compared with GSH from mice infected with *S. mansoni*, *Pb*A infected group of mice and *S. mansoni*- *Pb*A co-infected group of mice.

GSH buffers and standard solutions were prepared and loaded on a 96- well plate as described below;

3.9.1 Reagent preparation

5% Sulphosalicylic acid (SSA) was prepared by dissolving 1g of SSA in 19ml dH_2O and stored at 4°C. Aliquots of 100µl brain, liver, spleen, heart and kidney homogenates were separately mixed with 100µl solution containing sulphosalicylic acid (5% w/v) and 0.25mM ethylenediaminetetraacetic acid (EDTA). The homogenate samples were centrifuged at 8000 X *g* for 10 minutes at 4^{o}C. The resulting supernatant was transferred into new tubes for GSH assay.

The standard was prepared first by making 0.5% SSA (0.1g of SSA was dissolved in 19 ml of dH_2O), GSH stock solution was prepared by dissolving 10mg of the standard powder in 19 ml of 0.5% SSA to constitute 200 µM GSH standard solution. 100 µl of 200 µM GSH standard solution was double diluted in 100 µl of 0.5% SSA in 1.5 ml Eppendorf tubes, the following GSH standard solutions were made 100mM, 50mM, 25mM, 12.5mM, 6.25mM, 3.13mM, 1.56mM and 0.78mM.

DNTB (5, 5'-dithio-bis-2-(nitro benzoic acid) was prepared by dissolving 0.03g/30 ml KPE buffer. This solution was stored in 4°C for use within the same day.

3.9.2 Tissue homogenization and GSH concentration measurement

The brain, liver spleen, heart and kidney samples stored in a freezer were left to thaw at 4ºC. They were weighed using an analytical balance. Homogenization buffer was prepared by mixing 0.25M sucrose, 5Mm Hepes- Tris pH 7.4, with protease inhibitor cocktail to a concentration of 10%w/v.

The organs were crushed using a homogenizer in appropriate weight to volume of homogenization buffer and the homogenates stored in freezer.

To prepare 1M potassium phosphate buffer with 5 mM EDTA disodium salt, pH 7.5 (KPE) the following two solutions were prepared; Solution A was prepared by dissolving 6.8 g KH_2PO_4 in 500 ml dH_2O the resulting solution was stored at 4 °C.

Solution B was prepared by dissolving 8.5 g K_2HPO_4 in 500 ml dH_2O; also, the resulting solution was Stored at 4 °C. 0.1M phosphate buffer was prepared by adding 16 ml of solution A to 84 ml of solution B to make a 100 ml after which 0.327g EDTA disodium salt was added (normally EDTA dissolves at a pH of 8.0). The pH was then adjusted to 7.5 by adding few drops of solution A. This buffer henceforth was known as KPE (potassium phosphate–EDTA) buffer, the buffer was stored at 4 °C.

These were determined by adding 100 µl both standards and the supernatants separately into a 96 well plate followed by addition of 100µl of DNTB, the plate was incubated at 37°C for 10 minutes, then the absorbance was read at 405 nm using a multidetector micro titer plate reader (R&D Systems, Minneapolis, MN) at an interval of 30 sec i.e., 30sec, 60sec, 90sec and 120sec. The time interval that gave the best GSH standard graph was chosen as 90 seconds and were used for determining the GSH in the samples.

3.10 Histopathological analysis

Standard histopathology examination of the kidney, spleen, liver and the brain were done to determine the extent of injury and whether co- infection offered organ protection. To achieve this, kidney, spleen, liver and the brain harvested were washed in phosphate buffered saline, fixed in 4% formalin and refrigerated at -20º C. The organs were further processed by dehydration and

embedded using paraffin wax. Sections of 5µm thickness were cut and mounted on glass slides. The sections were transferred to xylene in two changes followed by serial rehydration (100%, 95%, 90%, 70%, and 50%) and then washed in tap water. Staining was done using Hematoxylin and Eosin (1% for 2min). Serial dehydration then followed in ascending concentrations of alcohol (70%, 80%, 95%, and 100%), and cleared in xylene. The slides were then be mounted in DPX and examined microscopically using x40 objective lens.

3.11 Effect of *S. mansoni* and/or *Pb*A infection on host protein quantity.

The protein concentration of sera was measured using Bio-Rad technique. Sera from naive mice was measured and compared with sera from mice infected with *S. mansoni*, *Pb*A infected group of mice and *S. mansoni*- *Pb*A co-infected group of mice.

3.11.1 Reagent preparation

Bovine Serum Albumin (BSA) standard stock was prepared at a concentration of 2mg/ml. 0.020g of BSA was weighed and mixed well with 10ml of phosphate buffered saline (PBS). Bio-Rad dye was diluted with distilled water in ratio 1:5 to make up 10 ml and was covered with aluminum foil since it is light sensitive.

3.11.2 Bio-Rad Procedure

Twenty microliters of the BSA standard stock solution were taken and dispensed onto an ELISA plate wells. The BSA standard was serial diluted two folds using PBS. The sera were put into their respective wells in duplicate. 200ul of the diluted dye was added to the well of standards and samples. The plate was vortexed and incubated at room temperature for 5 minutes. The absorbance

of the samples and standard were read at 630nm using the dye and PBS as the blank. A curve of absorbance against concentration of the standard was plotted and the absorbance of the unknown sample were read and used to calculate the protein concentration.

3.12 Data analysis

Statistical analysis was done using GraphPad prism software package (Version 5.0). The survival rate was analyzed with Log-rank (Mantel-Cox) Test. Images of sectioned organs were taken under a compound microscope (Zeis Axio scope) and examined for pathology. One way ANOVA was done to compare the treatment groups with controls. For internal comparisons, Turkey's post-hoc test was used. The results were given as a mean ± SEM with significance set at $p \leq 0.05$.

CHAPTER 4

RESULTS

4.1 Effects of *Pb*A infection on survival rate following *S. mansoni* infection

Survival analysis was performed for groups of mice that were infected with *S. mansoni,* PbA alone and with both *Pb*A and *S. mansoni*. It was observed from this study that groups of mice developed blood-stage infection. As shown in Figure 4.1, in *S. mansoni- Pb*A infected group of mice survived for 5 more days compared to the group of mice infected with *Pb*A alone. Overall, the group of mice infected with *Pb*A- *S. mansoni* significantly survived longer compared with mice infected with *Pb*A alone ($p< 0.05$). More importantly, on 15th day post co-infection, only one mouse (12.5%) infected with *Pb*A alone was still remaining. More than 60% of *S. mansoni- Pb*A co-infected mice were still alive 15 days post infection. Mice infected with *S. mansoni* alone survived all through the study. The experiment was terminated on the 25th day post coinfection as there was no other group to compare with and to prevent inhumane suffering of the mice.

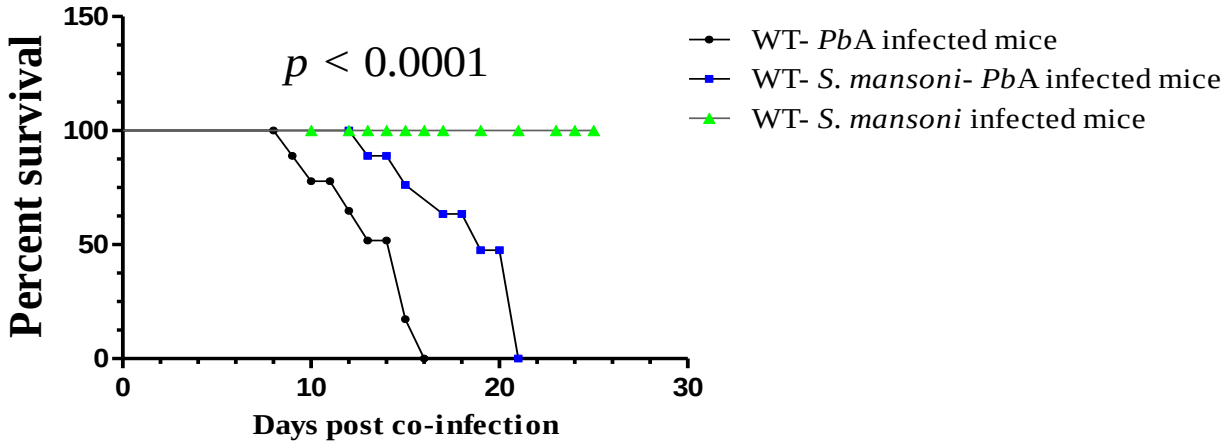

Figure 4.1: Survival analysis for mice with acute malaria infection
Data was analyzed using Log-rank (Mantel-Cox) Test (n=8)

4.2 Effects of *S. mansoni* infection on liver physiology

In this study, liver samples from the group of mice that were infected with *S. mansoni* were harvested, perfused and observed for the presence of adult worms (Figure 4.2A). Pictorial representation of the granuloma depicts the immunological response elicited by eggs lodged in the liver. Granuloma were characterized as white spots as indicated with arrows (Figure 4.2B). Also, the liver homogenates revealed the presence of eggs with lateral spine near the posterior end as shown with stars in Figure 4.2C.

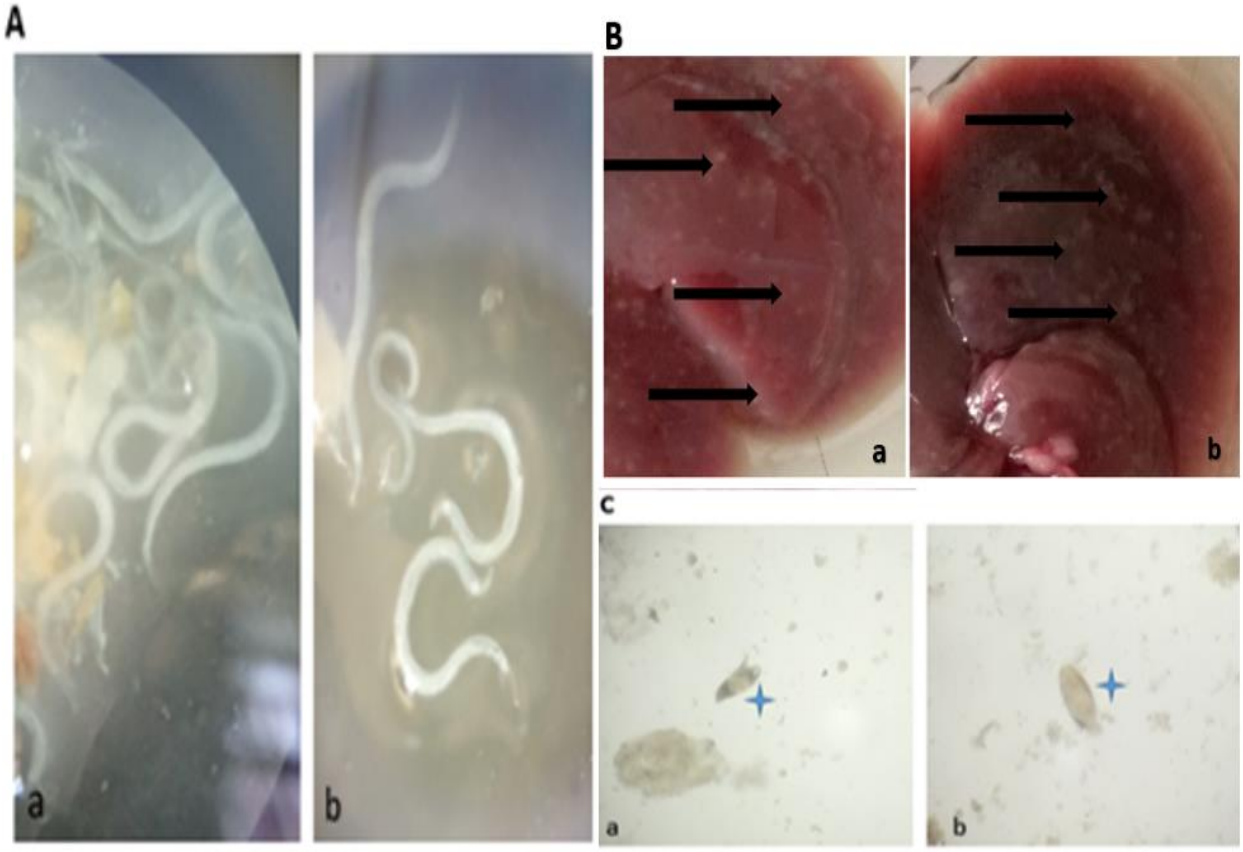

Figure 4.2: Pictorial representation of adult worms (A), liver with granuloma (shown with arrows; B) and eggs (shown by a star; C)
a- group 2, b- group 3.

4.3 Effects of *S. mansoni* infection on the parasitemia levels of *Pb*A

Parasitemia was done to determine if immune response induced during schistosomiasis would suppress or enhance the levels of *Pb*A in blood. The levels of parasitemia for the 8 days, was comparable in the two group (p= 0.2039; Figure 4.3A & B). At the eighth day post infection, the percentage levels of parasitemia in co-infected group of mice and *Pb*A alone infected mice were 28.1% and 36.6% respectively (Figure 4.3A). In addition, the levels of parasitemia on the 8 days, was comparable in the two groups (Figure 4.3B).

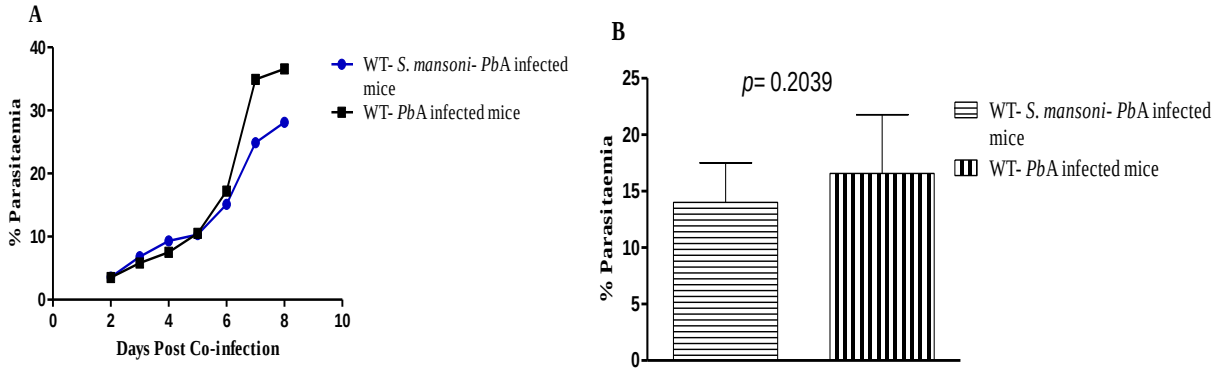

Figure 4.3: Parasitemia in co-infected mice and the one infected with *Pb*A alone
The analysis was conducted using student t-test, two tailed.

4.4 Effect of *S. mansoni* and/ or *Pb*A on mice behavior; exploratory behavior, hygiene related behavior, reflexes and self-preservation

This study assessed the health status of mice upon infections by employing Rapid Murine Coma and Behavioral Scale which is a quantitative tool. Mice infected with *Pb*A had a steady drop RMCBS scores from day two post infection (Figure 4.4). In contrast, RMCBS scores for co-infected group of mice was not significantly different compared to naive mice (Figure 4.4). On the contrary, for mice infected with *S. mansoni* alone, the total score remained constant in the course

of the study. However, a marginal reduction in the RMCBS in the infection groups was observed when compared to the normal control group mice although it was not statistically significant.

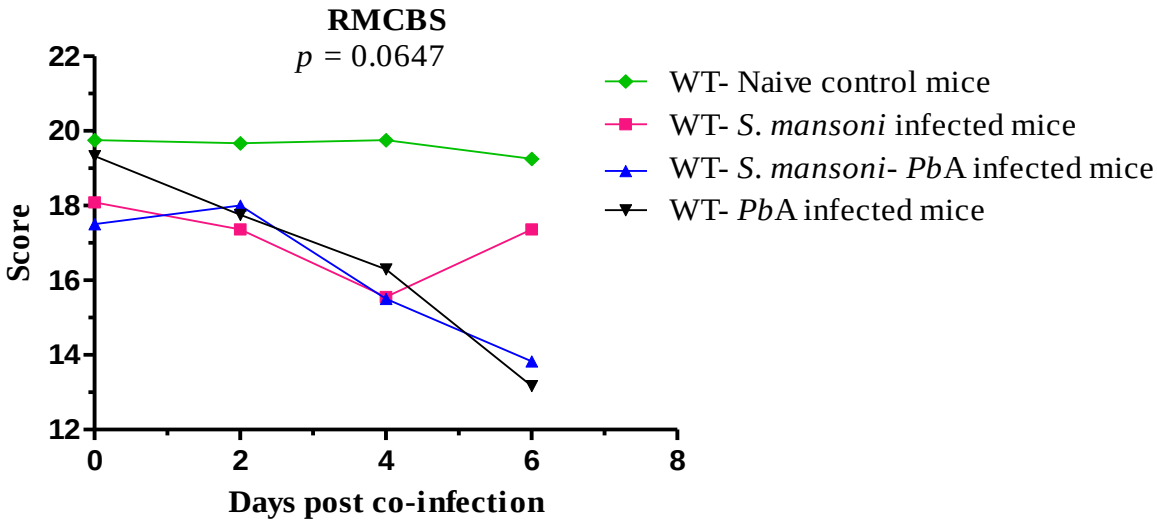

Figure 4.4: Effect of the infections on RMCBS trend in study groups
The analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated no statistical difference

4.4.1 Effects of *Pb*A or *S. mansoni* infection alone or co-infection on body groom and motor performance

When individual RMCBS parameters were analyzed in the course of the infection. There was a significant decrease in body groom ($p< 0.05$) in mice infected with *Pb*A or *S. mansoni* alone as well as co-infected group of mice when compared with the naive control mice (Figure 4.5A). There was no significant difference between co-infected group of mice and mice infected with *Pb*A or *S. mansoni* alone. The co-infected group of mice had comparable groom to *S. mansoni* infected mice (Figure 4.5A). In contrast, mice in co-infected group had lower groom score relative to *Pb*A infected mice. Similarly, there was a significant decrease in motor performance ($p< 0.05$) in mice infected with *S. mansoni* alone as well the *S. mansoni- Pb*A co-infected mice relative to the naive

control mice (Figure 4.5B). Motor performance of mice in co-infected group was comparably similar to mice singly infected with *S. mansoni* or *Pb*A.

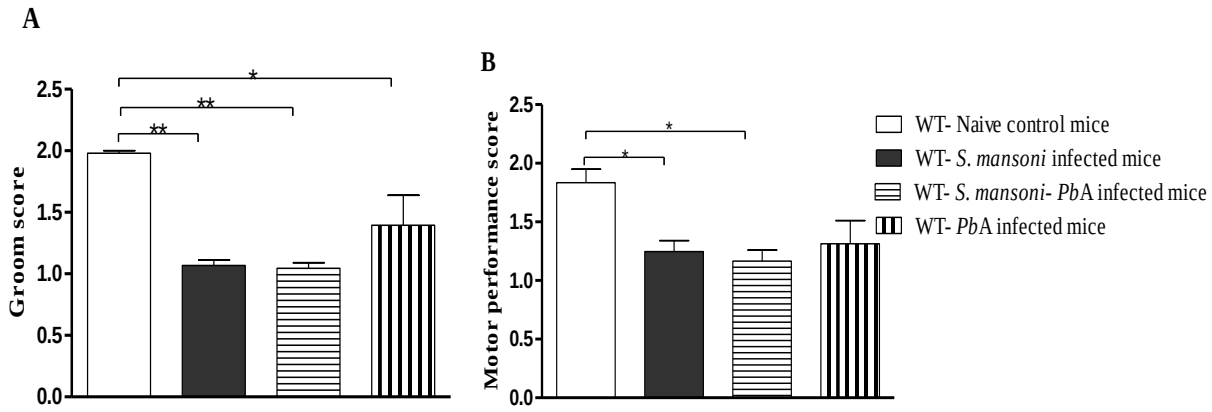

Figure 4.5: Effect of the infections on mice body groom (A) and motor performance (B)
The analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p< 0.05, **p< 0.01.

4.4.2 Effects of *Pb*A or *S. mansoni* infection alone or co-infection on aggression, touch, and pinna reflex scores

The naive control group of mice constantly maintained high scores for the three parameters of RMCBS that were under investigation in this experiment. Co-infected group of mice and mice infected with *Pb*A alone showed an increase of aggression at day 2 post co-infection (Figure 4.6A). Mice in the co-infected group had a varied aggression trend in comparison with mice infected with *S. mansoni* alone, as the latter had a drop in the score and later increased (Figure 4.6A). Mice infected with schistosomes only registered a decrease in aggression initially from day two post co-infection and was observed to be high at day 6 post infection (Figure 4.6A). Significant reduction in the pinna reflex scores was witnessed across all the infected groups of mice in the course of infection (Figure 4.6B) Mice infected with *S. mansoni* alone had a decrease in pinna reflex initially from day two post co-infection and increased at day 6 post infection (Figure 4.6B). Mice infected

with *Pb*A alone and those in co-infected group of mice had a decrease in pinna reflex score after 2 days post infection which was comparable. The group of mice infected with *S. mansoni* alone had pinna reflex score which was distinct from co-infected mice characterized by decrease and increase in the score (Figure 4.6B). Mice infected with *Pb*A alone and the co-infected group of mice had comparable trend of touch reflex. Mice infected with *S. mansoni* equally registered a decrease in touch reflex score though not comparable with the co-infected mice (Figure 4.6C)

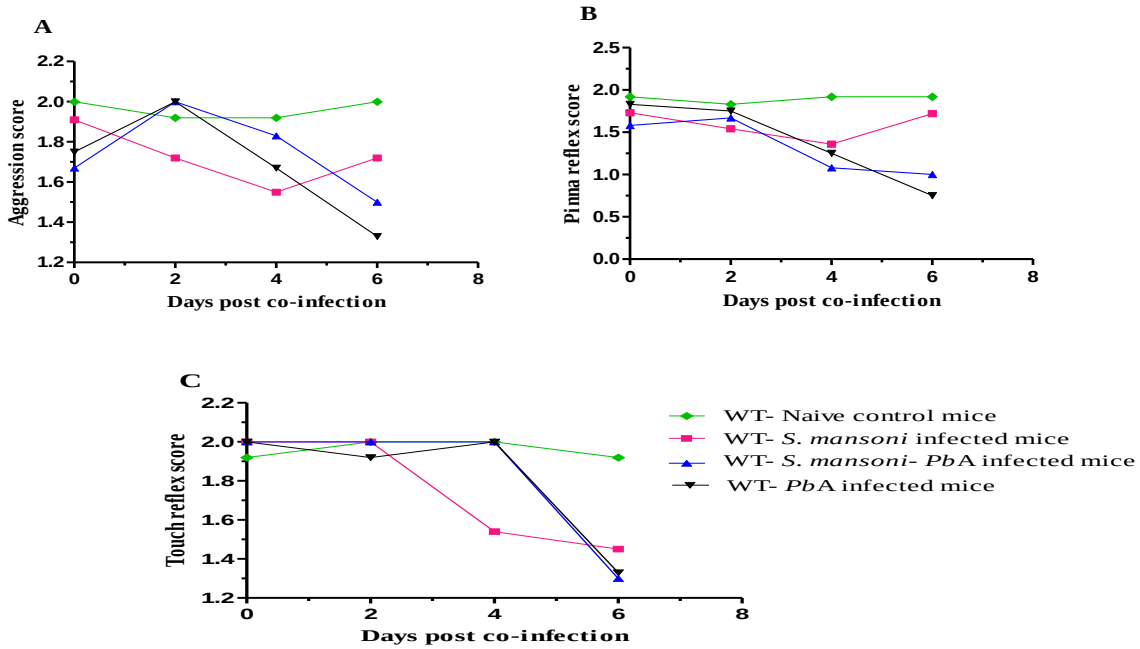

Figure 4.6: Effect of the infections on mice aggression (A), pinna reflex (B), and touch reflex (C) trends
The analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated no statistical difference.

4.5 Effects of *Pb*A or *S. mansoni* infection alone or co-infection, on general change in body weight

There was a significant elevation ($p < 0.05$) of body weight in both mice infected with *S. mansoni* and those co-infected with *S. mansoni- Pb*A in comparison to the naive control mice after 8 weeks

(Figure 4.7). However, the body weight for co-infected group of mice were significantly lower compared to *S. mansoni* infected alone group of mice ($p < 0.05$; Figure 4.7). Co-infected group of mice had significantly elevated body weight relative to mice infected with *Pb*A alone ($p < 0.05$). The body weight for mice infected with *Pb*A alone were unaffected by *Pb*A infection relative to naive control mice (Figure 4.7). The change in body weight was calculated by subtracting initial weight before infection from the final weight before euthanasia.

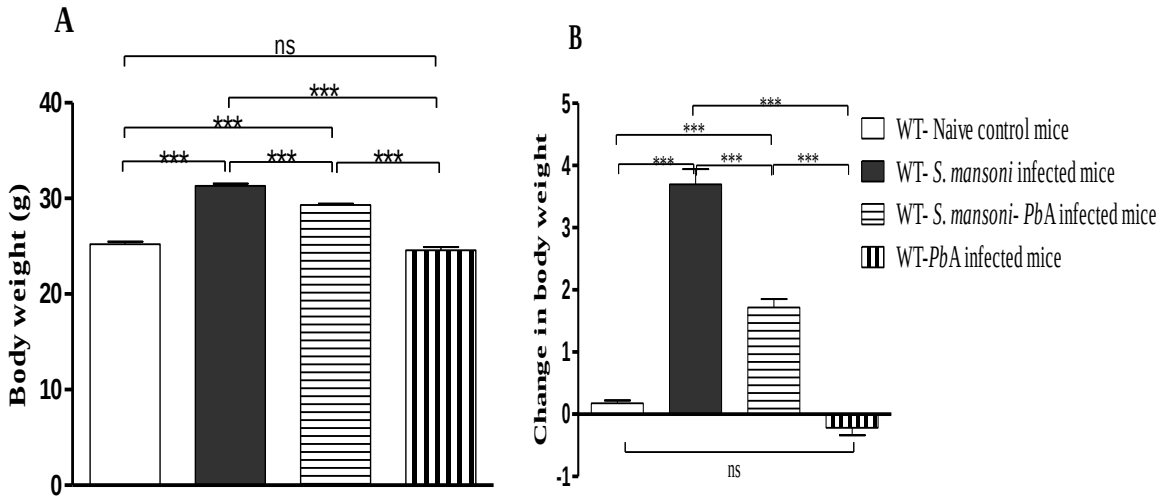

Figure 4.7: Effect of schistosomiasis, malaria, and co-infections on general body weight
The analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p< 0.05, ***p< 0.001.

4.6 Effects of *Pb*A or *S. mansoni* infection on relative organ weight

Following the variation in body weight due to the infection, further investigations were conducted to determine relative organ weight in order to confirm organ alteration as a result of the infections. Infection with *S. mansoni* alone resulted in a significantly higher relative spleen ($p < 0.0003$) and liver weights ($p < 0.0001$) when compared with naive control mice (Figure 4.8A &B). Mice infected with *Pb*A alone or co-infected group of mice had significantly lower ($p < 0.05$) spleen and

liver relative organ weights compared to *S. mansoni* alone mice and comparable as naive control mice (Figure 4.8A &B). The co-infected group of mice had similar spleen and liver weight relative to mice infected with *Pb*A alone (Figure 4.8A &B). The relative heart and brain weight for groups of mice infected with either *S. mansoni* alone or co-infected with *S. mansoni*- *Pb*A were significantly reduced ($p < 0.0001$) in comparison to naive control mice (Figure 4.8C&D). The heart and brain of mice in the co-infected group were comparable to ones from mice infected with *S. mansoni* and *Pb*A alone (Figure 4.8C&D). The relative organ weights for the kidneys and lungs were comparable across all the infected groups (Figure 4.8E&F). However, mice from the co-infected group had slightly lower relative lung weight compared to *S. mansoni* and *Pb*A infected mice (Figure 4.8F).

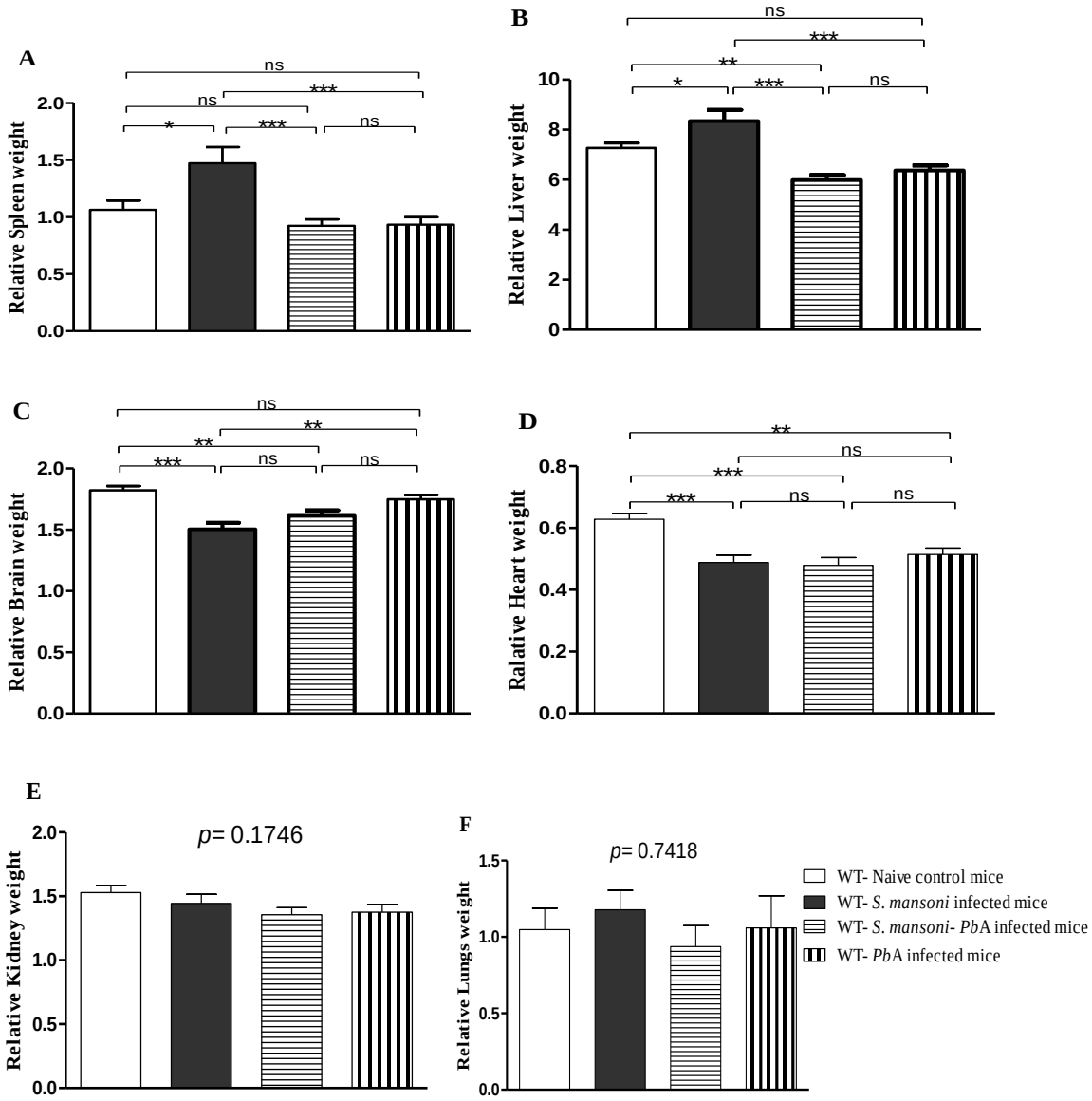

Figure 4.8: Relative organs weight post co- infection with *Pb*A and *S. mansoni*
Analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p<0.05, **p< 0.01**,** ***p< 0.001.

4.7 Reduction in splenomegaly among *S. mansoni* and *Pb*A co-infected mice

Spleen from the group of mice infected with *S. mansoni* alone was large and longer (3cm long) compared to the rest of the group which corroborates the relative organ weight, clear indication of splenomegaly (Figure 4.9). The group of mice infected with *Pb*A alone, the co-infected group of

mice had similar spleen length as the naive control mice with a range of 2.2- 2.5 cm. Mice from the co-infected group had a slender spleen than mice from *S. mansoni* alone group. On the other hand, co-infected mice had a longer spleen than mice infected with *Pb*A alone (Figure 4.9).

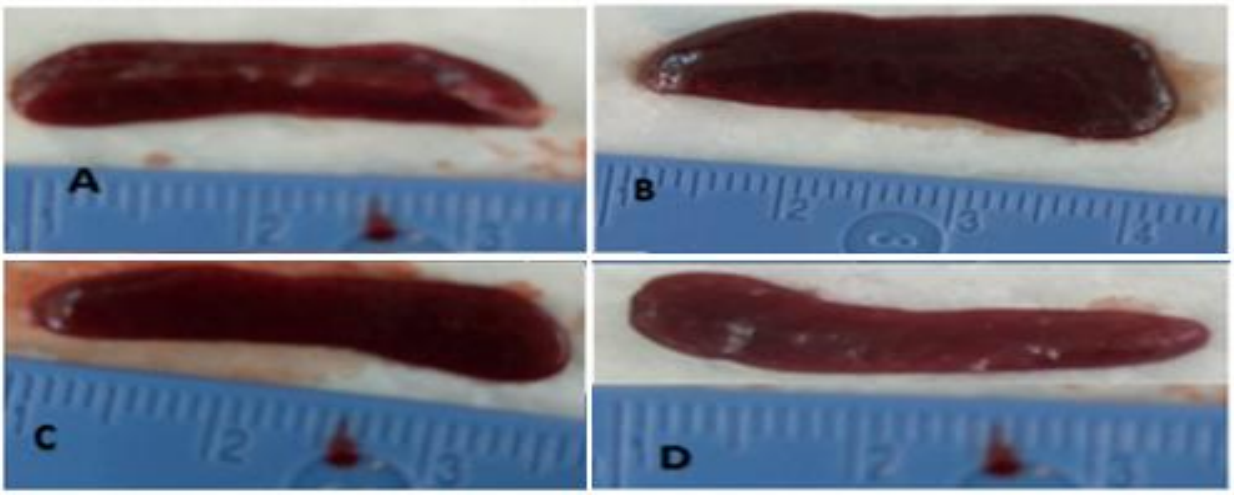

Figure 4.9: Images of spleen across the groups.
A- Spleen for naive mice, B-spleen for *S. mansoni* infected mice, C- spleen for *S. mansoni- Pb*A co-infected mice and D- spleen for mice infected with *Pb*A

4.8 Effects of *Pb*A or *S. mansoni* infection alone or co-infection on RBC count, and hemoglobin levels

Infection of mice with *Pb*A or *S. mansoni* alone or co-infection had comparable red blood cells count and hemoglobin levels relative to control group of mice (Figure 4.10A&B). Similarly, group of co-infected mice had comparable levels of red blood cells and hemoglobin relative to mice infected *S. mansoni* alone and those infected with *Pb*A alone (Figure 4.10A&B).

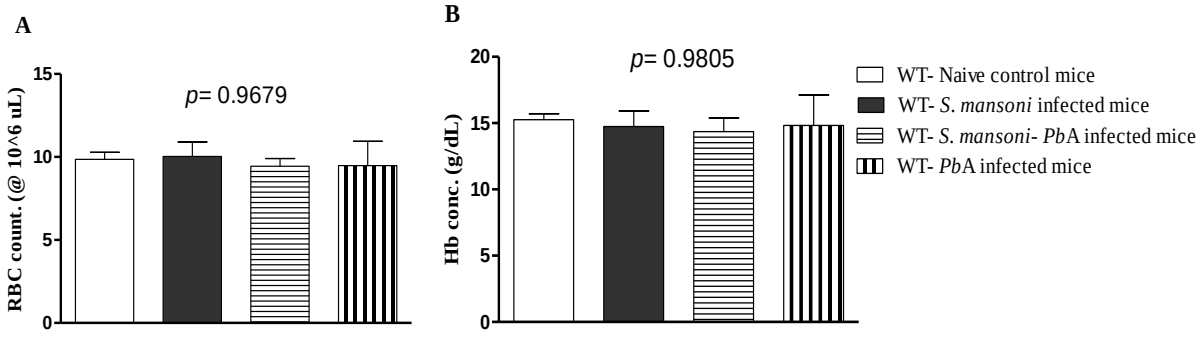

Figure 4.10: The red blood cells count and hemoglobin level infection study groups
The analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons.

4.9 Effects of *Pb*A or *S. mansoni* infection alone or co-infection on hematocrit

In this study, it was observed that infection of mice with *Pb*A or *S. mansoni* alone or co-infection with the two parasites had comparable levels hematocrit relative to control group of mice (Figure 4.11). Similarly, co-infected group of mice had comparable levels of hematocrit relative to mice infected *S. mansoni* alone and mice infected with *Pb*A alone (Figure 4.11).

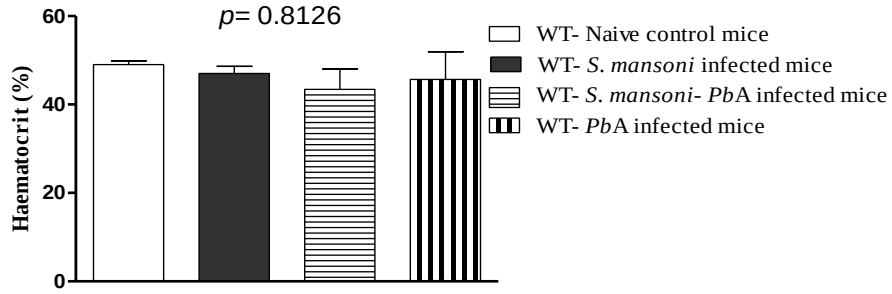

Figure 4.11: Effect of the infections on HCT levels in the study groups
The analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons.

4.10 Effects of *Pb*A or *S. mansoni* infection alone or co-infection on RBC indices

The levels of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width- standard deviation (RDW-SD), red cell distribution width- coefficient of variation (RDW- CV) in all the infected

mice were comparable with the naive control mice (Figure 4.12A-E). Group of co-infected mice had comparable levels of red blood indices relative to mice infected *S. mansoni* alone and those infected with *Pb*A alone (Figure 4.12A-E).

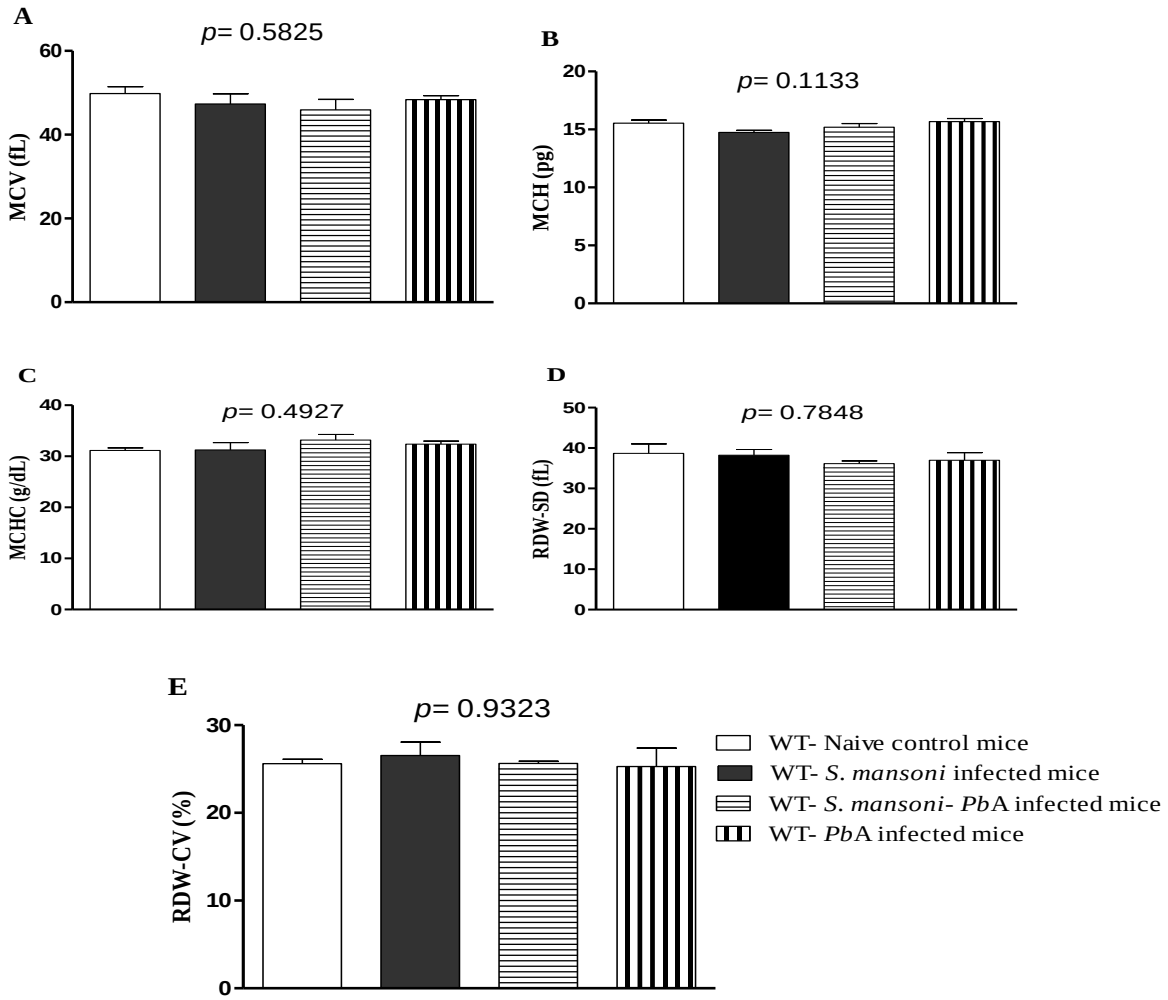

Figure 4.12: Red blood cell indices in *S. mansoni* and *Pb*A infections
The analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons.

4.11 The effect of *Pb*A infection on WBCs following schistosomiasis infection

There was elevation in the count of white blood cells in the mice co-infected with *S. mansoni- Pb*A compared to the naive mice, mice infected with *Pb*A alone and *S. mansoni* alone, though not

statistically significant (Figure 4.13). The white blood cells count in the mice infected with *Pb*A alone and *S. mansoni* alone were comparable to the naive control mice (Figure 4.13).

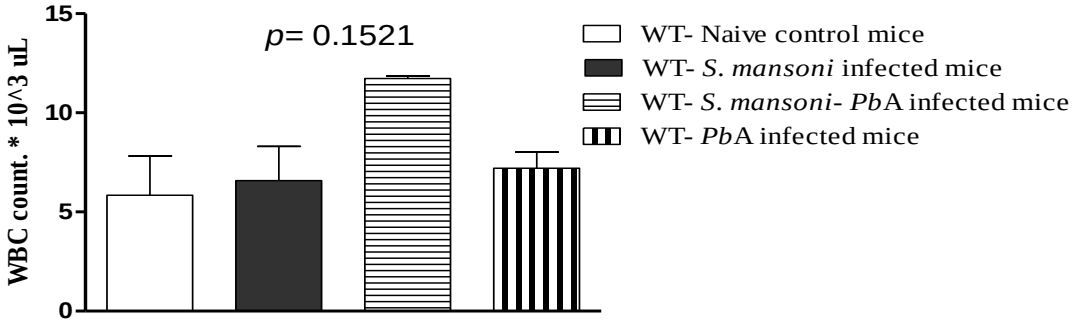

Figure 4.13: The effect of *S. mansoni* and *Pb*A infections on the white blood cell count
The analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons.

4.12 The effect of *Pb*A infection on differential WBCs following schistosomiasis infection

In this study mice co-infected with *S. mansoni* and *Pb*A had comparable count of neutrophils, basophils, lymphocytes, and monocytes relative to the naive control mice (Figure 4.14 A, B, C & E). Co-infected mice had slightly higher count of neutrophils, lymphocytes and monocytes relative to mice infected *S. mansoni* alone and those infected with *Pb*A alone. On the other hand, co-infected group of mice had slightly lower count of basophils relative to mice infected *S. mansoni* alone and those infected with *Pb*A alone though not statistically significant. (Figure 4.14A-E). Interestingly, there was a significant increase ($p < 0.05$) in the count of eosinophil in the group of mice co-infected with the two parasites in comparison to the naive mice, *S. mansoni* alone and *Pb*A alone infected mice (Figure 4.14D).

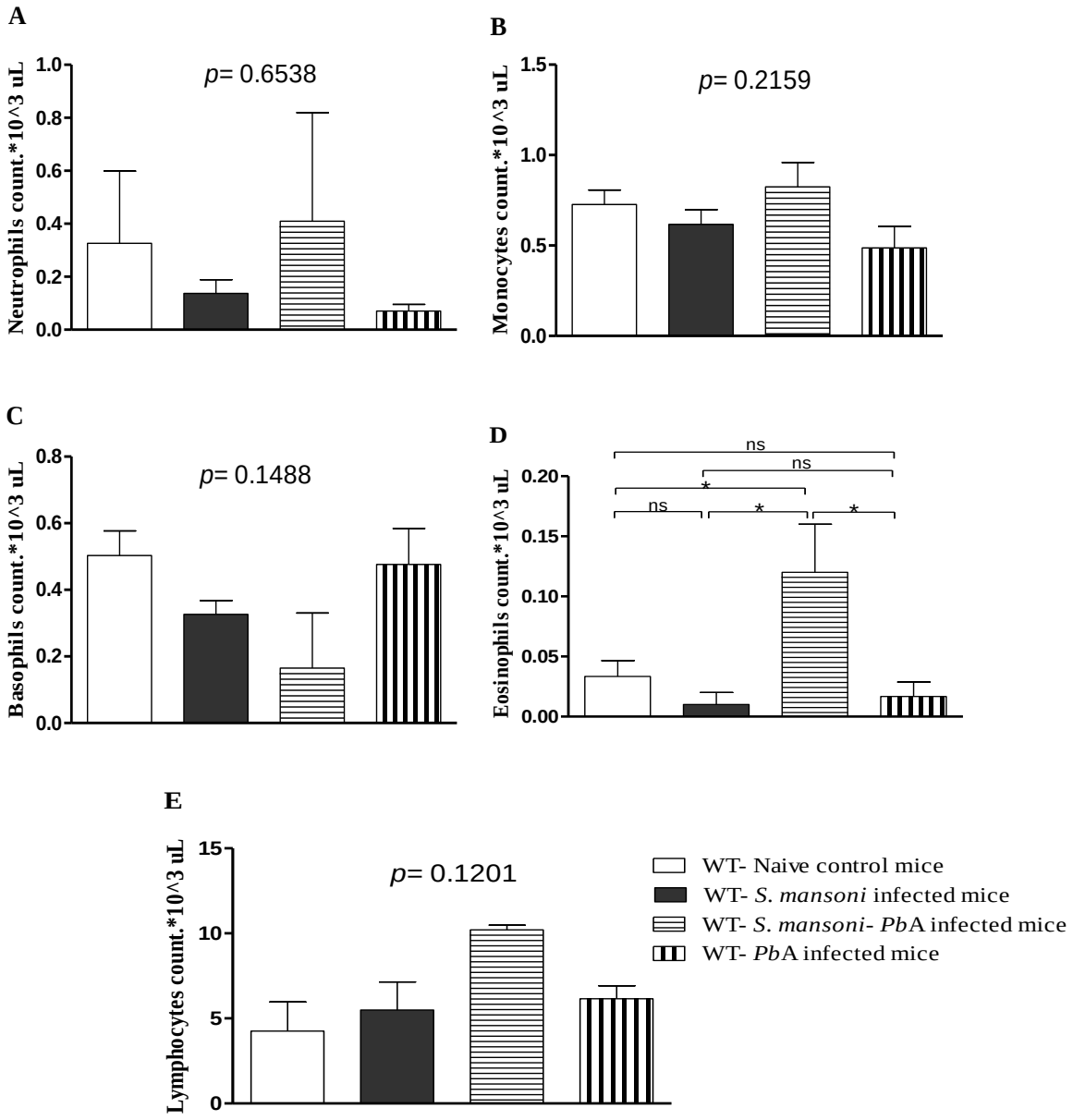

Figure 4.14: The effect of *S. mansoni* and *Pb*A infections on the differential WBCs count in the study groups

The analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p<0.05.

4.13 The effect of *Pb*A infection on platelets level following schistosomiasis infection

There was an elevation in the level of platelets in the infected mice compared to the naive control mice (Figure 4.15). Mice co-infected with *S. mansoni* and *Pb*A had slightly lower platelet level

compared to mice infected with *S. mansoni* alone and *Pb*A infected mice despite not being statistically different (Figure 4.15).

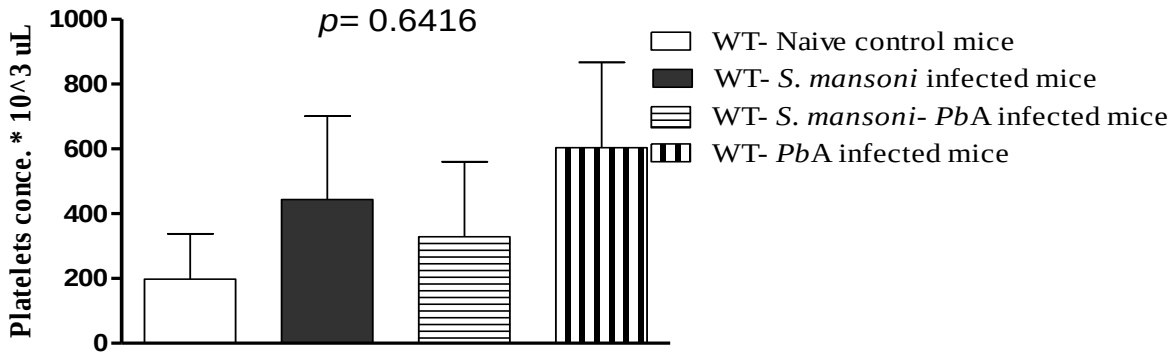

Figure 4.15: Platelets level in mice model upon *S. mansoni* and *Pb*A infections
Analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons.

4.14 Effects of *S. mansoni* and *Pb*A on liver function markers

There was a significant reduction ($p < 0.05$) in level of alkaline phosphatase (ALP) in both mice infected with *S. mansoni* alone and co-infected group of mice as compared to the naive control mice (Figure 4.16A). However, the levels for the co-infected group of mice were slightly higher compared to *S. mansoni* alone mice (Figure 4.16A). Notably, ALP levels in mice infected with *Pb*A alone were significantly elevated ($p < 0.05$) relative to co-infected group of mice. On the other hand, the levels of ALP in mice infected with *Pb*A alone was comparable relative to the naive control mice (Figure 4.16A). Additionally, the levels of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels was comparable across all the treatment groups (Figure 4.16B &C). Furthermore, to check if there was a disruption in the bile duct and liver functionalities, a ratio of the two essential enzymes were calculated. Mice infected with *Pb*A or *S. mansoni* alone or co-infected with the two parasites had comparable ratio levels of AST: ALT relative to control

group of mice. Similarly, group of co-infected mice had comparable ratio levels of AST: ALT relative to mice infected *S. mansoni* alone and mice infected with *Pb*A alone (Figure 4.16D).

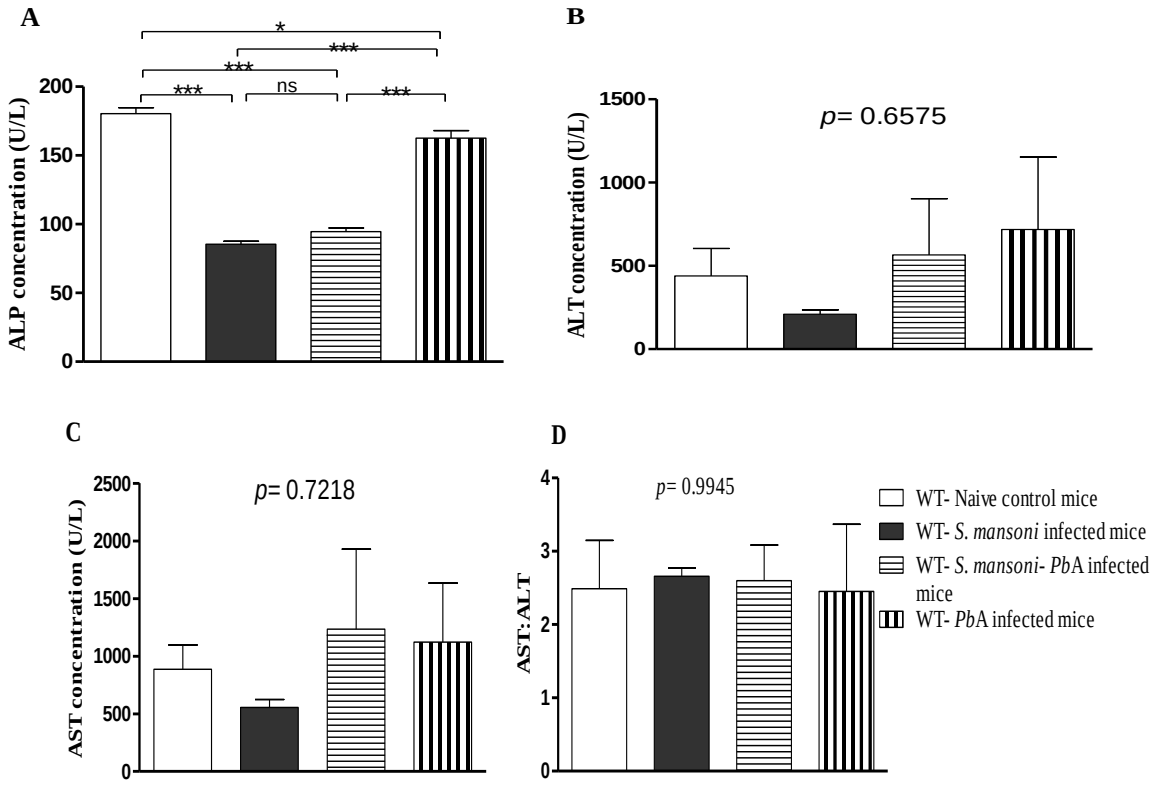

Figure 4.16: Effect of *S. mansoni* and *Pb*A infections on liver function markers in mice. Analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p<0.05, ***p< 0.001.

4.15 Effects of *Pb*A infection on bilirubin, GGT and albumin levels following schistosomiasis

There was a significant elevation ($p< 0.05$) in bilirubin levels in mice infected with *Pb*A alone compared to the naive control mice (Figure 4.17A). Bilirubin levels in the co-infected mice was comparable with *Pb*A infected mice and *S. mansoni* infected mice (Figure 4.17A). However, the levels in co-infected mice were lower and higher than in mice than *Pb*A infected mice and infected

with *S. mansoni* alone respectively (Figure 4.17A). The levels of Gamma- glutamyl transferase (GGT) were unaffected upon infection with either *S. mansoni* alone, *Pb*A alone or when co-infected with *S. mansoni* (Figure 4.17B). Introduction of *Pb*A in mice already infected with *S. mansoni* resulted in a significant increase ($p < 0.05$) in albumin, when compared to the *Pb*A group (Figure 4.17C). The co-infected group of mice had comparable levels of albumin relative to mice infected with *S. mansoni* alone (Figure 4.17C). The level of total bilirubin was unaffected by the infections (Figure 4.17D).

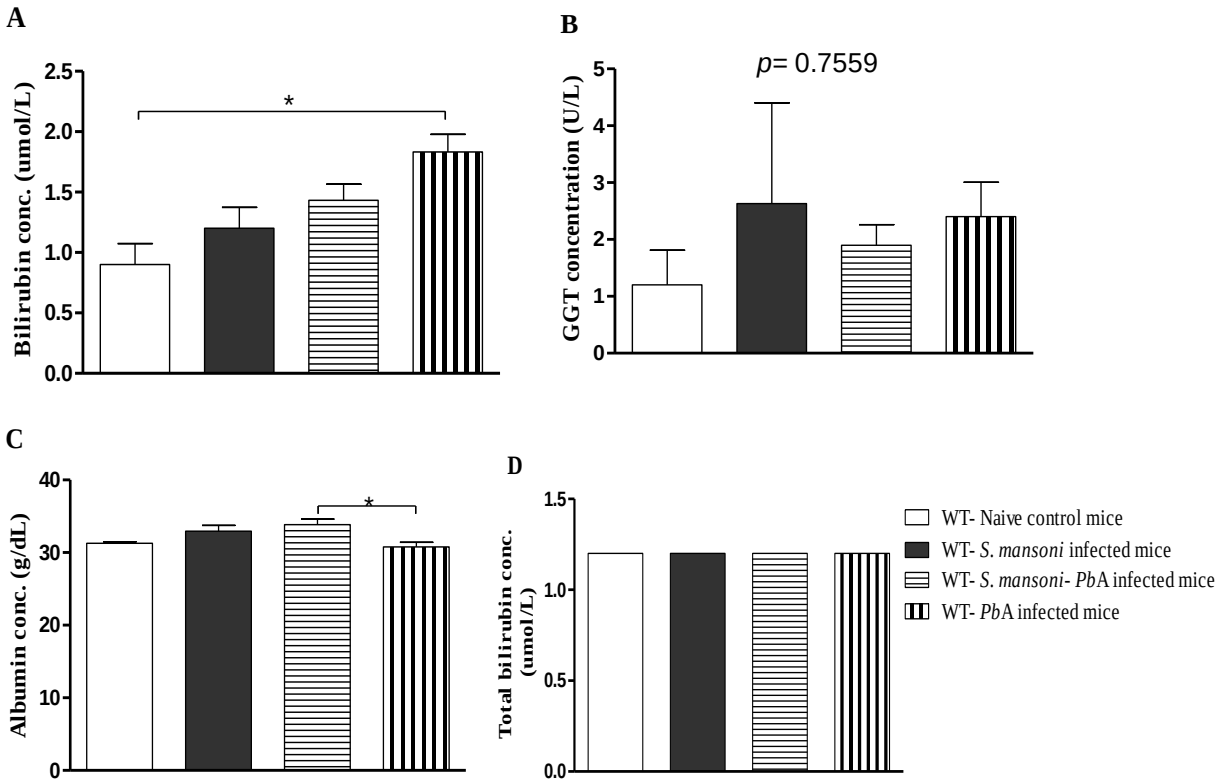

Figure 4.17: Effect of the infection on bilirubin, GGT and albumin levels in the mice infected with *S. mansoni* and *Pb*A

Analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p<0.05.

4.16 Effects of *Pb*A infection on lipid profile after induction of schistosomiasis

There was a significant reduction ($P = 0.05$) in total cholesterol levels in mice infected with *S. mansoni* alone, *Pb*A alone and those co-infected with *S. mansoni- Pb*A as compared to the naive control mice (Figure 4.18A). The total cholesterol level of co-infected mice was comparable with mice singly infected with either *Pb*A or *S. mansoni* (Figure 4.18A). An analysis of the lipid profile revealed that the levels of HDL were unaffected by the infections (Figure 4.18B). In addition, when infected alone, *S. mansoni* induced triglycerides suppression significantly relative to naive control mice (Figure 4.18C). Co-infected mice had statistically comparable levels of triglycerides with mice singly infected with either *Pb*A or *S. mansoni* (Figure 4.18C).

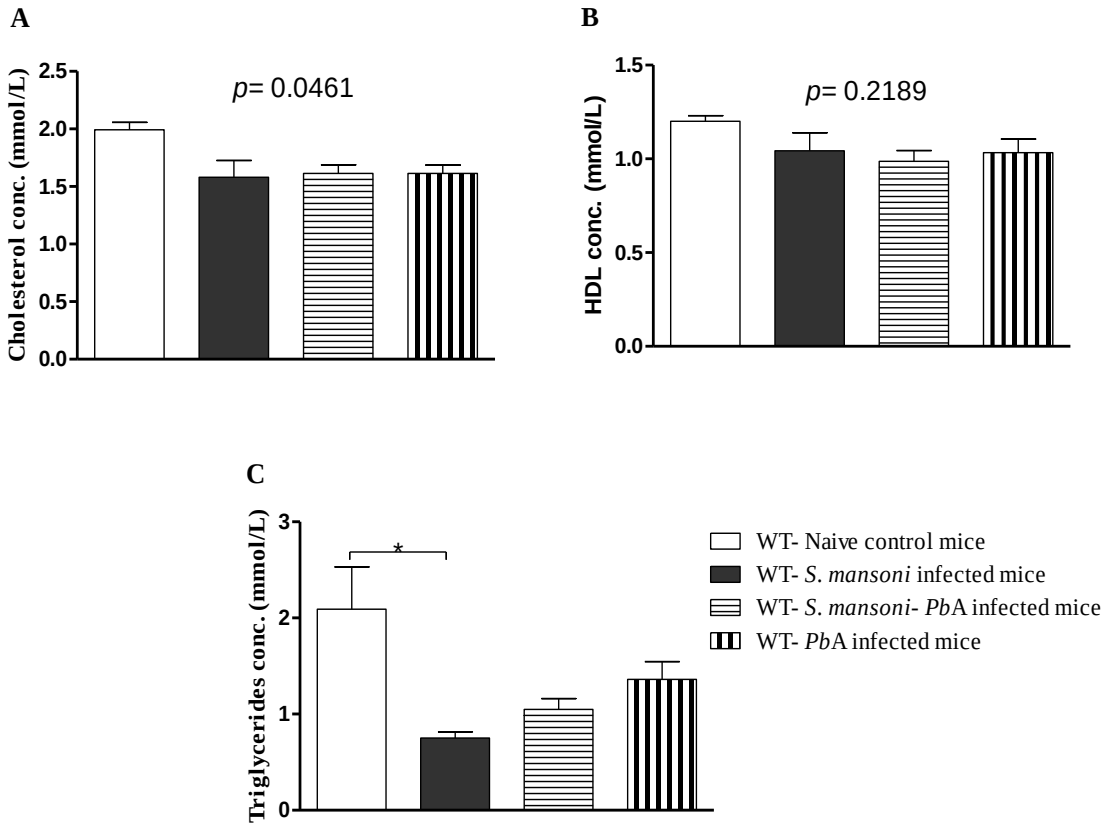

Figure 4.18: Lipid profile following the infections in mice infected with parasite.
Analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p<0.05.

4.17 Effects of *S. mansoni- Pb*A co-infection on creatinine, urea and uric acid levels

In the present study the levels of creatinine were quantified to assess the extent of kidney damage by the infections. *Pb*A and/ or *S. mansoni* did not affect the levels of creatinine, urea and uric acid (Figure 4.19A-C). Despite the marginal variations, the levels of creatinine, urea and uric acid in all the experimental groups were comparable.

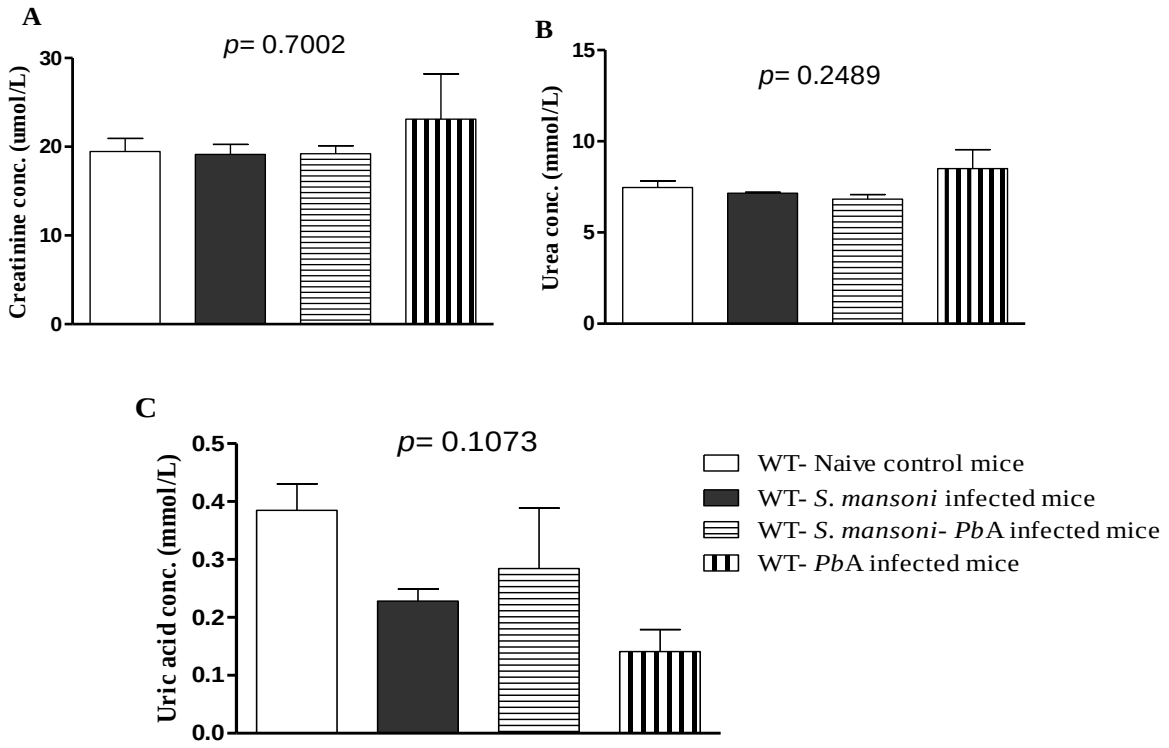

Figure 4.19: Effect of infections on creatinine, urea and uric acid levels
Analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons.

4.18 Effects of *Pb*A infection on cytokines level after induction of schistosomiasis

There was significant difference in the levels of TNF-α, IFN-γ and IL-10 in the experimental groups.

4.18.1 Effect of *S. mansoni* and *Pb*A infections on the pro-inflammatory cytokines

The serum levels of parasite induced specific cytokines were analyzed to determine the role of *Pb*A and/or *S. mansoni* in regulating inflammatory response. Infection with *Pb*A alone resulted in significant elevations ($p < 0.05$) in the pro-inflammatory cytokine TNF-α and IFN-γ (Figure 4.20A & B). Notably, the levels of these pro-inflammatory cytokines were down regulated in co-infected group of mice when compared with *Pb*A infected mice. Co-infected mice had comparable levels of TNF-α and IFN-γ relative to mice infected with *S. mansoni* alone. Pro-inflammatory TNF-α and IFN-γ were significantly elevated ($p < 0.05$) in mice infected with *Pb*A alone when compared to co-infected group of mice. Furthermore, *Pb*A infection had significant elevation ($p < 0.05$) of the TNF-α and IFN-γ relative to *S. mansoni* infected mice. In contrast, mice infected with *S. mansoni* alone had significantly elevated levels ($p < 0.05$) of TNF-α relative to naive control mice (Figure 4.20A).

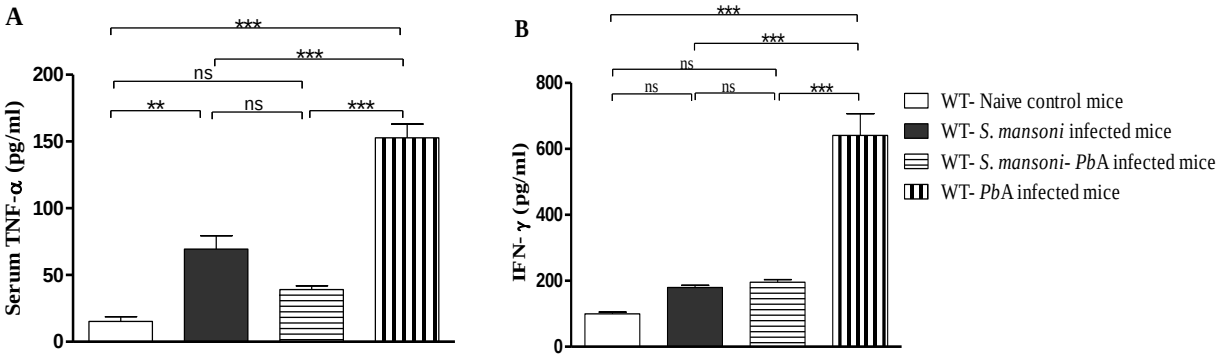

Figure 4.20: Effect of the infections on the levels pro-inflammatory cytokines in mice
Analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p<0.05, **p< 0.01, ***p< 0.001.

4.18.2 Effect of *S. mansoni* and *Pb*A infections on anti-inflammatory cytokines

The levels of the anti-inflammatory cytokine interleukin-10 (IL-10) were comparable in both *Pb*A and *S. mansoni* infected groups in comparison to naive control group (Figure 4.21). However, co-infected group of mice had significantly increased levels of IL-10 relative to naive control mice. The IL-10 levels remained marginally at the baseline when mice are infected with *Pb*A or *S. mansoni* alone.

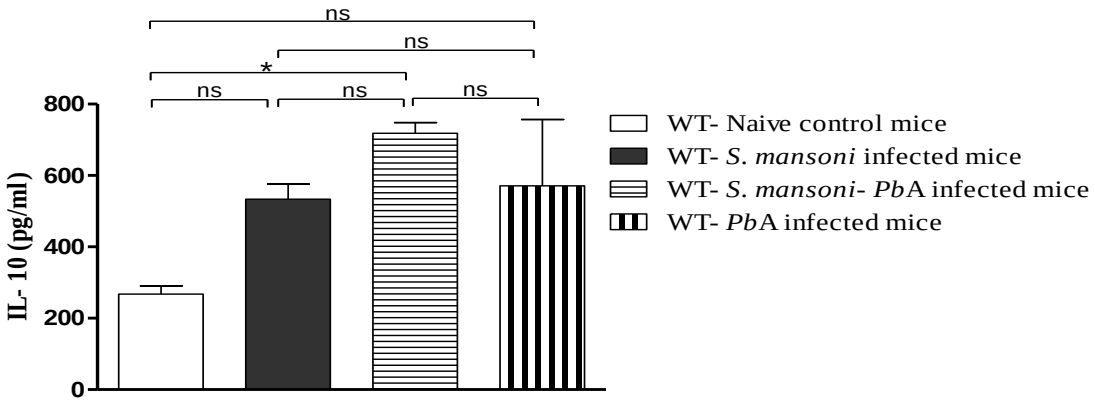

Figure 4.21: Effect of the infections on the levels anti-inflammatory cytokines in mice
Analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p<0.05.

4.18.3 Effect of co-infection with *S. mansoni* and *Pb*A pro inflammatory cytokines balance

The ratios TNF-α: IL-10 and IFN-ϒ: IL-10 were calculated to determine if there was an imbalance between pro-inflammatory and anti-inflammatory cytokines during the infection period. The ratios of TNF: IL-10 (p =0.0008; Figure 4.22A) and IFN: IL-10 (p< 0.0001; Figure 4.22B) in mice infected with *Pb*A alone were significantly higher relative to the ratio of naive control group, mice infected with *S. mansoni* alone and the co-infected group of mice. Co-infected group of mice had comparable TNF: IL-10 and IFN: IL-10 ratios relative to naive and group of mice infected with *S. mansoni* alone (Figure 4.22 A and B). Infection with *S. mansoni* alone or when co-infected with

*Pb*A significantly prevented the imbalance between the pro (TNF-α and IFN-ϒ) and anti-inflammatory (IL-10) cytokines compared to singular infection with *Pb*A ($p<0.05$; Figure 4.22 A and B).

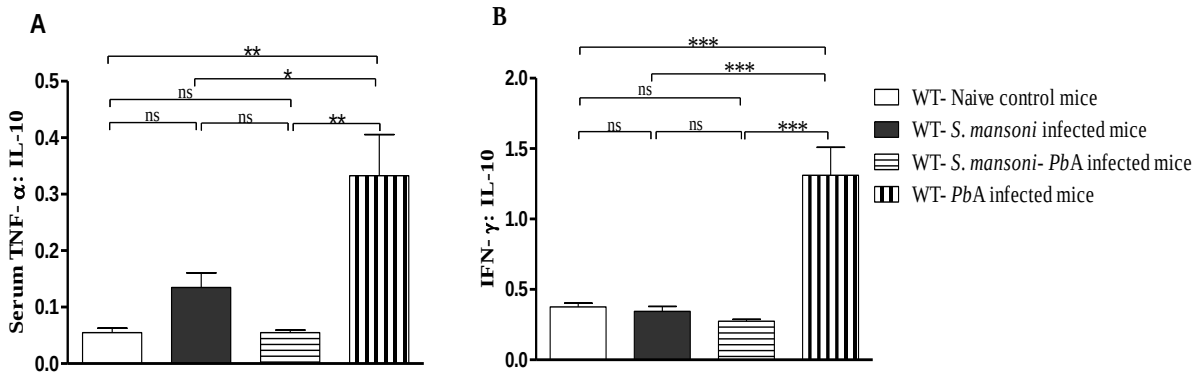

Figure 4.22: Effect of the infections on the balance of pro-inflammatory cytokines.
Analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p<0.05, **p< 0.01, ***p< 0.0001.

4.19 Effects of *Pb*A infection on protein quantity on mice already infected with *S. mansoni*

In this study, the effect of schistosomiasis and malaria on protein levels were determined following co-infection. There was a significant decrease ($P < 0.05$) of protein in mice infected with *S. mansoni* when compared to the levels in naive control mice (Figure 4.23). *S. mansoni- Pb*A co-infection significantly protected ($p < 0.05$) the mice from *S. mansoni* reduction of protein levels (Figure 4.23). Mice infected with *Pb*A alone had comparable levels of protein as the naive control mice. Co-infected mice had significantly elevated levels of protein ($p< 0.05$) when compared to mice infected with *S. mansoni* or *Pb*A alone.

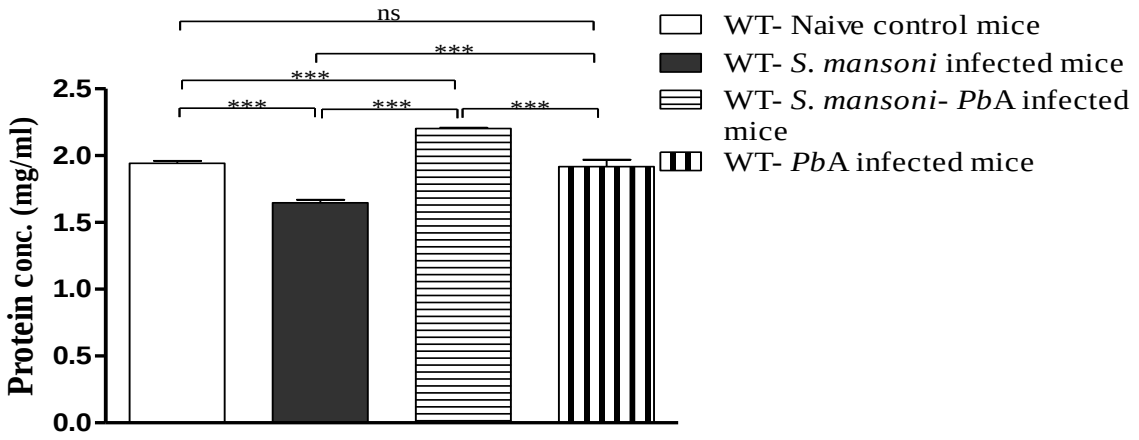

Figure 4.23: Serum protein concentration levels in the study groups following schistosomiasis and malaria infection.
Analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p<0.05, ***p< 0.001.

4.20 Effects of *Pb*A infection on reduced glutathione following induction of schistosomiasis

Heightened or extremely low levels of reduced glutathione (GSH) can be used to potently demonstrate the presence of active oxidative stress in vital organs. In the present study, Co-infection with both parasites restored *S. mansoni/ Pb*A depleted GSH in the brain whereas *Pb*A infection alone resulted in elevated levels of GSH in comparison to naive control (Figure 4.24A). Co-infected group of mice had comparable levels of spleen GSH relative to *S. mansoni* or *Pb*A alone mice (Figure 4.24B). Importantly, co-infection of mice resulted in restoration of *S. mansoni/ Pb*A depletion of cellular GSH in the liver (Figure 4.24C). Similarly, co-infection with the two parasites resulted in restoration of *S. mansoni/ Pb*A induced elevation of GSH in the kidney tissue (Figure 4.24D). Meanwhile the levels of cellular GSH in the heart tissue were comparable across all the experimental groups (Figure 4.24E).

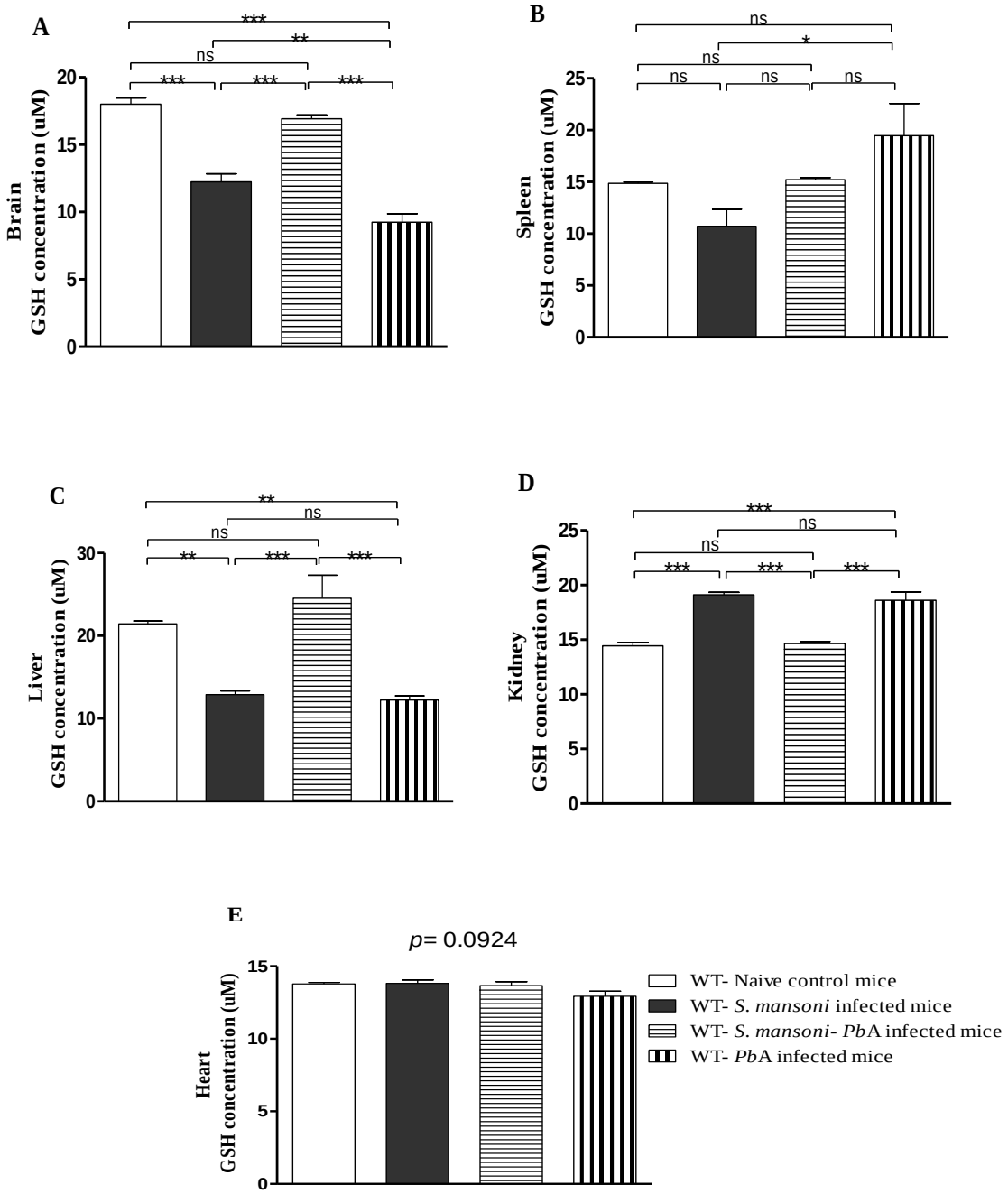

Figure 4.24: Effect of *S. mansoni*- *Pb*A co-infection on GSH levels in mice
Analysis was conducted using one-way ANOVA and Tukey's post hoc test for comparisons. Indicated statistical difference *p<0.05, **p< 0.01, ***p< 0.001.

4.21 Effects of *Pb*A infection on organ histology following *S. mansoni* infection

In the current study, chronic liver damage and necrosis in mice infected with *S. mansoni* alone was characterized by diffuse vascular congestion (C), multifocal areas of granulomatous reactions (G) as well as hepatocyte swelling in the focal area (S) (Figure 4.25b). Co-infected mice had mild liver injury characterized as focal areas of liver necrosis (star) and infiltration of the perivascular areas with mononuclear cells (arrowheads) (Figure 4.25c). Liver for mice infected with *Pb*A had liver injury associated with vascular congestion in the focal area (Figure 4.25d). Naive control mice had a normal liver tissue (Figure 4.25a). There was mild injury in liver of co-infected mice compared to chronic liver damage in mice infected with *S. mansoni* alone. Mice infected with *Pb*A alone were characterized with congestion only indicating a mild injury when compared to co-infected mice

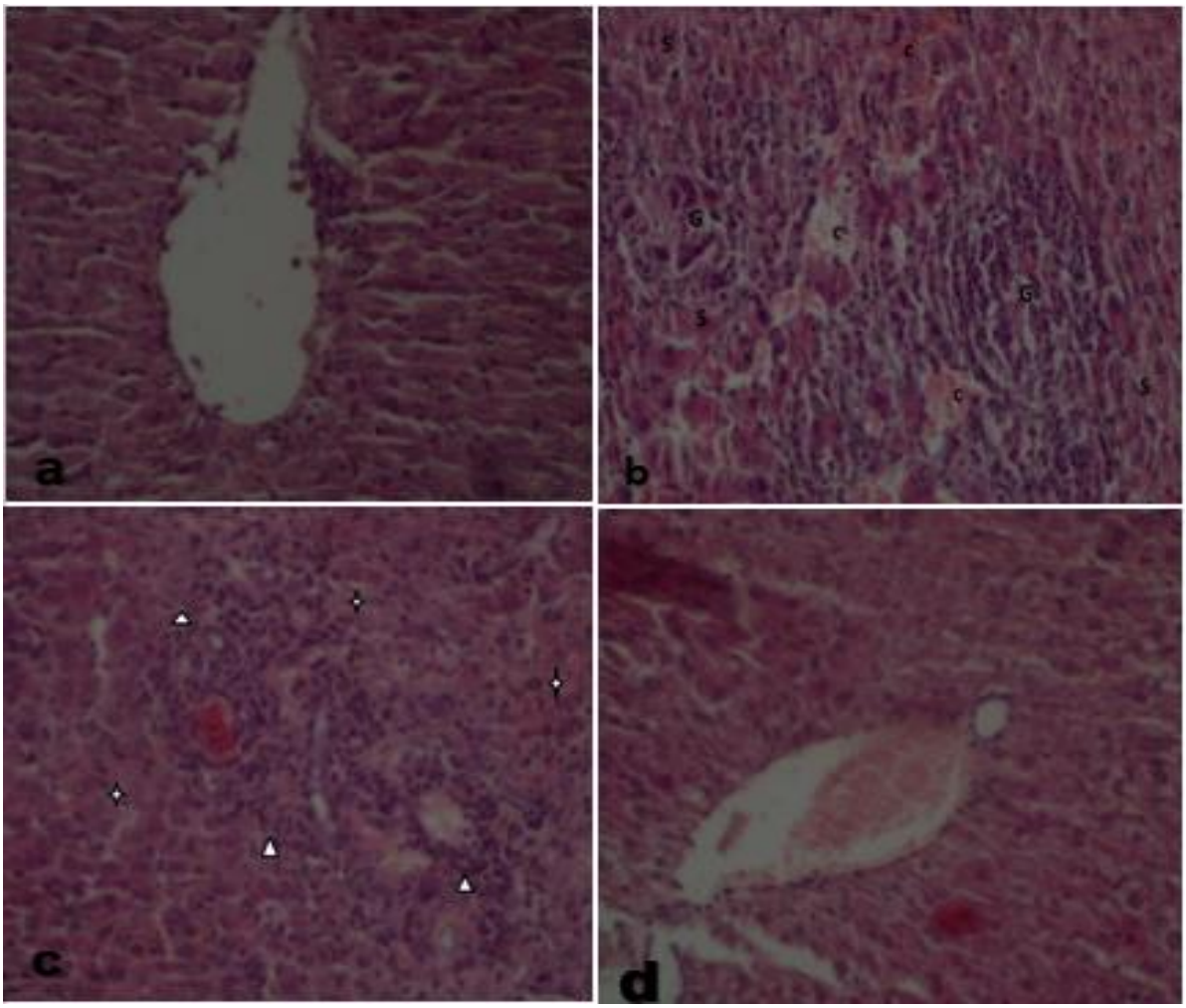

Figure 4.25: Comparison of the effect of the schistosomiasis and/ or malaria on the liver (×400, H&E)
a- WT Naive control mice, b- WT *S. mansoni* infected of mice, c- WT *S. mansoni* – *Pb*A infected mice, d- WT *Pb*A infected mice.

Kidneys of mice infected with schistosomiasis alone had kidney injury as the focal areas in the renal interstitial were infiltrated with mononuclear cells (arrow) and congestion (c) (Figure 4.26b). Mononuclear cells had infiltrated the focal area of the renal interstitial in the kidneys of co- infected group of mice (arrow; Figure 4.26 c). Kidney for mice infected with *Pb*A (Figure 4.26d), and naive control mice (Figure 4.26a) were normal at the end of the study period.

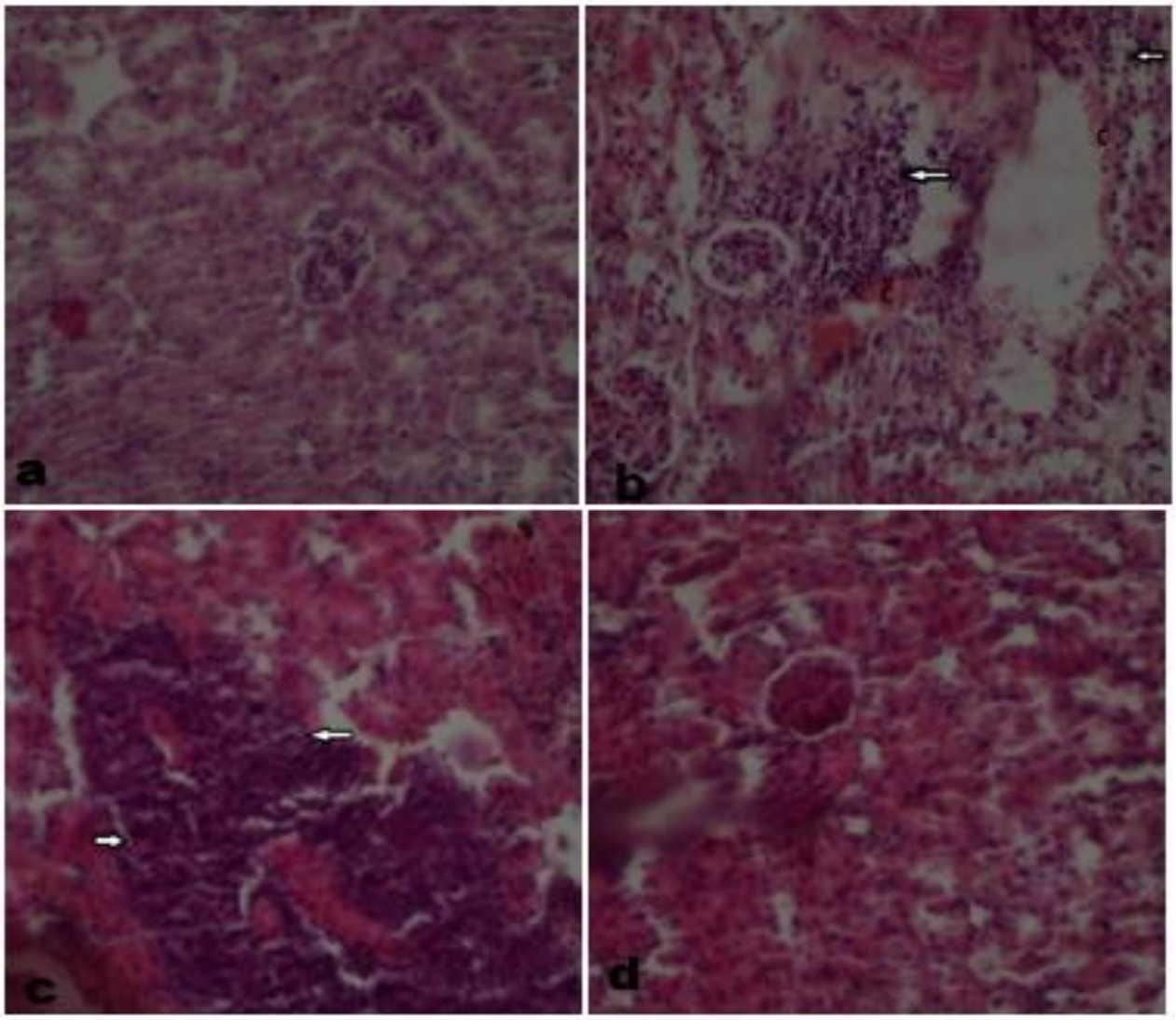

Figure 4.26: Comparison of the effect of the schistosomiasis and/ or malaria on the kidney (×400, H&E)
a- WT Naive control mice, b- WT *S. mansoni* infected of mice, c- WT *S. mansoni* – *Pb*A infected mice, d- WT *Pb*A infected mice.

Mice infected with *S. mansoni* and/ or *Pb*A as well as naive control mice had normal spleen tissues as shown in Figure 4.27a-d.

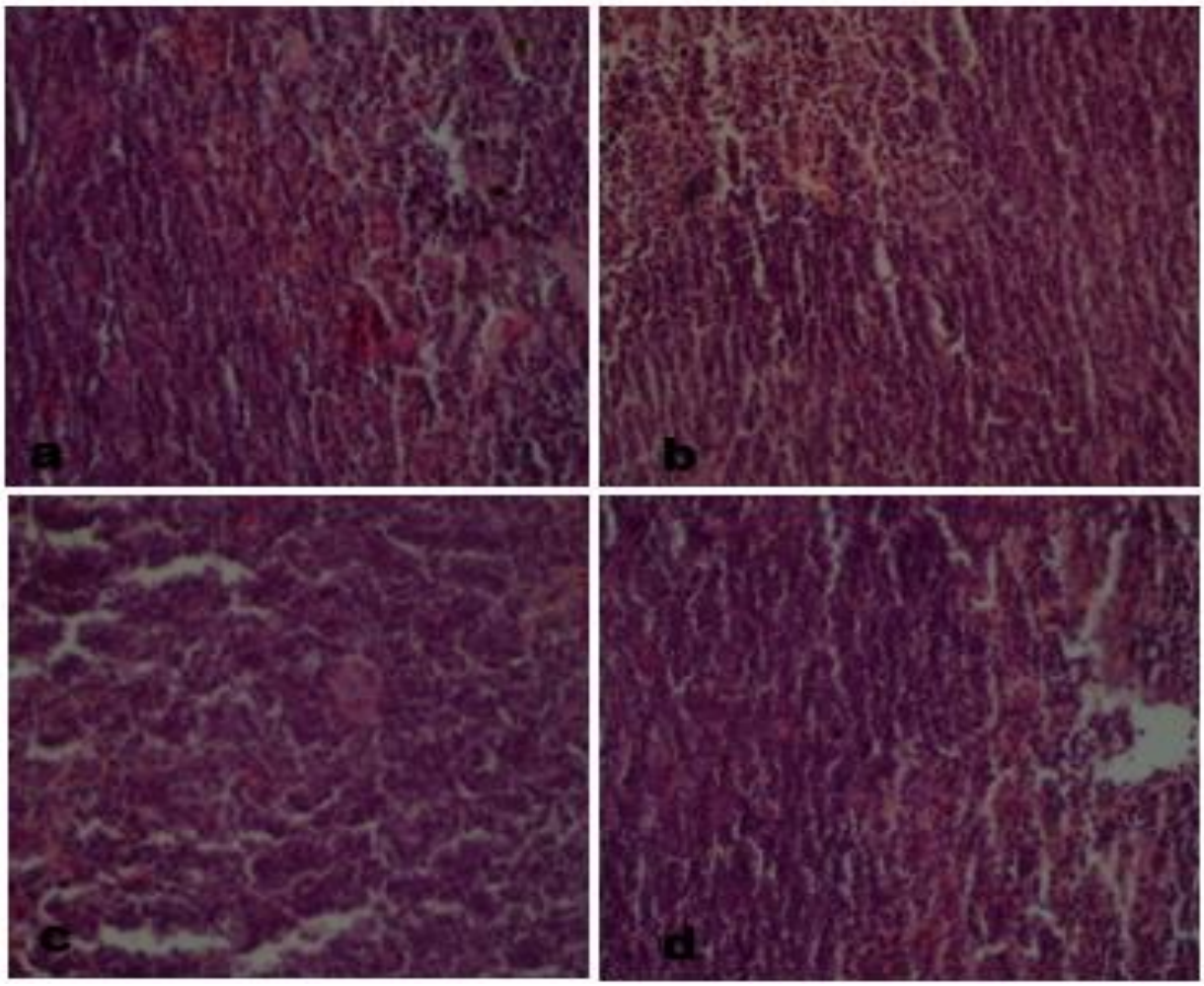

Figure 4.27: Comparison of the effect of the schistosomiasis and/ or malaria on the spleen (×400, H&E).
a- WT Naive control mice, b- WT *S. mansoni* infected of mice, c- WT *S. mansoni* – *Pb*A infected mice, d- WT *Pb*A infected mice.

Mice infected with *S. mansoni* and/ or *Pb*A as well as naive control mice had normal brain tissues as shown in Figure 4.28a-d, despite the red pigmentation in in Figure 4.28b.

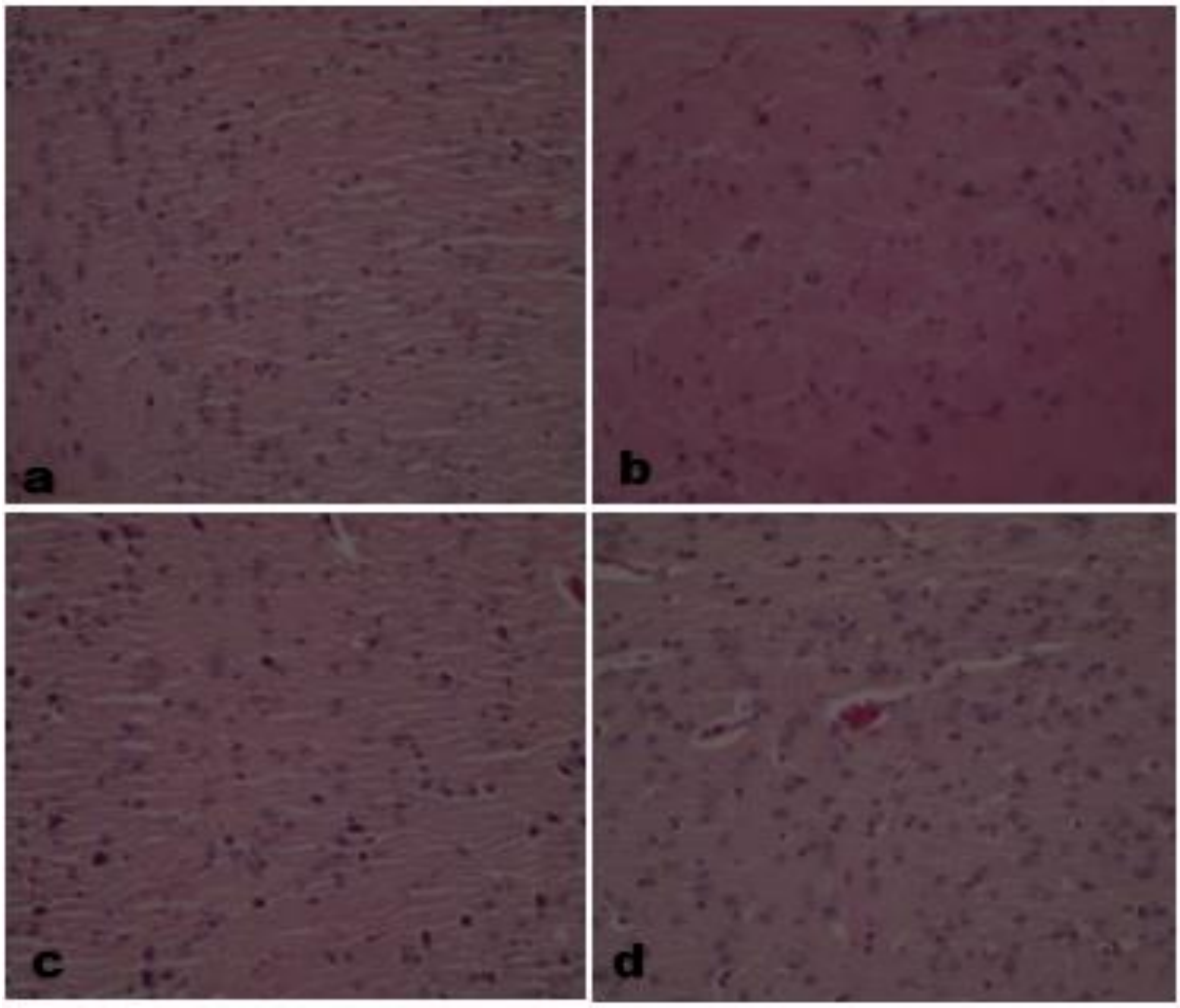

Figure 4.28: Comparison of the effect of the schistosomiasis and/ or malaria on the brain (×400, H&E)
a- WT Naive control mice, b- WT *S. mansoni* infected of mice, c- WT *S. mansoni* – *Pb*A infected mice, d- WT *Pb*A infected mice.

CHAPTER 5

DISCUSSION

5.1. General Outcome

In co-infection studies, epidemiological studies have often produced conflicting results; some studies indicate that *Schistosoma* infection increases disease intensity of *Plasmodium falciparum* infection, and co-infection contribute to high levels of anemia (Anchang-kimbi *et al.*, 2017). Whilst other studies and review contend that *Schistosoma* infection protect the host against malaria parasite density (Adegnika & Kremsner, 2012; Yves *et al*., 2014). Furthermore, *Schistosoma mansoni* infection suppresses *Plasmodium yoelii* growth in the liver (Moriyasu *et al*., 2018). Differences in study design, host or phylogenic variation presumably contribute to the conflicting outcome. This study has shown that both *S. mansoni* and/or *Pb*A infection have different attenuating effects on each other. Also, the host might experience either synergistic or antagonistic effect depending on the parameter under test. In this section, the effects are highlighted and distinguished as below;

5.2. The effect of chronic *S. mansoni* on selected physiological and hematological parameters during *Pb*A infection

In the current study, several parameters were carried out to check the variation and effects of *S. mansoni* and or *Pb*A on mice. Following co-infection, it was essential to check if presence of both infections would suppress or enhance the survival rate of the experimental mice. In the current study, *S. mansoni- Pb*A co- infected mice survived 5 days longer compared to mice infected with *Pb*A alone. In a similar study using a mouse model co-infected with *S. haematobium* and *P. berghei*, there was extended survival during chronic schistosomiasis (Paa *et al*., 2015). In a study

conducted by Donald *et al*., (2014), mice models in one group were infected with *Pb*A while another group of mice were infected with *Pb*A then inoculated with *S. mansoni* egg antigen. The study demonstrated that mice infected with *Pb*A alone had the least survival time than mice inoculated with *S. mansoni* egg antigen. These findings were similar to the current study, where *S. mansoni* infection protected the co-infected group of mice from succumbing early to malaria hence sustaining their survival.

Preliminary investigations were conducted to confirm if *S. mansoni* parasites had manifested in the chronic phase of infection before co- infecting mice with *Pb*A. In the present study, there were limited differences in the intensity of the granuloma in the study groups, but their presence was a confirmation of schistosomiasis infection. In *S. mansoni* infection, granulomas usually cause destruction of the ova but result in fibrotic depositions in the host tissues, mostly in the intestines and liver (WHO, 2018; Harvie *et al*., 2007). Granulomas are normally characterized as white spots, are as a result of the eggs lodging in the liver and immune cells surrounding them. This granulomatous reaction tends to protect hepatocytes from further destruction from released toxins of the schistosomes eggs such as Schistosomes egg antigen (SEA) (Abdulla *et al*., 2011). However, the intensity of granuloma is not directly associated with the infection level nor adult worm after perfusion, nor the number of eggs (Mola *et al*., 1999). Immature eggs have an immunologically inert eggshell hence do not recruit macrophages around them to form granuloma but mature eggs secrete antigen that promote egg granuloma formation (Takaki *et al*., 2020). To overrule the assumption that granulomatous reaction might have been caused by another infection, the liver was perfused and adult schistosomes were observed. Eggs collected from the liver by

sieve method had lateral spines near the posterior end confirming *S. mansoni* infection which is accordance to WHO, (2019).

In the present study, parasitemia in mice pre-exposed to *S. mansoni* was slightly lower compared to mice infected with *Pb*A alone though not statistically significant. Though the patent period was the same, it seemed like schistosomes counter *Plasmodium* effect hence the lower parasitemia. Several studies indicate that an underlying experimental schistosomiasis infection reduces the severity of subsequent malaria infection in co-infection studies (Donald *et al*., 2014; Mulei *et al.*, 2012). Data from this study indicates that schistosomes protected the mice from severe malaria hence the marginally lower parasitemia compared to *Pb*A alone infected mice though not statistically different (p= 0.2039). According to Donald *et al*., (2014) the progression of malaria in the mice without schistosomes intervention is rapid compared to the group intervened with schistosomes. In a co-infection study of *P. yoelli* and *S. mansoni,* the latter reduced the malaria parasite levels in the liver This reduction led to fewer schizonts that would rupture into blood phase hence reduced parasitemia (Moriyasu *et al*., 2018). However, in a study conducted in Senegal, the low intensity occurrence of *S. haematobium* was found to lower the malaria effect whereas high schistosomiasis intensity promoted the occurrence of malaria (Yves *et al*., 2014). This can be attributed to the different species of schistosome as compared to the current study.

Assessment of the health status of mice, appearance, and behavior was done by employing a quantitative tool of rapid murine coma and behavioral scale (RMCBS) scores. The test was carried out post co-infection with *Pb*A checking the effects of the infections, severity. This tool was a

determinant on which day to terminate the experiment. RMCBS labels affected host and links it with neuropathological injury (Carroll *et al*., 2010). In the present study mice were euthanized on day 7 post infection with *Pb*A when they registered RMCBS mean score of 13. This finding is in line with a malaria study where, infected mice showed signs of brain disorder (paralysis, ataxia, and seizures) with RMCBS score equal or less than 10 in 6-8 days as some mice died on day 6 (Adriana *et al*., 2017). The first mouse to succumb to malaria was on day 8 which is within 6-8 days according to Adriana *et al*., (2017). In this co-infection study, schistosomes not only improved survival, but also clinical outcome as per RMCBS score. Despite these variations in the mean of the experimental groups, there was no statistical difference in the current study. The measure of moderate experimental cerebral malaria has a RMCBS score of 10-15 (Hoffmann *et al*., 2016). In the current study, the RMCBS mean for mice infected with *Pb*A alone and co-infected group of mice was below 15 at the time of euthanasia. It was essential to confirm brain damage by histology later in the study. A decrease in RMCBS might be a confirmation of cerebral malaria in *Pb*A infection (Miranda *et al*., 2015). Motor performance and groom were affected significantly. These two parameters were similar for both co-infected group of mice and *S. mansoni* infected mice. This is an indication that presence of malaria did not correct *S. mansoni* induced poor groom in the co-infected group of mice. Motor performance of the *S. mansoni* infected mice reduced due to anomalies in the weight increase brought about by organ enlargement (Marr *et al.*, 2012). Co-infected group of mice reduced motor performance might be due to host adapting to the immune responses' changes elicited by secondary infection. *Pb*A infected mice had piloerection as both *S. mansoni* infected mice and co-infected group of mice mostly had ruffed; with swaths of hairs out of place labelling mouse as affected an indication of disease progression for therapy and tests (Abdulazeez *et al*., 2020). In mice infected with *S. mansoni* and

co-infected group of mice, hygiene and exploratory behaviors were highly affected at the time study. Furthermore, reflexes and co-ordination were affected though not significantly at the time of termination.

Onset of *S. mansoni* and/or *Pb*A parasitic infection led to variation in the body weight as the hosts were under stress, the organs were enlarged. *Plasmodium* parasite interferes with host's organs such as brain, kidney, liver, lungs, spleen and the central nervous system causing stress (Harvie *et al*., 2007; Ngozika, 2018). In the present study livers from *S. mansoni* infected mice were significantly enlarged. In schistosomiasis, the eggs have been seen to lodge in liver, spleen, kidney, brain hence the need to probe deeper by analyzing the changes in relative organ weight. These findings concur with Egal, (2006) and Lopes *et al*., (2006) who reported a significant increase in liver of mice infected with schistosomiasis after 8 weeks post infection that is attributed to hepatomegaly. Furthermore, Donald *et al*., (2014) indicated an increase in in body weight might be an effect on eggs lodged in the organs. In the present study, mice infected with *P. berghei* alone did not have a significant weight reduction when compared to naive mice. This finding is contradicting with a co-infection study of *P. berghei* and *T. brucei*, where mice infected with *P. berghei* had a significant reduction in body weight (Ademola *et al*., 2016). In *S. mansoni* infected group of mice, there was significant increase in liver relative to *S. mansoni* and *Pb*A co-infected group of mice. This could be attributed to an antagonizing effect of malaria to schistosomiasis. Relative liver weight from *Pb*A alone mice was comparable to naive mice, as a result of shorter experimental time. Most of these antagonistic effects elucidates that malaria parasite reduced the schistosomes effects in the host.

In this study, the spleen for mice infected with *S. mansoni* had a higher relative weight compared to naive mice. This was attributed to spleen enlargement. Additionally, co-infected group of mice had comparable spleen weight as *Pb*A infected mice which was lower than naive mice. This indicates that *P. berghei* had an attenuating effect on the splenomegaly brought about by schistosomiasis. The spleen is known to clear the parasite, by removal of malaria parasite from its red blood cell host as parasitized erythrocyte travel through the spleen (Spillman *et al*., 2015; Milner, 2017). Sequestering of splenocytes is a common occurrence in malaria infection (Id *et al*., 2019) and hepatosplenomegaly is a common indicator in schistosomiasis as a result of retention of activated cells (WHO, 2018b).

In the current finding the relative brain weight for mice infected with *S. mansoni* and co-infected group of mice was reduced when compared with naive mice. This finding confirms that schistosomiasis leads to destruction of brain and higher tropism for brain as highlighted Pinto-almeida *et al*., (2016).

The relative kidney weights remained unaffected by *S. mansoni* and/or *Pb*A in the current study. In contrast, there was marginal increase in the size of kidney of children with malaria which worsened with increasing severity of morbidity as a result of malaria (Atalabi *et al.*, 2013). Schistosomiasis is known to cause kidney damage (WHO, 2020b). Whilst the current study supported the kidney injury; as mononuclear cells infiltrated foal area of renal interstitium, the relative kidney weight was not significantly affected.

In the present study, the relative lung weights remained unaffected by *S. mansoni* and/or *Pb*A. In the lungs, chronic pulmonary schistosomiasis results in granuloma formation and fibrosis may lead to cor pulmonale (Niemann *et al*., 2010). In *Plasmodium* infection, pulmonary manifestation is

part of severe systemic illness and its involvement has a lower frequency compared to the other complications (Taylor *et al*., 2006). However, there is limited information to depict if this complication could result in change of lung size.

In the current study, a hematological analysis was performed as a marker of the infection levels. In the present study, the levels of RBCs in *S. mansoni* alone, *PbA* alone and *S. mansoni- Pb*A co-infected mice were comparable relative to the naive control mice hence no evident anemia. This was contrary to studies which indicate anemia in these two infections. Ingestion of red blood cells by schistosomes and phagocytosis of parasitized red cell which occurs in the spleen in malaria result in severe anemia in the host (Niikura *et al*., 2011; Marr *et al*., 2012; Leticia *et al*., 2014). Hematuria and lower levels of hemoglobin is one of the morbidity caused by schistosomiasis (WHO, 2018b). To support this analogy, hematocrit, and hemoglobin were unaffected across the study groups. Furthermore, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, as well as red cell distribution width, in the *S. mansoni* and/or *Pb*A infected mice were comparable relative to the naive control mice. Despite severe malaria infections being well known to cause anemia by reducing hemoglobin and hematocrit levels (Al-salahy *et al.*, 2016), This was not the case in the current study due to the shorter experimental time and mice model used.

WBC levels in *S. mansoni* or *Pb*A infections were comparable relative to the naive mice. However, in the co-infected group of mice, the levels were elevated. This findings are contrary to findings by Al-salahy *et al*., (2016), who recorded decrease. The elevation in co-infection is due to the hyper immune response that might destroy red blood cells as well as immunocompromised hosts (Leticia *et al*., 2014). Alteration and elevation of leukocytes especially eosinophil are considered a marker for Helminthes infection (Castro *et al*., 2018). Eosinophil levels were heightened

significantly in *S. mansoni*- *Pb*A co-infected group of mice and low in *S. mansoni* mice (p= 0.0162). Our findings were similar to the study done in Brazil by Castro *et al*., (2018), which indicated low eosinophil levels in schistosomiasis infection hence not being the best serological marker for the infection. Mulei, (2012) stated that eosinophilia was present in acute schistosomiasis and this explains the diminished levels in chronic phase as the host tends to elicit more adaptive immunity. Current study indicated reduction in neutrophil levels, which is contrary to a *P. falciparum* infection study which indicated an increase in this cell type (Al-salahy *et al*., 2016). The reduction in neutrophil and eosinophil count was evident in mice infected individually with *Pb*A or *S. mansoni*. These findings are in line with a study done by Leticia *et al*., (2014). Co-infected group of mice had heightened levels of both neutrophil and eosinophil and also had high monocyte levels which leads to macrophage formation. A synergistic response was seen in the lymphocyte levels of co-infected group of mice. The level of lymphocyte in mice infected with *Pb*A was elevated in the current study. This is contrary to a study conducted by Al-salahy *et al*., (2016) which showed a decrease in lymphocyte levels in *P. falciparum* infection. The white blood cell count in the co-infected group of mice cannot be attributed to either *S. mansoni* or *Pb*A infection, as the variations were independent to individual cells.

There was a marginal increase in the platelet levels in *Pb*A or *S. mansoni* individually infected mice in relative to the naive control mice. These findings were contrary with hematological profile of patients with malaria where the levels of platelets decrease with an increase in the parasite level (Al-salahy *et al*., 2016; Sakzabre *et al*., 2020). This current study is contrary to findings on schistosomiasis study which indicated decrease in platelet levels in the course of the infection (Duarte *et al*., 2014; Menezes *et al*., 2013). The co-infection of *Pb*A and *S. mansoni* corrected the

marginal elevated levels brought about by *Pb*A or *S. mansoni* infections hence the antagonizing effect.

5.3 Effect of chronic *S. mansoni* infection on immune regulation and metabolic changes during *Pb*A infection

In the present study, liver, and kidney function tests were performed to assess the damage levels and functionality of these two vital organs in presence of these infections either individually or in co-infection cases. Functionality was based on enzymes released by the liver and bile in response to their damage as well as the byproducts released. Alkaline phosphatase (ALP), for example, is an enzyme released by the bile duct and its reduction is due to damage of the bile duct in the liver (Letícia *et al*., 2018). These were observed in the current study where mice infected with *S. mansoni*, *Pb*A infected mice and mice co-infected with *S. mansoni*- *Pb*A had reduced ALP levels (p= 0.0363). There were contrary results in mice infected with *Pb*A which had significant reduction in the levels of ALP as malaria, was reported to increases the levels of ALP (Auta, 2018). Since schistosomes tend to migrate in the mesenteric veins, this could disrupt the functionality of the bile attached to the liver. Furthermore, the levels of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in *S. mansoni* mice were decreased. These two enzymes are used to detect hepatocyte damage. These findings were similar to a study conducted in Brazil, where ALT and AST levels were reduced in schistosomiasis infection (Letícia *et al*., 2018). These abnormalities are as a result of liver fibrosis and portal hypertension (Menezes *et al.*, 2013). However, in *S. mansoni*- *Pb*A co-infected group of mice had marginally elevated ALT and AST levels. Malaria tends to increase serum liver enzymes and the increase is linked to the intensity of malaria parasite (Al-salahy *et al*., 2016; Auta, 2018; Houmsou, 2018). As a result, *Pb*A had a

recovery effect in liver damage in co- infection since *S. mansoni* infected mice were decreased while elevated levels were observed in *Pb*A infected mice.

Schistosomes do not produce their cholesterol and long chain fatty acids *de novo* hence they depend and acquire the host cholesterol and fatty acids via the tegument (Marr *et al*., 2012). In the current study, the levels of cholesterol, triglycerides, and HDL, were reduced in *S. mansoni* and/or *Pb*A infected mice relative to the naive mice. Both mice infected with *S. mansoni* alone and co-infected with *Pb*A behaved similarly with an exception of triglycerides. Mice infected with *S. mansoni* alone had significantly reduced levels ($p = 0.0255$) of triglycerides than in *S. mansoni*-*Pb*A co-infected group of mice and comparative to the naive mice. Our study was similar to that of Letícia *et al*. (2018), which reported a decrease in the lipid profile of cholesterol, triglycerides, HDL for individuals infected with *S. mansoni*. Presence of malaria in the co-infection lowered the schistosomes effect on the levels and hence the level of triglycerides was comparably higher than in schistosomiasis mice alone.

In the current study, the direct bilirubin in mice infected with *Pb*A was elevated significantly relative to naive control mice as total bilirubin was the same across the experimental groups. With regards to direct bilirubin, this is in agreement with malaria studies using *P. falciparum*, which states that patients with malaria had higher bilirubin levels both direct and total than uninfected ones (Al-salahy *et al*., 2016; Houmsou, 2018). Despite that, there was contradicting result in a malaria study which indicated a decrease in serum bilirubin (Andrade *et al*., 2010), though it was by *Plasmodium vivax* which might have different response. Patients with hepatosplenic schistosomiasis recorded an elevated bilirubin levels (Menezes *et al.*, 2013). This finding is in line with the current study which had marginal increased level of bilirubin in mice infected with schistosomiasis compared to naive. There were some contrary findings by both Richter *et al*.,

(2015), and David *et al*., (2017) which indicated that bilirubin was not affected in schistosomiasis infection. In the co-infection study, the level was in between levels of mice infected with individual infections indicating an antagonizing effect of schistosomes on malaria.

In this study, Gamma- glutamyl transferase (GGT) concentration levels were marginally elevated in schistosomiasis infected mice. In the present study, albumin levels were slightly higher in schistosomiasis infected mice than naive mice. The albumin levels in mice infected with *Pb*A were slightly lower than naive mice. In a study conducted on patients with hepatosplenic schistosomiasis, there was an elevated levels of GGT and lowered albumin levels (Menezes *et al.*, 2013). Current findings are in line with study by Wokem *et al.*, (2018) which showed a decrease in albumin levels in children suffering from malaria. Co-infected mice had marginally elevated levels relative to *S. mansoni* or *Pb*A infected mice. Creatinine levels in the current study was comparable in *S. mansoni* infected mice, co-infected group of mice relative to naive mice. *Pb*A infected mice had marginally heightened creatinine levels, though not statistically significant, which was contrary to study by Andrade *et al*., (2010), which suggested a decrease in the level who used using *P. vivax* species.

The cytokine levels in a host are a key indicator of infections and/ or activation of the host immune system to respond to infectious pathogens. In this study, levels of IFN- γ, TNF- α, and IL- 10 were measured in naive, *S. mansoni*, *Pb*A, and co-infected group of mice. IFN- γ and TNF- α levels in *Pb*A infected mice were significantly heightened relative to naive control mice. This finding are in agreement with a study done in Sudan by Barkat *et al*. (2020), who reported that these pro-inflammatory cytokines in malaria infection are elevated more than in healthy control causing

inflammation. IL- 10 levels had a statistically significant association with parasite levels in co-infected group of mice. Present data on co-infected group of mice is in agreement with other studies showing the correlation between parasitemia and IL-10 (Barkat *et al.*, 2020). During a malaria infection, IL-10 is necessary for repressing hepatic pathology and suppressing the infection (Niikura *et al*., 2011).

In the present study TNF- α, was observed to be elevated as IFN-γ and IL- 10 levels were comparable in the group of mice infected with *S. mansoni* alone relative to naive mice. The finding corroborates with a study by Castro *et al*., (2018), which indicated that IFN-γ levels are elevated in acute phase of schistosomiasis, as the levels of TNF- α are elevated in chronic infection. However, in the co-infection study, TNF- α and IFN-γ levels were comparable with naïve control mice whereas IL- 10 was significantly elevated. This implicates that inflammation was suppressed in co-infected group of mice. Acute malaria elevates regulatory cytokines such as IL- 10 (Farrington *et al*., 2017). This is in agreement with our study and this explains the elevated levels in co-infected group of mice. IFN- γ levels in malaria infection are also elevated during the liver stage in hepatocytes (Maheshwari, 1990). This explains the lower levels in *S. mansoni- Pb*A mice since schistosomes' eggs are present in the liver with the protective schistosomes' egg antigen (SEA) protein.

A review by Niikura *et al*. (2011), indicates that high TNF- α /IL- 10 ratio is associated with severe malarial anemia. These results suggest that low IL-10 levels are associated with increased production of IFN-γ and TNF- α levels. This is contrary to our findings since the levels of IL- 10 were elevated in the *S. mansoni* and/or *Pb*A infected mice relative to naive mice. The cytokine levels in schistosomes infected mice were higher than in naive mice. These observations is in agreement with a previous study by Meurs *et al*. (2014), which states that as *Schistosoma* infection

intensity increases, Th1 cytokine responses decrease and the Th2 phenotype became more pronounced. This was exemplified by relatively higher IL-10 levels in co-infected group of mice and relatively lower IFN-γ, and TNF- α as compared to mice infected with *Pb*A alone. Furthermore, IFN-γ levels in *S. mansoni* infected mice was elevated relative to naive mice. These findings agreed with a study conducted in Kenya on baboons, which indicated that SEA induces gamma interferon production (Mola *et al*., 1999; Montenegro *et al*., 2016).

In the present study, the ratio of pro-inflammatory and anti-inflammatory cytokines was low in co-infected groups of mice relative to in *S. mansoni* or *Pb*A or naive control mice. This suggests that the already established immune response by the primary infection by S. mansoni modulated the Th1 cytokine levels following the secondary infection by *Pb*A. The down regulation of IFN- γ and TNF- α by IL- 10 was in line with a study by Montenegro *et al*. (2016) on acute and chronic infections as low TNF- α and IL- 10 are associated with severe hepatic fibrosis due to chronic *S. mansoni* (Mutengo *et al*., 2018).

The level of protein in sera was quantified for the experimental study mice. *Pb*A infected mice had a similar protein level as naive mice. The co-infected group of mice had a higher level whereas *S. mansoni* infected mice had the least level of sera protein. These findings were expected as *Plasmodium* spp. are known to interconvert amino acid of the host and not necessarily take it away from the host. *Plasmodium falciparum* for example, interconverts serine with cysteine amino acid leaving the quantity comparable (Marr *et al*., 2012). There was significant reduction in the protein level of mice infected with *S. mansoni.* The present results concur with Egal, (2006) reported a significant reduction in protein level, six weeks post infection with *S. mansoni.* This is attributed by decrease in protein anabolism, increase in catabolism and malabsorption during hepatic fibrosis in schistosomiasis (Egal, 2006). Schistosomes also are known to oxidize amino acids such as

glutamine to CO_2 through the citric acid cycle and respiratory chain (Marr *et al*., 2012). A combination of passive diffusion and a carrier mediated system are used by schistosomes to absorb amino acids such as methionine, glutamine, arginine and alanine across the tegument (Marr *et al*., 2012). The protein level in co-infected group of mice was elevated, and this is thought to be as an antagonistic effect of malaria to schistosomes. *Plasmodium berghei* parasite could have interconverted amino acids in the cell before they are taken up by schistosomes.

5.4 Effect of chronic *S. mansoni* on *Pb*A -driven oxidative stress and tissue inflammation

Inflammations, host immune response and infections result in release of reactive oxygen species by host cells as well the infections. In this study, glutathione levels in co-infected group of mice, naive control mice, and those infected with *Pb*A or *S. mansoni* individually, were assayed as a marker of oxidative stress. The levels of GSH in both brain and liver from the mice individually infected with *Pb*A or *S. mansoni* were lower compared to the naive control mice and co-infected group of mice. These observations are consistent with the study conducted in Nigeria on human serum antioxidants which indicates that the levels of reduced glutathione were lowered by the occurrence of malaria (Abubakar *et al*., 2016). These individual infections have been seen to immuno-compromise the host by increasing the free-radical levels like reactive oxygen species. GSH being an antioxidant quenches these free radicals and protect the host cell (Marr *et al*., 2012; Vega-rodriguez *et al*., 2015). Counteracting the reactive oxygen species among other free radicals might have triggered suppression of these antioxidant levels in the brain and liver in this study.

The decrease of GSH was also observed in the spleen (p= 0.0324) of mice infected with *S. mansoni* whereas *Pb*A mice had elevated levels. The co- infected group of mice had their levels of GSH

comparable to the naive mice. This could be counteraction effect of the co-infection indicating that either of the infection did not induce free radicals or the released free radicals by *Plasmodium* were countered by antioxidants elicited by schistosomes or vice versa. Similar study conducted in India which indicates that the parasitic clearance leads to a decrease in ROS generation by the parasite (Tyagi *et al*., 2017). On contrast, the parasite was not cleared in the present study. Therefore, host-parasite association in schistosomiasis results in the production of free radicals as a result of oxidative stress where the parasites struggle to evade the immune response of the host. Notaly, there was no change in heart GSH levels in the present study. As indicated in by Egal, (2006), these changes occur in the host liver, brain, kidney, and spleen antioxidants as a means to scavenge these radicals hence no changes in the heart.

Histopathological analysis revealed an abnormal vascular congestion, hepatocyte swelling, granulomatous reactions characterized by spotty necrosis in the liver and kidney tubular necrosis with mononuclear cells infiltration in mice infected with *S. mansoni* and the co-infected group of mice. In the current study organs from *P. berghei* infected mice were marginally affected. These findings concur with a study on *P. falciparum* infection as the morphology of hepatocytes and endothelial cells in liver were generally unaffected (Viriyavejakul *et al*., 2014). Congestion of hepatocytes in this study can linked with the higher bilirubin in *Pb*A infected mice. Liver cell necrosis was evident in *Pb*A infected mice as the changes in liver histology is associated with total bilirubin (Viriyavejakul *et al*., 2014). Other organs in this study were visualized as normal for the *Pb*A infected mice. These were contradicting findings which indicate lung damage (Valecha *et al*., 2009), both liver and lung damage (Brugat *et al*., 2014). Brugat *et al*., (2014) also indicates that malaria parasite sequesters mostly in the liver, lungs and spleen and not Kidney and brain.

Mice infected with *S. mansoni* had granulomatous reactions in the liver, hepatocyte swelling and congestion. Liver damage was chronic in these mice whereas mice co-infected with *Pb*A had mild liver damage in this study. These findings concur with granulomatous responses seen in livers of rodents infected with *S. mansoni* (Lopes *et al*., 2006; Amaral *et al*., 2017). Renal damage was evident in the mice infected with *S. mansoni* and the co-infected group of mice. The infiltration of mononuclear cells in the renal interstitial in both *S. mansoni* and co- infected group of mice signaled kidney damage in this study. Renal lesion in schistosomiasis indicates schistosomal glomerulopathy mostly in hosts with hepato- splenomegaly. These are characterized with type 1 membrane proliferation, glomerulonephritis and cellular proliferation (Geraldo *et al*., 2013). Renal damage is associated with its functionality as Duarte *et al.*,(2013) highlighted this in *S. mansoni* infection. Brain and spleen were not affected in the current study though hemozoin could have altered the color of brain and the limited cell. Notably, organs damage induced by *S. mansoni* were reduced by occurrence of malaria in the co-infected group of mice.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusions

The results from this study shows that *S. mansoni* or *Pb*A affects the host physiological, biological and hematological parameters in a varied manner with both infections exhibiting an interaction that is both antagonistic and synergistic.

1. Co-infection enhanced survival rate of mice which occurred independent of parasitemia thus preventing lethal experimental malaria in majority of mice, and prevented hepatosplenomegaly.
2. Enhanced immunomodulatory role of co-infection against pro-inflammatory cytokines at protein levels, and decelerated liver pathology as shown by significant decrease in Alkaline phosphatase enzyme that is associated with liver pathology.
3. Co-infection with *S. mansoni* and *Pb*A ameliorated oxidative stress with concomitant attenuation of organ injury hence reinforcing protection observed in experimental malaria in the current study.

Overall, this study demonstrates that chronic *S. mansoni* infection is critical in regulation of *Pb*A infection associated severity & pathological events in a mouse model

6.2 Recommendations

The following are the recommendation from this study:

1. To perform proteomic analysis to determine the differences in protein expressed profiles among the different experimental groups.
2. To check the differential gene expression during singular infection and in co-infections

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APPENDIX 1: ANTIPLAGIARISM REPORT

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