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Altered renal immune complexes deposition in female BWF1 lupus mice following *Plasmodium chabaudi* infection



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Abstract Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease that has a mysterious relationship with malaria infection. The current study was designated to compare between the effect of the live and the gamma irradiated *Plasmodium chabaudi* infection on BWF1 lupus murine model. A total of 30 female BWF1 mice were randomly divided into three groups (10 mice/group) as follows: group (I) lupus group (lupus non infected); group (II) live malaria infected group (lupus + live malaria infection); and group (III) irradiated malaria-infected group (lupus + gamma irradiated malaria infection). Live *P. chabaudi* infection was accompanied with a decrease in survival rate and food consumption in comparison to the control group of mice while gamma irradiated *P. chabaudi* -infection was unable to do this effect. Additionally, live *P. chabaudi* infection was accompanied with an increased level of proteinuria and increased rate of immune complexes deposition in kidney. Moreover, infection with live, but not gamma-irradiated *P. chabaudi* was accompanied with an increase in nitric oxide (NO), hydrogen peroxide (H₂O₂), and malondialdehyde (MDA) levels in plasma of lupus mice. The levels of both total cholesterol and triglycerides in plasma of lupus mice after live *P. chabaudi* infection were obviously decreased in comparison to the control group. On the other hand, gamma-irradiated *P. chabaudi* infection

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resembled the control group. Our data revealed that infection of lupus mice with live but not gamma-irradiated *P. chabaudi* has several histological and biochemical effects.

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1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease of unknown etiology, and for which approved therapies are inadequate (Perl, 2013). SLE is characterized by increased production of auto reactive antibodies and deposition of immune complexes in renal tissue, which represent the main pathological features of lupus (Yung et al., 2013). Increased proteinuria represents another characteristic feature of the disease (Birmingham et al., 2008). A key issue in the pathogenesis of lupus is how intracellular antigens become exposed and targeted by the immune system (Danченко et al., 2006). In this regard, excessive production of reactive oxygen species (ROS), redox imbalance (Graham and Utz, 2005) and a defect in apoptosis (Kurien and Scofield, 2003) are considered as imperative factors involved in the development of antibody flares and various other clinical features in SLE (Shah et al., 2011). ROS represent a part of the defense mechanisms against microbes in the body, but increased lipid peroxidation and ROS generation has the potential to initiate damage to lipids, proteins and DNA (Grimsrud et al., 2008), and they have been reported to promote inflammation, necrosis, and apoptosis in chronic kidney disease (Al-Quraishy et al., 2013). There is some evidence indicating that lipid peroxidation can also affect progression of atherosclerosis, cardiovascular diseases (CVDs) and glomerulonephritis in SLE patients (Bahrehmand et al., 2014). Triglycerides and cholesterol are both lipids that circulate in the bloodstream and are escorted through the blood vessels by lipoproteins. Indeed, lipids have a direct effect on coronary events in lupus patients (Nikpour et al., 2013). The lipid profile in lupus patients is characterized by increased total cholesterol and triglycerides and this lupus associated dyslipidemia is well-documented (Ortiz et al., 2013). Additionally, lipid metabolism is directly related to the immune status in SLE and it has been found that resetting lipid metabolism restores T cell function in SLE (Kidani and Bensinger, 2014).

Tropical infections, particularly malaria, have a mysterious relationship with SLE (Clatworthy et al., 2007). Clinical and epidemiological studies have revealed that SLE is rarely observed in rural tropical areas of Africa and Asia, where malarial infection is prevalent (Minaur et al., 2004), and it has therefore been hypothesized that SLE-susceptibility genes are beneficial in controlling severe malaria but promote inflammation in the absence of malaria (Greenwood and Corrah, 2001). In this regard, Greenwood et al. (1970) described a higher survival rate in young lupus-prone mice infected with *Plasmodium berghei yoelii*. Another study revealed that old BWF1 mice, when infected with *Plasmodium chabaudi* at the onset of clinical signs of lupus, and then subsequently treated with chloroquine, developed temporary remission of symptoms. Moreover, the injection of immunoglobulins isolated from *P. chabaudi*-infected BALB/c mice produced similar protective effects as the infection itself in BWF1 mice (Hentati et al., 1994). Our previous study

revealed that infecting lupus mice with *P. chabaudi* confers protection against lupus nephritis by altering the redox state in the kidneys and the liver (Al-Quraishy et al., 2013). The redox state change in plasma is to some extent different, however, and hence this study aimed to investigate the possible beneficial and harmful effects of *P. chabaudi* infection upon the renal histological, lipid profile and oxidative stress changes induced by SLE in the murine lupus model.

2. Materials and methods

2.1. Animals

A total of 30 female BWF1 29-week-old mice were purchased from Jackson Laboratory (Bar Harbor, USA) and maintained and monitored in an environment which is specific pathogen-free (SPF). All animal procedures were performed in accordance with the standards set out in the Guidelines for the Care and Use of Experimental Animals issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the Animal Ethics Committee at King Saud University. All animals were allowed to acclimatize in plastic cages (five animals per cage) inside a well-ventilated room for one week prior to the experiment. The animals were maintained under standard laboratory conditions (temperature of 23 °C, relative humidity of 60–70% and a 12-h light/dark cycle), fed a diet of standard commercial pellets and given water *ad libitum*.

2.2. Malarial infection

The blood stage forms of *P. chabaudi* parasites were stored in liquid nitrogen after *in vivo* passage in 3-month-old BALB/c mice according to a previously described protocol (Hentati et al., 1991). Female BWF1 mice (30-week-old) were infected by an intraperitoneal (i.p) injection of 10^6 parasitized erythrocytes obtained from an infected mouse of the same strain as previously described (Hentati et al., 1994). For monitoring of parasitemia, Giemsa-stained thin blood smears were prepared and investigated under the light microscope. Experimental animals were divided into three groups (10 mice/group) as follows: group (I) Control group (lupus uninfected with *P. chabaudi*); group (II) live *P. chabaudi* – infected group (Lupus + live *P. chabaudi* infection); and group (III) irradiated *P. chabaudi* – infected group (Lupus + irradiated *P. chabaudi* infection). Group III was infected i.p. with 10^6 gamma-irradiated RBCs infected with *P. chabaudi*. Prior to injection, the blood cells were exposed to a dose of 200 Gy gamma-radiation from a Gamma Cell 200 Irradiator (Atomic Energy of Canada, Ltd., Ottawa, Canada) utilizing a ^{60}Co source located at the Research Center of the College of Science, King Saud University, Saudi Arabia. The chosen radiation dose was applied based on experiments conducted by Ferreira-da-Cruz

Mde et al. (1997) that provided evidence that a 200-Gy gamma-irradiation dose was able to abolish the original replication of erythrocytic forms of the Palo Alto *P. falciparum* strain, most likely by inactivating their infectivity. According to their data, 100- or 150-Gy irradiation doses were unable to inactivate the parasite, despite a reduction in the parasitemia. All animals were sacrificed on day 14 post-infection.

2.3. Sample collection

For plasma samples preparation, blood was collected from the heart in heparinized tubes and plasma was obtained for biochemical investigations. Plasma was stored at -80°C until use. For histological investigation, the kidneys were removed and cut into small pieces in sterile saline. The pieces were fixed in 10% neutral buffered formalin and then embedded in paraffin.

2.4. Immunohistochemical detection of IgG immune complexes in kidney

For Immunohistochemical localization of IgG immune deposits, paraffin sections were cleared in xylene, rehydrated in descending grade ethanol solutions (100–70%), immersed in water for 5–10 min, and incubated in 0.3% H_2O_2 /70% methanol for 20 min in order to inhibit endogenous peroxidase activity. The specimens were then rinsed in phosphate buffered saline (PBS) three times ($3\times$) for 5 min. Epitopes were unmasked by boiling in a citrate buffer (pH 6.0) for 10–15 min. Sections were blocked for 30–60 min in 3% bovine serum albumin (BSA) dissolved in PBS followed by incubation with anti-IgG primary antibody (Sigma–Aldrich, USA) in PBS-buffered 0.1% BSA overnight at 4°C in a humidified chamber. The samples were then rinsed in PBS and incubated with 7.5 g/ml of the biotinylated secondary antibody in PBS buffered 0.1% BSA for 1 h at room temperature, followed by avidin/biotin amplification (ABC Elite) for 30 min, before developing with 3,3-diaminobenzidine peroxidase substrate. Sections were counterstained with Mayer hematoxylin for between 2 and 5 min.

2.5. Assessment of food consumption and survival rate

Daily food intake was measured (in grams) for each animal in each experimental group using metabolic cages (Tecniplast®) that are designed for the immediate and complete separation of urine and feces, allowing their quantitative and reliable collection over 24 h periods. Clinical progression of symptoms of infection was monitored daily and the survival pattern for all groups was calculated and compared with age-matched *P. chabaudi*-infected female BALB/c mice using sufficient number of animals in each group (20 animal/group).

2.6. Assessment of proteinuria

For the quantitative determination of proteinuria, the Biuret method for total protein determination was applied. Cupric ions react with protein in alkaline solution to form a purple complex the absorbance of which is directly proportional to the protein concentration in the sample. Briefly, 20 μl of urine and 20 μl

of standard solution (provided by the kit) were put into tubes, and 1.0 ml of color reagent solution was then added. The tubes were mixed well and incubated at 25°C for 10 min. The color development was detected at 500 nm with a Beckman DU 640 spectrophotometer (Beckman Coulter, Fullerton, CA).

2.7. Assessment of cholesterol and triglyceride levels in plasma

Total cholesterol and triglyceride levels were measured by an enzymatic colorimetric kit (Wako Chemicals USA, Inc.). Briefly, 10 μl of plasma were put into tubes and 1 ml of color reagent solution was then added. 10 μl of standard solution (provided by in the kit), were put into tubes and 1 ml of color reagent solution was then added. The solution was mixed well and incubated at 37°C for 5 min. The color development was detected at 500 nm with a Beckman DU 640 spectrophotometer.

2.8. Oxidative stress assessment

Oxidative stress markers were measured in plasma using commercial kits (Biodiagnostic, Dokki, Giza, Egypt) as follows:

Nitrite/nitrate was assayed according to the technique of Berkels et al. (2004). In brief, nitrous acid is formed in an acid medium and, in the presence of nitrite, diazotizes sulfanilamide, which is coupled with N-(1-naphthyl) ethylene diamine, and the resulting azo dye is then measured at 540 nm.

Lipid peroxidation was determined by the method of Ohkawa et al. (1979). The plasma was suspended in 1 ml of 10% trichloroacetic acid and 1 ml of 0.67% thiobarbituric acid boiled in a water bath for 30 min. Thiobarbituric acid reactive substances are measured at 535 nm and expressed as malondialdehyde (MDA) equivalents formed.

Hydrogen peroxide (H_2O_2) was assayed according to Aebi (1984). In the presence of horse radish peroxidase (HRP), H_2O_2 in plasma reacts with 3,5-dichloro-2-hydroxybenzenesulfonic (DHBS) acid and 4-aminophenazone (AAP) to form a chromophore that can be quantified at 240 nm.

2.9. Statistical analysis

Prior to further statistical analysis, the data were tested for normality using the Anderson–Darling test, as well as for homogeneity variances. The data were normally distributed and are expressed as mean \pm standard error of the mean (SEM). Significant differences among the groups were analyzed by a one-way ANOVA followed by Bonferroni's test for multiple comparisons using PRISM statistical software (GraphPad Software). The data were also reanalyzed by a one- or two-way ANOVA followed by Tukey's post-test using SPSS software, version 17. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Female BWF1 mice have a lower survival rate after infection with live *P. chabaudi* in comparison to both the control and the gamma irradiated *P. chabaudi*-infected groups

As shown in Fig. 1, infection of female BWF1 mice with live *P. chabaudi* was accompanied by an increase in survival rate

compared to the live *P. chabaudi*-infected female BALB/c strain. The gamma irradiated *P. chabaudi*-infected group (group III), and the control group (group I), however, each had higher survival rates than the live *P. chabaudi*-infected group (group II).

3.2. Infection of female BWF1 mice with live *P. chabaudi* was accompanied by a reduction in food consumption

In the current study, food consumption was severely impaired in the live *P. chabaudi*-infected group in comparison to both the control and the gamma irradiated *P. chabaudi*-infected group, as shown in Fig. 2. Indeed, by day 13 p.i. there was complete anorexia (no food intake), and severe hypokinesia was obvious in all the animals in the live *P. chabaudi*-infected group. On the other hand, the gamma irradiated *P. chabaudi*-infected group has exhibited a normal pattern of food consumption that was similar to that of the control group.

3.3. Lupus associated proteinuria in BWF1 mice increased after infection with live *P. chabaudi*

The quantitatively determined protein concentration in the urine of the three groups is shown in Fig. 3. Proteinuria was significantly ($P < 0.05$) higher in the live *P. chabaudi* infected group (group II) (278.96 ± 12.7 mg/dl) compared to both the control group (191.55 ± 71.6 mg/dl) and the gamma irradiated *P. chabaudi*-infected group (193.8 ± 37.3 mg/dl). Proteinuria was similar in the gamma irradiated *P. chabaudi*-infected

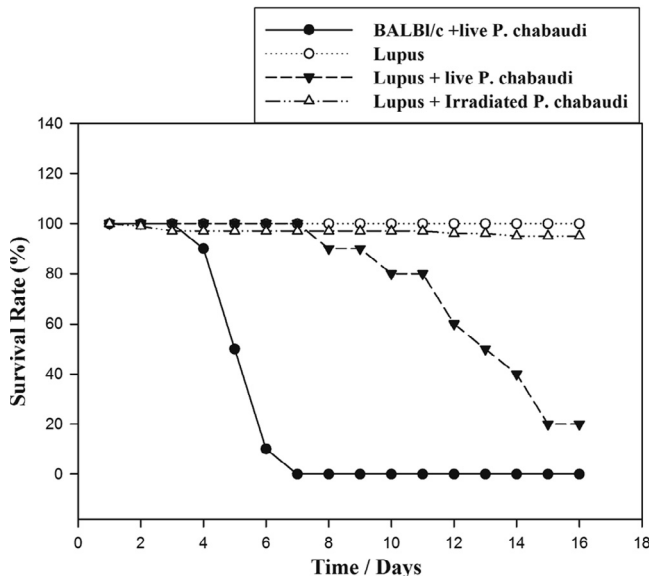


Figure 1 Effect of *P. chabaudi* infection on survival rate of female BWF1 mice. Follow up started at the first day of *P. chabaudi* infection for both BALB/c and BWF1 strains. From day 4 to day 14, all of control, live *P. chabaudi* infected and gamma irradiated *P. chabaudi* infected BWF1 groups exhibited a higher survival rate than BALB/c group. Among BWF1 groups, live *P. chabaudi* infected group exhibited the lowest survival pattern in comparison to either control or gamma irradiated *P. chabaudi* infected group. The accumulated data from 10 individual female mice from each group are expressed.

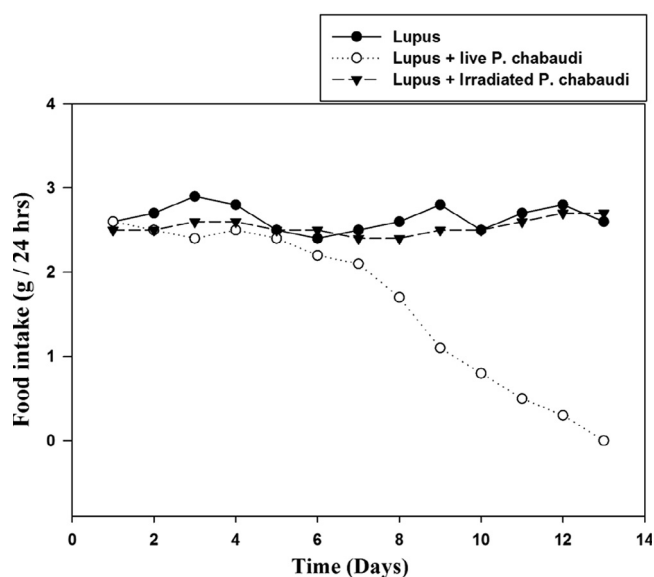


Figure 2 Effect of *P. chabaudi* infection on food intake in female BWF1 mice. Beginning from the third day of *P. chabaudi* infection, food consumption (g/24 h) in live *P. chabaudi* infected group was lower than that in either control or gamma irradiated *P. chabaudi* infected group. By the day 13 post infection, food intake has reached zero level in live *P. chabaudi* infected group. The accumulated data from 10 individual female mice from each group are expressed.

group (group III) to that of the control group (group I), even though the control group is already characterized by increased proteinuria as a clinical sign of the disease.

3.4. Changed pattern of immune complexes deposition in renal tissue following infection of BWF1 mice with live *P. chabaudi*

Since autoantibody-induced immune complexes deposition in the kidneys is considered to be the primary cause of lupus nephritis, IgG deposits were measured in the kidney. IgG immune complexes deposition in the glomerulus of the control group was clearly detected (Fig. 4a). When compared with the control group, IgG immune complexes deposition in the glomerulus of the live *P. chabaudi* infected group was higher (Fig. 4b). However, the gamma irradiated *P. chabaudi* infected group showed a similar degree of glomerular IgG immune complexes deposition to that of the control group (Fig. 4c).

3.5. Live *P. chabaudi* infection caused hypocholesterolemia and hypotriglyceridemia in female BWF1 mice

Fig. 5a demonstrates the cholesterol lowering effect associated with infection with live *P. chabaudi* (228.99 ± 16 mg/dl) in comparison to the control group (263.71 ± 8.6 mg/dl). This significant ($P < 0.05$) effect was not observed in the group infected with gamma irradiated *P. chabaudi* (260.04 ± 3.6 mg/dl). Also, the triglyceride level was significantly ($P < 0.05$) decreased in the group infected with live *P. chabaudi* (165.69 ± 6.6 mg/dl) in comparison to the control group (189.08 ± 5.5 mg/dl), while in the group infected with gamma irradiated *P. chabaudi*, the level of triglycerides (184.87

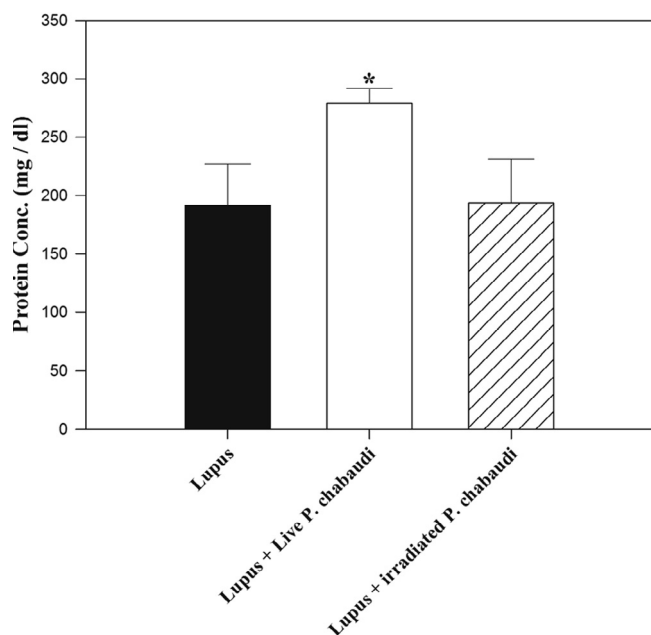


Figure 3 Effect of *P. chabaudi* infection on proteinuria in female BWF1 mice. Protein concentration (mg/dl) in urine was determined quantitatively through Biuret method. Urine samples were taken from control, live *P. chabaudi* infected and gamma irradiated *P. chabaudi* infected group. The data are given as mean \pm SEM for 10 mice per group. * $P < 0.05$ for live *P. chabaudi* infected BWF1 mice vs. control.

± 4.9 mg/dl) still resembled that of the control group (Fig. 5b).

3.6. Live *P. chabaudi* increased lipid peroxidation and oxidative stress in plasma of BWF1 mice

The effect of malarial infection on oxidative stress markers in the SLE experimental model was measured by determining the levels of NO, H₂O₂, and MDA in plasma from the three experimental groups of female BWF1 mice. A significant increase ($P < 0.05$) in the level of NO was observed in the group infected with live *P. chabaudi* compared to the control group, but there was a significant decrease ($P < 0.05$) in the level of NO in the group infected with gamma-irradiated *P. chabaudi*, compared to the group infected with live *P. chabaudi* (Fig. 6c). On the other hand, the levels of both MDA (Fig. 6a) and H₂O₂ (Fig. 6b) were each slightly increased in the group infected with live *P. chabaudi* compared to the control group, while in the group infected with gamma-irradiated *P. chabaudi*, the level of both parameters resembled that in the control group, with no significant change.

4. Discussion

SLE is a prototypic autoimmune disease that can affect essentially any organ or tissue (Doria and Gatto, 2012). One of its most severe manifestations is lupus nephritis (LN), which remains a cause of substantial morbidity and mortality (Watson et al., 2012). Malaria, meanwhile, is one of the major tropical diseases. This parasitic infection results in 200–300

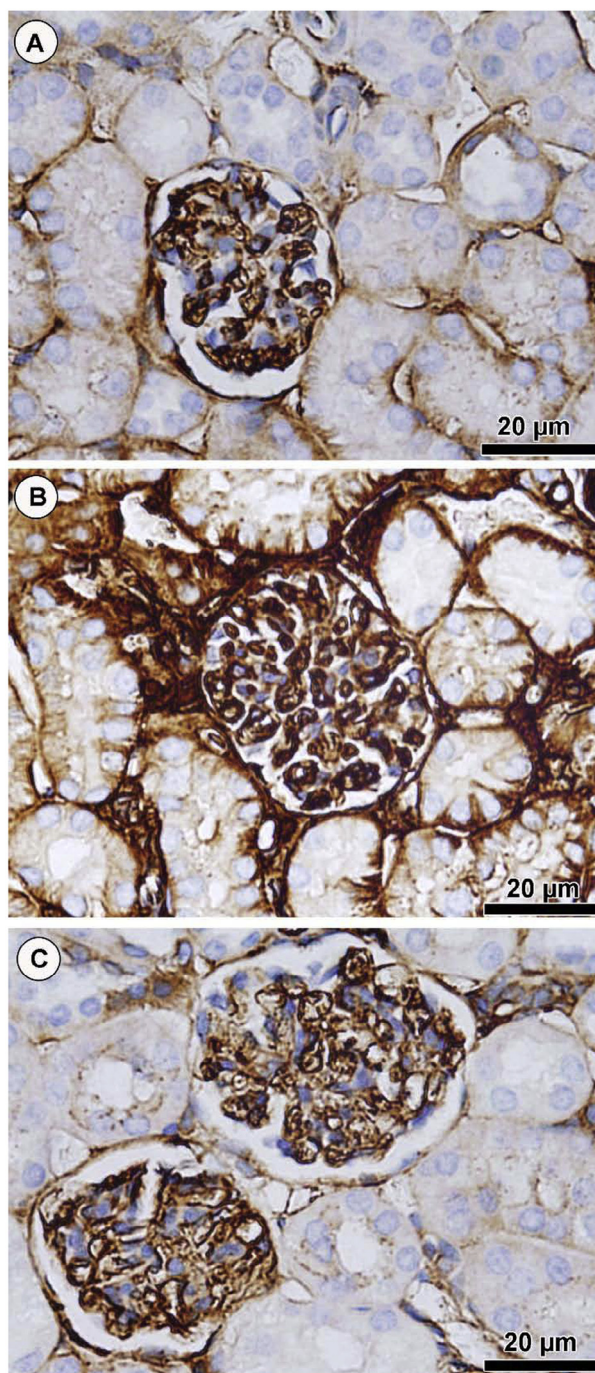


Figure 4 Renal histological alterations due to *P. chabaudi* infection in BWF1 mice. Immunohistochemical staining of renal tissue sections of uninfected control (A), live *P. chabaudi* infected (B), and gamma irradiated *P. chabaudi* infected (C) group ($n = 10$). Sections were stained with anti-IgG antibodies and analyzed under light microscopy.

million clinical cases and 1–2 million deaths annually (Kesteman et al., 2014). Most studies have targeted infectious agents and their role in the development of autoimmune diseases (Muñoz-Grajales and Pineda, 2015; Anaya et al., 2016). Recently, however, many studies have suggested the opposite outcome, which is the prevention or amelioration of

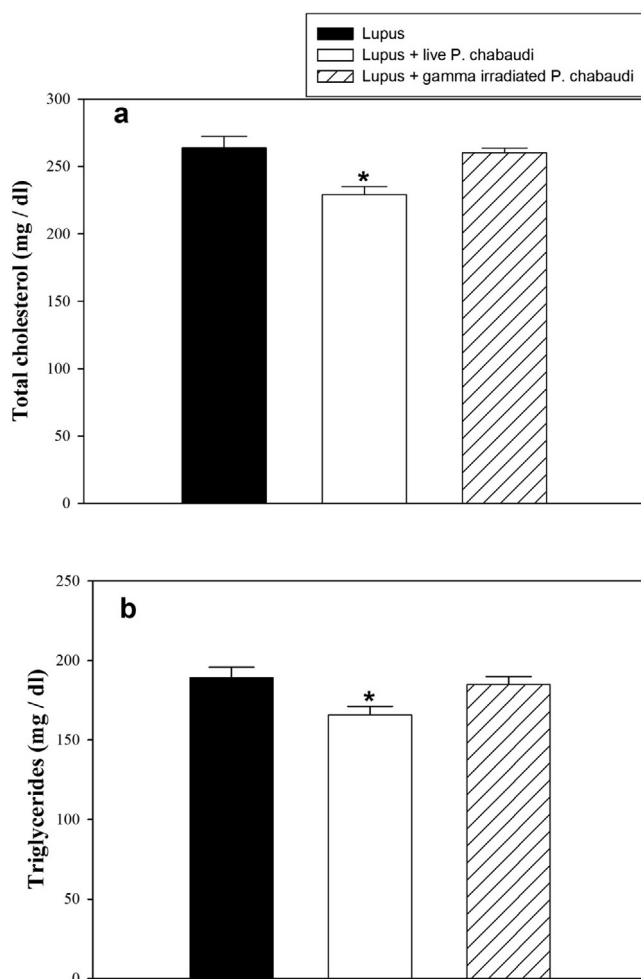


Figure 5 Effect of *P. chabaudi* infection on lipid profile in plasma of female BWF1 mice. (a) Cholesterol concentration (mg/dl). (b) Triglycerides concentration (mg/dl) in plasma samples of control, live *P. chabaudi* infected and gamma irradiated *P. chabaudi* infected group. The data are given as mean \pm SEM for 10 mice per group. * $P < 0.05$ for live malaria infected BWF1 mice vs. control.

autoimmune processes following microbial infection (Praprotnik et al., 2008; Kivity et al., 2009). In the present study, we have clearly demonstrated that female BWF1 mice infected with live *P. chabaudi* have a higher survival rate than similarly infected BALB/c mice. Waisberg et al. (2011) have reported that a genetic susceptibility to SLE protects against cerebral malaria in mice and that protection appears to be by immune mechanisms that allow SLE-prone mice better to control their overall inflammatory responses to parasite infections. Hentati et al. (1991) have reported that infection of BALB/c mice with the parasite *P. chabaudi* induces a high production of natural auto antibodies. Here, clinical infection of lupus-prone female BWF1 mice with live malaria may decrease the plasma level of these auto reactive antibodies and consequently increase their survival. It seems, however, that gamma irradiation of the parasite serves to diminish its fecundity because the group infected with gamma-irradiated *P. chabaudi* exhibited nearly the same survival pattern as the lupus control

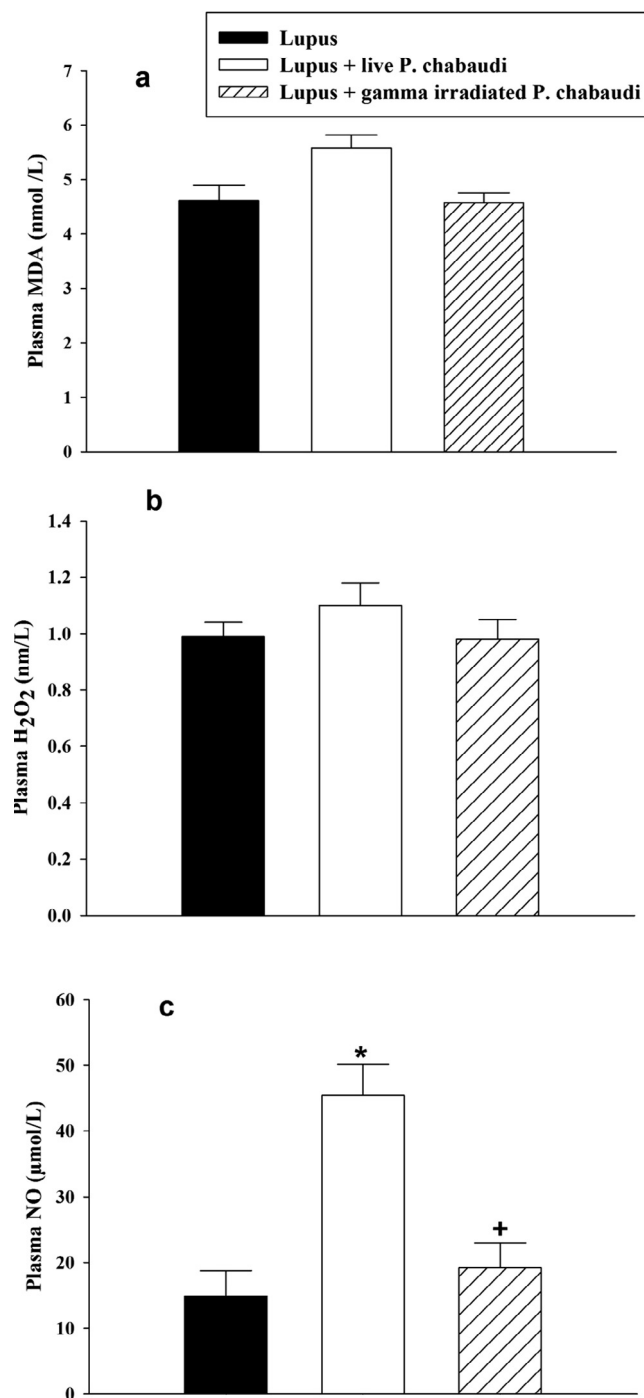


Figure 6 Effect of *P. chabaudi* infection on the plasma level of MDA, NO and H₂O₂ in female BWF1 mice. The levels of MDA (a), H₂O₂ (b) and NO (c) in plasma of BWF1 lupus mice after experimental infection with either live or gamma irradiated *P. chabaudi* in comparison to the lupus uninfected group. The data are given as mean \pm SEM for 10 mice per group * $P < 0.05$ for live *P. chabaudi*-infected BWF1 mice versus lupus; + $p < 0.05$ for gamma-irradiated *P. chabaudi*-infected BWF1 mice versus lupus mice.

group. Following infection with live *P. chabaudi* reduced food intake was observed in the BWF1 mice, which is a well-known symptom of rodent malaria. Basir et al. (2012) reported a

decrease in body weight of malarial mice that was clearly evident from the third day of infection, and this was attributed in part to the decrease in food intake. Again, however, the group infected with gamma-irradiated *P. chabaudi* did not suffer this decrease in food intake and showed a normal pattern of food consumption.

Kidney function (as indicated by proteinuria assessment) was not significantly improved after infection with live *P. chabaudi*, in agreement with the study of Greenwood et al. (1970), who reported a slight decrease in kidney function post malaria infection. Once again, the group infected with gamma-irradiated *P. chabaudi* still resembled the control group data. Deposition of immune complexes (ICs) is considered to be an important pathogenic mechanism in SLE (De Zubiria Salgado and Herrera-Diaz, 2012). Granular deposits of immunoglobulins and complementary components are presumptive evidence of IC and are frequently found in the renal glomeruli of SLE patients. There is a direct correlation between immune complex levels and clinical disease activity (Cozzani et al., 2014). In the present study, the live *P. chabaudi* infected group exhibited an increased deposition of IgG antibodies in renal tissue sections as compared to the control. In contrast, the group infected with gamma irradiated *P. chabaudi* showed a similar level of ICs to that of the control. Since female BWF1 mice have elevated triglycerides compared to non-lupus mice (Jacobson et al., 1989), hypertriglyceridemia is considered to be a pathological sign in this model of murine lupus. In the present study, infection with live *P. chabaudi* decreased the level of plasma triglycerides while infection with gamma irradiated *P. chabaudi* resulted in plasma levels of triglycerides that were similar to that of the control group. Mishra et al. (2010) have postulated that increased cholesterol synthesis and increased triglyceride secretion are the molecular mechanisms of dyslipidemia in lupus mice. On the other hand, hypercholesterolemia and hypertriglyceridemia have been observed in both uncomplicated and complicated malaria (Bansal et al., 2005). In the present study, during live malaria infection, the increase in resting energy expenditure (REE) combined with a low energy intake due to anorexia are important factors that may explain the observed decrease in the plasma levels of both cholesterol and triglyceride in the live *P. chabaudi* infected group of mice in comparison to the control group. Increased oxidative stress has emerged as playing a central role in the initiation and progression of many autoimmune diseases. Therefore, several studies have provided evidences for successful use of natural antioxidants in the prevention and treatment of the oxidative stress-mediating immune complications (Badr et al., 2012). The production of ROS and RNS during lupus is considered to be an important part of the innate immune response against the disease (Oates and Gilkeson, 2006), but they also represent an important aspect of the host defense against a variety of pathogens (Zahrt and Deretic, 2002). In the current study, infection with live *P. chabaudi* increased the level of NO, H₂O₂ and MDA in plasma of BWF1 lupus mice and this can be considered as anti malarial defense mechanism (Aguilar et al., 2014). Conversely, in the group infected with gamma irradiated *P. chabaudi*, the levels of these molecules were not significantly changed in comparison to that of control group which means that parasite viability is necessary for such malaria-induced changes. In our previous study (Al-Quraishy et al., 2013), the change in hepatic level of MDA, NO and H₂O₂ after live *P. chabaudi* infection

had, to some extent, a similar pattern to that in plasma samples in the current study whereas both of MDA and H₂O₂ have shown insignificant changes in comparison to the control group. As the life cycle of malaria occurs in liver and blood, not surprisingly the response of both of them is to some extent similar. Taken together, our data illustrate that infection of female BWF1 lupic mice with live *P. chabaudi* has several effects on the pathological signs of SLE while the parasite gamma irradiation can abolish these effects.

5. Conclusion

Our data demonstrate that infection with live *P. chabaudi* can affect, the nutritional status, the kidney function, the renal IgG immune complexes deposition, the plasma lipid profile and the plasma levels of oxidative stress parameters. Such effects are totally different when using Gamma irradiated parasite instead of a live one.

Authors' contributions

MAM put the design of the experiment and carried out all the lab work, preparing the figures and drafted the manuscript. FAG participated in the design of the study and helped to draft the manuscript. AEA participated in the design of the study and helped to draft the manuscript. GB participated in the design of the study, participated in the figures preparation and helped to draft and edit the manuscript. SQ participated in the design of the study, helped to perform the statistical analysis, funded the animal model and helped to draft the manuscript. All authors read and approved the final manuscript.

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