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LETROZOLE ATTENUATES CARDIOMETABOLIC RISK IN *PLASMODIUM BERGHEI*-INFECTED MICE

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Malaria is associated with cardiometabolic disorders, promoting the risk of cardiovascular disease (CVD). We recently demonstrated letrozole's cardioprotective effects in fructose-exposed male rats. Hence, in this study, we investigated the effect of a low-dose letrozole against cardiometabolic risk in Plasmodium berghei-infected female mice. Twenty female mice were randomly grouped into four (n=5/group): uninfected, infected, letrozole (0.24 mg/kg, p.o/day, without infection), and infected + letrozole. Weekly percentage parasitaemia was recorded. At the end of the 21-day exposure, blood and liver were collected and processed for biochemical analyses. P. berghei infection decreased serum estradiol level after 21-day infection and increased serum and liver levels of malondialdehyde, very low-density lipoprotein cholesterol, triglycerides, and triglycerides/high-density lipoprotein cholesterol (TG/HDL-c) index. Similarly, P. berghei elevated serum uric acid and hepatic total cholesterol levels. Meanwhile, the administered dose of letrozole did not significantly affect serum estradiol but lowered lipid peroxidation, attenuated lipid alterations, and reduced serum uric acid level. We reveal that P. berghei-infection lowered serum estradiol and promoted cardiometabolic risk in female mice, while letrozole lowered parasitaemia and mitigated the associated cardiometabolic risk. Thus, this study is suggestive of letrozole's potential as an adjuvant therapy for improved management of malaria-induced cardiometabolic complications. Further study is recommended to investigate the anti-malaria potency of letrozole, independent of gender.

***Key words**: Letrozole; malaria; cardiovascular risk; cardiometabolic disorder; CVD*

INTRODUCTION

The increased incidence of cardiometabolic disorders (CMD) poses a serious threat to public health, leading to more global mortality rate[1]. Changes in diet and lifestyle are the major causes of CMD, promoting metabolic predisposing factors for cardiovascular disease (CVD)[2&3]. The prevalence of CVD has almost doubled from 271 million in 1990 to 523 in 2019, accounting for more death and disability than any other disease throughout the world[4]. While CVD is common to both males and females, epidemiological data show that premenopausal women have less incidence of CVD than men[5].

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This advantage is largely attributed to the female estrogen as disease risk is higher in postmenopausal women than men of the same age[6].

Many observational studies have established a link between malaria and cardiometabolic risk[7-9]. Malaria is a life-threatening, mosquito-borne parasitic infection with an increasingly widespread burden. Symptoms of malaria vary from mild uncomplicated conditions to severely complicated states and even death. The severity of infection largely depends on factors such as *Plasmodium* sp. involved, age, and host immunity[10]. According to WHO[11], malaria alone accounted for about 400,000 deaths worldwide in 2018, about 85 % of which occurred in sub-Saharan Africa and Asia continent where *P. falciparum* is more common. Specifically, infection with *P. falciparum* causes serious damage to several body organs such as the liver, lung, kidney, and brain[11]. Similar to CVD, sex difference exists in malaria. Men have higher malaria-related deaths than their counterpart women[12]. This sexual dimorphism in malaria reflects differences in immune responses to infection, which are influenced by sex hormones as women generally show a better immune response to parasites than males[12], suggesting a protective role for oestrogen against malaria.

Despite the increasing report of concomitant cardiovascular deaths in malaria-endemic regions, the relationship between malaria and cardiometabolic diseases is poorly investigated[13]. Nonetheless, the involvement of oxidative stress in malaria is well documented[14&15], while we and others have recently reported hyperuricemia as well as dyslipidemia in both rodent and human subjects of malaria[15-17]. Oxidative stress, dyslipidemia, and hyperuricemia are all major factors for cardiometabolic diseases; thus, these previous studies have uncovered the importance of cardiovascular risk in malaria.

Letrozole is a third-generation, highly selective aromatase inhibitor that is considered for breast cancer treatment[18]. Although the cardiovascular consequence of letrozole treatment remains unresolved[19&20], we recently showed that it ameliorates fructose-induced hyperlipidemia, hyperuricemia, hepatic oxidative stress, and hepatic lipid accumulations[21&22]. We, therefore, hypothesized that letrozole may be protective against malaria-associated cardiometabolic risk in female mice infected with *P. berghei*. Considering the positive effect of estrogen on the cardiovascular system and in female malaria subjects, we used a low dose of letrozole that was postulated not to alter estrogen biosynthesis but which would, at the same time, be beneficial in rodent malarial infection.

MATERIALS AND METHODS

Experimental animals

Twenty *Plasmodium*-free female albino mice (age = 16 weeks; average weight = 24g) were used for this study. The mice were obtained from the Department of Biochemistry, and housed in the animal house facility, Department of Zoology, University of Ilorin, Ilorin, Nigeria. They were maintained under standard environmental conditions and allowed to acclimatize for 14 days, with free access to food and water. The Animals were carefully handled following the rules and regulations of the Animals Care and Use Committee (ACUC) and in conformity with the guidelines provided by the Institutional Ethical Review Board (UERC/ASN/2019/513).

Parasite inoculation

Plasmodium berghei (NK-65)-donor mice were procured from the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. Blood from the donor mice was mixed with normal saline in the ratio 1:10, respectively, as previously reported[23]. *Plasmodium*-free mice were then inoculated intraperitoneally with 0.2ml of stock inoculum ($1x10^7$ infected erythrocytes). Parasite estimation was done by preparing thin blood film from the caudal tip of the animals, stained with Giemsa stain, and viewed under ×100 objective of a light microscope to confirm parasite growth. Estimation of parasitemia was done according to the previous study[24]. Treatments commenced upon confirmation of sufficient parasite growth (Week 0) and continued for 3 weeks.

Letrozole preparation

Letrozole (Sun Pharma, India) was commercially obtained, powdered, and

prepared in distilled water at a dose of 0.24mg/kg body weight.

Animal grouping and treatment

The mice were randomly grouped into four groups (n=5/group) as follows: uninfected (received 0.2ml distilled water), infected (infected with *P. berghei* and received distilled water), letrozole only (received 0.24 mg/kg letrozole) and infected + letrozole (infected with *P. berghei* and treated with 0.24 mg/kg letrozole).

Sample preparation

At the end of the 3-week experiment, three mice from each group were sacrificed by cervical dislocation, and blood was collected through the cardiac puncture into the plain bottle and centrifuged at 3000 revolutions per minute for 5 minutes. The serum was stored frozen until needed for biochemical assays. The liver was excised, rinsed with normal saline, and homogenized mechanically in ice-cold 0.25 M sucrose solution. The resulting homogenates were kept frozen overnight before being processed further for biochemical assays.

Estimation of serum 17β-estradiol level

The concentration of 17β-oestradiol was estimated in the serum of each mouse by enzyme-link immunosorbent assay (ELISA), using a kit (CAlbiotech company, USA, Catalog No: ES180S-100). The assay's procedures were carried out in accordance with the manufacturer's instructions.

Lipid profile test and malondialdehyde estimation

Serum and tissue total cholesterol (TC) and triglycerides (TG) concentrations were measured using enzymatic analysis, using an assay kit obtained from Fortress Diagnostics Ltd. (Antrim, United Kingdom). High-density lipoprotein-cholesterol (HDL-C) was measured by enzymatic clearance assay (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) in both serum and tissue, while concentrations of low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-c) were determined using modified Friedewald's formula[25]. Serum and tissue levels of malondialdehyde (MDA) were estimated by colorimetric method using an assay kit obtained from Fortress Diagnostics, Ltd. (Antrim, United Kingdom), following the manufacturer's instruction

Estimation of uric acid and xanthine oxidase activity

A nonenzymatic colorimetric assay kit, obtained from Oxford Biomedical Research Inc., Oxford, USA, was used to estimate serum uric acid (UA) following the manufacturer's instructions, while the activity of xanthine oxidase (XO) was determined by the standard enzymatic colorimetric method using reagents obtained from Fortress Diagnostics Limited, Antrim, UK.

Statistical analysis

All data were subjected to statistical analysis using GraphPad Prism software version 8.0 (GraphPad Software, USA) and presented as mean ± SEM. One-way analysis of variance (ANOVA), followed by Tukey post-hoc, was used to compare the mean values among the groups. The significant difference was determined at a 95% confidence level and $p < 0.05$, $p < 0.01$, and $p < 0.001$ were considered statistically significant.

RESULTS AND DISCUSSION

Results

Anti-plasmodial test

The pattern of percentage parasitemia, as well as parasite inhibition, is shown in Table 1. There was a progressive increase in parasitemia in the infected group (untreated) from Week 0 to Week 3 (19.4 % Week 0 vs 53.2 % Week 3), while letrozole suppressed the parasite growth steadily (20.1% vs 8.3% Week3), with percentage parasite inhibition of 58.7 %.

Effect of letrozole on serum oestradiol in female Plasmodium berghei-infected mice

There is evidence that estrogen is protective against CVD and malaria in women [6, 12], hence, we used a low dose of letrozole that might not suppress estrogen synthesis. As presented in Fig. 1, 0.24 mg/kg letrozole did not significantly alter serum oestradiol as compared with the uninfected group. In contrast, infection with *P. berghei* lowered ($p < 0.001$) serum estradiol when compared with uninfected mice as well as mice that received letrozole only.

Table 1: Anti-plasmodial effect of letrozole in female *Plasmodium berghei*-infected mice.

GROUPS	Parasitaemia (%)				Parasite Inhibition (%)
	Week 0	Week 1	Week 2	Week 3	
Uninfected	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.0
Infected	19.4 ± 0.35	24.9 ± 0.46***	44.8 ± 0.29***	53.2 ± 0.29***	0.0
Letrozole only	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.0
Infected +letrozole	20.1± 0.29	15.0 ± 0.17***	11.5 ± 0.19***	8.3 ± 0.34***	58.7

Data were expressed as mean ± SEM (n = 3). Data were analyzed by One-way analysis of variance (ANOVA) followed by the Tukey post hoc test. (***$p < 0.001$ vs Week 0 of the same group).

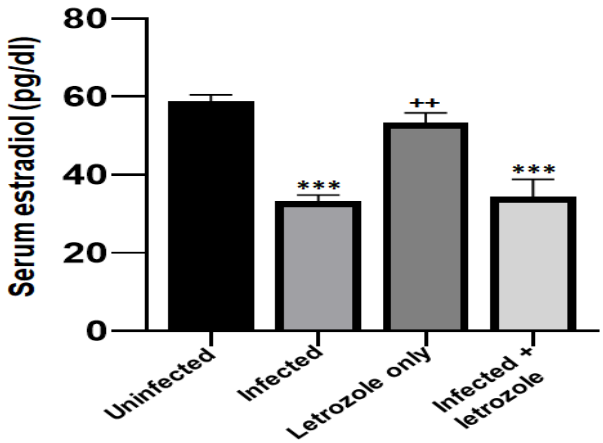

Fig. 1: Effect of *P. berghei* and letrozole on serum estradiol in female Albino mice. *P. berghei* lowered serum estradiol ($p < 0.001$). In contrast, 0.24 mg/kg letrozole had no significant effect on serum estradiol level. Data were expressed as mean ± SEM. n = 3. Data were analyzed by One-way analysis of variance (ANOVA) followed by the Tukey post hoc test. (***$p < 0.001$ vs uninfected; ++$p < 0.01$ vs infected).

Infection with P. berghei increases serum malondialdehyde in female mice

Oxidative stress is implicated in the pathogenesis of cardiometabolic disorders, therefore, the level of malondialdehyde (MDA) was assessed in both serum and liver of female *P. berghei*-infected mice, a marker of lipid peroxidation. As compared with the untreated group, our data showed that *P. berghei* but not letrozole significantly increased ($p < 0.05$) serum MDA and raised ($p > 0.05$) hepatic MDA (Fig. 2), while treatment with letrozole suppressed ($p < 0.05$) *P. berghei*-induced elevated MDA.

Effects of P. berghei and letrozole on serum and liver TC, HDL-c and LDL-c in female mice

Fig. 3 shows that neither *P. berghei* nor letrozole significantly changed ($p > 0.05$) serum total cholesterol (TC), serum or liver high-density lipoprotein cholesterol (HDL-c), and serum or liver low-density lipoprotein cholesterol (LDL-c) levels in female Albino mice. In contrast, *P. berghei* infection increased ($p < 0.01$) liver TC, while letrozole treatment in infected mice lowered ($p < 0.05$) liver TC.

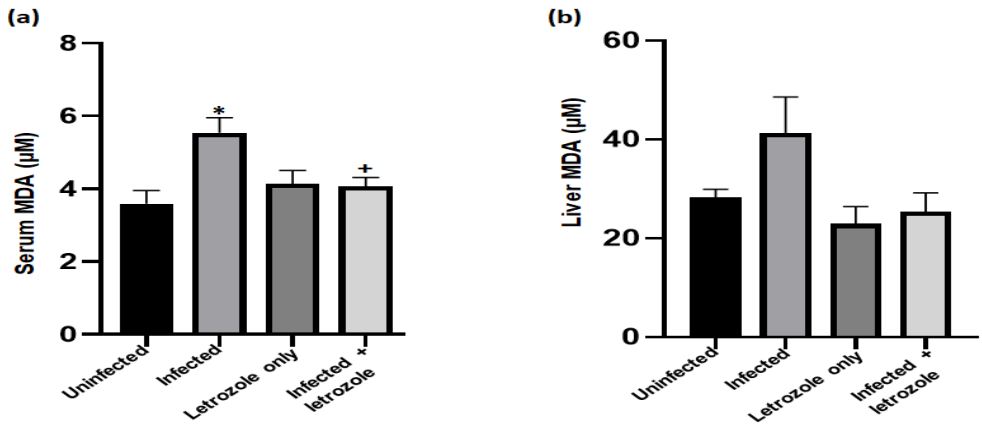

Fig. 2: Effect of *Plasmodium berghei* and letrozole on (a) serum malondialdehyde (MDA), and (b) liver MDA in female Albino mice. *P. berghei* but not letrozole elevated serum MDA ($p < 0.05$) as well as liver MDA ($p > 0.05$), whereas treatment with letrozole attenuated *P. berghei*-induced lipid peroxidation. Data were expressed as mean ± SEM. n = 3. Data were analyzed by One-way analysis of variance (ANOVA) followed by the Tukey post hoc test. (*$p < 0.05$ vs uninfected; +$p < 0.05$ vs infected).

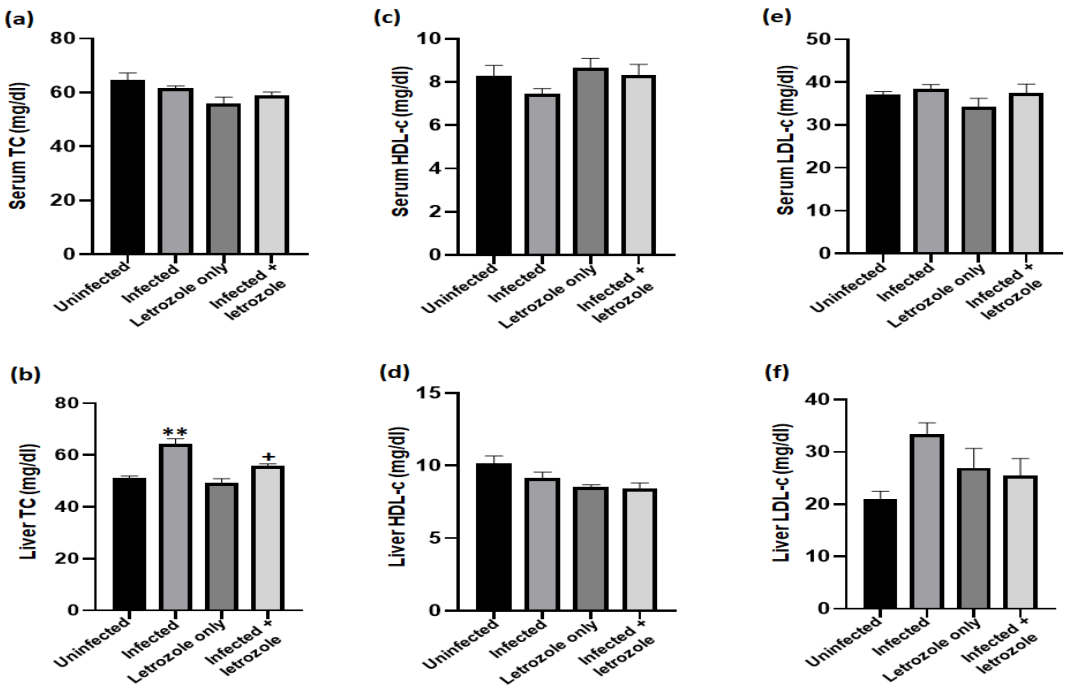

Fig. 3: Effect of *Plasmodium berghei* and letrozole on (a) serum total cholesterol (TC), (b) liver TC, (c) serum high-density lipoprotein cholesterol (HDL-c), (d) Liver HDL-c, (e) serum low-density lipoprotein cholesterol (LDL-c), and (f) liver LDL-c in female Albino mice. Both *P. berghei* infection and letrozole treatment did not significantly alter serum TC, serum and liver HDL-c, as well as serum and liver LDL-c. However, *P. berghei* infection significantly increased liver TC which was attenuated by letrozole treatment. Data were expressed as mean ± SEM. n = 3. Data were analyzed by One-way analysis of variance (ANOVA) followed by the Tukey post hoc test. (**$p < 0.01$ vs uninfected; +$p < 0.05$ vs infected).

Letrozole lowers VLDL-c and triglycerides in both serum and liver of female P. berghei-infected mice

Increased levels of very low-density lipoprotein cholesterol (VLDL-c) and triglycerides (TG) are important components of cardiometabolic syndrome. As shown in Fig. 4, infection with *P. berghei* significantly elevated serum VLDL-c ($p < 0.05$), liver VLDL-c ($p < 0.001$), serum TG ($p < 0.01$), and liver TG ($p < 0.001$), while letrozole, administered alone, did not alter serum or liver VLDL-c and TG. In contrast, letrozole treatment in infected mice attenuated ($p < 0.05$) *P. berghei*-induced elevation in serum or liver VLDL-c and TG.

Letrozole lowers TG/HDL-c index in female P. berghei-infected mice

The ratio of TG/HDL-c is a potential predictor for cardiometabolic risk; thus, we evaluated TG/HDL-c index in all the animal groups. As revealed in Fig. 5, our data show increased levels of TG/HDL-c index in both serum and liver of female mice infected with *P. berghei* when compared with uninfected mice. However, the increased levels of TG/HDL-c observed in infected mice were mitigated following letrozole treatment.

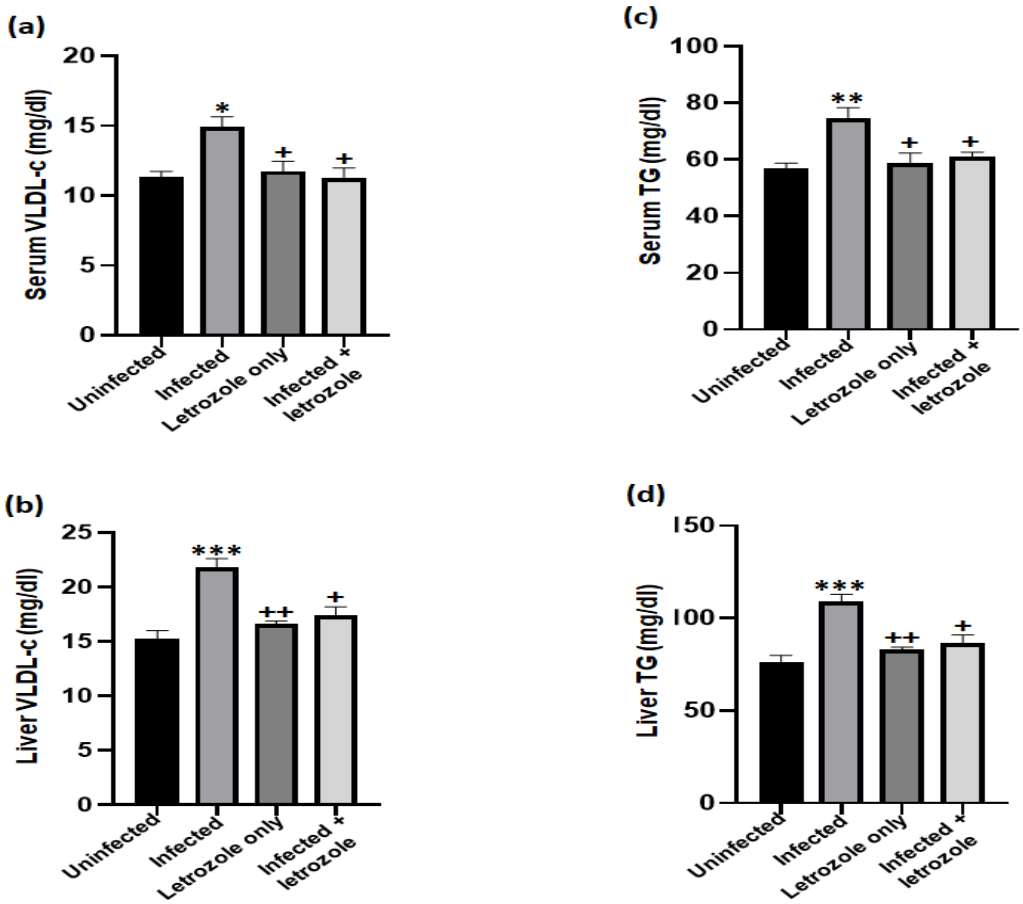

Fig. 4: Effect of *Plasmodium berghei* and letrozole on (a) serum very low-density lipoprotein cholesterol (VLDL-c), (b) liver VLDL-c, (c) serum triglycerides (TG), and (d) Liver TG in female Albino mice. *P. berghei* infection significantly elevated serum and liver VLDL-c as well as serum and liver TG. In contrast, letrozole treatment decreased serum and liver VLDL-c as well as serum and liver TG in *P. berghei*-infected mice. Data were expressed as mean ± SEM. n = 3. Data were analyzed by one-way ANOVA followed by the Tukey post hoc test. (*$p < 0.05$ vs uninfected; **$p < 0.01$ vs uninfected; ***$p < 0.001$ vs uninfected; +$p < 0.05$ vs infected; ++$p < 0.01$ vs infected).

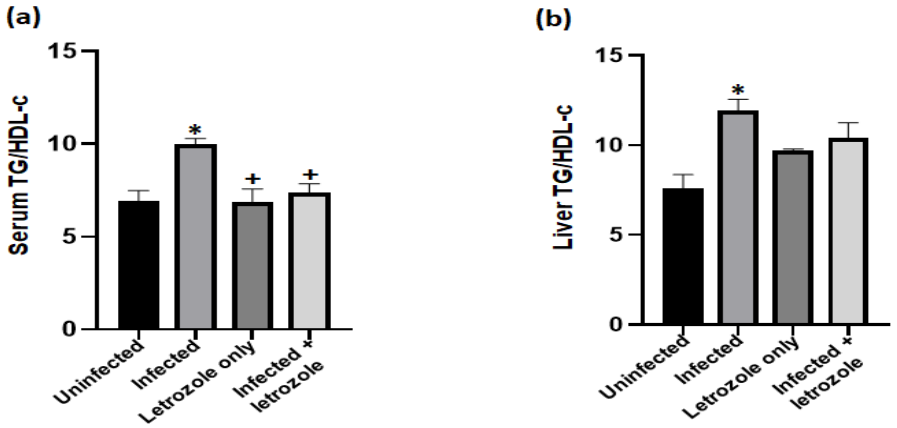

Fig. 5: Effect of *Plasmodium berghei* and letrozole on (a) serum TG/HDL-c (b) liver TG/HDL-c in female Albino mice. *P. berghei* infection significantly increased both serum and liver TG/HDL-c index. Whereas letrozole treatment reduced TG/HDL-c index *P. berghei*-infected mice. Data were expressed as mean ± SEM. n = 3. Data were analyzed by one-way ANOVA followed by the Tukey post hoc test. (*$p < 0.05$ vs uninfected; +$p < 0.05$ vs infected).

Letrozole decreases serum uric acid in female P. berghei-infected mice

Hyperuricaemia is commonly associated with malaria, while the relationship between hyperuricemia and cardiovascular disease is well established. Hence, we assessed the effect of *P. berghei* on serum uric acid. Fig. 6 of our data revealed that *P. berghei* but not letrozole (administered alone) elevated ($p < 0.001$) serum uric acid when compared with the uninfected group while letrozole treatment attenuated ($p < 0.01$) *P. berghei*-induced hyperuricemia. In contrast, both *P. berghei* and letrozole did not significantly alter serum and liver levels of xanthine oxidase.

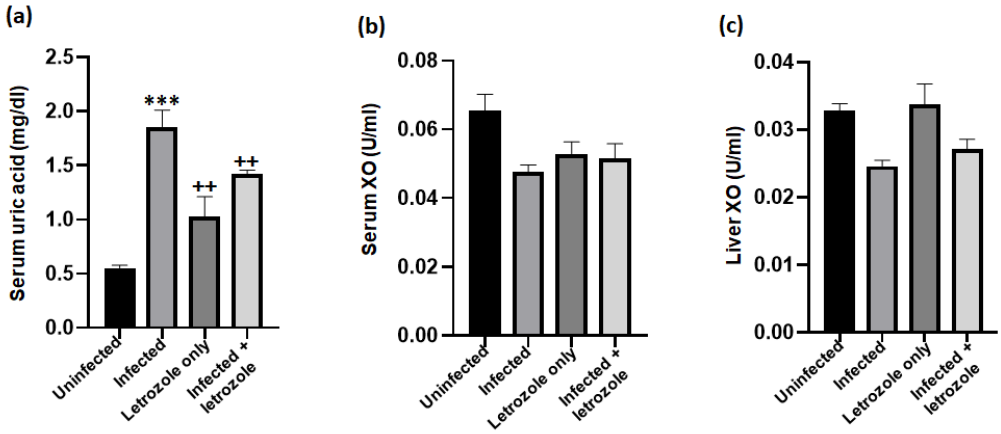

Fig. 6: Effect of *Plasmodium berghei* and letrozole on (a) serum uric acid, (b) serum xanthine oxide (XO), and (c) Liver XO in female Albino mice. *P. berghei* infection significantly increased serum uric acid but did not significantly affect serum and liver XO. Meanwhile, letrozole treatment reduced *P. berghei*-induced hyperuricemia. Data were expressed as mean ± SEM. n = 3. Data were analyzed by one-way ANOVA followed by the Tukey post hoc test. (***$p < 0.001$ vs uninfected; ++$p < 0.01$ vs infected).

Discussion

Prolonged and severe malaria results in serious complications that promote the risk of cardiometabolic disorders[26]. The pathogenesis of such complications is better studied using murine parasites for a better therapeutic approach. The conservation of genetic processes between murine and human malaria parasites as well as the proximity of early culture of *P. berghei* (an African rodent malaria parasite) with *P. falciparum*[27], validates the use of *P. berghei* in testing drug efficacy in *P. falciparum*-like infection. In the present study, we demonstrated the protective effect of letrozole against malaria-induced cardiometabolic risk in *P. berghei*-infected mice. We reported that after a 3-week infection, *P. berghei* induced lipid peroxidation, caused dyslipidaemia, increased hepatic lipid accumulation, and elevated serum uric acid level, all of which play significant roles in the pathogenesis of the cardiometabolic disease. Our study also showed that the dose of letrozole used reduced parasitaemia and attenuated lipid peroxidation, dyslipidaemia, hepatic lipid accumulation, and hyperuricaemia in *P. berghei*-infected female mice, suggesting that letrozole suppressed parasite invasion of red blood cell and internalization which then resulted in attenuation of malaria-induced cardiometabolic risk.

Considering the protective effect of oestrogen in females against CVD and malaria, we used a low dose of letrozole (0.2 mg/kg) that would not affect oestrogen biosynthesis but might be therapeutically potent. This was influenced by our earlier observed cardioprotective effect of letrozole in fructose-exposed male rats. As postulated, our data showed that 0.2 mg/kg letrozole did not alter serum oestradiol in both *P. berghei*-infected and uninfected female mice. This is in contrast to the previous study[28] where 1 mg/kg letrozole was reported to have significantly reduced plasma oestradiol concentration in mice. On the other hand, *P. berghei* significantly suppressed serum oestradiol level at the end of the 3-week experiment, suggesting that inhibition of estrogen biosynthesis is implicated in malaria pathophysiology, probably in a prolonged state of infection.

Oxidative stress has long been identified as a common consequence of malaria, largely due to the high metabolic rate of the rapidly proliferating erythrocytic stage of *Plasmodium* and the immune response of the host to the infection[29]. In response to parasite invasion, the host immune system secretes specific cytokines that may activate the host's monocytes, $CD4^+$ and $CD8^+$ T lymphocytes, natural killer (NK) cells, and neutrophils, promoting inflammatory reactions that further generate oxidative stress[14,30]. Malondialdehyde, a product of lipid peroxidation, is a strong marker of oxidative stress in severe malaria[31]. Therefore, we investigated its serum and hepatic levels. In agreement with the previous study[32], our analysis revealed increased levels of serum and hepatic MDA following *P. berghei* infection, suggesting increased oxidative stress in our model. Excessive generation of oxidative stress escalates pathology that eventually results in metabolic complications[14]. Thus, alleviation of oxidative stress may provide better malaria treatment and associated complications. In this study, letrozole attenuated MDA levels which may suggest that letrozole can complement and improve the efficacy of already existing antimalarial drugs, which are already facing resistance by *Plasmodium*.

Alteration in lipid profile is a characteristic feature of malaria[33]. Although the plausible mechanism for this alteration is not clear, it may depend on *Plasmodium* sp., host, or host-parasite interaction[34]. In this study, serum TC serum or liver LDL-c and HDL-c were not significantly altered by both *P. berghei* infection and letrozole treatment. However, *P. berghei* infection increased serum, VLDL-c, and TG. Hepatic levels of TC, VLDL-c, and TG were also elevated in infected mice that were not treated with letrozole. Elevated VLDL-c is a major type of dyslipidemia that is known to promote atherosclerotic cardiovascular disease (ACVD)[35]. More so, the significance of VLDL-c attenuation in preventing ACVD is well recognized and its superiority over LDL-c is now acknowledged[35-36]. Hence, elevated levels of VLDL-c reported in this study may be suggestive of ACVD risk in prolonged malarial infection. This assertion is further strengthened by the observed elevated serum TG level in infected untreated mice, as hypertriglyceridemia independently promotes the risk of CVD, especially in non-obese and

non-diabetic individuals[37]. Increased serum levels of VLDL-c and TG reported in *P. berghei*-infected mice in this study concurs with the previous report in children with *P. vivax* malaria[26]. Our findings which show that letrozole treatment reduced serum VLDL-c level and attenuated hypertriglyceridaemia support our hypothesis that letrozole may be beneficial in malaria treatment, mitigating the risk of CVD in a severe or prolonged malaria case.

We demonstrated that *P. berghei* increased hepatic levels of TC, VLDL-c, and TG, suggesting stimulation of hepatic lipogenesis by *P. berghei* which characterizes a metabolic syndrome called non-alcoholic fatty liver disease[38]. Our findings agree with the previous studies that reported lipid droplets as well as accumulation of TG and cholesterol esters in the liver of *P. berghei*-infected mice[39, 32]. Since oxidative stress plays a major role in the pathogenesis of hepatic manifestation of metabolic syndrome[40], there is a possibility that the hepatic lipid accumulations observed in infected mice were due to elevated MDA level in the liver, which although not significant, may be biologically relevant. To further validate the possible involvement of metabolic disorder in *Plasmodium* infection, we assessed serum and hepatic levels of TG/HDL-c index which is a potential predictor for cardiometabolic risk[41]. Our findings revealed an elevation of the TG/HDL-c index in both the serum and liver of the infected mice, which is indicative of cardiometabolic risk in malaria. Our study shows that letrozole treatment did not only reduce dyslipidemia and hepatic lipid accumulation in *P. berghei*-infected mice but also lowered TG/HDL-c index. Altogether, these findings suggest the potency and promising effects of letrozole in mitigating malaria-associated cardiometabolic risks and agree with our previous findings[21-22].

Uric acid has emerged as a central inflammatory molecule in malaria, playing a critical role in the pathogenesis of malaria and serving as a molecular marker for severe infection[42]. Our finding revealed hyperuricemia in *P. berghei*-infected mice, which was attenuated by letrozole treatment. Analysis of xanthine oxidase activity showed that the observed hyperuricemia was not likely due to increased uric acid formation but probably due to renal impairment that resulted in insufficient clearance of excess uric acid in the serum[43]. Interestingly, both hyperuricemia and acute kidney injury have been associated with increased cardiometabolic risk, suggesting hyperuricemia as another probable mediator of malaria-induced cardiometabolic risk. More so, reduction in serum uric acid level following letrozole treatment further suggests the potential efficacy of letrozole in preventing the risk of cardiometabolic disorder in malaria.

Conclusion

This study reveals that malarial infection is characterized by reduced serum estradiol level and increased cardiometabolic risk in female mice. Further, we uncovered the beneficial effects of letrozole in rodent malaria. Our study has several limitations among which is that we could not establish the possible link between oestradiol reduction and the induced metabolic risk in *Plasmodium* infection. Nonetheless, the findings of this study are important. The study provides evidence that letrozole lowered parasite load and attenuated cardiometabolic risk in rodent malarial infection. Thus, it may be suitably repurposed as an adjuvant drug in malaria treatment. Our next study will focus on the role of estrogen in malaria-associated complications in male as well as +/- oestradiol female mice. Dose-dependent effects of letrozole will also be considered in our future study.

Acknowledgments

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عقار ليتروزول يخفف من مخاطر التمثيل الغذائي القلبي في الفئران المصابة بالبلازموديوم بيرغي

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ترتبط الملاريا باضطرابات القلب والأوعية الدموية ، مما يزيد من خطر الإصابة بأمراض القلب والأوعية الدموية .(CVD) لقد أظهرنا مؤخرا تأثيرات ليتروزول الوقائية للقلب في ذكور الفئران المعرضة للفركتوز. ومن ثم ، في هذه الدراسة ، قمنا بالتحقيق في تأثير جرعة منخفضة من ليتروزول ضد مخاطر القلب والأوعية الدموية في إناث الفئران المصابة بالبلازموديوم بيرغي. تم تجميع عشرين فأرا أنثى عشوائيا في أربعة مجموعات (ن = ٥ / مجموعة): غير مصابة ، مصابة ، غير مصابة وتعطى ليتروزول (٠,٢٤ مجم / كجم/ يوم عن طريق الفم) ، مصابة و تعطى ليتروزول. تم تسجيل نسبة طفيلية الدم الأسبوعية. في نهاية التعرض لمدة ٢١ يوما ، تم جمع الدم والكبد ومعالجتهما لإجراء تحليلات كيميائية حيوية. أظهرت النتائج أن البلازموديوم بيرغي زاد تدريجيا من تطفل الدم. كما خفضت عدوى بالبلازموديوم بيرغي مستوى استراديول المصل بعد الإصابة لمدة ٢١ يوما وزادت مستويات المصل والكبد من المونوالدهايد ، وكوليسترول البروتين الدهني منخفض الكثافة ، والدهون الثلاثية ، والدهون الثلاثية / كوليسترول البروتين الدهني عالي الكثافة .(TG / HDL-c) وبالمثل ، رفع البلازموديوم بيرغي حمض اليوريك في الدم ومستويات الكوليسترول الكلي الكبدي. في المقابل ، لم تؤثر الجرعة المعطاة من ليتروزول بشكل كبير على استراديول المصل ولكنها خفضت بيروكسيد الدهون ، وتغيرات الدهون الموهنة ، و خفضت مستوى حمض اليوريك في الدم. لقد كشفنا أن عدوى البلازموديوم بيرغي خفضت استراديول المصل وعززت مخاطر القلب والأوعية الدموية في إناث الفئران ، بينما قلل ليتروزول من تطفل الدم وخفف من مخاطر القلب والأوعية الدموية المرتبطة به.

وبالتالي ، فإن هذه الدراسة تشير إلى إمكانات ليتروزول كعلاج مساعد لتحسين إدارة مضاعفات القلب والأوعية الدموية الناجمة عن الملاريا. يوصى بإجراء مزيد من الدراسة للتحقيق في فعالية ليتـــروزول المضادة للملاريا ، بغض النظر عن الجنس.