

Adam O. Abdulkareem*, Abdulkareem O. Babamale, Lucky O. Owolusi, Simbiat A. Busari and Lawrence A. Olatunji

Anti-plasmodial activity of sodium acetate in *Plasmodium berghei*-infected mice

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Abstract

Background: Continuous increase in drug resistance has hindered the control of malaria infection and resulted in multi-drug-resistant parasite strains. This, therefore, intensifies the search for alternative treatments with no or less side effects. Several histone deacetylase inhibitors have been characterised to possess anti-malaria activity; however, their further development as anti-malaria agents has not recorded much success. The present study investigated the anti-plasmodial activity of sodium acetate in *Plasmodium berghei*-infected mice, aiming at finding a better alternative source of malaria chemotherapy.

Methods: Thirty female Swiss albino mice were randomly distributed into six groups. Groups A (uninfected control) and B (infected control) received only distilled water. Group C (artesunate control) were infected and treated orally with 4 mg/kg artesunate on the first day, and subsequently 2 mg/kg artesunate. Groups D, E and F were infected and orally treated with 50, 100 and 200 mg/kg sodium acetate, respectively.

Results: Sodium acetate significantly lowered parasitaemia ($p < 0.05$) after 4 days post-treatment, and the parasite inhibition rate of 68.5% at 50 mg/kg compared favourably with the 73.3% rate of artesunate. Similarly, administration of 50 mg/kg sodium acetate improved serum total cholesterol relatively better than artesunate. Our results also revealed that sodium acetate does not interfere with liver function, as there was no significant difference ($p > 0.05$)

in the serum activities of aspartate aminotransferase and alanine aminotransferase in both infected treated and uninfected mice.

Conclusions: This study shows that sodium acetate may be a safe alternative source of anti-malaria drugs. Its effect on the serum total cholesterol also predicts its ability in correcting malaria-induced metabolic syndromes.

Keywords: anti-plasmodial; HDACs; malaria; *Plasmodium berghei*; sodium acetate.

Introduction

In spite of the global efforts at malaria control, the infection continues to pose enormous challenges in the public health sector worldwide [1, 2]. This infectious disease accounts for millions of clinical cases and >1 million annual deaths, the majority of which are attributable to falciparum malaria infection [3]. Many reports have associated these high morbidities and mortalities to the resistance of plasmodium to most of the available drugs such as chloroquine, atovaquone, pyrimethamine, sulfadoxine and the current widely used artemisinin combination therapies [4, 5]. This results in multi-drug-resistant strains of malaria parasites, especially in developing countries of the world including Nigeria [6], thus necessitating the search for novel malaria treatments. In this regard, drugs with multiple targets and anti-proliferative properties are extremely exigent [7, 8].

Recently, Andrews et al. [9] suggested histone as a molecular target in mitigating parasite proliferation in the infected host. Histone regulates gene expression via the continuous acetylation and deacetylation of the ϵ -amino group of specific lysine residues with the aids of histone acetyl transferases and histone deacetylases (HDACs), respectively [10]. It has been observed that balance in the activities of these enzymes is essential for proper DNA transcription and cellular function [11]. Therefore, disruption of HDAC activity by small-molecule inhibitors such as short-chain fatty acids may cause hyperacetylation of histones and hence, alterations in gene transcription, apoptosis, cell differentiation and cell cycle progression [7].

*Corresponding author: Adam O. Abdulkareem, Animal Physiology Unit, Department of Zoology, University of Ilorin, Ilorin, Nigeria, Phone: +2348066528548, E-mail: abdulkareem.ao@unilorin.edu.ng

Abdulkareem O. Babamale: Parasitology Unit, Department of Zoology, University of Ilorin, Ilorin, Nigeria

Lucky O. Owolusi and Simbiat A. Busari: Animal Physiology Unit, Department of Zoology, University of Ilorin, Ilorin, Nigeria

Lawrence A. Olatunji: Cardiometabolic Research Unit, Department of Physiology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria

Previous studies, both *in vitro* and *in vivo*, have identified compounds such as apicidin, suberohydroxamic acid, azelaic bishydroxamic acid, trichostatin A and salicylhydroxamic acid as potential HDAC inhibitors (HDACIs) in malaria parasites leading to a reduction in parasite proliferation [6, 11, 12]. However, these compounds are either cytotoxic to the host or not potent enough to clear parasites appreciably, making their further development as anti-malaria agents unrealistic. Therefore, continuous screening for more readily available and safe HDACIs becomes imperative.

Sodium acetate is a sodium salt of acetic acid (a short-chain fatty acid) commonly used as a seasoning [13]. Previous studies have reported the anti-tumour and HDAC inhibitory effects of sodium acetate, inducing growth arrest and cell death in colon cancer cells via mitochondria-mediated apoptosis or necrosis [14–16]. However, this substance has not been screened for its anti-plasmodial property. Therefore, in this study, we investigated the role of sodium acetate (an HDACI) on the proliferation of malaria parasite in *Plasmodium berghei*-infected mice.

Materials and methods

Sodium acetate (a product of Science Company, TX, USA) was purchased from Bridge Biotechnology Nigeria Limited (Ilorin, Nigeria), and 0.0272 g of it was dissolved in 200 mL distilled water to prepare a stock solution. The solution was stored in airtight container and stored at 4 °C for further use.

Experimental animals

A total of 30 female Swiss albino mice weighing 18 g on average were purchased from the animal house, Central Research Laboratory, University of Ilorin, Ilorin. All animals were housed in plastic cages with sawdust beddings and kept in the animal house, Zoology Department, University of Ilorin, Ilorin. They were maintained under standard laboratory conditions (temperature of 22 ± 3 °C and 12 h light/12 h dark cycle), fed twice a day (morning and evening) and acclimatised for 14 days. The animals were kept in accordance with the regulation of the University of Ilorin Ethical Committee (approval no. UERC/ASN/2016/513) and in conformity to the National Institutes of Health guidelines on the care and use of laboratory animals [17].

Inoculation of parasites and estimation of parasitaemia

A chloroquine-sensitive *P. berghei* (ANKA strain) was obtained from Biochemistry Department, University of Ilorin, Ilorin, Kwara State, Nigeria. One millilitre of blood was taken from donor mice and diluted with normal saline, such that 0.2 mL of the infected blood

contained inocula of 1×10^7 infected red blood cells. The mice were first screened for malaria parasites, after which 25 parasite-free mice were induced with standard inocula of 1×10^7 *P. berghei* via the intraperitoneal route. The parasite was then allowed to incubate in the mice for 72 h, after which thin blood film was prepared from the caudal tip, stained using Giemsa stain and viewed under $\times 100$ objective of a light microscope. Estimation of parasitaemia was done according to Babamale and Ugbomoiko [18].

Animal grouping

Thirty acclimatised mice were randomly divided into six groups of five mice each. Group A mice were not infected and taken as the uninfected group. Group B (untreated infected group) mice were inoculated with *P. berghei* and received no treatment. Group C mice, which served as the artesunate controls, were inoculated with *P. berghei* and treated with standard drug (artesunate) at 4 mg/kg body weight (b.w) on the first day, and 2 mg/kg subsequently through oral administration. Groups D, E and F were orally treated with 50, 100 and 200 mg/kg sodium acetate, respectively. Treatment commenced on day 0 and lasted for 4 days. Blood was collected daily for blood film examination.

Blood collection

On Day 4, the mice were sacrificed by cervical dislocation. The jugular vein was exposed and cut with a sterile scalpel blade. The mice were bled into plain specimen bottles.

Lipid profile test

Serum total cholesterol (TC) concentration was determined by enzymatic analysis [19], and plasma triglyceride (TG) concentration was determined by the Hantzsch condensation reaction [20]. High-density lipoprotein (HDL)-cholesterol concentration was estimated using the precipitation method of Warmick et al. [21].

Determination of liver function test

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using Randox diagnostic kits (Randox Laboratory Limited, Antrim, UK).

Statistical analysis

Results were expressed as means $\pm$ standard error of measurements. Significance was determined by analysis of variance, using SPSS version 10.0 (SPSS, Chicago, IL, USA). Post-test analyses were done using the Duncan multiple range test, and p values < 0.05 . Bar charts were generated using Sigma Plot, version 10.0 (Systat Software Inc., San Jose, CA, USA).

Table 1: Effect of sodium acetate on parasitaemia (parasite/ μ L of blood).

Groups	Day 0	Day 1	Day 2	Day 3	Day 4
A (uninfected)	0.000 ^a	0.000 ^a	0.000 ^a	0.000 ^a	0.000 ^a
B (infected untreated)	64.67 $\pm$ 16.67 ^a	70.65 $\pm$ 15.62 ^b	76.67 $\pm$ 37.81 ^b	106.33 $\pm$ 16.59 ^b	136.00 $\pm$ 39.40 ^c
C (artesunate group)	124.67 $\pm$ 28.50 ^a	78.33 $\pm$ 16.25 ^b	32.00 $\pm$ 5.77 ^c	33.00 $\pm$ 2.65 ^c	33.67 $\pm$ 4.33 ^c
D (50 mg acetate)	89.33 $\pm$ 18.32 ^a	53.00 $\pm$ 14.90 ^b	29.33 $\pm$ 4.81 ^c	28.67 $\pm$ 3.48 ^c	28.34 $\pm$ 2.85 ^d
E (100 mg acetate)	27.50 $\pm$ 2.50 ^a	23.25 $\pm$ 1.25 ^{ab}	18.50 $\pm$ 0.50 ^{bc}	15.50 $\pm$ 1.50 ^c	12.50 $\pm$ 2.50 ^c
F (200 mg acetate)	56.67 $\pm$ 4.51 ^a	49.50 $\pm$ 14.27 ^{ab}	42.33 $\pm$ 12.08 ^b	40.33 $\pm$ 11.90 ^b	38.33 $\pm$ 16.90 ^b

Superscripts across a row depict a significant difference ($p < 0.05$).

Results

Anti-plasmodia (Rane's) test

As shown in Table 1 and Figure 1, there was a significant daily increase in parasitaemia level in infected untreated mice from day 0 to day 4 ($p < 0.05$). Administration of artesunate and sodium acetate at test doses, however, significantly lowered the parasitaemia ($p < 0.05$). The percentage of parasite inhibition progressively increased on days 1, 2 and 4 in all treated groups with a slight drop on day 3 in the artesunate group. Throughout the treatment period, the artesunate group recorded the highest percentage of

parasite inhibition except on day 1, when 50 mg/kg sodium acetate had the highest inhibition. At the end of Day 4 post-treatment, the highest anti-plasmodial effect observed in mice treated with artesunate (72.99% parasite inhibition) compared favourably with the 68.28% inhibition observed in the 50 mg/kg b.w sodium acetate group.

Lipid profile and enzyme (AST and ALT) activities

There was no significant difference ($p > 0.05$) in the lipid profile (HDL, TG and TC) in all the experimental mice. However, there were slight decreases in the

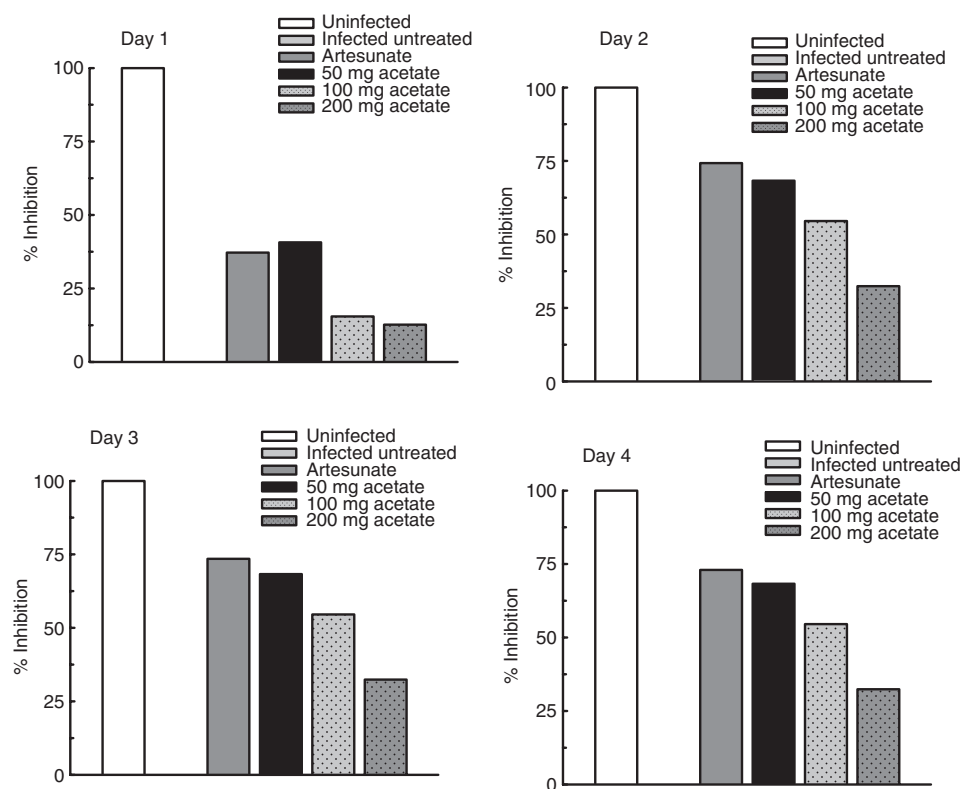
**Figure 1:** Daily percentage parasite inhibition.

Table 2: Effect of sodium acetate on lipid profile.

Parameters	Uninfected control	Infected untreated control	Artesunate control	Acetate 50 mg/kg	Acetate 100 mg/kg	Acetate 200 mg/kg
HDL-C, mg/dL	62.78 ± 3.98 ^a	60.34 ± 2.76 ^a	59.44 ± 0.55 ^a	59.64 ± 1.71 ^a	62.27 ± 0.00 ^a	58.59 ± 5.82 ^a
TG, mg/dL	175.14 ± 37.18 ^{ab}	132.66 ± 39.40 ^{ab}	157.89 ± 12.13 ^{ab}	175.14 ± 37.18 ^{ab}	118.89 ± 0.00 ^a	111.15 ± 3.82 ^a
TC, mg/dL	106.73 ± 44.24 ^a	84.22 ± 17.51 ^a	92.74 ± 6.31 ^a	97.55 ± 22.67 ^a	74.21 ± 0.00 ^a	75.00 ± 0.66 ^a

Superscripts across a row depict a significant difference ($p < 0.05$). HDL-C, high-density lipoprotein-cholesterol; TG, triglyceride; TC, total cholesterol.

Table 3: Effect of sodium acetate on AST and ALT.

Parameters	Uninfected control	Infected untreated control	Artesunate control	Acetate 50 mg/kg	Acetate 100 mg/kg	Acetate 200 mg/kg
AST, IU/L	44.53 ± 39.51 ^a	28.66 ± 31.19 ^a	37.76 ± 7.96 ^a	40.21 ± 12.74 ^a	39.61 ± 0.001 ^a	39.17 ± 20.69 ^a
ALT, IU/L	47.15 ± 47.78 ^a	55.9 ± 22.29 ^a	30.55 ± 38.43 ^a	51.23 ± 37.28 ^a	10.63 ± 0.0031 ^a	29.90 ± 24.87 ^a

Superscripts across a row depict a significant difference ($p < 0.05$). AST, aspartate transaminase; ALT, alanine aminotransferase.

HDL (60.34 ± 2.76 mg/dL), TG (132.66 ± 39.40 mg/dL) and TC (84.22 ± 17.51 mg/dL) of the infected untreated mice as compared to 62.78 ± 3.98, 175.14 ± 37.18 and 106.73 ± 44.24 mg/dL recorded for HDL, TG and TC, respectively, in the uninfected group. Both artesunate and sodium acetate improved the serum levels of TG and TC, with the highest effect observed in the 50 mg/kg sodium acetate group (Table 2). All doses of sodium acetate caused no significant change ($p > 0.05$) in serum AST and ALT as compared to the controls (Table 3).

Discussion

Histone deacetylation is very essential in proper cell functioning, differentiation and growth [10–12]. The multifunctional benefits of pharmacological inhibition of HDACs in the treatment of many diseases have rekindled the scientific search for more potent therapeutic HDACIs [22, 23]. Currently, HDACIs are widely used in medicine for curing different diseases such as sickle cell anaemia, cutaneous T-cell leukaemia and β -thalassemia [24, 25], but none has been established for successful malaria treatment. Many of the identified HDACIs are either faced with toxicity or parasite clearance challenges [6, 8, 12].

In this study, we observed that sodium acetate impressively inhibited parasite growth in *P. berghei*-infected mice. The pattern of result on the chemosuppressive property ranks the lowest dose of sodium acetate (50 mg/kg b.w) next to artesunate, indicating that, the salt inhibits parasite growth in a reversed dose-dependent manner.

This observation may be attributable to the HDAC inhibitory activity of the salt as previously reported by [13, 14, 26, 27], which probably resulted to an increase in *P. berghei* histone acetylation and concomitantly reducing parasite proliferation [28, 29]. The mechanism for this reduction is not yet known; however, Agbor-Enoh et al. [30] has postulated that the trophozoite and schizont stages of malaria parasites are most susceptible to HDACI-induced cell death simply because DNA replication and synthesis of essential macromolecules necessary for parasite multiplication occur during these stages of development. The potential of sodium acetate in reducing proliferation of parasite in the infected host is evidenced by the 68% parasite inhibition observed in the infected mice treated with 50 mg/kg sodium acetate for 4 days, compared with the group treated with standard drug (artesunate).

Furthermore, the slight decrease in serum cholesterol obtained in this study among the infected untreated group is similar to the reports of previous studies [31, 32], indicating reduced serum cholesterol in the malaria condition. Cholesterol is an essential component of cell membrane required by all organisms, including malaria parasites [33, 34]. The latter require it for membrane formation in maintaining intraerythrocytic infection, hence competing with host for this essential lipid [31, 35]. Upon administration of sodium acetate at the highest effective dose (50 mg/kg), serum TC relatively improved as compared to the controls. Although the mechanism was not investigated, it may be that host cholesterol synthesis improved after most of the parasites were cleared, as evidenced in our results (Table 2).

Our analysis of toxicity revealed no observable clinical side effects of sodium acetate. Also, the integrity and normal functioning of the liver were not compromised, as shown by the insignificant changes in the serum activities of AST and ALT of the sodium acetate-treated mice when compared to controls [36]. This may imply that sodium acetate showed selective toxicity against growing parasites [14].

Conclusions

The outcome of this study underscores the parasite-inhibiting potential of sodium acetate, thus serving as an alternative that may be exploited in the development of anti-malaria drug. It does not only compare favourably well with the standard drug but also less toxic to the body system. Its effect on the serum TC also predicts its ability in correcting malaria-induced metabolic syndromes. However, the mechanisms of action of this chemical need to be elucidated for effective exploitation in anti-malaria drug formation.

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