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Antiplasmodial activities of selected artemisinin-based combination therapy (ACT) drugs co-administered with beer in *Plasmodium berghei*-infected albino rats.

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Abstract

Alcoholic beverages, particularly beer, are widely consumed in Nigeria despite its contribution to most disability, deaths, and some non-communicable diseases. Based on unpublished reports, some individuals consume alcoholic beverages while treating malaria, which raises concerns about potential interactions between alcoholic beverage and antimalaria drugs. However, there is paucity of information on the effect of alcoholic beverage consumption on the management of malaria. Hence, this study investigated the effect of alcoholic beverage co-administered with Artemisinin-based Combination Therapy (ACT) drugs on parasitaemia in an established malaria infection of albino rats infected intraperitoneally with Chloroquine-sensitive *Plasmodium berghei*. Different sub-groups of the experimental animals (except the positive and negative controls) were orally administered 0.5mL of beer (5.2% alcohol) either 1 hour before, concurrently, or 1 hour after malaria treatment with 0.2 mL of selected ACT medicines. All experimental animals (except the negative control) were treated with only one of the ACTs: artemether + lumefantrine (AL), dihydroartemisinin + piperazine (DP), and artesunate + amodiaquine (AA) for three days. Parasitaemia was determined from microscopy of Giemsa-stained thin blood films of experimental animals. Result showed that parasitaemia reduced significantly ($p<0.05$) in the positive control groups treated with ACTs only compared to other experimental groups (beer-administered and negative control). Parasitaemia reduced from 50.7%, 39.27%, and 29.4% to 6.07%, 5.63%, and 9% in the AL, DP, and AA groups respectively. Parasitaemia also reduced significantly ($p<0.05$) in the beer-administered groups treated with ACT compared to the negative control only. In the group of infected animals administered beer without antimalarial treatment, parasitaemia reduced significantly ($p<0.05$) compared to the negative control only. These findings suggest possible drug-alcohol interactions that has the potential to reduce the therapeutic efficacy of ACTs.

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1.0 Introduction

The health, social and economic costs of alcohol-related harm and diseases globally are well-documented [1]; [2]; [3]. In Nigeria, alcohol is the sixth leading risk factor contributing to most death and disability [4].

When alcohol is ingested, approximately 10% of it undergoes initial processing in the stomach, intestines, and liver [5]. One of the key enzymes responsible for alcohol metabolism is alcohol dehydrogenase (ADH), which transforms alcohol into acetaldehyde, a harmful substance subsequently broken down into acetate by aldehyde dehydrogenase (ALDH) [6]. After this initial metabolic phase, alcohol spreads throughout various body tissues, exerting its effects. It is then transported back to the liver for further processing and elimination. Besides further metabolism by ADH within the liver, alcohol is also metabolized by a group of enzymes known as CYP450, with CYP2E1 being the primary enzyme involved in this process [7].

Most people who consume alcohol, whether in moderate or large quantities, also take medications, at least occasionally [8]. As a result, many people ingest alcohol while a medication is present in their body or vice versa [9]. Many medications that are either available only by prescription or Over-the-Counter (OTC) have the potential to interact with alcohol [10]. Those interactions can alter the metabolism or activity of the medication and/or alcohol metabolism, resulting in potentially serious medical consequences. This may particularly apply to antimalarial medications especially the World Health Organization (WHO) recommended ACT drugs for treatment of uncomplicated malaria cases [11]. Beer is a widely consumed alcoholic beverage, and its consumption has been reported to consistently increase human attractiveness to *Anopheles* mosquito [12]. Ethanol had been reported to significantly inhibit *in vitro* growth of *Plasmodium falciparum* [13]. However, there is relatively no information on the effect of alcohol-drug interactions (ADI) on the efficacy of antimalaria medications. This study was conducted to investigate the effect of co-administration of beer with ACTs on the efficacy of the latter in albino rats infected with Chloroquine-sensitive *Plasmodium berghei*.

2.0 Methods

2.1 Experimental design

The experiment was set up in a completely randomized design. Sixty (60) Swiss male albino rats were randomly divided into 15 sub-groups that represented 15 different treatments. Each subgroup (treatment) had four replicates, represented by four albino rats.

2.2 Brand of 'Beer' used for the study

The brand of beer used for this study was Gulder lager and it was sourced from a local supermarket in Afikpo, Ebonyi State, Nigeria. The alcohol content of a bottle of the beer is 5.2%. A bottle of beer served for each day of the study, any left-over was discarded and a new bottle purchased for the next day.

2.3 Antimalarial drugs used for the study

Three (3) different Artemisinin combination therapy (ACT) derivative drugs (tablets) were used in this study, and they are: Atmal-plus® containing artemether + lumefantrine (AL), Fanmet® containing Dihydroartemisinin + piperaquine (DP), and Camosunate® containing artesunate + amodiaquine (AA). These three ACT drugs were used in this study because they were the only ACTs derivative drugs available in registered pharmacies as at the time of the study. The antimalarial drugs were sourced from a registered pharmacy in Afikpo, Ebonyi State, Nigeria.

2.4 Experimental animals used for the study

In-bred male Swiss albino rats weighing between 150g and 265g obtained from the Animal House of Department of Veterinary Medicine, University of Nigeria, Nsukka, were used for the protocols. The animals were housed in clean gauzed cages under 12 hours light/dark cycles and fed with pelletized feed (Guinea feeds Nigeria Plc) and clean water *ad libitum* during the 10 days acclimatization period. The caring and experimental uses of the rats were according to the guidelines of National institute of health guidelines for cares of laboratory animals.

2.5 Parasite used for the study

Chloroquine sensitive strain of *P. berghei* (ANKA 65) obtained from the Department of Veterinary Medicine, University of Nigeria, Nsukka were used. The parasite has been maintained successfully in albino rats. The parasites were acquired few days to the start of the experiment.

2.6 Parasite inoculation

Parasitized blood from donor rat was collected by cardiac puncture into heparinized tubes containing 0.5% trisodium citrate. Donor albino rats previously infected with *P. berghei* (ANKA 65 chloroquine sensitive strain) and having parasitaemia level of 25-35% obtained from the Department of Veterinary Medicine, University of Nigeria, Nsukka were used. Parasitaemia was established by microscopy of Giemsa-stained thin blood film using X100 magnification of the objective lens and measured as percentage of total red blood cells. *P. berghei* infection of experimental animals was initiated by intraperitoneally inoculating 0.2mL physiological saline (0.9%) containing approximately 10^6 parasitized erythrocytes prepared from donor mouse into each animal.

2.7 Administration of beer and antimalarial drugs

Experimental animals were orally administered 0.5mL of the beer daily (1hour before, or concurrently with, or 1hour after administration of the antimalarial drug). Clinical doses [14] of the test drugs: Artemether/Lumefantrine (1/7mg/kg), Dihydroartemisinin/piperaquine (6/54mg/kg), and Artesunate/Amodiaquine (3/9mg/kg) were prepared as aqueous suspension (using distilled water) and 0.2ml administered orally to the experimental animals. The different sub-groups are shown below:

Sixty (60) Swiss male albino rats were divided into fifteen (15) groups with four rat per group (n= 4) as described below.

Sub-group I: *P. berghei* infected animals, treated with AL at 1 hour before administering beer.

Sub-group II: *P. berghei* infected animals, treated with AL concurrently with beer administration.

Sub-group III: *P. berghei* infected animals, treated with AL at 1 hour after administering beer.

Sub-group IV: *P. berghei* infected animals, treated with DP at 1 hour before administering beer.

Sub-group V: *P. berghei* infected animals, treated with DP concurrently with beer administration.

Sub-group VI: *P. berghei* infected animals, treated with DP at 1 hour after administering beer.

Sub-group VII: *P. berghei* infected animals, treated with AA at 1 hour before administering beer.

Sub-group VIII: *P. berghei* infected animals, treated with AA concurrently with beer administration.

Sub-group IX: *P. berghei* infected animals, treated with AA at 1 hour after administering beer.

Sub-group X: *P. berghei* infected animals, administered beer only.

Sub-group XI: *P. berghei* infected animals, administered AL only (positive control).

Sub-group XII: *P. berghei* infected animals, administered DP only (positive control).

Sub-group XIII: *P. berghei* infected animals, administered AA only (positive control).

Sub-group XIV: *P. berghei* infected animals, administered distilled water only (negative control).

Sub-group XV: Normal uninfected animals administered distilled water only (control).

After 10 days acclimatization, the first 10 groups (I to X) were administered 0.5mL/kg body weight of beer orally for 25 days for further acclimatization to beer. After this, all experimental animals except those in Group XV were passaged with *P. berghei* and 4 days later, infection was established. Treatment with antimalarial drugs commenced in all experimental animals on the fifth day except Groups XIV and XV that were not administered test drugs.

2.8 Estimation of parasitaemia

On each day, blood collected from tail of animals were used to prepare thin blood films on microscope glass slides. Thin blood films were fixed with absolute methanol for one minute, air-dried, stained with Giemsa, and allowed to dry in room temperature. Tail end of blood films were viewed under X100 oil immersion objective lens of a light microscope. Percentage parasitaemia was determined by estimating the number of parasitized red blood cells in 100 red blood cells under the light microscope [15].

2.9 Data analysis

The data obtained were analyzed using One Way Analysis of Variance (ANOVA). The results were expressed as mean ± SD. *Post hoc* test was used to compare the group means with control groups. Significant differences were obtained at $p<0.05$.

3.0 Results

Result showed gradual reduction in parasitaemia in all the experimental groups (except the negative control) after the first day of treatment (Day 1) till Day 1 post treatment (P-T) (Table 1).

3.1 Effect of 'beer only' on parasitaemia

Result showed a significant ($p<0.05$) reduction in mean parasitaemia (44.43% to 35.97%) in the animals administered beer only when compared to the negative control (Figure 1). However, the reduced parasitaemia observed in the 'beer only' group (35.97%) on day 1 post treatment is significantly higher ($p<0.05$) than parasitaemia in the positive control groups treated with ACTs only. Parasitaemia in the positive control groups only- AL, DP, and AA on day 1 post treatment were 6.07%, 5.63% and 9.00% respectively, significantly lower parasitaemia compared to the negative control and pre-treatment (day 0).

Table 1: Percentage Parasitaemia in *Plasmodium berghei* infected animals treated with antimalarial drug and beer.

		Parasitaemia (%) on days of treatment				
Groups	**Sub-groups**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 1 (P-T)**
AL	1hr b4 beer	43.87 ± 2.73^{b}	48.23 ± 1.90^{b}	32.63 ± 2.55^{a}	18.83 ± 1.62^{a}	10.27 ± 1.00^{ab}
	C.W. beer	35.67 ± 2.97^{b}	44.20 ± 3.93^{b}	22.73 ± 2.47^{ab}	19.93 ± 1.56^{a}	9.50 ± 1.42^{ab}
	1hr after beer	29.23 ± 4.53^{b}	36.83 ± 1.52^{ab}	25.80 ± 1.40^{ab}	20.80 ± 1.50^{a}	15.87 ± 0.38^{ab}
	Atmal only	50.70 ± 4.60^{a}	59.40 ± 1.00^{a}	36.03 ± 0.35^{a}	19.73 ± 0.99^{a}	6.07 ± 1.10^{a}
DP	1hr b4 beer	53.03 ± 3.88^{ac}	50.80 ± 7.01^{c}	34.13 ± 3.66^{ac}	22.87 ± 2.60^{ac}	14.43 ± 1.63^{ac}
	C.W. beer	29.97 ± 2.66^{ac}	33.63 ± 1.39^{ac}	25.07 ± 1.82^{ac}	17.80 ± 1.40^{a}	8.97 ± 1.50^{ac}
	1hr after beer	38.50 ± 9.07	42.27 ± 9.05^{a}	24.87 ± 1.33^{ac}	20.03 ± 0.72^{ac}	15.07 ± 1.00^{ac}
	Fanmet only	39.27 ± 1.81	43.33 ± 1.11^{a}	20.10 ± 1.05^{a}	15.20 ± 1.05^{a}	5.63 ± 0.42^{a}
AA	1hr b4 beer	28.00 ± 3.00^{a}	31.97 ± 2.46^{ad}	30.23 ± 1.68^{a}	33.80 ± 7.96^{ad}	30.50 ± 2.46^{ad}
	C.W. beer	39.77 ± 4.61^{d}	43.70 ± 5.11^{a}	42.07 ± 2.20^{ad}	37.13 ± 2.40^{ad}	30.50 ± 2.46^{ad}
	1hr after beer	50.33 ± 8.08^{ad}	55.40 ± 8.03^{d}	41.23 ± 3.73^{ad}	34.70 ± 3.29^{ad}	25.53 ± 2.62^{ad}
	Cam. only	29.40 ± 0.00^{a}	46.70 ± 0.00^{a}	28.07 ± 0.06^{a}	16.20 ± 0.00^{a}	9.00 ± 0.00^{a}
Beer only		35.17 ± 3.43^{b}	44.43 ± 4.15^{b}	41.53 ± 1.36^{abcd}	39.10 ± 1.95^{abcd}	35.97 ± 3.70^{abcd}
Negative control		39.07 ± 1.00^{bd}	48.97 ± 0.45^{b}	51.37 ± 1.26^{bcd}	56.90 ± 1.55^{bcd}	65.10 ± 2.05^{bcd}

n=4; Data presented in mean±SD. $^{a}p<0.05$ significant compared with the negative control. $^{b}p<0.05$ significant compared with the standard drug (AL). $^{c}p<0.05$ significant compared with the standard drug (DP). $^{d}p<0.05$

significant compared with the standard drug (AA); mean values without superscripts: no significant difference. AL: Arthemeter/Lumefantrine; DP: Dihydroartemisinin/Piperaquine; AA: Artesunate/Amodiaquine; C.W.: concurrently with; (P-T): post-treatment.

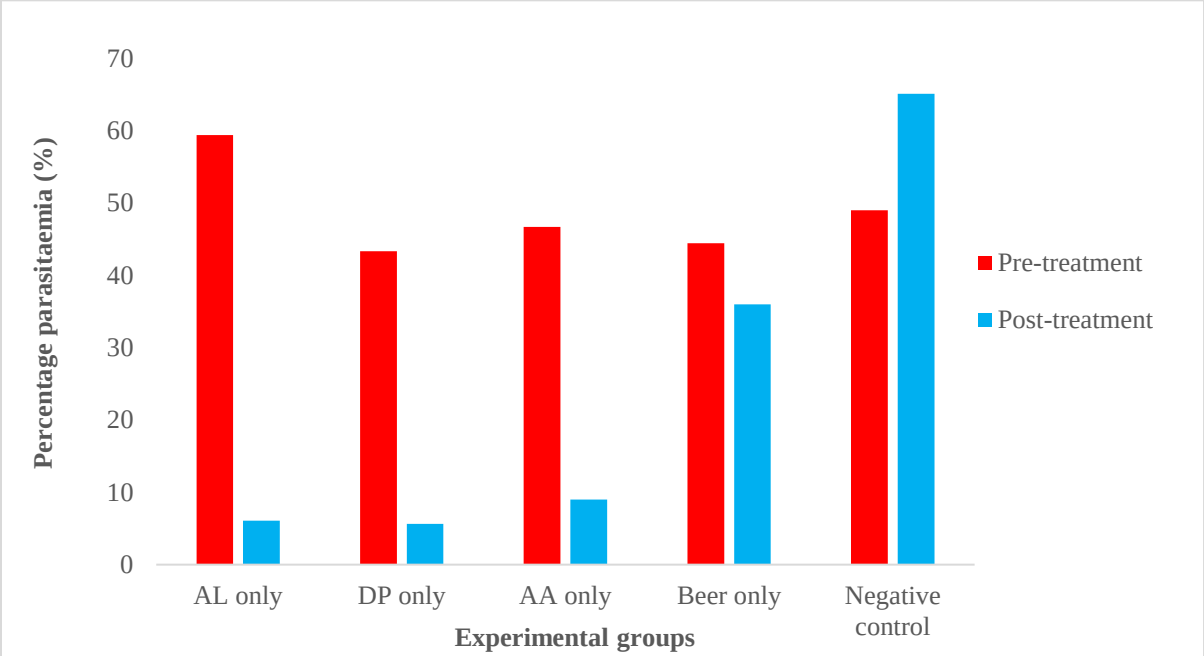

Fig. 1: The effect of 'beer only' on parasitaemia compared with the chemotherapeutic activities of the control drugs in *Plasmodium berghei*-infected rats before treatment (Day 0) and after treatment (Day 1 post treatment).

3.2 Effect of co-administering Artemether lumefantrine and beer on parasitaemia

Result showed that treatment with artemether lumefantrine (AL) only reduced parasitaemia significantly ($p<0.05$) compared to the negative control and other groups where beer was combined with AL. On day 1 (post treatment), parasitaemia in ascending order were 6.07%, 9.50%, 10.27%, and 15.87% in the groups treated with AL only, AL concurrently with beer, AL treatment at 1hour before beer, and AL treatment at 1 hour after beer respectively (Figure 2).

3.3 Effect of co-administering Dihydroartemisinin/Piperaquine and beer on parasitaemia

Result showed that treatment with Dihydroartemisinin/Piperaquine (DP) only reduced parasitaemia significantly ($p<0.05$) compared to the negative control and other groups where beer was combined with DP. On day 1 (post treatment), parasitaemia in ascending order were 5.63%, 8.97%, 14.43%, and 15.07% in the groups treated with DP only, DP concurrently with beer, DP treatment at 1hour before beer, and DP treatment at 1hour after beer respectively (Figure 3).

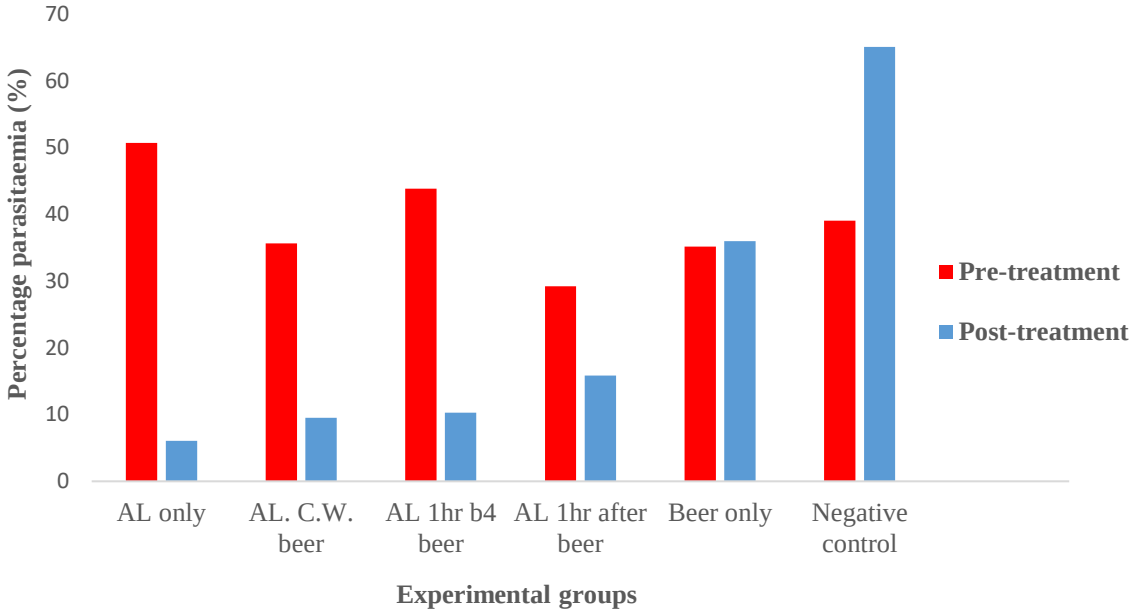

Fig. 2: The effect of co-administering Artemether lumefantrine and beer on parasitaemia compared with the negative control on Day 0 and Day 1 post treatment

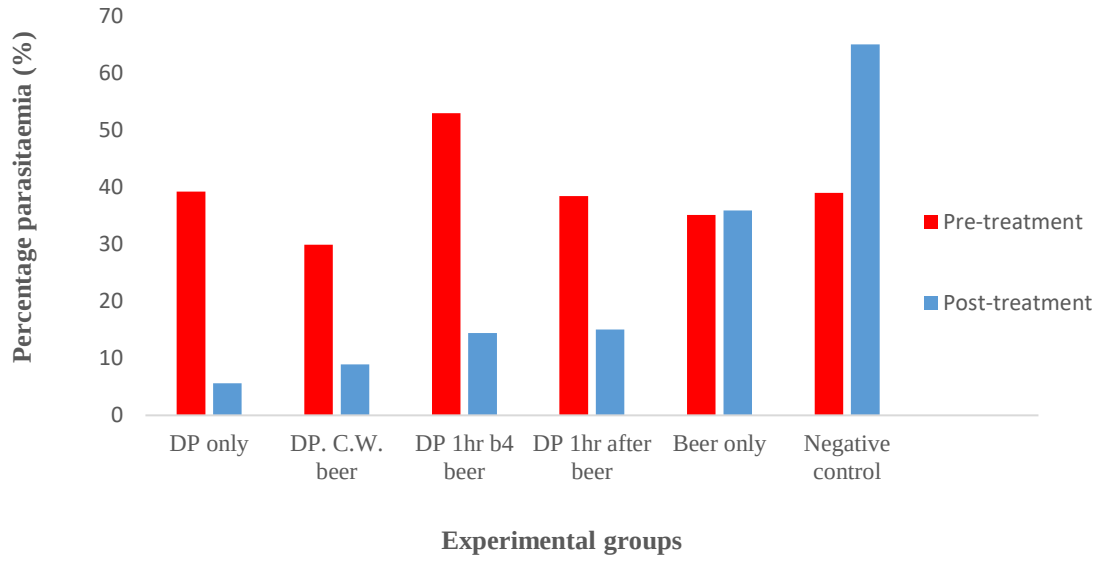

Fig. 3: The effect of co-administering Dihydroartemisinin/Piperaquine and beer on parasitaemia compared with the negative control on Day 0 and Day 1 post treatment

3.4 Effect of co-administering Artesunate/Amodiaquine and beer on parasitaemia

Result showed that treatment with Artesunate/Amodiaquine (AA) only reduced parasitaemia significantly ($p<0.05$) compared to the negative control and other groups where beer was combined with AA. On Day 1 post treatment (Day 1 P-T), parasitaemia in ascending order were 9.00%, 25.53%, 30.50%, and 30.50% in the groups treated with AA only, AA treatment at 1hour after beer, AA concurrently with beer, and AA treatment at 1hour before beer respectively (Figure 4).

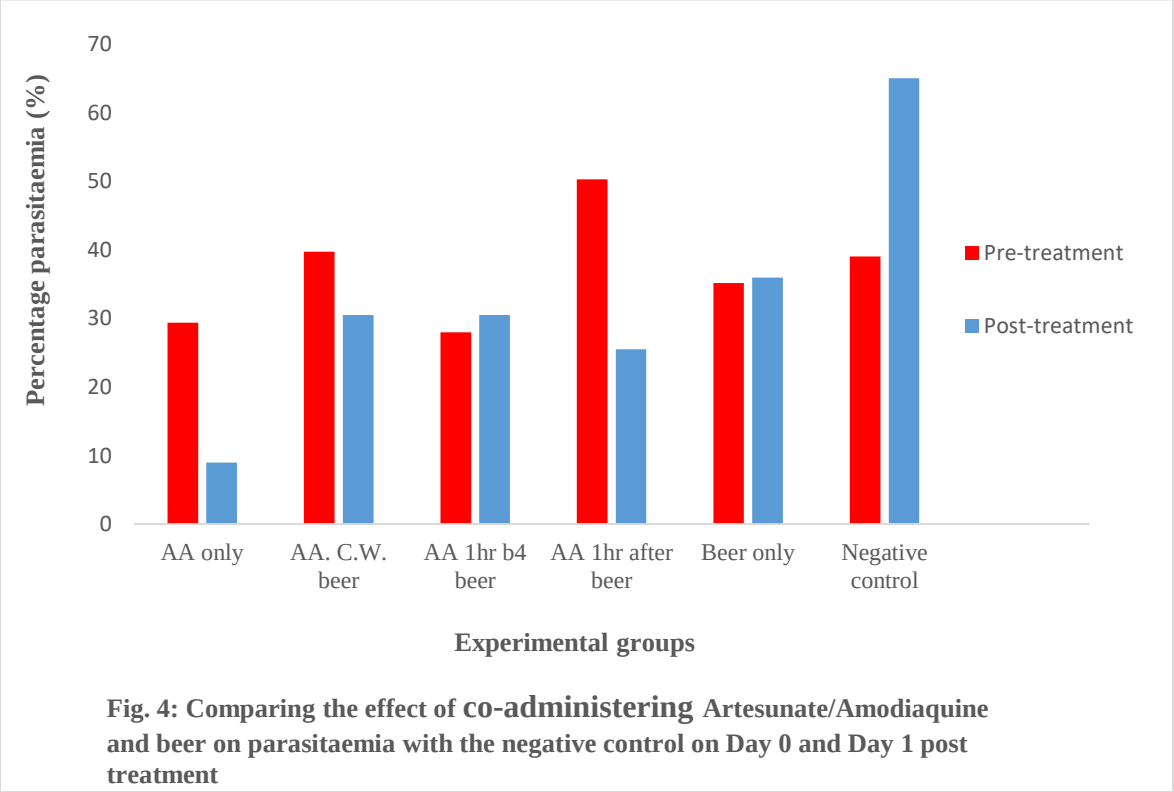

Fig. 4: Comparing the effect of co-administering Artesunate/Amodiaquine and beer on parasitaemia with the negative control on Day 0 and Day 1 post treatment

4.0 Discussion

The coexistence of alcohol and pharmaceuticals within the human body can lead to a myriad of interactions [16], some of which may impact the effectiveness of medical treatments. In the context of malaria, a deadly mosquito-borne disease that affects millions of people globally [17] the interaction between alcohol, specifically beer, and anti-malarial drugs is a subject of critical importance. This study's focus is on the interaction between alcohol and anti-malarial drugs when co-administered.

The study's initial observation is intriguing: the group administered only beer exhibited a notable reduction in parasitaemia compared to the negative control group. This finding raises an important question: can beer, a beverage often associated with relaxation and social gatherings, possess anti-malarial properties?

However, a deeper analysis reveals that while beer consumption on its own does demonstrate some anti-malarial effect, it falls short when compared to the artemisinin-based combination therapy (ACT) drugs used as the positive control. In fact, the low parasitaemia observed in the 'beer only' group on day 4 (day 1 post treatment) was significantly higher than parasitaemia seen in the groups treated with ACT drugs alone (AL, DP, and AA). This implies that while beer may have some modest anti-malarial potential, it cannot match the effectiveness of the dedicated anti-malarial drugs.

However, when the same drugs were co-administered with beer, a complex interaction emerged. The results showed a significant reduction in parasitaemia compared to the negative control, suggesting that the anti-malarial effect of the drugs was not entirely nullified by the presence of alcohol. However, the parasitaemia levels in these co-administration groups were significantly higher than in the groups that received ACT drugs alone.

In essence, co-administering beer with ACT drugs still retained some anti-malarial effect, but it was notably less effective than administering the drugs in isolation. This highlights the potential interference of alcohol, specifically beer, with the full therapeutic potential of these critical anti-malarial medications.

One of the intriguing aspects of the study was its investigation into the timing of co-administration, particularly in the case of AL and DP where parasitaemia reduced significantly when it was administered concurrently with beer compared to when administered 1hour after consumption of beer. This observation is different from what was observed in the AA where parasitaemia increased when the drug was administered concurrently with beer and 1hour after beer.

The anti-malarial drugs mentioned in the study, such as artemether and dihydroartemisinin, are metabolized in part by CYP2B6 and CYP3A4 enzymes [18]. When alcohol (contained in beer) is co-administered with these drugs, it can lead to reduced enzyme activity due to inhibition [19]. As a result, the metabolism of the anti-malarial drugs is slowed down, leading to reduced drug efficacy. Hence, the anti-malarial effect of the ACT drugs in this study was compromised.

The results also showed that concurrent administration of DP and beer led to a significant reduction in parasitaemia compared to administering DP either one hour before or after beer consumption. This outcome indicates that the timing of alcohol consumption concerning drug administration can significantly influence the outcome of the interaction. When DP was administered either before or after beer consumption, the anti-malarial effect was compromised. This suggests that the timing of alcohol consumption relative to drug administration is crucial. Administering the drug before alcohol might result in more rapid metabolism (and reduction in parasitaemia observed in the AL group) and reduced drug bioavailability due to the presence of active, un-inhibited CYP enzymes. Conversely, administering the drug after alcohol may inhibit the optimum absorption of the drug to the expected body concentration before metabolism occurs.

However, it's worth noting that similar results were not observed in the groups receiving other ACT drugs like AL and AA, suggesting that the timing of co-administration may have varying effects depending on the specific drug being used.

The findings of this study had shown: (i) the ACTs are still effective in the treatment of malaria, and (ii) that in an established malaria infection that is not treated with an ACT drug, consumption of beer inhibited

multiplication of *Plasmodium* in peripheral blood but not total clearance. This latter evidence supports a previous *in vitro* study where ethanol concentrations were reported to strongly inhibit malaria parasites [13]. Interestingly, palm wine (an alcoholic beverage) is also believed to possess prophylactic agents against malaria by some consumers in Nigeria [20]. On the other hand, we also observed that the efficacy of ACT on parasitaemia reduced when administered with beer at varying intervals of time. This is likely an indication of beer-drug interaction on parasitaemia when ACT is used in the treatment of malaria. However, the possible mechanism for such interaction was not evaluated in the present study.

In these two scenarios where parasitaemia is not totally cleared and if symptoms are no longer present, such individuals become asymptomatic carriers of the parasite thereby contributing to continuous malaria transmission especially in endemic settings. This may pose a public health challenge to efforts at eliminating malaria.

5.0 Conclusion

These findings shed light on the intricate nature of drug-alcohol interactions, especially in the context of malaria treatment. Beer consumption alone does appear to have some anti-malarial properties, albeit less potent than dedicated anti-malarial drugs. However, when co-administered with these antimalarial drugs, the anti-malarial effect of the drugs on the parasite is compromised. In summary, the observed drug-alcohol interactions in the study can be explained through the inhibition of CYP2B6 and CYP3A4 metabolizing enzymes by alcohol. The timing of alcohol consumption relative to drug administration plays a critical role in the outcomes. Concurrent administration may lead to enhanced drug efficacy due to reduced enzyme activity and slower drug metabolism, whereas administration before or after alcohol can result in compromised drug effectiveness. These mechanisms illustrate the complex interplay between alcohol, anti-malarial drugs, and drug-metabolizing enzymes, underscoring the importance of understanding these interactions for safe and effective malaria treatment.

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Author contributions: OTS and SSO conceived the study; OTS, SSO and JBS contributed to the experimental design; OTS and SSO played significant parts in data collection, OTS did the analysis of data; and OTS wrote the main manuscript text including tables and figures. All authors read, reviewed, and approved the manuscript.

Competing interests: The author(s) declare no competing interests.

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