African Scientist Vol. 11, No. 3, September 30, 2010
Printed in Nigeria

1595-6881/2010 $12.00 + 0.00

http://www.klobex.org/afs

AFS 2010081/11306

Behavioural patterns of rats in an open field following treatment with Artesunate *plus* Amodiaquine combination

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(Received May 31, 2010; Accepted July 8, 2010)

ABSTRACT: Artesunate (AS) *plus* amodiaquine (AQ) combination is a potent artemisinin-base antimalrial drug is commercially available with different trade names. Several adverse effects including neurotoxic complaints have been ascribed to this combination. This study was to determine the behavioral pattern of rats treated with this drug in an open field. Forty matured male Wistar rats weighing 150-180g were equally grouped into four. Group A was the control and the animals received tap water placebo, while groups B, C and D were the experimental. Groups B and C were treated respectively with 2.86mg/kg *plus* 8.75mg/kg (therapeutic dose, TD), and 5.71mg/kg *plus* 17.50mg/kg (high pharmacologic dose, HPD) of AS *plus* AQ combination per day for 3 days, while group D was treated with 2.86mg/kg *plus* 8.75`mg/kg (long duration therapeutic dose, LDTD) of AS *plus* AQ combination per day for 6 days. The open field test was carried out 12 hours after their last treatments. No significant difference existed between the experimental groups and the control in total locomotor activity, central square frequencies and duration, and stretch attend. There was no urination in all the groups, while the HPD group had a significant higher defecation than the control and the TD and LDTD groups. This study revealed no significant change in behavior of the rats after treatment with this drug, indicating that this drug may not have altered locomotion, and may neither be anxiolytic nor anxiogenic at these doses and time.

Keywords: Artesunate, Amodiaquine, Behavioural patterns, Wistar rats.

Introduction

The endemic nature of malaria in sub-Saharan Africa including Nigeria, and the resistance of the malarial parasites to conventional antimalarial drugs brought about the need for change in the regimens of antimalarial drugs used for treatment. Hence the artemisinin-based combination therapies (ACTs) was introduced (1,2).

ACTs provide the highest effectiveness with reduction in the ability of the parasites to offer resistance (3,4). These, and the awareness created by care givers (5) have resulted in the rise in usage of ACTs.

Artesunate (AS) and amodiaquine (AQ) combination is one of the different ACTs currently recommended by the World Health Organization (WHO), and is commercially available as Larimal®. AS is a water-soluble hemisuccinate derivative of artemisinin derived from the leaves of a Chinese tree, *Artemisia annua.* It is a blood schizonticide which is effective as a monotherapy drug, and this efficacy is enhanced in combination with conventional drugs like AQ (1,6). Its mechanism of action involves the heme-mediated decomposition of the endoperoxide bond to produce carbon-centered free radicals, which kills the malaria

parasite if accumulated in the erythrocytes (7). Adverse effects include neurotoxicity, which has been observed in animal studies but not in humans (8).

AQ is a 4-aminoquinoline antimalarial drug with schizonticidal activity and, possesses antipyretic and anti-inflammatory properties. AQ generates free radicals in the form of AQ quinone immine and semi quinone immine which kills the parasites in blood (9). In therapeutic doses used for prophylaxis and for malaria therapy, AQ have been reported to occasionally cause peripheral neuropathy, and occasionally nausea, vomiting, diarrhoea, vertigo and lethargy (10).

This combination has been implicated in several adverse behavioural effects (8,10,11). Behavioural activities of animals in normal and adverse conditions vary in different environments. In a novel environment, animals behave in unpredictable ways (12). On exposure to exogenous substances, like treatment with a drug, these either stimulate, modulate or inhibit these behavioural patterns. This study seeks to determine the behavioral pattern of Wistar rats in an open field on treatment with the therapeutic and pharmacological doses of the AS *plus* AQ combinations.

Materials and Methods

Forty adult male Wistar rats weighing between 150-180g procured from the animal house of the Department of Anatomy were handled in accordance with the International regulation governing the use and care of laboratory animals, and ethical approval was sort from the institution. The animals were randomly assigned into four groups (A, B, C, and D) of ten animals each. Group A served as the control, while groups B, C and D were the experimental.

Two packets of Larimal® was bought from a reputable pharmacy in Calabar, Nigeria. Each packet of Larimal® contained twelve blistered tablets of AS (50mg) and AQ (153.1mg) each. These were dissolved in clean tap water and the mg/kg body weight was calculated using the weight of a physiologic man (70kg), which was regarded as the therapeutic dose in the animals (2.86mg/kg *plus* 8.75mg/kg of AS and AQ respectively).

Group A animals received tap water placebo, and groups B and C were treated respectively with 2.86mg/kg *plus* 8.75mg/kg (therapeutic dose, TD) and 5.71mg/kg *plus* 17.50mg/kg (high pharmacologic dose, HPD) of AS *plus* AQ combination per day for 3 days, while group D was treated with 2.86mg/kg *plus* 8.75mg/kg (long duration therapeutic dose, LDTD) of AS *plus* AQ combination per day for 6 days, all by oro-gastric tubes. The animals were treated twelve hourly (twice daily). The treatments is shown in Table 1. The open field test was carried out 12 hours after their last treatments using the methods of Walsh and Cummins (1976) as modified by Brown et al (1999). Briefly, it involved an apparatus constructed of white plywood of 72×72cm with 36cm walls. One of the walls was clear Plexiglas, so the animals would be visible, and the floor lined with clear Plexiglas. Blue lines were drawn on the floor with a marker and this was visible through the clear Plexiglas floor. These lines divided the floor into sixteen 18×18cm squares. A central square of 18×18cm was drawn in the middle of the open field.

Rats were carried to the test room in home cages and were handled by the base of their tails at all times. Each rat was placed in the proximal right-hand corner of the maze and allowed to explore the apparatus for five minutes. After the five minute test, the rat was returned in its home cage and the open field was cleaned with 70% ethyl alcohol and permitted to dry before introduction of the next rat. Behavior was scored manually, and each trial was recorded for latter analysis using a video camera positioned above the apparatus. The counting was done manually.

The following activities were carried out: frequency of line crossing; frequency of central square entry (CSF), central square duration (CSD); frequency of rearing; frequencies of stretch-attend (SA); urination and defecation.

Statistical analysis using a one-way analysis of variance (ANOVA) was used to compare the group's mean for the open field parameters for treatment and their interactions. Thereafter post-hoc test using Tukey-Kramer Multiple Comparative Test was carried out to find the level of significance at $p<0.05$. All the results were expressed as mean ± standard error of mean.

Table 1: Schedule of the drug administration.

Group	Dosage per day of AS	Duration (days)
A	Control (tap water)	3
B (TD)	*2.86mg/kg *plus* 8.75mg/kg	3
C (HPD)	*5.71mg/kg *plus* 17.50mg/kg	3
D (LDTD)	*2.86mg/kg *plus* 8.75mg/kg	6

n = 10
*The drug was administered twice daily. The dose per day is the sum of the treatment in a day (morning and evening). Therefore, half of these values were actually administered per treatment

TD - Therapeutic Dose
HPD - High Pharmacologic Dose
LDTD - Long Duration Therapeutic dose

Results

Body weight

There was no significant (p= 0.0292) difference in the body weights of the animals in the experimental groups compared with the control, while the TD group was significantly higher than the LDTD group. This is shown in Table 2.

Behavioural activity

Total locomotor activity (TLA):

There was no significant difference (p=0.0469) between the experimental groups and the control, as well as no difference among the experimental groups. This is shown in Fig. 1.

Central square frequency (CSF):

There was no significant difference (p=0.8385) between the experimental groups and the control, as well as, among the experimental groups. This is shown in Fig. 2.

Central square duration (CSD):

There was no significant difference (p=0.6046) between the experimental groups and the control, as well as, among the experimental groups. This is shown in Fig. 3.

Stretch attend (SA):

There was no significant difference (p=0.5322) between the experimental groups and the control, as well as, among the experimental groups. This is shown in Fig. 4.

Urination:

There was no urination in all the groups, hence the reason it was not represented.

Defecation:

There was no significant difference (p=0128) between the experimental groups and the control, but the LDTD group was significantly (p<0.01) lower than the TD group. This is shown in Fig. 5.

Table 2: The weight distribution of the Control, TD, HPD and LDTD groups.

Groups	Body weight (g)
A(Control)	189.38±11.51
B (TD)	198.60±5.56^{NS}
C (HPD)	172.40±8.26^{NS}
D (LDTD)	165.80±6.31^{b}

n = 10
Result are presented as Mean±Standard error of mean
^{b} - Significantly different from the B at p<0.05
^{NS} - Not significantly different from Control at p<0.05
TD - Therapeutic Dose
HPD - High Pharmacologic Dose
LDTD - Long Duration Therapeutic dose

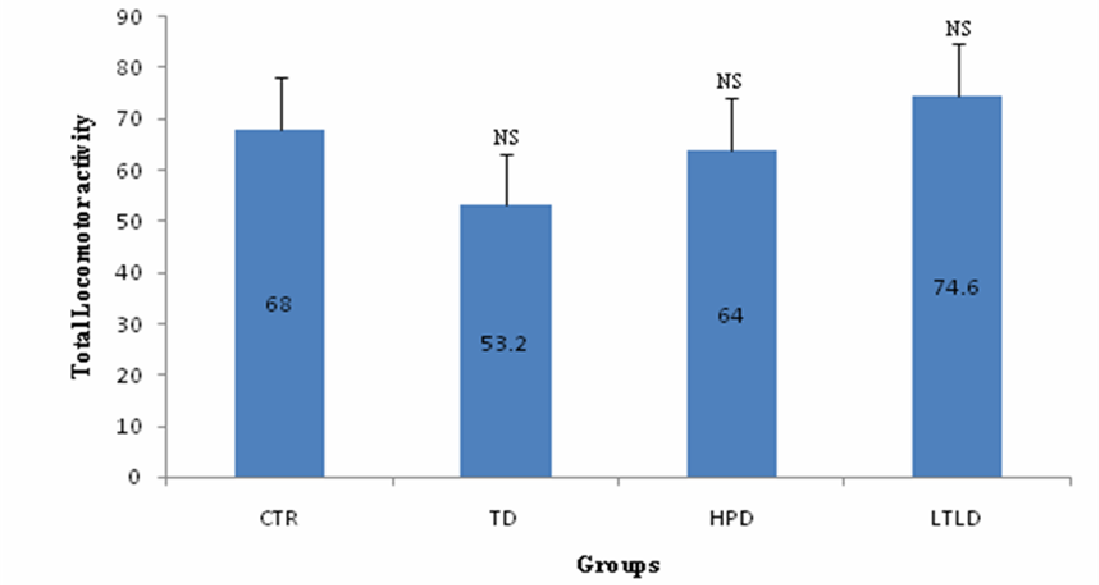

Fig. 1: Total locomotor activities of the control, TD, HPD and LDTD groups

n = 10
^{NS} - Not significantly different from A at p<0.05
Result are presented as Mean±Standard error of mean

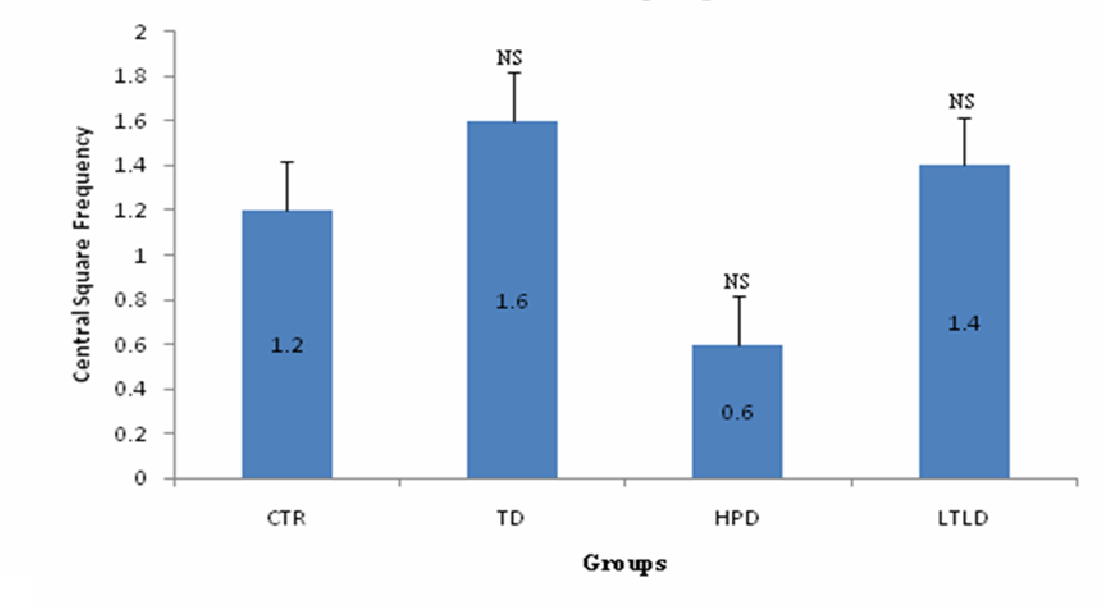

Fig. 2: Central square frequencies of the control, TD, HPD and LDTD groups

n = 10
NS - Not significantly different from A at p<0.05
Result are presented as Mean±Standard error of mean

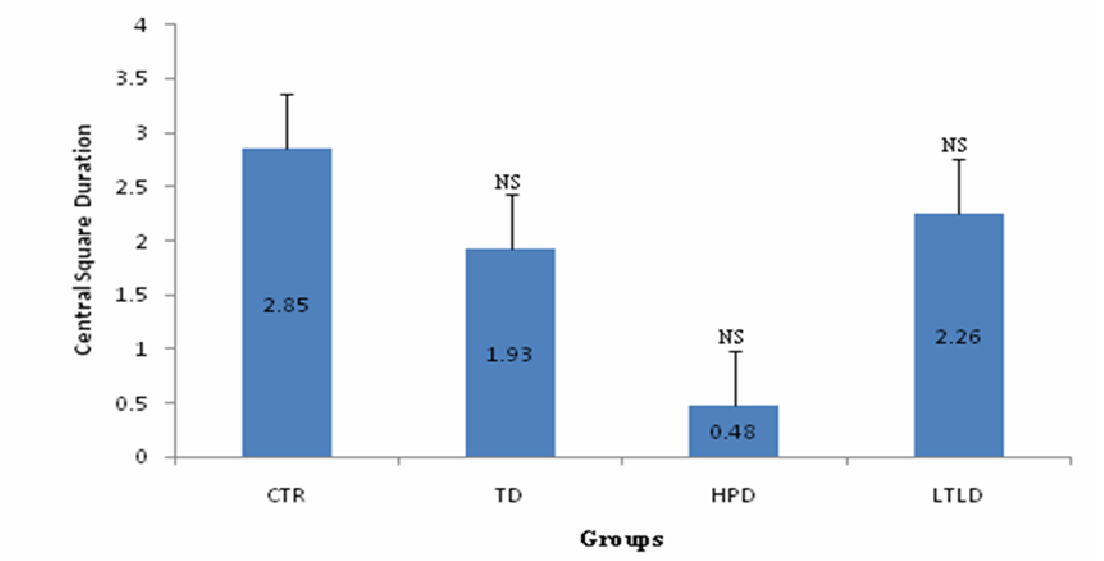

Fig. 3: Central square durations of the control, TD, HPD and LDTD groups

n = 10
NS - Not significantly different from A at p<0.05
Result are presented as Mean±Standard error of mean

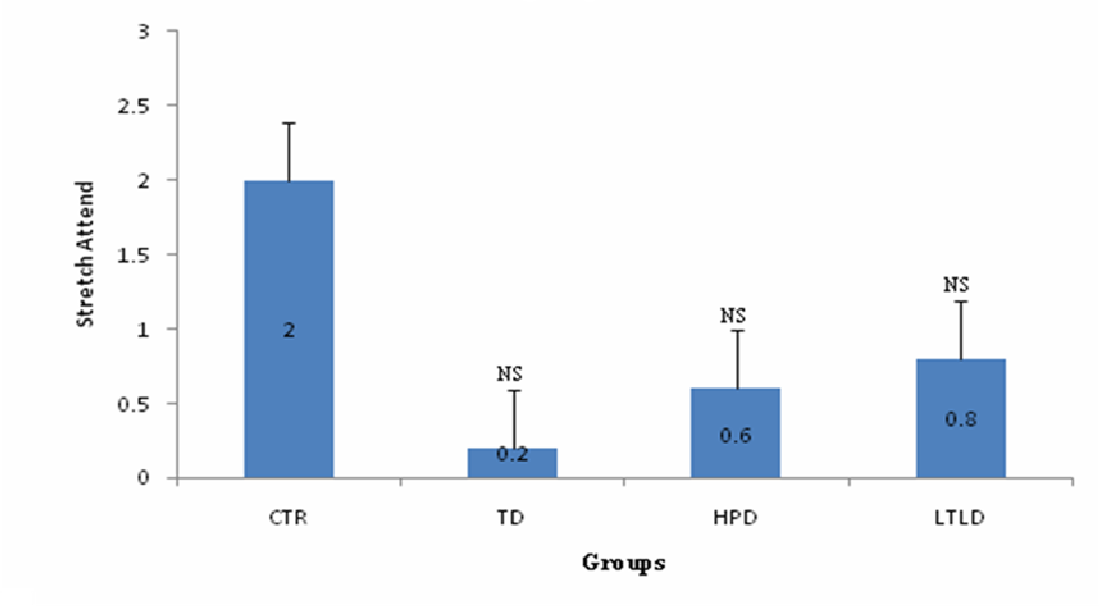

Fig. 4: Stretch attends of the control, TD, HPD and LDTD groups

n = 10
NS - Not significantly different from A at p<0.05
Result are presented as Mean±Standard error of mean

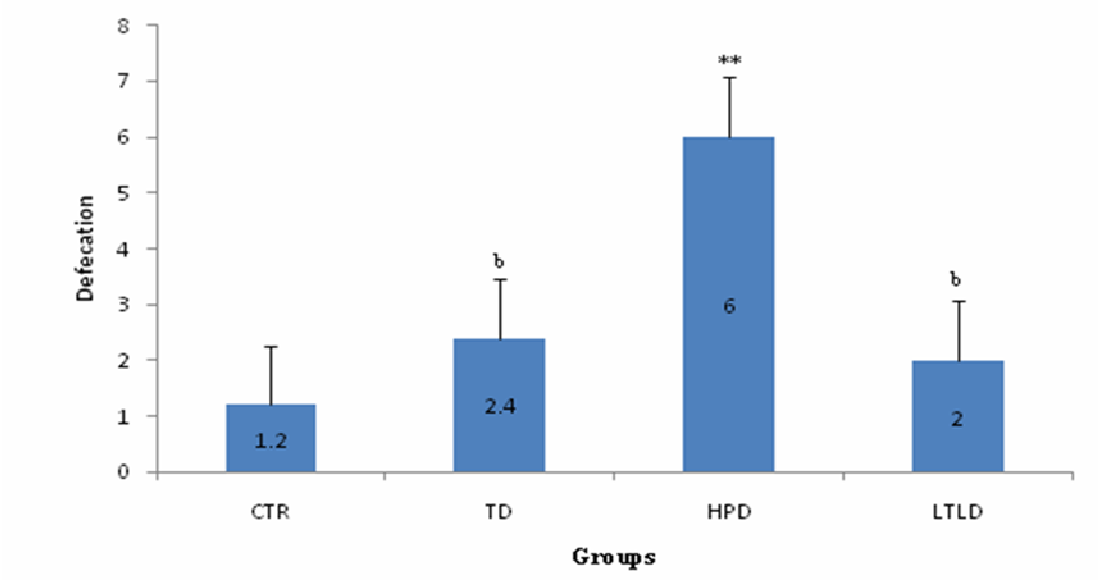

Fig. 5: Defecations of the control, TD, HPD and LDTD groups

n = 10
** - Significantly different from A at p<0.01
b - Significantly different from B at p<0.05
NS - Not significantly different from A at p<0.05
Result are presented as Mean±Standard error of mean

Discussion

Behavioural patterns of rats in an open field following treatment with amodiaquine *plus* artesunate combination was carried out. In this study, there was no significant difference between the body weights of the experimental groups and the control group. This indicates that the experimental and control groups were within the same age range and body weight. The body weight of the TD group was however significantly (p=0.0292) higher than the LDTD group. This difference may be due to the size of the individual animals that constituted each of these groups.

Total locomotor activity (TLA) measures locomotor activity, exploration and anxiety, central square frequencies (CSF) and central square durations (CSD) measure exploration and anxiety, while stretch attends (SA) measure anxiety (13,14,15,16). High frequencies of TLA, CSF and CSD, indicate increased locomotion, exploration and decreased anxiety, whereas the high frequencies of SA, urination, and defecation, indicate increased anxiety. The results of this study showed no significant difference between the experimental groups and the control, as well as, among the experimental groups in TLA, CSF, CSD and SA. These may indicate that the drugs at the given treatments may not have affected locomotion, exploration and anxiety.

There was no urination in all the groups. This also infer that the animals may not have been affected either by the open field nor the treatment drugs. In defecation, the HPD group had a significant higher fecal boli than the control, the TD and LDTD groups. This may indicate high anxiety among the animals in the experimental groups compared with the control group. Defecation and urination was reported to be indices of anxiety in rodents (15), whose reliability have been question (17,18). These conflict limited our reliance on data from urination and defecation as parameters for behavioural study.

Novel environment have been reported to influence the behavioural pattern of animals. This has been shown to be further complicated when substances are taken by the animals, which may modify the already altered behavior (12). This study revealed no change in behavior of the rats after treatment with these drugs, indicating that this drug may not have altered locomotion, and may not have been anxiolytic nor anxiogenic at these doses and time. This is in line with a previous work by Ekong et al (19), who reported that 2.86mg/kg and 5.71mg/kg of AS alone did not affect the behavioural activities of treated rats. The additional AQ component of the drug in this study, may not have had a substantial effect of its own on the behavioural parameters measured in this study.

This study is at variance with earlier works on other antimalarial drugs using the open field. Odo et al (20) reported decreased locomotor and exploratory behaviours on rats treated with 1.42mg/kg and 4.26mg/kg of AS. Ekong et al (21) reported that 17.50mg/kg and 8.75mg/kg body weights of AQ increased locomotor and exploratory behaviours. Adjene and Ezenwanne (22) reported that chronic administration of 2mg/kg body weight of chloroquine reduces the locomotor activities in adult Wistar rats. Quinine was reported to decreased total locomotion at doses of 50 and 60 mg/kg in mice (23). Mefloquine at threshold dose of 187 mg/kg of body weight in female rats induced dose-related changes in endpoints associated with spontaneous activity and impairment of motor function (24), while Nontprasert et al (11) earlier reported gradual decrease in locomotor activities in rats treated with doses up to 50 mg/kg/day of artemether.

Studies with other types of drugs in the open field also contradict our results. Antipsychotic drug, risperidone attenuates MK-801-induced hyperlocomotion in mice (25). The indole alkaloid alstonine, an antipsychotic drug, partially reversed the increase in locomotion in mice (26). Treatment with 200 mg/kg of hydroalcoholic extract of *Euphorbia hirta* showed anti-anxiety activity in chronic immobilization stressed rats (27). *Eury*coma longifolia Jack increased locomotion and reduced anxiety in mice (28), while *Cardiospermum halicacabum* induces anxiety behavior in mice (29).

This study revealed no significant change in behavior of the rats after treatment with this drug, indicating that this drug may not have altered locomotion, and may neither be anxiolytic nor anxiogenic at these doses and time. We may state though cautiously, that AS and AQ combination may not be neurotoxic at these administered doses and duration as it did not alter the measured parameters significantly.

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