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Effect of Oral Artemether on Anxiety and Fear in Mice

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Abstract

This study assessed the effect of oral artemether on anxiety and fear in mice. Mice were treated with 30mg/kgbwt and 60mg/kgbwt of oral artesunate for 17 days. Behavioural tests employed were open field, elevated plus and light/dark transition box tests. Parameters measured were stretch attend posture, frequency and duration of grooming, centre square duration, open arm duration, head dips, number of transition and time in light chamber. There was significant reduction in grooming duration and frequency, stretch attend posture as well as the number of transitions. Reduced frequency and duration of these parameters is indicative of reduced anxiety and vice versa. In conclusion, this study showed that higher doses of oral artemether cause reduce anxiety and fear. This effect was more marked in the 60mg/kgbwt group.

Keywords: Anxiety, fear, artemether, mice and neurobehaviour.

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Background Information

The Qinghaosu (artemisinin) and its derivatives are a group of antimalarial drugs isolated from the leaves of a medicinal herb, the Artemisia annua[1]. Artermisinin is the most rapid acting class of antimalarial drugs for both uncomplicated and severe malaria [2] Treatments containing an artemisinin derivative (artemisinin-combination therapies, ACTs) are now standard treatment worldwide for *P. falciparum* malaria. falciparum malaria. Although artemisinins have been widely reported to be safe clinically, some studies (animal studies) have shown that these drugs are neurotoxic. Neurotoxicity is commonly seen with parenteral route and prolonged

administration. Oil soluble derivatives of artemisimins are reported to be more toxic than the water soluble forms. Artemisinins produce unusual selective pattern of damage to certain brainstem nuclei particularly those involved in auditory processing and vestibular functions. These nuclei include trapezoid nucleus, the gigantocellular reticular nucleus and the inferior cerebeller peduncle [3][4][5]. In the rat, the target brainstem nucleus consistently and most severely affected is the nucleus of the trapezoid body [6]. Changes in the affected neurons were loss of Nissl substance, perikaryonal swelling, margination of the nucleus (nucleus accentricity), nucleolar changes and increased perikaryonal eosinophilia with occasional clumping of eosinophilic debris [6].

Anxiety is defined a psychological, physiological and behavioural state induced in human and animals by a threat to well being or survival, either actual or potential. Features of anxiety include increased arousal, expectancy, autonomic, neuroendocrine activation, and specific behaviour patterns, often with a behavioural transition from ongoing behaviours (e.g. exploration, feeding) to an escape (e.g. flight) or other defensive behaviours. The aim of these changes is to facilitate coping with an adverse or unexpected situation [7]. However, if the adaptive function of anxiety is not successful, anxiety can become a pathological state which may later on interfere with the ability to cope with various challenges or stressful events in daily life and even alter body condition [7].

Anxiety test is based on the contrasting tendency of mice to explore a novel environment against the aversive properties of an open, brightly lit, or elevated space [8][9]. In addition, in the open field, anxiety behaviour may be triggered by 2 factors namely, individual testing (the animal separated from its social group) and agoraphobia (as the area is very large relative to the animal) [9].

As mentioned above, there have been many detailed reports on artemisinins toxicity in animals. In contrast, there are little or no studies of artemisinins on neurobehaviour such as anxiety and fear. Recently we showed that artesunate at 60mg/kg has anti-anxiety properties [10]. The goal of this study therefore is to assess the effect of artesunate, one of the most commonly available lipid soluble derivatives of artemisinin, on anxiety.(Davies & Udoh, 2016)

Materials and Methods

Animal care: Adult albino mice, thirty in number were housed individually in single cages under standard laboratory conditions and maintained under 12h light/12h dark cycle and were fed with pellet feed (Vital feed and flour mill limited, Edo, Nigeria). All animals were given *ad libitum* access to feed and water. Permission and approval for animal studies were obtained from College of Health Sciences Animal Ethics committee, University of Uyo and experiments were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Drug Preparation Oral Artemether suspension "Gvither" manufactured by Bliss GVS Pharmaceutical limited of India was purchased from a reputable pharmacy in Uyo, Akwa Ibom State, Nigeria. The suspension was reconstituted by adding 100ml of water to 300mg of artemether to produce a stock solution of 3mg of artemether per ml. Each reconstituted suspension was stored in a cool dry place at room temperature (25^0C) and used within 7 days.

Animals' treatment: Thirty (30) Albino Mice were randomly separated into 3 groups. Group 1 served as control and received only feed and water. Groups 2 and 3 respectively received 30mg/kg (low dose) and 60mg/kg (high dose) of oral artemether suspension daily for 17 days by gavage.

Behavioural assay

Behavioural test employed in this study were Open field maze, Elevated plus maze and light/dark transition box. The tests are described below.

Open field maze (OFM)

The open field is constructed of plywood and measures 72 x 72 cm with 36 cm high walls. The floor and three walls of the OF are made from 2-cm thick plywood that has been painted white.

The fourth wall is made of clear Plexiglas so that the mice can be observed from the front of the apparatus as well as from the top. Blue lines painted on the floor divide the open field into forty-nine 5 x 5 cm squares, and these lines are used to assess locomotor activity. The centre square (15 x 15 cm) is formed from the four inner squares and this square is highlighted with a black marker. A sheet of clear Plexiglas covers the floor. All animal testing is conducted under diffuse lighting conditions via a 60-Watt white light bulb.

Procedure

Mice were carried to the test room in their home cages and tested one at a time. The mice were scooped up in a small plastic container from their home cages and placed randomly into one of the four corners of the open field. They were allowed to explore the apparatus for 5-minutes while taking scores of their behaviours. After the 5-minutes test, the mice were scooped up from the open field with the plastic container and returned to their home cages. The open field was cleaned with 70% ethyl alcohol and permitted to dry between trials.

The behaviours scored included:

1. Stretch attend posture

2. Grooming frequency and duration

Elevated Plus-Maze (EPM)

The Elevated Plus-Maze was built according to the description of Lister (1987) [9]. The apparatus is in the configuration of a + and comprised two open arms (25 x 5 x 0.5 cm) across from each other and perpendicular to two closed arms (25 x 5 x 16 cm) with a center platform (5 x 5 x 0.5 cm). The open arms had a very small (0.5 cm) wall to decrease the number of falls, whereas the closed arms had a high (16 cm) wall to enclose the arm [9]. The entire apparatus was 50 cm above the floor. The apparatus was made of white transparent Plexiglas materials.

Procedure

Mice were carried into the test room in their home cages and were handled by the base of their tails at all times. Mice were placed in the central square of the Plus-Maze facing an open arm and

were then allowed to explore the apparatus for 5 minutes. The maze was then cleaned with a solution of 70% ethyl alcohol and allowed to dry between tests.

Behaviours scored were:

i. Open Arm Entries: Frequency with which the animal entered the Open arms. All four of the mouse's paws should be in the open arms to be regarded as an entry.

ii. Open Arm Duration: Length of time the animal spent in the open arms.

iii. Grooming frequency and duration

iv. Frequency of stretch attend posture

Light-dark box (LDB)

The light-dark box (45 x 27 x 27 cm) is made of plywood and consists of two compartments of unequal size as described by Costall et al. [11]. The small compartment (18 x 27 cm) is painted black (2/5 of the box) and the larger compartment (27 x 27 cm) is painted white (3/5 of the box). These compartments are connected by a door (7.5 x 7.5 cm) located at floor level in the center of the wall between the two compartments. The floor is divided into 9 x 9 cm squares and is covered with Plexiglas. Both compartments are covered with lids of clear Plexiglas. A 60-Watt table lamp located 40-cm above the center of the white compartment provides bright illumination of white light. The apparatus was located in a 2 x 5 m laboratory room.

Procedure:

Mice were carried into the test room in their home cages. The procedure was as described by Costall *et al.* [11]. Mice were picked up by the base of their tail and placed in the center of the white compartment facing the door and allowed to explore the apparatus for 5-minutes.The maze was then cleaned with a solution of 70% ethyl alcohol and allowed to dry.

Behaviours scored were:

1. Light box duration: length of time the animal spent in the light side of the box
2. Stretch-attend postures: frequency with which the animal demonstrates forward elongation of head and shoulders followed by retraction to original position.

3. Transitions: number of times the animal passes into the opposite compartment (all four paws of the mouse must have moved into the new compartment for a transition to be scored and for that compartment to be considered entered).

Statistical analysis:

Data collected during the study were expressed as mean + standard error of mean (SEM), analysis of variance (ANOVA) was used for analysis. Values of $P<0.05$ were regarded as significant. Statistical analysis was done with the aid of computer software SPSS and Excel from Windows XP (Brain Series, China).

RESULTS

Open field maze

There was significant reduction in grooming frequency and duration in mice treated with 30mg/kgbwt ($p< 0.05$) and ($p< 0.01$) of artemether compared to the control mice. Oral artemether also caused reduced grooming frequency which was statistically significant at ($p< 0.05$) and ($p< 0.01$) respectively, in the low dose and high dose groups.

Elevated plus maze

There was significant reduction ($p< 0.05$) in grooming frequency and duration in mice treated with 60mg/kgbwt of artemether compared to the control mice. Grooming frequency and duration were not significantly different between the group treated with 30mg/kgbwt of artemether and control. There was a significant reduction in stretch attend posture ($p< 0.01$) in the treated groups compared to the control. Open arm duration was not significantly different between test groups and the control.

Light/dark box

There was a significant reduction in stretch attend posture($p < 0.01$), grooming frequency($p < 0.001$) and duration($p < 0.01$) between the high dose group and the control. However, the above parameters were not significantly different between low dose group and the control. Transitions was significantly reduced in the low dose group ($p < 0.05$) compared to the control. The number of transitions was not significantly reduced in the high dose group. Light box duration was not significantly different between test groups and control.

Open Field Maze	Control	Low dose	High dose
Duration of grooming	11.36 ± 1.82	4.42 ± 2.1[b]	1.4 ± 0.82[c]
Frequency of grooming	5.81 ± 0.94	2.4 ± 0.98[b]	0.8 ± 0.37[c]
Elevated Plus Maze			
Duration of grooming	14.40 ± 3.69	5.76 ± 2.46	3.88 ± 0.47[a]
Frequency of grooming	4.36 ± 0.88	2.6 ± 0.87	1.6 ± 0.24[a]
Stretch attend posture	5.18 ± 1.02	1.2 ± 0.49[b]	1.0 ± 0.77[b]
Open arm duration	9.91 ± 0.90	10.2 ± 1.1	11 ± 2.07
Light/Dark transition box			
Duration of grooming	36.45 ± 12.36	31.24 ± 7.57	7.56 ± 3.02[b]
Frequency of grooming	9.55 ± 1.40	7.4 ± 1.3	2.8 ± 0.97[c]
Stretch attend posture	5.18 ± 0.50	3.6 ± 1.08	2 ± 0.32[b]
Light chamber duration	36.13 ± 3.06	50.73 ± 8.69	42.32 ± 10.98
Transitions	23.45 ± 1.93	18.2 ± 2.24[a]	20.2 ± 3.83

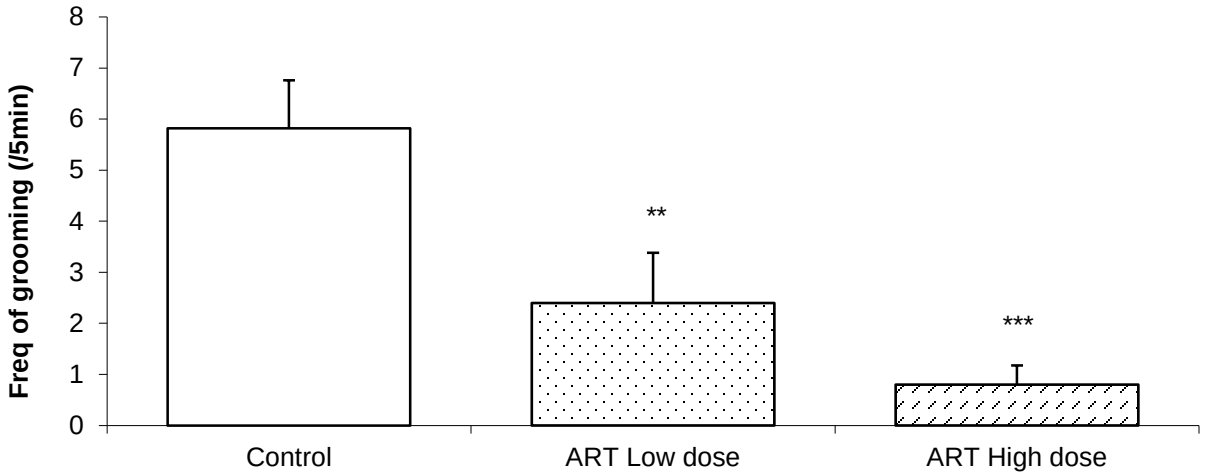

Fig. 1: Comparison of the frequency of grooming in the open field test following oral administration of 30mg/kg and 60mg/kg of artemether in mice.

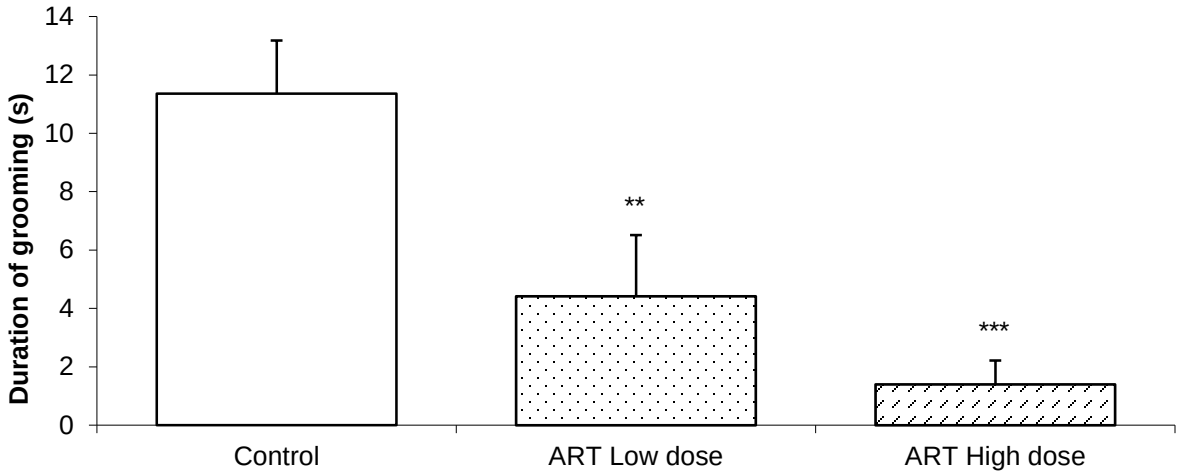

Fig. 2: Comparison of the duration of grooming in the open field test following oral administration of 30mg/kg and 60mg/kg of artemether in mice.

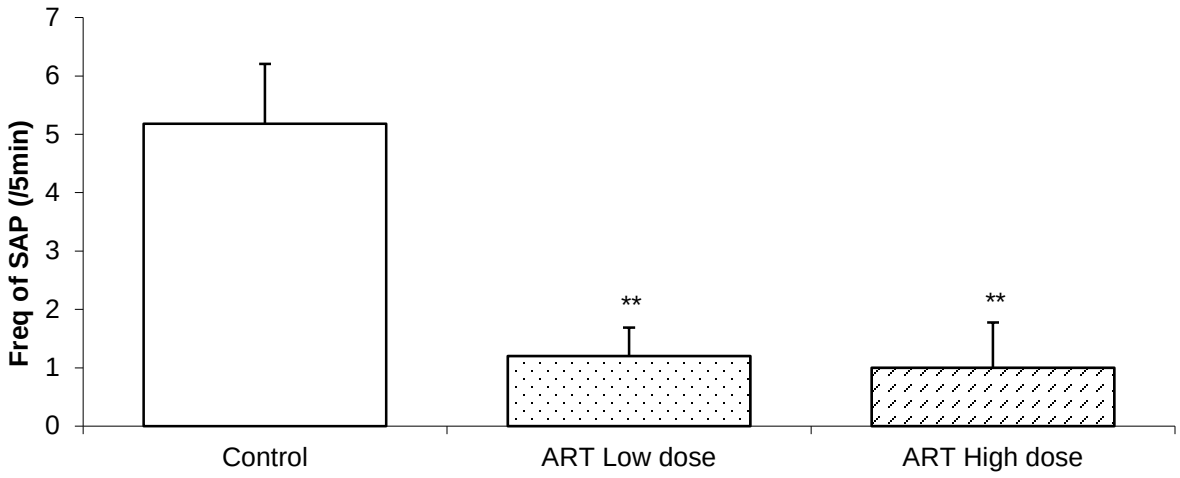

Fig. 3: Comparison of SAP in EPM following oral administration of 30mg/kg and 60mg/kg of artemether in mice

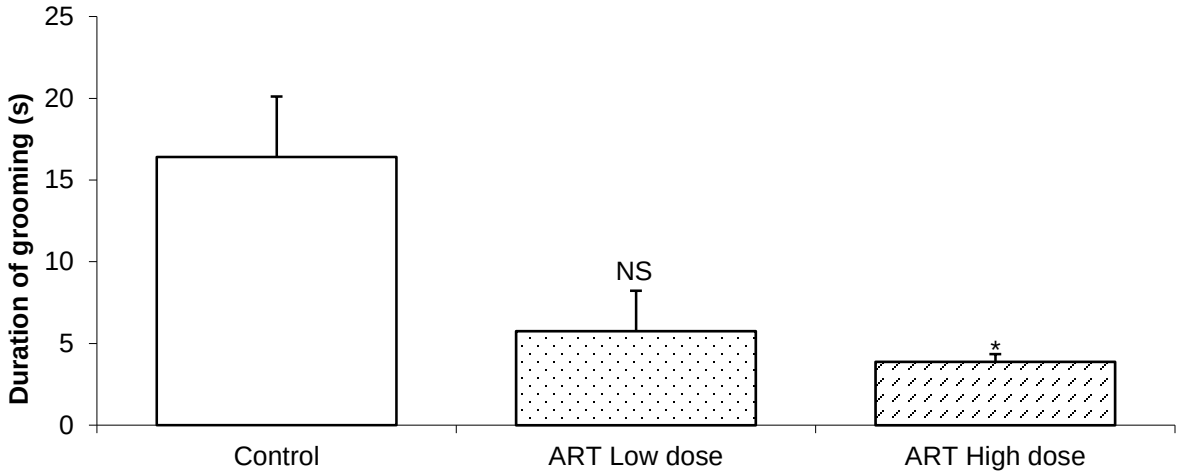

Fig. 4: Comparison of duration of grooming EPM following oral administration of 30mg/kg and 60mg/kg of artemether in mice

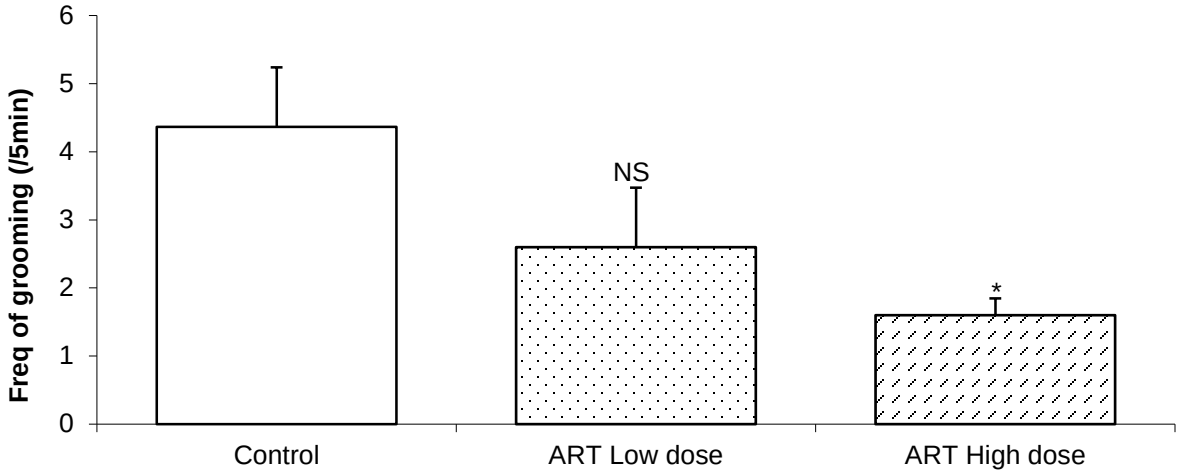

Fig. 5: Comparison of duration of grooming EPM following oral administration of 30mg/kg and 60mg/kg of artemether in mice

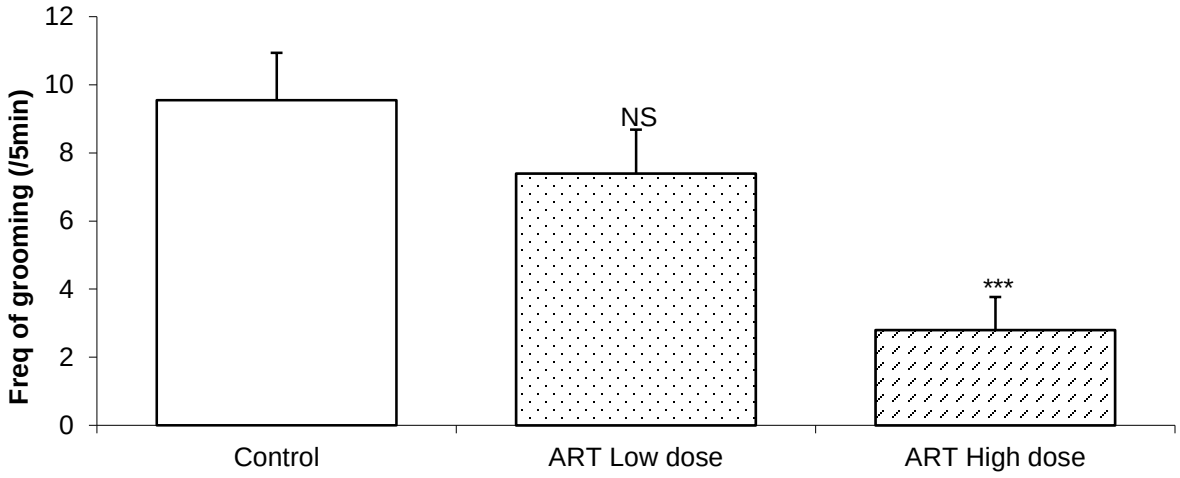

Fig. 6: Comparison of the frequency of grooming in Light/Dark Box following oral administration of 30mg/kg and 60mg/kg of artemether in mice.

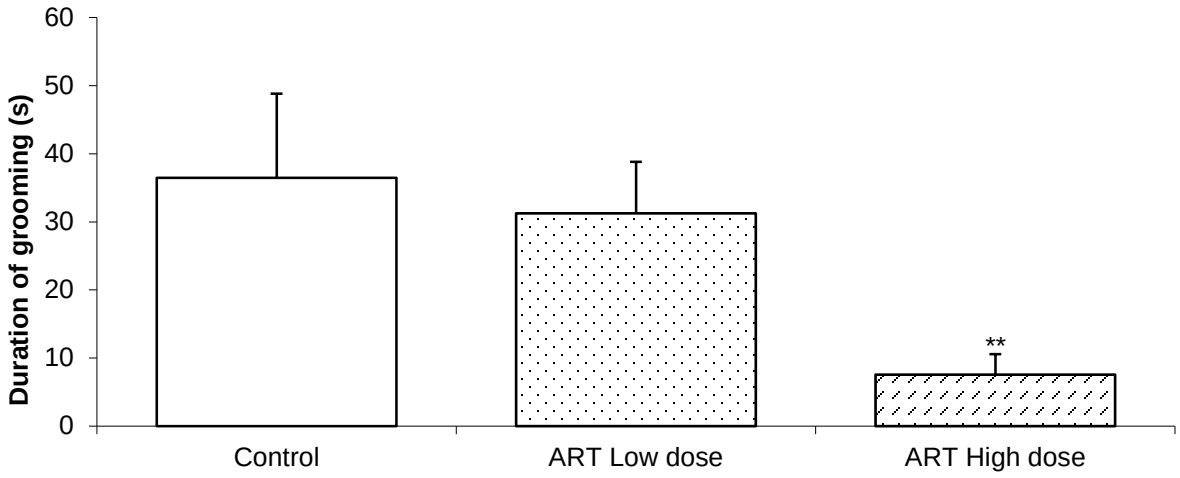

Fig. 7: Comparison of the duration of grooming in Light/Dark Box following oral administration of 30mg/kg and 60mg/kg of artemether in mice.

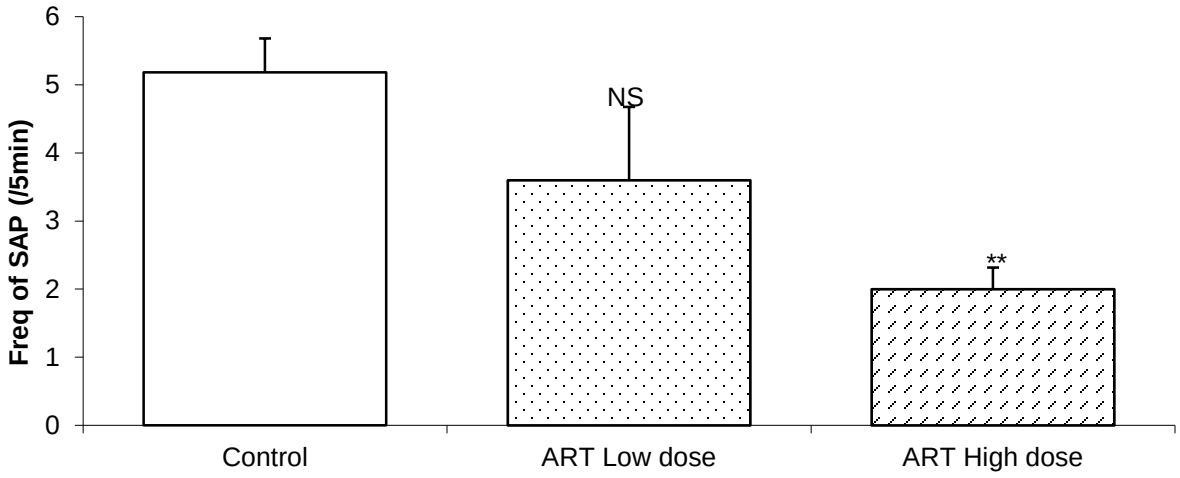

Fig. 8: Comparison of frequency of SAP in Light/Dark Box following oral administration of 30mg/kg and 60mg/kg of artemether in mice.

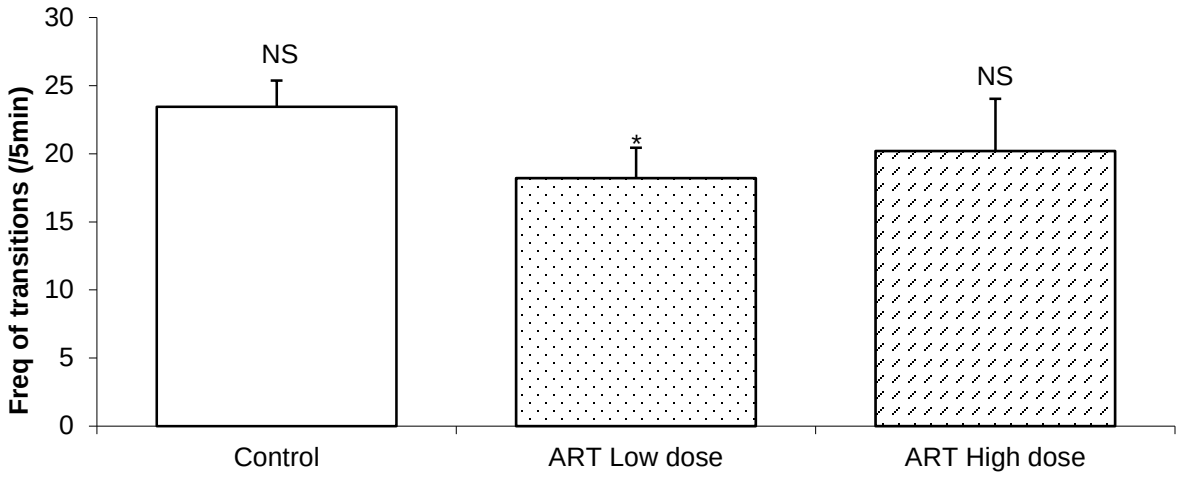

Fig. 9: Comparison of frequency of transition in Light/Dark Box following oral administration of 30mg/kg and 60mg/kg of artemether in mice.

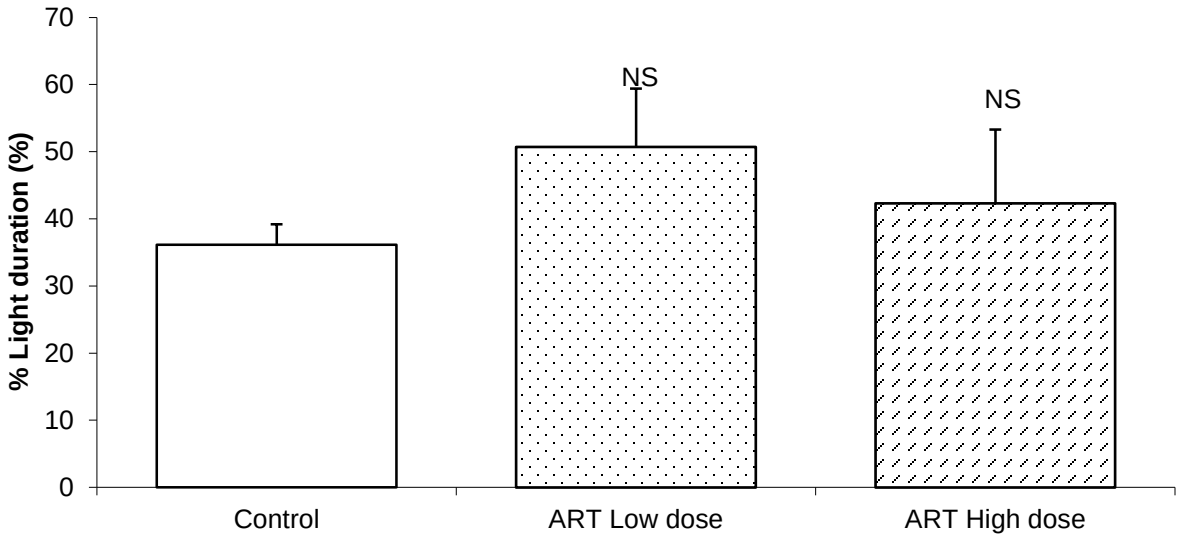

Fig. 6: Comparison of % Light Duration in Light/Dark Box following oral administration of 30mg/kg and 60mg/kg of artemether in mice.

DISCUSSION

The results showed that oral artemether at 30mg/kgbwt and 60mg/kgbwt caused reduction in Stretch attend posture, reduce grooming frequency/duration and reduce transitions.

Stretch attend posture is categorised as a risk assessment behaviour. It reflects an approach avoidance conflict. Stretch attend posture is increased in anxiety states and by anxiogenic drugs. On the other hand SAP is reduced by anxiety reducing drugs [9][12]. The most common being the GABAergic stimulants such as the benzodiazepines are known to reduce SAP. Reduction of in the treated groups indicated that artemether at high doses cause reduce anxiety.

Grooming is a displacement response and it is associated with anxiety in animals when they are introduced into a novel environment [8]. It has been showed to be induced by stress or anxiety. Modulators associated with stress such as cortisol, ACTH, corticotrophin releasing hormone, prolactin and bombesin been shown to correlate positively with grooming. Increased number and duration of this parameter is indicative of increased anxiety and vice versa [12][13]. Grooming and anxiety has been shown to be mediated by GABAergic mechanism. Stimulators of this pathway such as benzodiazepines cause reduce grooming while blockers such as pentylenetetrazole cause reduce grooming [14][15]. Though some authors have disputed that grooming is not a reliable marker of anxiety [16], it is conceivable, however, that the reductions in stretch attend posture taken together with reduce grooming indices in this study is an indication of reduced anxiety caused by artemether. Thus artemether may be causing reduce grooming by stimulating the GABAergic pathway. This effect was more marked in the high dose compared low dose group and indicates dose dependency.

Transition in the LDB is a measure of anxiety state. Increase transition indicates reduce anxiety, increase exploration and vice versa. The reason why transition frequency was reduced in the low dose group is not clear.

In our recently published work, artesunate at 60mgkg showed an anti-anxiety potential. The effect was less than that of artemether at the same dose levels [10]. The reason for the higher anti-anxiety effect over artesunate is not clear. However, it is conceivable that artemether being lipid soluble is more rapidly absorbed and can achieve a higher serum concentration than artesunate and also clearance from the tissue slower than that of artesunate [11]. These factors may likely contribute to its increased bioavailability of artemether compared to artesunate and therefore a higher anxiolytic effect.

REFERENCES

[1] Tu Y. The discovery of (ginghaosu) and gifts from Chinese Medicine. *Nature Medicine*, 2011; 17(10): 1217-1220.

[2] Adekunle AS, Falade CO, Agbedana EO, and Egbe A. Assessment of side effects of administration of artemether in humans. *Biology and medicine*, 2009; 1(3): 15-19.

[3] White NJ. Assessement of the neurotoxicity of parental artemisinin derivatives in mice. *Transaction of the Royal Society of Tropical Medicine and Hygeine*, 2011; 90(5): 553-555.

[4] Nontprasert A, Pukviltayakanee S, Dondrop AM, Clemens R, LovaveeSuwan S, and White NJ. Neuropathologic Toxicity of Artemisinin Derivatives in a Mouse Model.*American Journal of Tropical Medicine and Hygiene*, 2000; 62(3): 409 – 412.

[5] Li GQ, Mog SR., Si YZ, Gettayacamin M, and Mihous K. Neurotoxicity and efficacy of arteether related to its exposure times and exposure levels in rodents. *American Journal of Tropical Medicine and Hygiene*, 2002; 66(5):516-25.

[6] Abdulazeez AA, Owoeye O, and Ejiwunmi AB. The Neurotoxic Effects of Artemether on the cytoarchitecture of the Trapezoid Nuclei of Adult male Wistar rats (Rattusnovegicus). *International Journal of Morphology*, 2006; 24(4):535-540.

[7] Steimer T. The Biology of Fear and Anxiety-related Behaviour. *Dialogues in Clinical Neuroscience*, 2000; 4:123 – 137.

[8] Bourin M, and Hascoet, M. The Mouse Light/Dark Box Test. *European Journal Pharmacology*, 2003; 463(1-3):55 ─65.

[9] Lister RG. The Use of a Plus-Maze to Measure Anxiety in the Mouse. *Psychopharmacology*, 1987; 92: 180 ─185.

[10] Davies KG and Udoh, U. G. (2016). Effects of oral artesunate on anxiety and fear in mice. *Journal of Disease and Global Health*, 8(2), 48-54.

[11] Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J. and Tomkins, D.M. Exploration of Mice in a Black and White Test Box: Validation as a Model of Anxiety. *Pharmacology, Biochemistry and Behavior*, 1989; 32:777 ─785.

[12] Blanchard, DC., Griebel, G., Blanchard, RJ. 2001. Mouse defensive behaviors: Pharmacological and behavioral assays for anxiety and panic. *Neuroscience and Biobehavioral Reviews*, 25, 205-218.

[13] Kalueff, A.V., Tuohimaa, P., 2004a. Grooming analysis algorithm for neurobehavioural stress research. *Brain Res. Protoc*. 13, 151– 158.

[14] Kalueff, A.V., Nutt, D.J., 1997. Role of GABA in memory and anxiety. *Depression Anxiety* 4, 100–110.

[15] Barros, H.M., Tannhauser, S.L., Tannhauser, M.A., Tannhauser, M., 1994.The effects of GABAergic drugs on grooming behaviour in the open field. *Pharmacol. Toxicol*. 74, 339–344.

[16] Rodgers, R.J., Davies, B., Shore, R., 2002. Absence of anxiolytic response to chlordiazepoxide in two common background strains exposed to the elevated plus-maze: importance and implications of behavioural baseline. *Genes Brain Behav*. 1, 242– 251