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Research Article

Evaluation of the effect of resveratrol on parasitaemia in *Plasmodium berghei*-induced malaria in diabetic male Wistar rats

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Keywords:

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ABSTRACT

Background: Metabolic syndrome (MS); which is mostly caused by a high-carbohydrate high-fat diet (HCHFD) as well as a sedentary lifestyle; is associated with an increased risk of cardiovascular and hepatic complications. In this study, we investigated the effect of supplementation with ursolic acid (UA) on MS parameters induced by HCHFD in male Wistar rats. **Methods:** Twenty male Wistar rats, aged 8-9 weeks old, weighing 120 - 170 grams, and randomly divided into 4 groups (n =5) were used. Group I received normal diet (ND) and distilled water (DW); group II received ND and UA; group III received HCHFD and DW; group IV received HCHFD and UA. HCHFD was formulated in-house and the drinking water was augmented with 20% fructose. The animals were fed their respective diets daily for 20 weeks. A dose of 250 mg/kg body weight of ursolic acid was adopted and administered orally to UA-treated groups starting 12 weeks after initiation of the HCHFD for a further 8 weeks. Body weight, body mass index (BMI), and fasting blood glucose (FBG) were measured every four weeks and percentage increases were determined. An oral glucose tolerance test (OGTT) was performed and the area under the curve (AUC) was determined. Blood samples were obtained for serum insulin and lipid profile. Insulin resistance was determined using the homeostatic model assessment for insulin resistance (HOMA-IR). Histopathological evaluation of liver tissue was performed using the hematoxylin and eosin staining technique. **Results:** The increase in BMI and FBG of the HCHFD+UA group was significantly lower ($P<0.05$) compared to the HCHFD+DW group. The HCHFD+DW group had a higher ($P<0.05$) HOMA-IR and AUC for OGTT compared to HCHFD+UA. There was a significant decrease ($P<0.05$) in serum insulin, cholesterol, triglyceride, and LDL-C in the HCHFD+UA group compared to the HCHFD+DW group, while HDL-C significantly ($P<0.05$) increased in the HCHFD+UA group compared to HCHFD+DW group. **Conclusion:** In this study, UA supplementation prevented the development of MS in male Wistar rats fed with HCHFD for 20 weeks. This suggests that UA has the potential to be considered for the management of MS.

INTRODUCTION

Despite tremendous reduction in the burden of malaria in the last decade, world health organization (WHO) in 2017, reiterates that malaria remains a public health problem in most malaria endemic countries and accounted for 445,000 deaths in 2016 with 91% of cases occurring in sub–Saharan Africa (Thera *et al*., 2018). These epidemiological shifts clearly suggest that replication of *Plasmodium* species depends on host's blood glucose concentration and that the drugs which reduce the blood glucose levels may interfere with growth of the parasites. In uncontrolled type 2 diabetes, patients possess elevated blood glucose and profound level of oxidative stress (Thera *et al*., 2018).

If diabetes increases malaria parasitaemia, as reported by Danquah *et al.* (2010), gestational diabetes might increase the incidence, morbidity, and mortality of pregnancy-associated malaria (Danquah, Bedu-Addo and Mockenhaupt, 2010). Such an association would be relevant, especially in the light of the rising global incidence of gestational diabetes (van Crevel *et al.*, 2017). About 60% of hospital attendance is due to febrile illnesses, many of which are malaria affects children performance in schools because it is a major cause of absenteeism (WHO, 2015). In Northern Nigeria, malaria is the most prevalent disease with incidence of 56% in patients attending clinics in Northern Nigeria tertiary institutions. The prevalence of malaria might increase in the region due to long term insurgency that led to death of thousands and displacement of over a million people (WHO, 2017). Apart from this, adults can also become infected with the disease by visiting places where the malaria is widespread like in Sub-Saharan Africa where the disease claims the life of one child every 30 seconds. WHO estimates that every week, around 8,500 people are infected with the disease in North Eastern States of Nigeria (Enato and Okhamafe, 2004; WHO, 2017).

As malarial parasites cannot store energy in the form of glycogen, they rely entirely on an exogenous supply of glucose. The infected erythrocyte exhibits a substantial increase in its permeability to low molecular weight sugar (Humeida, *et al*., 2011). The incidence of malaria is more in children and pregnant women and this incidence correlates well with the blood glucose concentration (Raghunath, 2017), reported oxidative stress mediates impairment in malaria parasitaemia development. Holistically, these data show the probable antagonism between oxidative stress in uncontrolled diabetes and parasite susceptibility while, hinting on the possible unintended synergistic effect of medication for diabetes on parasitaemia development. (Matough, *et al.*, 2012; Zuzarte-Luis, *et al.* 2017)

Resveratrol is capable of inducing a wide variety of effects in different tissues and this pleiotropic action leads to the therapeutic effect in the whole organism (Szkudelski, *et al*., 2015). Lee, *et al*. (2008), reported methanol extracts from the dried roots of *Pleuropterus ciliinervis* were found to have high antimalarial activity against *P. falciparum* in vitro, this activity being largely attributable to a (E)-resveratrol-3-O-a-Lrhamnopyranosyl-(1-2)-b-D-xylopyranoside (RRX) and encouragingly, RRX was also found to have moderate antimalarial activity in vivo, when tested against *P. berghei* in mice (Vaughan *et al*., 2008), Although, the results indicate that RRX possesses useful blood schizontocidal action when used at doses that cause no marked toxicity in mice, the mechanism of action of this compound has not been elucidated (Moon and Sim, 2008). Resveratrol is a natural host-targeted supplement used by diabetics for its antioxidant activity (Glennon, *et al*., 2018).

Resveratrol (RSV) is a 3, 4, 5-trihydroxystilbene, a naturally occurring polyphenol found in different plant species. Enormous amounts of resveratrol are found in berries, grapes, peanuts and can be used in tablets form as a dietary supplement (Soliman *et al*., 2017). RSV has been reported to have cardio-protector, neuro-protector, antioxidant, anti-inflammatory, anti-cancer and anti-diabetic activities (Elshaer, *et al*., 2018; Jimoh *et al.*, 2018).

The elevated blood glucose levels in those with uncontrolled type 2 diabetes may result in glycosylation of immune cells. The immune cells are activated in the absence of an infection and become exhausted and desensitized. Glycosylated immune cells are thus unable to respond effectively to infection and the immune system is weakened. Hence, the incidence of infections including malaria is increased in

patients with type 2 diabetes mellitus (Raghunath, 2017).

There are four parasite species that cause malaria in rodent, vis *Plasmodium berghei*, *Plasmodium chabaudi*, *Plasmodium vinckei*, and *Plasmodium yoelii* (Otto, *et al.*, 2014). *Plasmodium berghei* is a widely used mouse malaria model and a dominant tool for reverse genetic studies in malaria (Jambou *et al.*, 2011). The *P. berghei* genome is largely homologous to the genome of the human parasites, which permits experiments that are difficult to perform with human subjects (Kooij, *et al*., 2005).

Malaria parasites release a large quantity of reactive oxygen species (ROS) in the infected RBC in the process of converting heme to hemozoin for heme detoxification. This is suggested to be a factor in decreased erythropoiesis and malaria induced anemia (Foldes, *et al*., 1994; Omodeo-Sale, *et al*., 2005;). *Plasmodium berghei* ANKA, a strain of the *P. berghei* transmitted by the bite of Anopheles durene is the most widely used model of malaria. These parasites of rodents are practical model organisms in the laboratory for the study of human malaria aimed at the development of new vaccines and treatments (Basir, *et al*., 2012; Otto, *et al.*, 2014).

This study attempted to investigate the effects of resveratrol on *P. berghei-induced* parasitaemia in a prevailing comorbidity of diabetes in male Wistar rats.

MATERIALS AND METHODS

Materials

Light microscope, methanol (99%), distilled water, RSV, vitamin C, artesunate, streptozotocin, Computer, Beakers, Specimen bottles, weighing balance, 1ml and 5ml syringes and injecting needles, dissecting tray, Dissecting kit, pLDH ELISA Kit, Chloroform. All chemicals and reagents used for this research were of analytical grade.

Ethical approval

Animals were handled according to the ethics guiding the use of laboratory animal in Ahmadu Bello University, Zaria, Nigeria. Ethical clearance was granted with the approval no.: ABUCAUC/2021/026

Source of P. berghei and experimental animals

The parasite (*P. berghei*) was obtained from Animal House of Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria-Nigeria. A total of thirty-five male Wistar rats 6-8 weeks old, weighing 150-180 grams were purchased from the Department of Pharmacology and Therapeutics' Animal house, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were housed in plastic cages fed with feed and clean water *ad libitium* in accordance with the rules governing the use and care of laboratory animals in Ahmadu Bello University, Zaria, Nigeria. The rats were allocated to groups as shown in figure 1.

Preparation of High Fat Diet (HFD) HFD was prepared by mixing the grower mash (constitutes; fats 18%, proteins 54% and carbohydrates 28%) with margarine (99.9% fats) and groundnut meal in the (percentage) ratio of (50%) 500g of feed to (25%) 250g of margarine and (25%) 250g of ground nut meal, a modification of the composition described by Okoduwa, *et al*., (2017) and Alex *et al*. (2019).

Induction of Type 2 Diabetes Mellitus Type II diabetes was induced according to the method described by Alex *et al.* (2019). The animals were fed with HFD along with 20% fructose solution as drinking water for eight weeks, after which they were fasted overnight and injected intraperitoneally with a single, low dose of streptozotocin (STZ), at a dose of 30 mg/kg diluted in 0.1 M citrate-buffered saline (pH 4.5). Diabetes was confirmed by determining blood glucose concentrations 72 hours and on day 5 after STZ administration. Only rats with blood glucose levels ≥200 mg/dL were considered diabetic.

Malaria Inoculation

The inoculation of malaria was by the method described by Fernanda *et al*. (2010). Blood from a donor mouse with a parasitemia level above 20% was drawn into a heparinized syringe and diluted with phosphate buffered saline (pH 7.2). The infection was initiated by injecting 0.2 ml of the parasite preparation from the donor mouse to the experimental rats via the intraperitoneal route. The Wistar rats were divided into respective groups with four rats per group (cage). From day 3 (72 hours after inoculation), daily thin blood

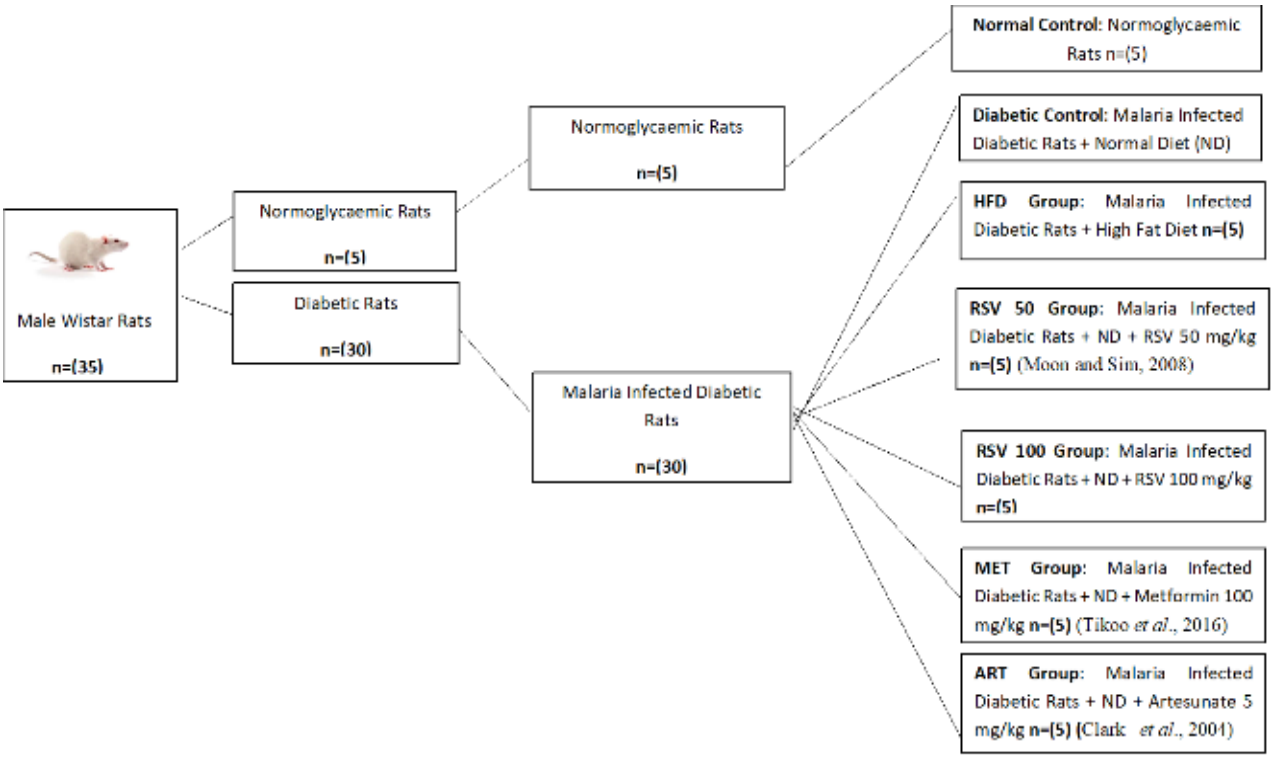

Figure 1: Experimental animal groupings, RSV = resveratrol, HFD = high fat diet, MET = metformin, ART = artesunate

films stained with Giemsa were prepared from tail blood of each rat till day 7, to monitor the parasitemia level. The packed cell volume (PCV) was also measured on day 0 prior to inoculation and day 6. Male Wistar rats were chosen in *lieu* of mice and female rats for this study due to the concomitant stress from STZ-induced diabetes and *P-berghei*-induced malaria which might lead to high fatality in mice and oestrous hormonal fluctuations in female rats which might interfere with readings from biochemical assays.

Estimation of Parasitaemia

The blood sample was collected from tail snip of each rat as previously described by Fernanda, *et al*. (2010). The smears were then applied on microscope slides fixed with absolute methanol for 15 min and stained with 15% Geimsa stain at pH 7.2 for 15 min. The stained slides were then washed gently using distilled water and air dried at room- temperature. Then, each stained slide was examined under Olympus microscope (CHK2-F-GS, Taiwan) with an oil immersion objective of 100x magnification power to investigate parasite load and PCV. The number of parasitized erythrocytes in about 10-50 fields was counted twice and the average computed to give the parasitemia of each rat (Fernanda, *et al*., 2010). The parasitemia level was determined by counting minimum of five fields per slide. Percentage parasitemia was calculated as: (%) Parasitemia = (Number of parasitized RBC/ Total number of RBC) x 100

Weight determination

The weights of the animals were determined on day 0, 3 and 6 of the experiment using electronic weighing scale (Model: EK3052, Zhongshan, China).

Determination of Blood Glucose Level

Tail vein blood was used for monitoring the blood glucose level using glucose test strips and digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany). The result was expressed in mg/dL of blood (Rheney and Kirk, 2000).

Collection of Sample

After the expected experimental period, all the animals were anaesthetized through cervical dislocation using 100 mg/kg body weight of ketamine and 5mg/kg body weight of Diazepam. Blood samples were collected in plain and potassium EDTA bottles.

Haematological Indices
Estimation of total RB was done using standard technique (Neubauer hemocytometer; Feinoptik, Germany) and differential WBC count was done by Leishman's staining method. The PCV was estimated by centrifuging blood in hematocrit tube and reading percentage RBC in a hematocrit reader (Ghai, 1999).

Estimation of lipid peroxidation
Levels of the lipid peroxidation biomarker, malondialdehyde (MDA) concentrations were quantified according to the method described by Yagi, (1984).

Plasmodium Lactate Dehydrogenase (PLDH) assay
Plasmodium lactate dehydrogenase (pLDH) enzyme was determined in the rat serum and tissue homogenate using Shanghai Coon Biotech Rat Plasmodium Lactate Dehydrogenase (PLDH) ELISA kit (Catalogue number: CK-bio-20369, Standard Curve Range: 1ng/mL-20ng/mL, Sensitivity: 0.1ng/mL). The kit is based on the principle of double antibody sandwich technology enzyme linked immunosorbent assay (ELISA).

Standard and sample were added to wells that were pre-coated with objective antibody, then HRP-Conjugate reagent was added to form an immune complex. The samples were incubation, and washing, removal of unbound enzyme, and then the substrate A and B were added. Then the solution turned blue and finally changed into yellow at the effect of acid. The color depth or light was positively correlated with the concentration of pLDH.

Statistical Analysis
All data were analyzed using one-way analysis of variance (ANOVA) followed by *Tukeys* post hoc test. Results were expressed as mean ± S.E.M. The Probability that P values less than 0.05 ($P < 0.05$) were considered as accepted level of significant difference between the groups.

RESULT
Figure 2 revealed a significant ($p < 0.05$) decrease in the body weight with induction of diabetes and inoculation of malaria across all the groups in comparison with the normal control group. Within the treatment groups, body weight did not significantly ($p > 0.05$) change when compared after day six of treatment.
Figure 3 highlights the result for the blood glucose levels (mg/dl) across groups from before diabetes induction, on the day of malaria inoculation and days 3 and 6 after the malaria inoculation. On day 6, significantly ($p < 0.05$) decreased in blood glucose level in resveratrol 50 mg/kg group (87.20 ± 3.44 mg/dL), resveratrol 100 mg/kg group (184.60 ± 51.22 mg/dL), metformin group (93.20 ± 4.25 mg/dL) and artesunate group (161.20 ± 33.52 mg/dL), when compared to the HFD group (315.20 ± 25.50mg/dL) and diabetic control (408.00 ± 85.79 mg/dL) groups, respectively.

As shown in Figure 4, the main effect of time and treatment were significant on blood glucose changes in the treated groups $F(2.557) = 45.886$, $P = 0.0005$ and $F(7.00) = 5.687$, $P = 0.0005$. The interaction between time and treatment was also significant $F(17.897) = 5.439$, $P = 0.000$. The mean blood glucose levels on day 0 (137.45 ± 13.19 mg/dL), day 3 (245.93 ± 17.80 mg/dL) and day 6 (225.90 ± 15.48 mg/dL) were significantly higher, compared to the baseline before diabetes induction (87.80 ± 2.45 mg/dL).

Values for the average RBC are depicted in Figure 5, Resveratrol 50 mg/kg (550.80 ± 1.59) and 100 mg/kg (533.76 ± 8.25) groups significantly ($p < 0.05$) increased in RBC, when compared with diabetes control (437.80 ± 9.05) and HFD (440.48 ± 16.34) groups on days 4, 5 and 6.

The main effect of time and treatment (Figure.6) were significant in the treated groups $F(2.901) = 6.775$, $P = 0.0005$ and $F(7.00) = 32.403$, $P = 0.0005$. The interaction between time and treatment was also significant $F(20.306) = 7.070$,

P= 0.0005. The mean average RBC on day 6 (477.16 ± 4.94) was significant, compared to day 4 (459.317 ± 4.87). There was also a significant difference between day 5 (447.88 ± 4.81) and day 6.

The PCV values revealed that significant ($p < 0.05$) increase was recorded in resveratrol 50 mg kg and 100 mg/kg, when compared with the diabetes control and HFD. Figure 7

Figure 8 shows the values for average infected RBC. The values for Resveratrol 50 mg/kg (1.34 ± 0.15) and Resveratrol 100 mg/kg (1.56 ± 0.07) were significantly ($p < 0.05$) lower when compared with values from the diabetic control (2.16 ± 0.20) group on day 6. Artesunate and Metformin recorded significant ($p < 0.05$) decrease in infection, when compared with the diabetes control and HFD groups.

Figure 9 shows the main effect of time and treatment were significant in the treated groups F (2.786) = 36.993, *P*= 0.0005 and F (7.00) = 63.399, *P* = 0.0005. The interaction between time and treatment was also statistically significant F (19.499) = 8.345, *P* = 0.0005. The effect of time was significant on days 4, 5 and 6 compared to day 3; and on day 6, compared to days 4 and 5.

The average parasites in 5 fields are depicted in Figure 10. Both Resveratrol 50 mg/kg (6.46 ± 0.21) and 100 mg/kg (8.48 ± 0.46) showed significant ($p < 0.05$) decrease in the parasites, when compared with values from diabetic control (13.68 ± 1.33) and HFD (10.04 ± 0.59), respectively on day 6 after malaria inoculation.

Figure 11 shows the main effect of time and treatment were significant in the treated groups F (2.482) = 92.037, *P* = 0.0005 and F (7.00) = 106.85, *P* = 0.0005. The interaction between time and treatment was also statistically significant F (17.376) = 14.423, *P* = 0.0005. The effect of time was significant on days 4, 5 and 6, compared to day 3; and on day 6, compared to days 4 and 5.

The serum pLDH (ng/ml) and Liver pLDH (ng/ml) values were represented in Figures 12 and 13, respectively. There was a significant ($P < 0.05$) decrease in the values of pLDH across the treatment groups, when compared with the diabetes control and HFD groups.

The MDA concentrations in RSV 50 mg/kg (135.00 ± 2.18 Umol/ml) and RSV 100 mg/kg (166.02 ± 16.58Umol/ml) to be statistically significant ($p < 0.05$) decreased when compared with the diabetes control (188.40 ± 5.73Umol/ml) and HFD (175.82 ± 6.95Umol/ml) groups. Treatment groups (artesunate and metformin) also recorded a significant ($p < 0.05$) decrease in MDA concentrations, when compared with the diabetes control and HFD groups. Table 2.

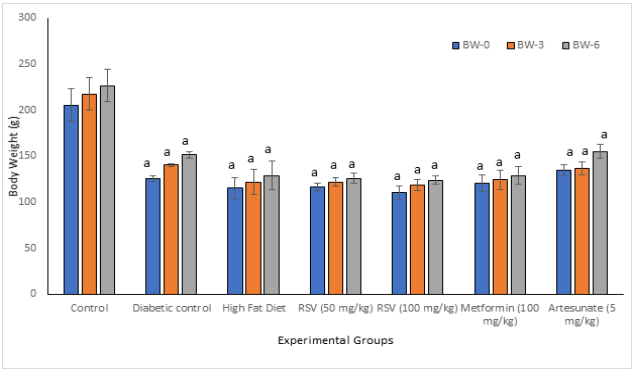

Figure 2: Body weight for diabetes and malaria comorbidity in male Wistar rats. Superscripts [a] indicate statistically significant difference compared to control, RSV= Resveratrol, HFD = High fat diet, BW = Body weight.

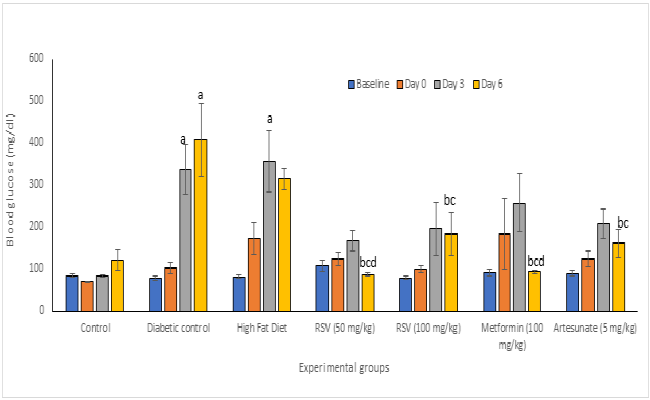

Figure 3: Blood glucose for diabetes and malaria comorbidity in male Wistar rats. Superscripts [a,b,c and d] indicate statistically significant difference compared to control, Diabetic control, and HFD groups respectively, RSV= Resveratrol, HFD = High fat diet

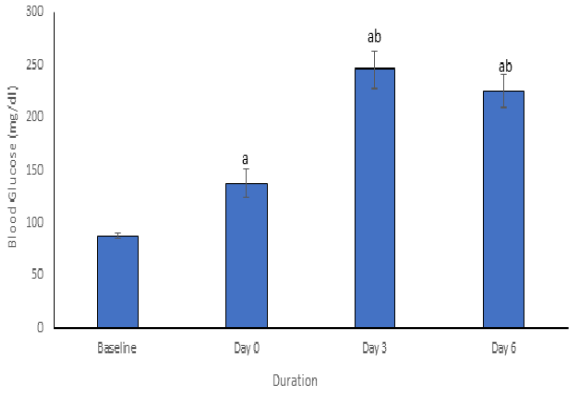

Figure 4: Variation in blood glucose concentrations for diabetes and malaria comorbidity in male Wistar rats. Superscripts [a and b] represents statistically significant effect of time on blood glucose level compared to the baseline and day 0 respectively

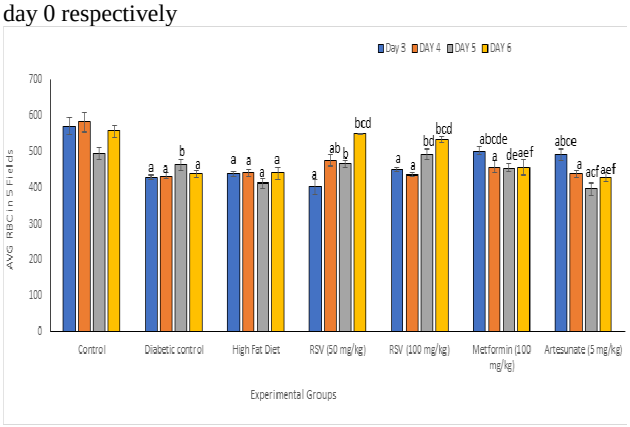

Figure 5: Daily variation in average RBC in male Wistar rats with diabetes and malaria comorbidity.
RBC= Red Blood Cell, VIT C= Vitamin C, RSV= Resveratrol. Superscripts [a,b,c,d,e and f] indicate statistically significant difference compared to control, DC, HFD, RSV (50 mg/kg) and RSV (100 mg/kg).

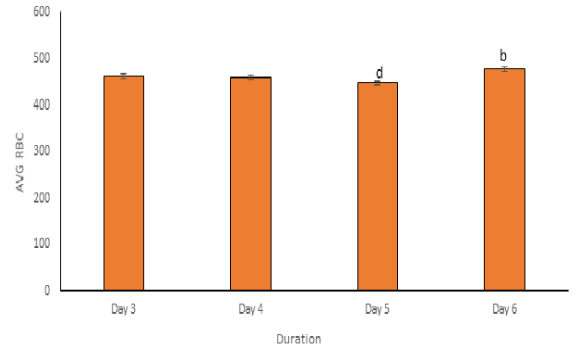

Figure 6: Daily changes in average RBC in five field in male Wistar rats with diabetes and malaria comorbidity. Superscripts [b and d] represents statistically significant effect of time compared to day 4 and day 6 respectively.

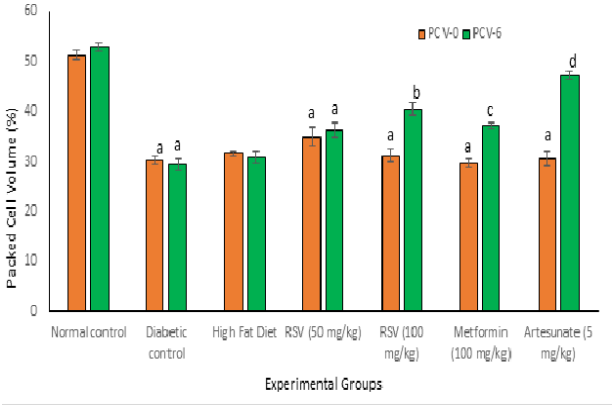

Figure 7: Changes in packed cell volume for diabetes and malaria comorbidity in male Wistar rats. Superscripts [a,b,c and d] indicate statistically significant difference compared to control, Diabetic control, and HFD groups respectively, RSV= Resveratrol, HFD = High fat diet.

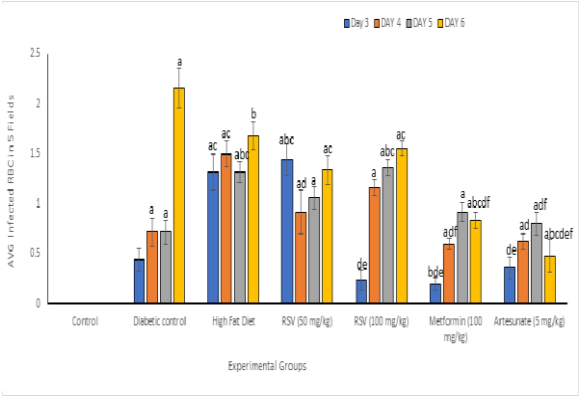

Figure 8: Average infected RBC in male Wistar rats with diabetes and malaria comorbidity. RSV= Resveratrol. Superscripts [a,b,c,d,e and f] indicate statistically significant difference compared to control, RSV (50 mg/kg) and RSV (100 mg/kg).

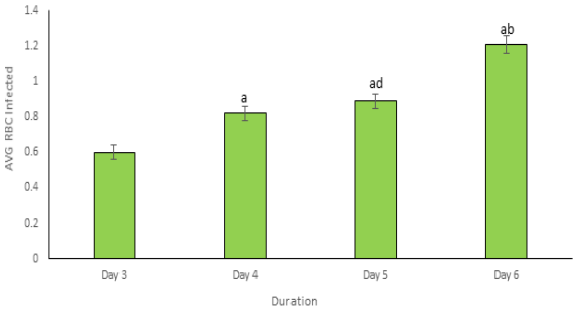

Figure 9: Daily changes in average infected RBC in male Wistar rats with diabetes and malaria comorbidity. Superscripts [a, b and d] represents statistically significant effect of time compared to day 3, day 4 and day 6 respectively

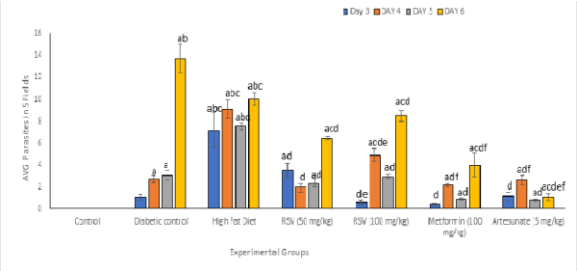

Figure 10: Average parasites in RBC of male Wistar rats with diabetes and malaria comorbidity. RSV= Resveratrol. Superscripts [a,b,c,d,e and f] indicate statistically significant difference compared to control, RSV (50 mg/kg) and RSV (100 mg/kg), BW = Body weight in day

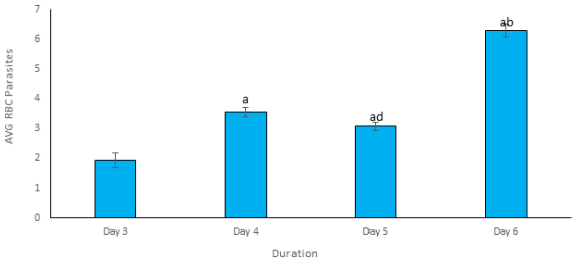

Figure 11: Daily fluctuations in the average parasites in RBC of male Wistar rats with diabetes and malaria comorbidity. Superscripts [a, b and d] represents effect of time compared to day 3, day 4 and day 6 respectively.

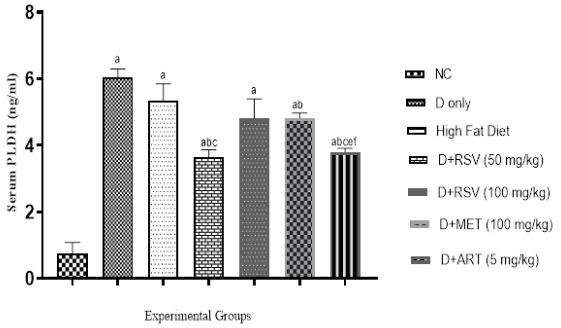

Figure 12: Serum pLDH in male Wistar rats with diabetes and malaria comorbidity. Superscripts [a,b,c,d,e and f] indicate statistically significant difference compared to control, DC, HFD, RSV (50 mg/kg) and RSV (100 mg/kg) respectively.DC= Diabetic control, HFD= High fat diet, RSV= Resveratrol, MET= Metformin, ART= Artesunate, pLDH = plasmodium lactate dehydrogenase

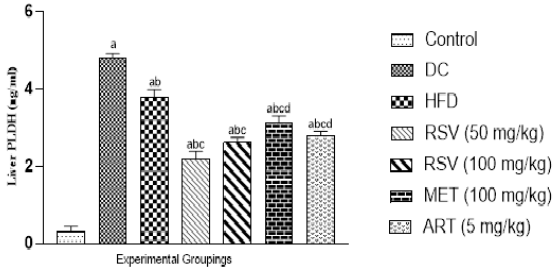

Figure 13: Liver pLDH in male Wistar rats with diabetes and malaria comorbidity. Superscripts [a,b,c,d,e and f] indicate statistically significant difference compared to control, DC, HFD, RSV (50 mg/kg) and RSV (100 mg/kg) respectively. DC= Diabetic control, HFD= High fat diet, RSV= Resveratrol, MET= Metformin, ART= Artesunate, pLDH = plasmodium lactatae dehydrogenase

Table 1: Eosinophils in Wistar rats with diabetes and malaria comorbidity administered with resveratrol.

Experimental Groups (n=5)	Eosinophils (%)
Normal control	3.00 ± 0.55
Diabetic control	6.40 ± 0.25[a]
High Fat Diet	5.60 ± 0.25
RSV (50 mg/kg)	3.20 ± 0.37[b]
RSV (100 mg/kg)	3.20 ± 0.58
Metformin (100 mg/kg)	3.40 ± 0.60
Artesunate (5 mg/kg)	2.80 ± 0.25[c]
F (P value)	2.239 (0.057)

Control= Normal Control, RSV= Resveratrol Superscripts [a,b,c,d,e and f] indicate statistically significant difference compared to control, DC, HFD, RSV (50 mg/kg) and RSV (100 mg/kg), respectively.

Table 2: MDA concentrations in Wistar rats with diabetes and malaria comorbidity administered with resveratrol.

Experimental Groups	MDA (Umol/ml)
Control	159.12 ± 14.09
Diabetic control	188.40 ± 5.73
High Fat Diet	175.82 ± 6.95
RSV (50 mg/kg)	135.00 ± 2.18
RSV (100 mg/kg)	126.02 ± 16.58[bde]
Metformin (100 mg/kg)	137.02 ± 11.07
Artesunate (5 mg/kg)	121.64 ± 5.72[bcdeg]
F (P value)	8.855 (0.001)

Control= Normal Control, RSV= Resveratrol Superscripts [a,b,c,d,e and f] indicate statistically significant difference compared to control, DC, HFD, RSV (50 mg/kg) and RSV (100 mg/kg), respectively.

DISCUSSION

The results of the experiment showed that the blood glucose levels in resveratrol groups significantly decreased on the sixth day post malaria inoculation (day of sacrifice), when compared with diabetes control; and when resveratrol 50 mg/kg was compared with the high fat diet (HFD) group. The findings agree with many studies that reported the anti-hyperglycaemic effect of resveratrol (Szkudelski and Szkudelska, 2011; Jimoh *et al*., 2018)

However, the observed hypoglycaemic effect of resveratrol in this study was not dose dependent. Furthermore, the effect of time and treatment were significant on the changes in blood glucose level across the treated groups, demonstrating a staircase increase from baseline (day of STZ injection) through days 0 - 3 post-malaria inoculation, but a slight fall on day 6 in the HFD, resveratrol, metformin and artesunate groups only. Nevertheless, the significant decrease in body weight recorded in all the treatment groups may probably be due to the disturbed metabolism which is an effect of malaria infection as reported by Basir *et al*. (2012). Although it was expected that the HFD alone group may cause increase in body weight, probably the time taken for the feeding was inadequate to cause increase in visceral adiposity and, subsequently, increase in body weight. According to Youn *et al*. (2014), increase in body weight from consumption of diet rich in fat in rodents may take up to six months and above. The inhibitory effect of metformin on hepatic gluconeogenesis (Horakova *et al*., 2019) and Artesunate's probably exerted inhibitory effect on glycogenolysis via G6Pase activity inhibition (Alagbonsi *et al*., 2020). The increase in eosinophil counts in diabetes control and HFD groups showed the manifestation of malaria parasites, which are usually higher with infection. This concurred with the result of the study on malaria by Aziakpono *et al*. (2021), who reported increased levels of eosinophils counts. McKenzie *et al*. (2005) described this finding to be due to localization of the white blood cells in the spleen and other organs, away from the peripheral circulation common in the acute malaria infection. However, the RSV groups were lower than the diabetic control and HFD groups, respectively. The decrease in average eosinophil count per full field in resveratrol groups agreed with the study by Ola-Davis. *et al*. (2018), who postulated the ameliorative effects of resveratrol's hypoglycaemic action on the desensitized immune system.

Increase in differentials white blood cells was also reported by Raghunath, (2017), who revealed that a possible outcome of a desensitized immune system was due to a state of frank hyperglycaemia before the malaria infection. This postulate could be true due to the significant hperglycaemia observed in diabetes control and HFD groups. Scores of findings and that of Kurtzhals, *et al*. (1998) reported eosinophil count to be increased in malaria-infected rats.

The result for the packed cell volume (PCV) showed significant decrease in all the groups when compared with the normal control alone group. The low PCV on day 0 of malaria inoculation may be attributed to the effect of STZ-induced anaemia, resulting from the hyperglycaemic non-enzymatic glycation of RBC membranes as described by Oyedemi *et al*. (2011). Moreover, the effect of time on the PCV from day of malaria inoculation to day 6 post inoculation showed a non-significant decrease across the groups which concurred with the findings of Osonuga *et al*. (2006), who reported that anaemia in malaria is multifactorial; due to lysis of parasitized erythrocytes, immune-mediated haemolysis and reduction in the lifespan of the erythrocytes. Furthermore, improvement after treatment takes time due to the stages and processes of haemopoiesis (Osonuga *et al*. (2006).

Result for the average infected RBC in five fields showed that the diabetic control group had the highest value, when compared across all treatment groups. This may be as a result of increased free glucose in the blood with the absence of exogeneous source of oxidative stress, supporting high proliferation of the Plasmodium parasite as described by Danquah *et al.* (2010). On the other hand, the RSV groups were significantly lower than the diabetic control group on day 6, which was an evidence of blood

schizontocidal action of RSV as reported by Moon and Sim (2008). The interaction between time and treatment showed that there was a significant increase in average-infected RBC over time, with the diabetic control groups showing highest increase, followed by the HFD, and resveratrol groups with little increase in average infected RBCs. The Metformin and Artesunate groups showed declines in average infected RBCs on the sixth day, suggestive of functions of the effect of hyperglyceamia, efficacy of intervention and phagocytic clearance by leukocytes as explained by White (2017).

Furthermore, average parasites in five fields showed that the diabetic control group had the highest value when compared across the groups, although, the values of the RSV groups were significantly lower when compared with the diabetic control and HFD groups, respectively on the sixth day (Ganiyu *et al.*, (2012)). There was a significant increase across the groups when the effect of time and treatment were assessed. The resulting outcomes for RSV and HFD groups agreed with the studies of Moon and Sim (2008), Ganiyu *et al.* (2012) and Zuzarte-luis (2017), who demonstrated that RSV exerted schizontocidal effect.

Multiple trials have reported globally that pLDH test is an effective diagnostic test for malaria (Moody *et al*., 2000). The pLDH is a glycolytic pathway enzyme secreted by the different Plasmodium species. The enzyme possesses species-specific isomers and the enzyme disappears within few days of effective malaria treatment. As a result, the pLDH antigen is considered a specific marker for the presence of viable Plasmodium spp. in blood, and is used for screening in malaria-endemic countries. Unfortunately, false positive rate of up to 97.8% have been reported to be evident with some kits (Atchade *et al*., 2013; Jang *et al*., 2013). Significant increase in pLDH in all the groups inoculated with malaria parasites compared with the control group alone indicates the presence of the parasites in the blood. Pakpour *et al.* (2016), demonstrated that type 2 diabetes rodent infected with malaria are more efficient at infecting mosquitoes and that malaria is also associated with higher mortality in diabetes. Having the pLDH value above 0.8 ng/ml in all the inoculated groups confirmed parasitic infections and value below 0.8 ng/ml in control group confirming no parasites. The result from this study showed that administration of RSV decreases the pLDH level, both in the serum and liver, and the values for pLDH in the liver of groups administered with resveratrol 50 mg/kg, metformin and artesunate significantly decreased when compared with the other experimental groups. The decrease in blood glucose level and serum level of pLDH in metformin-treated group conform with the accepted theory that metformin is widely used oral glucose-lowering drug to possess an anti-malaria property (Garcia *et al.,* 1950; Patade *et al.,* 2014). The findings of the present study further support the results of Sanjay *et al*. (2017), who demonstrated that metformin could be considered an appropriate primary prevention strategy against malaria, in subject with diabetes. although, specific recommendations were not made.

CONCLUSION

Resveratrol suppressed the proliferation of *P. berghei* parasites in diabetic Wistar rats and improved the RBC counts in a dose dependent manner. The findings of this study suggest that RSV exerted schizontocidal and ameliorative effects on RBCs.

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CONFLICT OF INTEREST

The authors have no conflicts of interest. The authors are solely responsible for the contents and writing of the paper

Author contribution

All the authors extensively worked in drafting of the article, study for test, and consented to the final version of the manuscript.

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