Oral Artesunate-Amodiaquine Combination Causes Anxiolysis and Impaired Cognition in Healthy Swiss Mice.

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Abstract: *The present study examined the effects of artesunate –amodiaquine combination therapy on spatial memory and anxiety- related behaviors in healthy Swiss mice. Neurobehavioural models used were the Y maze and elevated plus maze. Selected for the experiments were eighty adult mice weighing 20-25 g. Forty animals were used in each behavioral model; they were randomly assigned into five groups A, B, C, D and E. Group A served as control and received normal saline, groups B, C and D received artesunate (4 mg/kg), amodiaquine (10 mg/kg) and artesunate- amodiaquine combination(4 mg/kg and10 mg/kg) respectively while group E animals were given diazepam (5mg/kg). Drugs and vehicle were given orally for three days. The animals were exposed to the elevated plus maze (EPM) and the Y-maze on day 1 immediately after administering the first dose and day 3 immediately after the last dose, the number of entries and percentage time spent in the open and closed arms after five minutes' exposure to the plus maze and Y–maze spontaneous alternations over a five minute period was scored for each animal. Statistical analysis was carried out using a one way ANOVA followed by a post-hoc test. Results of elevated plus maze tests revealed a significant increase in number of open arm entries and percentage time spent in the open arm and a significant reduction in the total number of arm entries compared to control; Y-maze task performance showed a significant reduction in percentage correct alternations compared to control .In conclusion artesunate- amodiaquine administered over a three day period had anxiolytic and memory retarding effects in healthy mice thereby giving an insight into the possible behavioral effects in humans.*
Keywords: *Neurobehavior, Artesunate, Amodiaquine, Antimalaria, Anxiety, Memory*

I. Introduction

Malaria, a disease caused by parasitic protozoa of the genus *Plasmodium* is endemic in tropical regions of the world (1, 2). While the story of malaria as a disease is not new, the issue of parasite resistance to medications has always been a never-ending battle; hence, newer drugs are always being looked for each with their individual peculiarities. Artemisinin based combination antimalaria therapy is what is being presently advocated to deal with resistance to antimalarial (3, 4). One of such combinations is that of an artemisinin with amodiaquine. Artemisinin is a sesquiterpene lactone endoperoxide derived from the weed *qing hao* (*Artemisia annua*), also called sweet wormwood or annual wormwood(5, 6). Its medicinal value had been known by the Chinese for more than 2000 years (7). By 1972, scientists had extracted and crystallized the major antimalarial ingredient, *qinghaosu,* now known as *artemisinin.* Three semisynthetic derivatives with improved potency and bioavailability were later synthesised. These include *dihydroartemisinin,* a reduced product; *artemether,* an oil-soluble methyl ether; and *artesunate* the water-soluble hemisuccinate ester of dihydroartemisinin. Lipophilic derivatives of artemisinins have been known to cross easily into the brain(8). Artemisinin-based combination therapy (ACT) may slow the development of resistance and reduce malaria transmission (9). We assume that if the efficacy of the combination depends on the individual components, so does the behavioral effects. Despite accumulating clinical experience and increasing use of ACT, aspects of the safety of these regimens remain a concern, for instance, high doses of parenteral artemisin derivatives may cause toxicity in areas of the brain stem involved in coordination and balance in animals (10).In humans, reports of abnormal neurological signs in individual cases have been ascribed to artemisinin-based treatment in adults. (11).

Amodiaquine, a 4-aminoquinoline compound related to chloroquine, is used as an antimalarial.In many cases, it is preferred to chloroquine since it is not strongly associated with pruritus as seen with chloroquine. Presently, it has become an important drug in the combination therapy for malaria treatment in Africa (12). Nevertheless, amodiaquine has its side effects, some of which could be life-threatening. Among such are

neutropenia, hepatitis and fulminant hepatic failure (13), which may lead to hepatic encephalopathy, implying that amodiaquine therapy may affect the brain in ways that may not be envisaged at the commencement of therapy.

Genarally, there is lack of data on neurological or neurobehavioral effects of artemisinin combination drugs in healthy subjects in the first instance and then in the presence of malaria parasite. Studies examining neurobehavioral effects of antimalarials are not common, as most studies tend to concentrate on the effects of these drugs on malaria parasite alone. However, the fact that antimalarials such as artesunate amodiaquine combination can cross into the brain from the plasma necessitates a proper examination of their neurobehavioraleffects.

II. Materials And Method

2.1 Equipments And Apparatus
Electronic precision balance, plastic animal cages, sterile disposable syringes (1, 5 and 10 ml) and needles, cotton wool, stop watch, Elevated Plus-maze and Y-maze.

2.2 Reagents And Drugs
Normal Saline, Amodiaquine, Artesunate (Camosunate®) Geneith Pharmaceuticals limited and Diazepam (Valium®) were purchased from the local pharmacy crushed and dissolved in measured volume of isotonic saline solution to get desired concentrations. Drugs were administered orally using a cannula.

2.3 Animals
Healthy adult Swiss albino mice purchased from the Empire Animal farms, Osogbo, Osun State, Nigeria with weights ranging between 20 to 25 g were used. The animals were housed in plastic cages measuring 16"x12"x10" (10 mice in each cage). All animals had access to food and water *ad libitum*. They were maintained under standard laboratory conditions in a well aerated room with alternating light and dark cycles of 12 h each and at room temperature of 25°C. The experimental protocol was approved by the Ladoke Akintola University Animal Ethics Committee. All rules applying to animal safety and care were observed.

2.4 Experimental Method
A total of 80 animals were used in this study; forty animals for each of the models in the behavioural study. The animals were randomly assigned into five groups A, B, C, D and E. Group A received normal saline.

Groups B, C and D received artesunate (4 mg/kg), amodiaquine (10 mg/kg) and artesunate –amodiaquine combination (4 mg/kg+10 mg/kg) respectively, while group E animals received diazepam (5 mg/kg), drugs or vehicle were administered over a for a 3 day period; the animals were exposed to the elevated plus maze model and the Y-maze after the first and last dose of drug or vehicle. The behavioral tests were conducted in a large quite room between the hours of 8 a.m. and 4 p.m. Anxiolytic or anxiogenic effects of artesunate amodiaquine combination or vehicle were evaluated using the elevated plus maze while locomotor activity and spatial memory were assessed using the Y-maze. Behaviors were scored by the authors using a stop watch; all animals in one group were tested on the same day. All events were observed and recorded manually as previously described (14).

2.5 Statistical Analysis
All data were analyzed using one way analysis of variance (ANOVA) followed by post hoc tests (Student Newman Keul's) carried out to determine the source of a significant effect. Results were expressed as Mean ± S.E.M., $p < 0.05$ was taken as accepted level of significant difference from control.

III. Results

3.1 Effect Of Artesunate–Amodiaquine On Locomotor Activity In The Y-Maze.
Figure 1 shows the effect of artesunate – amodiaquine on locomotor activity following 5mins of exploration in the Y maze. On day 1, there was a significant (f=8.71, $p < .05$, degree of freedom 45) increase in locomotor activity in the group that received amodiaquine artesunate combination (D) compared to control (group A), locomotor activity was also significantly increased in this group compared to animals in groups B, C and E. On day three there was a slight increase in locomotor activity in group D compared to the other groups (A, B, C and E) this difference was however not statistically significant.
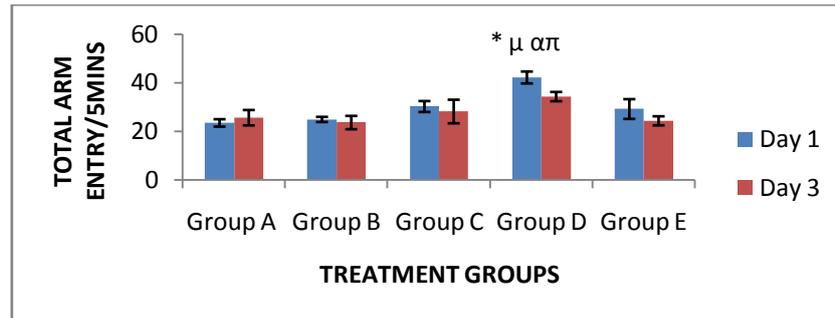
Fig 1: Effect of artesunate – amodiaquine on locomotor activity following 5 minutes' of exploration in the Y-maze. Each bar represents Mean ±S.E.M, $*^{\pi\,\mu\,\alpha}$ p ≤ 0.05 compared to the control and other groups, n=10. A is control, B - Artesunate alone, C –Amodiaquine alone, D – Artesunate –Amodiaquine combination and E - Diazepam.

3.2 Effect Of Artesunate–Amodiaquine On Spatial Working Memory In The Y-Maze.

Figure 2 shows the effect of artesunate – amodiaquine on spatial working memory following 5mins of exploration in the Y maze. On day 1, there was a significant (f=12.26, p<.05, degree of freedom=45) reduction in spatial memory in groups B, C, and D compared to control, compared to animals in groups B and C animals in group D that received artesunate amodiaquine showed a significant improvement in memory tasks performance. On day 3 there was a significant (f=7.59, p<.05, degree of freedom 45) increase in memory task performance in animals that received artesunate amodiaquine combination (group D) compared to animals in groups B, C and E.
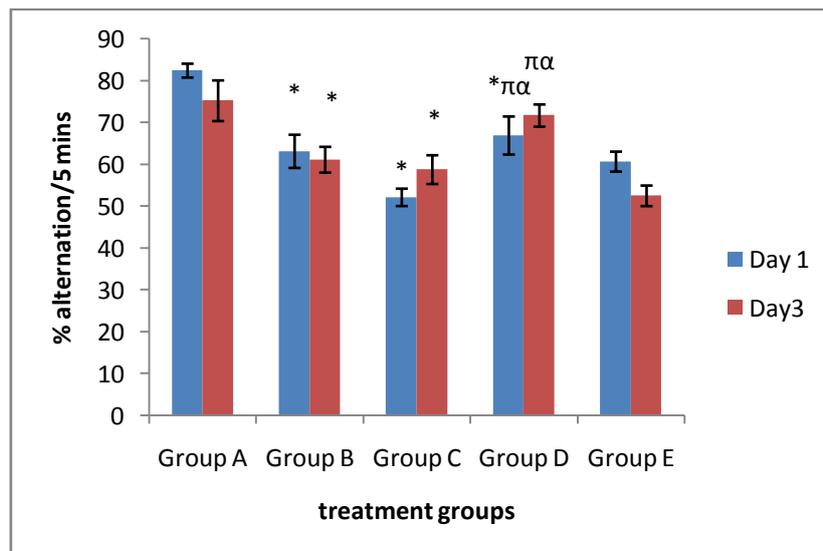

Fig 2: Effect of Artesunate – Amodiaquine combination on spatial memory following 5 minutes of exploration in the Y-maze. Each bar represents Mean ±S.E.M, $*^{\,\pi\,\alpha}$ p ≤ 0.05 compared to the control, n=10. A is control, B - Artesunate alone, C –Amodiaquine alone D – Artesunate –Amodiaquine combination and E – Diazepam

3.3 Effect Of Artesunate - Amodiaquine On Percentage Time Spent In The Arms.

Figure 3 shows the effects of artesunate – amodiaquine on percentage time spent in the open and closed arms following 5 minutes' of exposure to the elevated plus maze. On day1 there was no significant difference in open or closed arm entry in any ofthe groups compared to control, whereas on day 3 there was a gradual but significant (f=12.30, p<.05, degree of freedom 45) reduction in time spent in the closed arm in groups B, C, D and E and a significant (f=12.90, p<.05) increase in time spent in the open arm in these same groups compared to control. Animals in group D that received artesunate amodiaquine combination showed significant reduction in the time spent in the closed arm and a significant increase in open arm exploration time compared to animals that received artesunate alone (group B). The open and closed arm response seen in group D was comparable to that seen in groups E animals that received diazepam alone. Administration of amodiaquine alone (group C) also resulted in significant reduction in closed arm entry and an increase open arm entry compared to animals in group B (artesunate alone)animals.
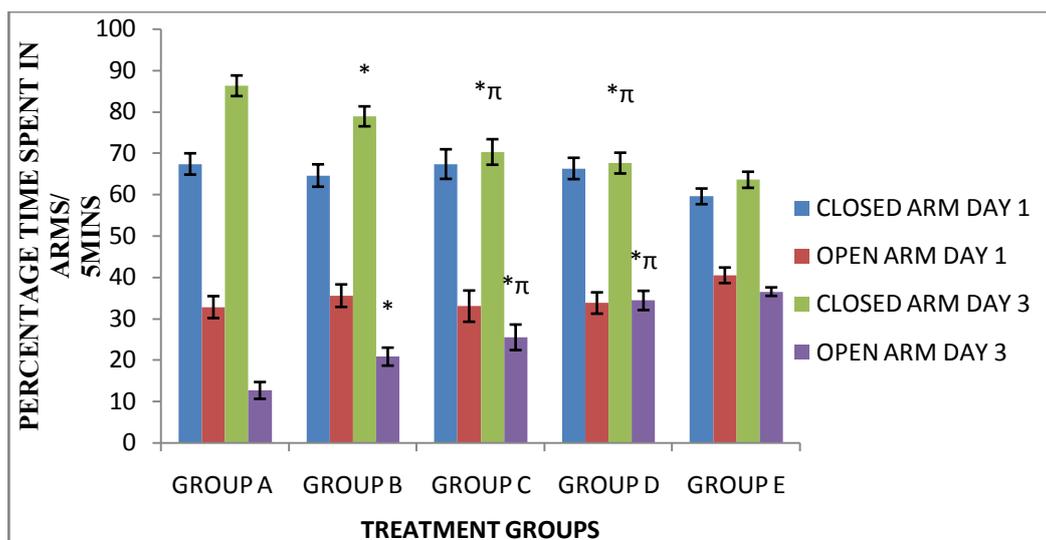
Fig 3: Effect of Artesunate – Amodiaquine combination on percentage time spent in the open and closed arms following 5 minutes of exploration in the elevated plus maze. Each bar represents Mean ±S.E.M, *$^\mu$ p ≤ 0.05 compared to the control, n=10. A is control, B - Artesunate alone, C –Amodiaquine alone D – Artesunate – Amodiaquine combination and E – Diazepam

3.4 Effect Of Artesunate Amodiaquine On Number Of Arm Entries

Figure 4 shows the effect of artesunate – amodiaquine combination the number of closed or open arm entries following 5 minutes of exposure to the elevated plus maze. On day 1 there was no significant difference in number of arm entries in any of the groups compared to control whereas on day 3 there was a significant (f=5.43, p-0.002, degree of freedom 45) increase in number of closed and open arm entries in groups C(amodiaquine alone) and D (artesunate amodiaquine)compared to animals in groups A (control) and B (artesunate alone).
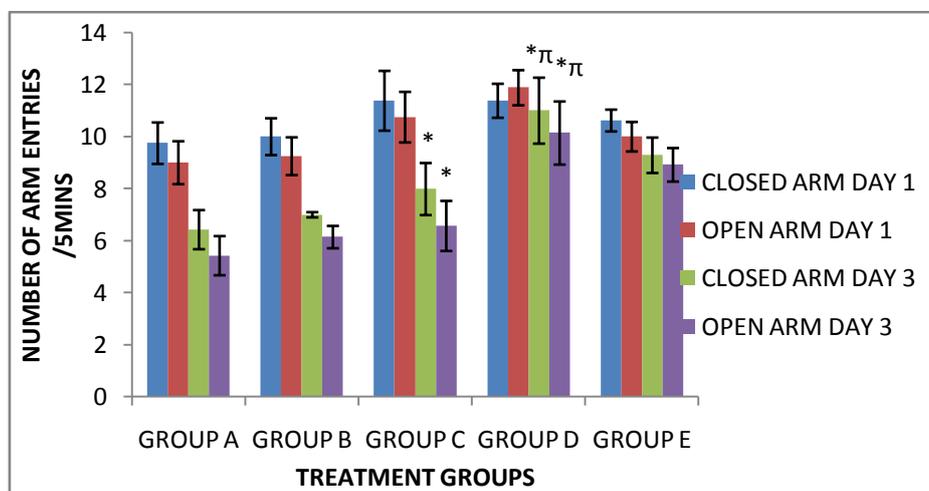

Fig 4: Effect of artesunate – amodiaquine combination on the number of closed arm entries following 5 minutes of exploration in the elevated plus maze. Each bar represents Mean ±S.E.M, *$^\mu$ p ≤ 0.05 compared to the control, n=10. A is control, B - Artesunate alone, C –Amodiaquine alone D – Artesunate –Amodiaquine combination and E – Diazepam

3.5 Effect Of Artesunate Amodiaquine On Total Arm Entry In The Elevated Plus Maze.

Figure 5 shows the effects of artesunate – amodiaquine on total arm entry following 5 minutes' of exposure to the elevated plus maze. On day 1 there was no significant difference in total arm entry in any of the groups compared to control whereas on day 3 there was a significant (f=5.41, p=0.002, degree of freedom 45) increase in total arm entry in the group D (artesunate amodiaquine combination) compared to animals in group A (control),groups B (artesunate alone) and C (amodiaquine alone).
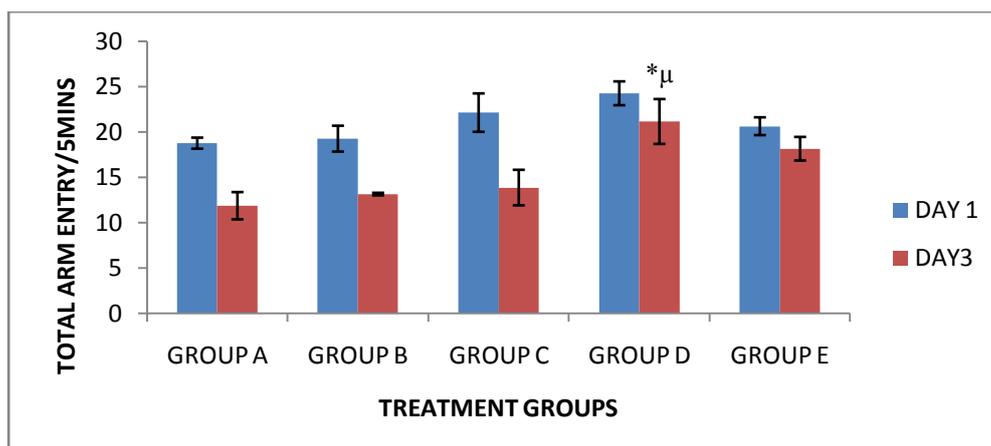
Fig 5: Effect of Artesunate – Amodiaquine combination on total arm entry following 5 minutes of exploration in the elevated plus maze. Each bar represents Mean ±S.E.M, *$^{\mu}$ p ≤ .05 compared to the control, n=10. A is control, B - Artesunate alone, C –Amodiaquine alone D – Artesunate –Amodiaquine combination and E – Diazepam

IV.   Discussion

The present study set out to assess the effects of artesunate amodiaquine combination on anxiety and memory related behaviours in healthy Swiss albino mice. The increasing endemicity of malaria and resistance to treatment has resulted in the use of combination therapies (3. 4); one of such is artesunate amodiaquine combination. This study is based on the assumption that artesunate amodiaquine combination can cross into the brain from the plasma and may therefore exert neurobehavioral effects. Our search shows that studies examining the neurobehavioral effects of medications used in the treatment of malaria are very few as most studies tend to concentrate on the efficacy of these drugs as antimalarial agents. In the study, we first examined the effects of these drugs after acute administration and then after a three day daily dosing regimen.

The Y- maze is a behavioral model that can be used to study locomotion and spatial memory in rodentsbased on the innate tendency of rats to explore novel environments (15),it assesseshippocampus dependent navigational behaviors of rodents (16). In the Y-maze, artesunate amodiaquine in combination when compared to other groups, led to significant increases in locomotor activity on day 1, increases seen over other groups on day 3 were not significant. It will be observed that locomotor activities seen in other groups on both days 1 and 3 are comparable with that of diazepam. The reason behind the increased locomotor activity in the artesunate amodiaquine group is not known.

In working spatial memory tasks, on day1, animals that were given artesunate, amodiaquine, artesunate-amodiaqiune or diazepam exhibited a significant reduction in Y-maze performance compared to control, curiously, those given artesunate –amodiaquine combination showed better Y- maze performance, in fact, significantly better performance in comparison to the amodiaquine group. Day 3 memory tasks showed the artesunate-amodiaquine group exhibiting a significantly better performance compared to artesunate, amodiaquine or diazepam group. This effect of artesunate-amodiaquine combination on spatial memory task is for us a source of curiosity as it is obvious from the study that when combined, these drugs show less impairment of cognition than when used singly.

The Elevated plus maze is a model for studying anxiety-related behaviors in rodents (17, 18). Behavior in the elevated plus maze is based on the natural aversion of rodents for elevated and open spaces. Naturally, rodents avoid open spaces and prefer enclosed spaces, and by extension, the closed arms of the maze are preferred over the open arms. This may however change when the animal is under the influence of certain drugs; drugs with anxiolytic effects such as Diazepam caused shift in the behavioral response of animals toward exploration of the open arms (19). Our observations from the study are that artesunate and amodiaquine either alone or in combination altered the relative percentages of the time spent in the open versus closed arms, however, significant changes in the time spent were not seen until after the third dose was administered. On day 3, significant reduction in closed arm time was seen in all test groups relative to the control. Animals that received artesunate- amodiaquine combination showed significant decrease in time spent in the closed arm compared to animals that received artesunate alone; there was however no significant difference seen compared to animals that received amodiaquine or diazepam. Conversely, time spent in the open arm increased on day 3 when compared to the control, artesunate and amodiaquine groups, but not in comparison to the diazepam group. This shows that artesunate-amodiaquine exhibits an anxiolytic effect that is comparable to that of diazepam on the third day of administration. An important observation regarding the effects of artesunate –

amodiaquine in the elevated plus maze is that significant changes became obvious after 3 days of dosing suggesting a cumulative effect of artesunate-amodiaquine on anxiety.

V.   Conclusion

This study shows the ability of artesunate-amodiaquine to alter neurobehavior in normal mice by affecting cognition and anxiety. While the emphasis of research relating to these drugs continues to be their antiparasite effect, we now know that neurobehavioral effects of antimalaria medications may also be worth looking into.

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