Locomotor and Exploratory Behaviour in Mice treated with Oral Artesunate

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Abstract

Previous studies with intramuscular Artemisinins reveal dose dependent neurotoxicity with specific concern on the nuclei of the brain stem. This study was aimed at assessing the effect of oral Artesunate on locomotor and exploratory behaviour in mice. The study was conducted using a total of 30 mice randomly divided into 3 groups. Group 1 served as the control and was administered with neither drug nor placebo. Groups 2 and 3 were administered with 30mg/kg/day (low dose) and 60mg/kg/day (high dose) respectively of oral Artesunate for 17 days. Behavioural tools used were Open Field Maze, Elevated Plus maze and Light/Dark transition box. Parameters measured were center square duration/ entries, frequency of rearing, frequency of line crosses, open arm duration / entries and head dips. In the Open Field test, the frequency of rearing, frequency of line crosses, center square duration and entries were not significantly different between the control and the experimental groups. In the Elevated Plus maze, frequency of rearing, head dips and open arm duration / entries were similar between control and the experimental groups. In the Light/Dark transition box, the frequency of rearing was significantly lower in the low dose group compared to the control group. However, there was no significant difference between the high dose group and the control in this parameter. The frequency of line crosses in the light/dark transition box was similar between the tested groups and the control. In conclusion, oral Artesunate up to 60mg/kg does not affect exploratory and locomotor activity.

Key Words: Locomotor activity, Exploratory activity, Artemether, Anti-malarial, Mice, Open Field Maze

1.0 Introduction

1.1 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 (Tu, 2011). Artermisinin is the most rapid acting class of antimalarial drugs for both uncomplicated and severe malaria (Adekunle et al, 2009).The discovery of artermisinin for malaria therapy by Chinese scientists in the 1970s was one of the greatest discoveries in medicine in the 20th century. Representing a new class of antimalarial agents, artemisinin is a sesquiterpene lactone characterized by an endoperoxide bridge essential for its antimalarial activity (Cui and Su, 2010) with a chemical structure different from any other drug. It acts against the asexual stage gametocytes and block the sexual stage (Adekunle et al, 2009). Chemical modifications of artemisinin (reduction plus esterification) have enabled more potent and more soluble derivatives to be obtained, with improved bioavailability (Basra, 2005; Chekem et al, 2006). Among these different derivatives is artesunate, a hemisuccinate derivative.

Artesunate administered by intravenous or intramuscular injection has proven superior to quinine in large, randomised controlled trials in both adults (Pasvol, 2005) and children (McIntosh and Olliaro, 2000). Combining all trials comparing these two drugs, artesunate is associated with a mortality rate that is approximately 30% lower than that of quinine (McIntosh and Olliaro, 2000). Reasons for this difference include reduced incidence of hypoglycaemia, easier administration and more rapid action against circulating and sequestered parasites. Artesunate is now recommended by the WHO for treatment of all cases of severe 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. These compounds have been shown to produce an unusual selective pattern of damage to certain brain stem nuclei i.e. precerebellar nuclei of the medulla oblongata and particularly those involved in auditory processing and vestibular functions i.e. the trapezoid nucleus, the gigantocellular reticular nucleus and the inferior Cerebellar peduncle (Abdulazeez et al, 2006). In the rat, the target brainstem nucleus consistently and most severely affected is the nucleus of the trapezoid body (Li et al., 2002 and Nontprasert, 2000). 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 (Abdulazeez et al, 2006).

Exploratory behavior refers to the tendency to investigate a novel environment. It is considered a motivation not clearly distinguishable from curiosity. The concept of exploration is closely associated with that of novelty (Barnett and Cowan, 1976) which may involve some quality never previously experienced or familiar items arranged in unfamiliar way. Okeefe and Nadel (1978) defined novelty within the framework of their cognitive map theory as follows "an item or place is novel if it does not have a representation in the locale system" and exploration as a direct response of the animal to the detection of a mismatch by the locale system". The locale system being the cognitive mapping system, presumably located in the hippocampus that contain mental representations of stimuli previously perceived. In other words, the hippocampal system supposedly signals a lack of information about the current environment. Consequently, one of the processes thought to be associated with exploratory activity is what is called latent learning or exploratory learning (O'Keefe and Nadel, 1978; Renner, 1988). Thus the animal acquire information about their environment (Crusio, 2001)

Till date however, there has not been any study evaluating effects of prolonged administration of Artemether on Locomotor and Exploratory behaviour. The aim of this study therefore, was to evaluate the effects of Artesunate on Locomotor and Exploratory behaviour in mice.

3.0 Materials and methods

3.1 Animal care: Adult albino mice, thirty in number were housed singly in metabolic cages under standard laboratory conditions and were fed with pellet feed (Vital feed and flour mill limited, Edo, Nigeria). All animals were housed in cross ventilated room (temp 22±2.5; humidity 65±5% and 12h light/12h dark cycle). A period of one week was allowed for acclimatization.

3.2 Drug Preparation Oral Artesunate marketed under the brand name "ARTESUNAT^{R}" manufactured by Neros Pharmaceutical limited, Lagos, Nigeria was purchased from a reputable pharmacy in Uyo, Akwa Ibom State, Nigeria. The drug was reconstituted by adding distil water to produce the desired stock solution. Each freshly reconstituted suspension was used for a day.

3.3 Animal 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 artesunate daily for 17 days by gavage.

3.4 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.

3.41 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 behaviors scored included:

1. Number of line crossing; frequency with which the mice crossed one of the grid lines with all four paws.
2. Center square entries; frequency with which the mice crossed one of the red lines with all four paws into the central square.
3. Duration of stay in the central square.
4. Rearing frequency and duration.

3.5 Elevated Plus-Maze (EPM)

The Elevated Plus-Maze was built according to the description of Lister (1987). 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. (Guy et al, 2004). The entire apparatus was 50 cm above the floor. The apparatus was made of white transparent Plexiglas materials.

3.51 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. Behaviors 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. Head Dipping: Frequency with which the animal lowered its head over the sides of the open arms towards the floor.
iv. Rearing: Frequency with which the animal stands on its hind legs or leans against wall of the maze with front paws.

3.6 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*. (1989). 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

3.61 Procedure:

Mice were carried into the test room in their home cages. The procedure was as described by Costall *et al*. (1989). 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. Line crosses: number of times the animal crossed a line drawn on the floor.
2. Rearing: frequency with which the animal stands on hind legs or leans against walls of the box with front paws.

3.7 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).

4.0 Results

4.1 Open Field Maze (OFM): There was no significant difference in the centre square duration/entries, frequency of rearing and line crosses between the experimental groups of mice and the control.

4.2 Elevated Plus Maze (EPM): The frequency of rearing, head dips, open arm entries and duration in the experimental group was comparable to that of the control.

4.3 Light and Dark transition box (LDB): The frequency of rearing was significantly higher in low dose group compared to control ($p<0.05$). There was no difference between the high dose group and the control. There was no significant difference between the experimental group and the control in the frequency of line crosses.

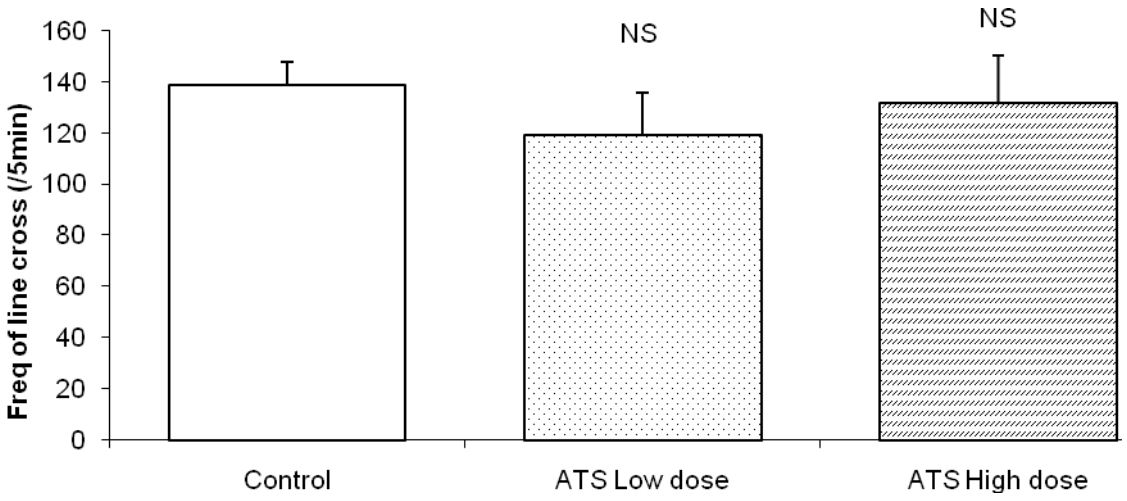

Fig. 4.1: Comparison of the frequency of line crosses in the open field test in mice following oral administration of 30mg/kg (low dose) and 60mg/kg Artesunate (ATS).

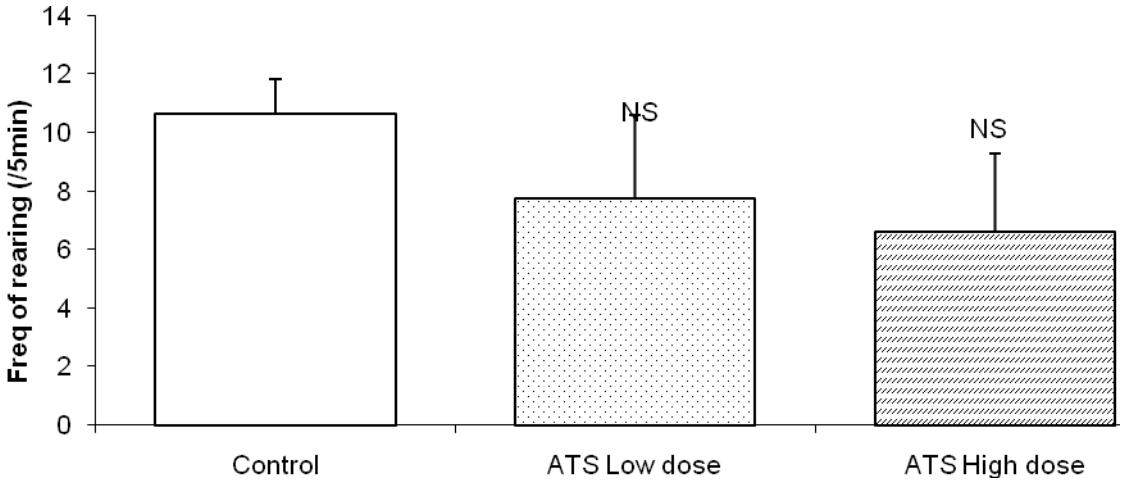

Fig. 4.2: Comparison of the frequency of rearing in the open field test in mice following oral administration of 30mg/kg (low dose) and 60mg/kg Artesunate (ATS).

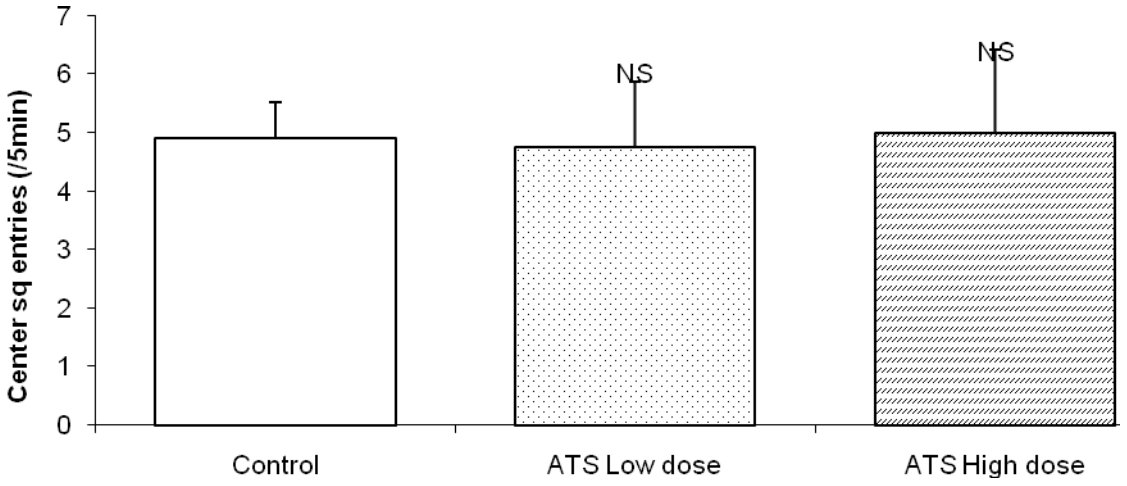

Fig. 4.3: Comparison of the frequency of centre square entries in the open field test in mice following oral administration of 30mg/kg (low dose) and 60mg/kg Artesunate (ATS).

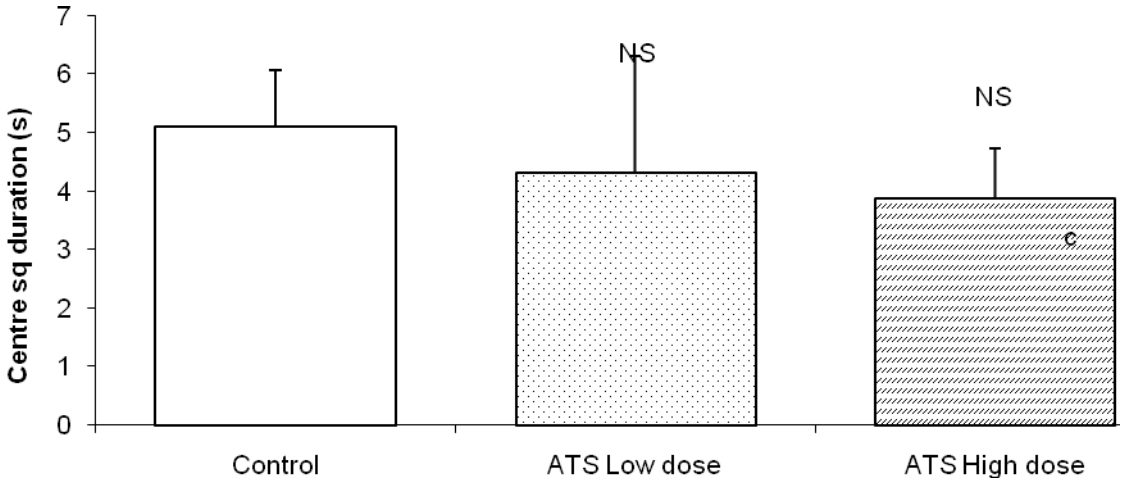

Fig. 4.4: Comparison of centre square duration in the open field test in mice following oral administration of 30mg/kg (low dose) and 60mg/kg Artesunate (ATS).

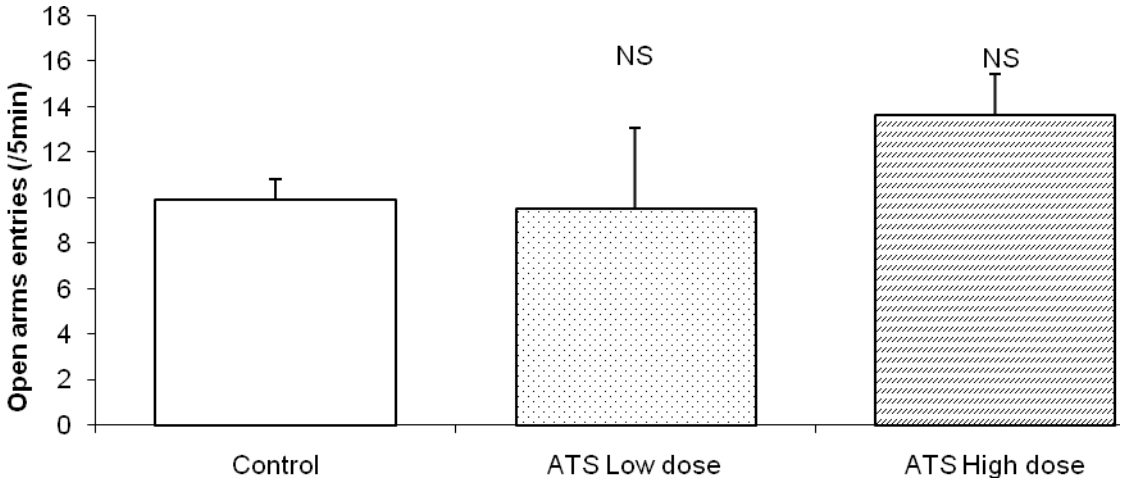

Fig. 4.5: Comparison of the frequency of open arm entries in the Elevated Plus Maze in mice following oral administration of 30mg/kg (low dose) and 60mg/kg (high dose) Artesunate (ATS).

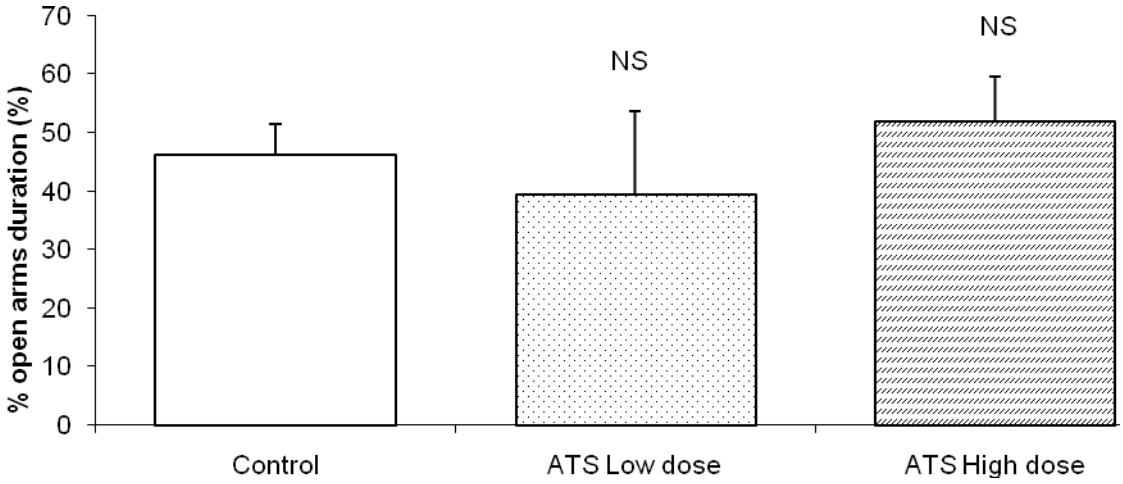

Fig. 4.6: Comparison of duration of open arm duration in the Elevated Plus Maze in mice following oral administration of 30mg/kg (low dose) and 60mg/kg (high dose) Artesunate (ATS).

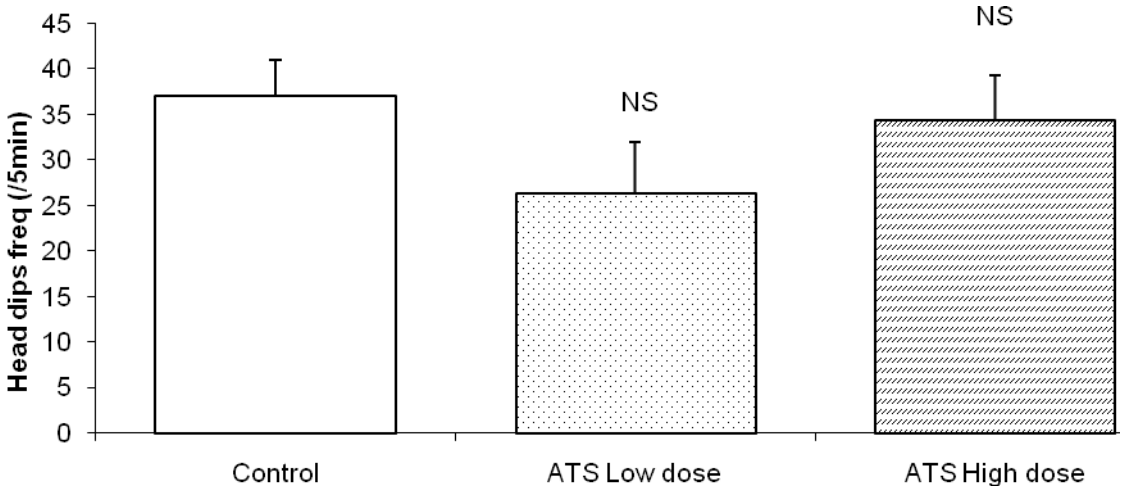

Fig. 4.7: Comparison of the frequency of Head dips in the Elevated Plus Maze in mice following oral administration of 30mg/kg (low dose) and 60mg/kg (high dose) Artesunate (ATS).

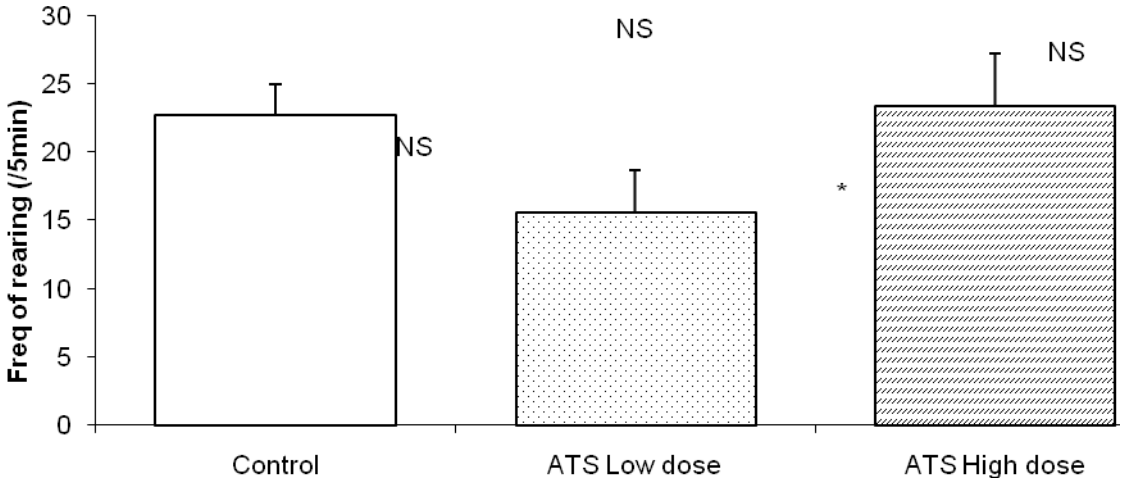

Fig. 4.8: Comparison of the frequency of rearing in the Elevated Plus Maze in mice following oral administration of 30mg/kg (low dose) and 60mg/kg (high dose) Artesunate (ATS).

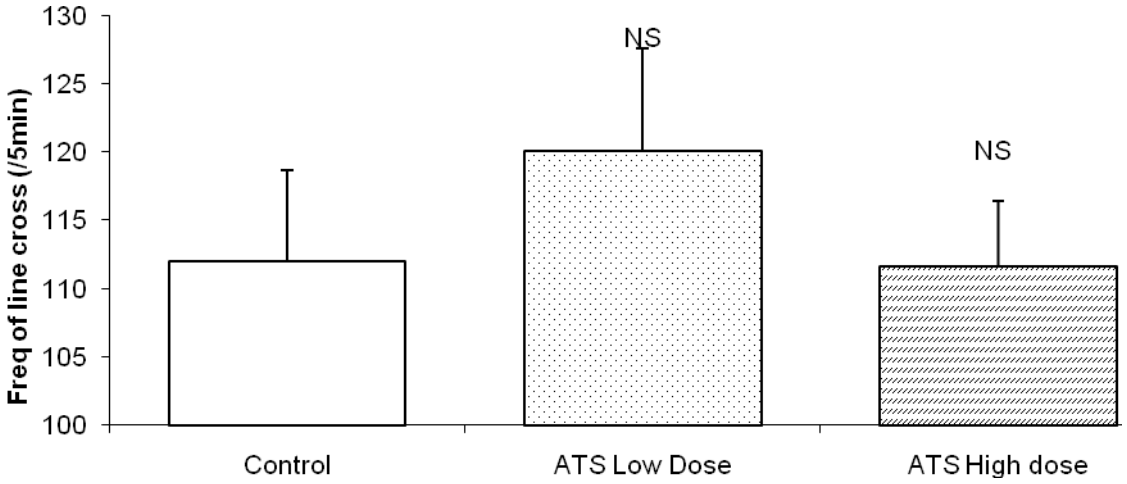

Fig. 4.9: Comparison of the frequency of line crosses in the Light and Dark Transition box in mice following oral administration of 30mg/kg (low dose) and 60mg/kg (high dose) of Artesunate (ATS).

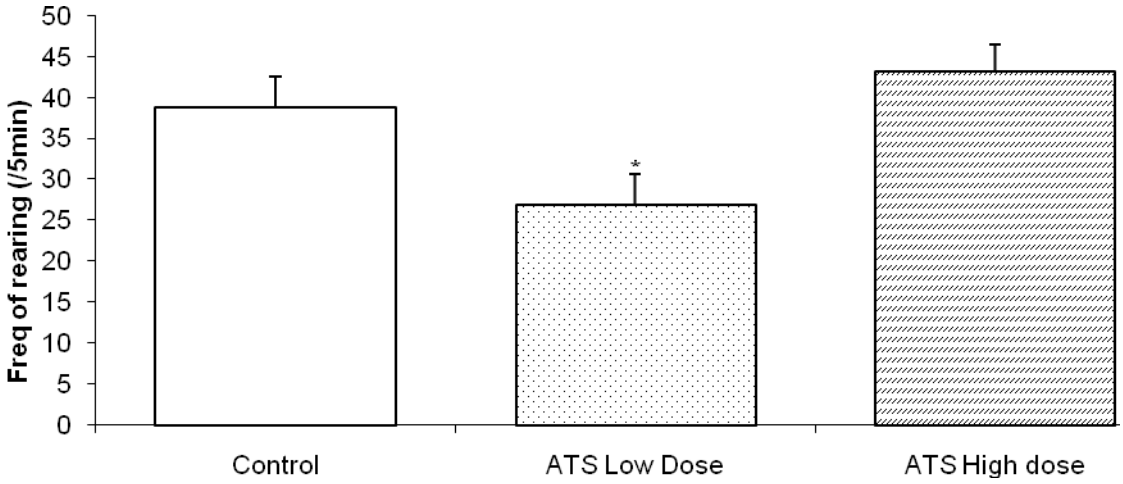

Fig. 4.10: Comparison of the frequency of rearing in the Light and Dark Transition box in mice following oral administration of 30mg/kg (low dose) and 60mg/kg (high dose) of Artesunate (ATS).

5.0 Discussion

This is the first study assessing the effects of long-term administration of oral Artesunate on Locomotor and exploratory behaviour of Mice. Locomotor and exploratory behaviour are usually tested for using Open field Maze, Light /Dark transition box and Elevated Plus Maze. The above behavioural tools also simultaneously assess anxiety (Walsh and Cummins, 1976). Behaviour such as frequency of line crosses, frequency of rearing, centre square entries/duration, open arm entries/duration and frequency of head dips are used as measures of Locomotion and Exploratory activity. A high frequency of these parameters indicates increased locomotion and Exploration. On the other hand, a reduced frequency of these parameters indicates decreased locomotion and Exploration. While the frequency of line crosses measures the horizontal locomotor behavior and represents the horizontal covered (Kelly *et al.,* 1989), other parameters are used to assess exploration.

The frequency of rearing was significantly higher in the low dose group compared to the control in the light/dark transition (LDB). This finding is difficult to interpret for the following reasons: Firstly, the frequency of rearing was not reduced in the high dose group. If rearing was due to the effect of the drug, then naturally the high group would have been affected even more. Secondly, this finding was observed only in one of the three behavioural tools employed in this study. Therefore, this finding may not be attributed to the drug. Apart from the above observation, there was no change in any other parameter used in the assessment of locomotor and exploratory behaviour in this study.

The no significant differences between the control and the experimental group in almost all the locomotor and exploratory behaviour tested for in this study indicates that oral Artesunate up to the dose of 60mg/kg does not impair exploration and locomotion. On the other hand, in a study similar to this one (performed by us), Artemether, a lipid soluble Artemisinin caused reduced locomotion and exploratory activity (Davies *et al.,* 2012). This is not surprising but further supports the earlier reports that water soluble derivatives of Artemisinin is less toxic compared to the lipid soluble forms, irrespective of the route of administration (Nontprasert et al., 2000). In their study, these authors showed the ED_{50} for neurotoxicity or death to be 300mg/kg for oral Artesunate administered daily for 28 days (Nontprasert et al., 2000).

Another reason for our findings could be due to the route of administration. Many studies have demonstrated that neurotoxicity of Artemisinins is seen more with parenteral route of administration than oral route (Petras *et al*., 1993; Nontprasert et al., 2000).

In conclusion oral Artesunate up to 60mg/kg does not affect exploratory or locomotor activity in mice.

References

Abdulazeez, A. A., Owoeye, O. and Ejiwunmi, A. B. (2006). The Neurotoxic Effects of Artemether on the cytoarchitecture of the Trapezoid Nuclei of Adult male Wistar rats (Rattus novegicus). *International Journal of Morphology*, 24(4):535-540.

Adekunle, A. S., Falade, C. O., Agbedana, E. O. and Egbe, A. (2009). Assessment of side-effects of administration of artemether in humans. *Biology and medicine*, 1(3): 15-19.

Barnett, S.A. and Cowan, P.E. (1976) Activity, exploration, curiosity and fear: An ethological study, *Interdisc. Sci. Rev.* 1: 43-62.

Basra, A. S. (2005). Handbook of medical Plants, 2nd end. CRC Press

Chekem L. Wierueki, S. (2006). Extraction of artemisinins and synthesis of it derivatives artesunate and artemether. *Med Trop. (Mars)*, 66(6): 602-5.

Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J. & Tomkins, D.M. (1989). Exploration of mice in a black and white test box: Validation as a model of anxiety. *Pharmacology, Biochemistry and Behavior*, 32:777-785.

Crusio, Wim E. (1995) Natural Selection on Hippocampal Circuitry Underlying Exploratory Behaviour in Mice: Quantitative-Genetic Analysis. In: E. Alleva, A. Fasolo, H.-P. Lipp, L. Nadel and L. Ricceri (eds.), *Behavioural Brain Research in Naturalistic and Seminaturalistic Settings.* NATO Advanced Study Institutes Series D, Behavioural and Social Sciences, Kluwer Academic Press, Dordrecht, THE NETHERLANDS, p. 323-342, 1995.

Cui, L. and Su, X. (2010). Discovery, Mechanisms of action and combinatino therapy of artemisinin. *Expert Review of Antiinfectious therapy*, 7(8): 999-1013.

Davies K. G., Edagha, I. A., Peter, I. P., John , E. O. and Eme E. Osim (2012). Effect of oral artemether suspension on spatial memory. *European Journal of Scientific Research.*82(4):499-505

Guy B Mulder, Kathleen Pritchett in Contemporary topics in laboratory animal

Kelley, A.E., Cador, M., and Stinus, L. (1989) Exploration and its measurement. A psychopharmacological perspective, in A.B. Boulton, G.B. Baker, and A.J. Greenshaw (eds.), *Neuromethods, Volume 13: Psychopharmacology*, Humana Press, Clifton, pp. 95-144.

Li, G. Q., Mog, S. R., Si, Y. Z., Kyle; Gettayacamin, M. and Mihous, K. (2002). Neurotoxicity and efficacy of arteether related to its exposure times and exposure levels in rodents. *American Journal of Tropical Medicine and Hygiene*, 66(5): 516-25.

McIntosh, H. M. and Olliaro, P. (2000). Artemisinin derivatives for treating severe malaria. Cochrane Database System Review (2)

Nontprasert, A., Pukviltayakanee, S., Dondrop, A. M., Clemens, R., Lovavee Suwan, S., White, N. J. (2000). Neuropathologic toxicity of artemisinin deriviatives in a mouse model. *American Journal of Tropical Medicine and Hygiene*, 62(3): 409 – 412.

O'Keefe, J. and Nadel, L. (1978). *The Hippocampus as a Cognitive Map*, Clarendon Press, Oxford.

Pasvol, G. (2005). The treatment of complicated and severe malaria.Br Med Bull.75(76): 29–47.

Petras, J. M., Brewer, T. O., Peggins J. O., (1993). Brain injury induced in Rathus rattus by the antimalarial drug arteether (AE). Journal of Anti-microbial Agent Chemotherapy 4(6): 78-80.

Renner, M. J. (1988). The role of behavioural topography during exploration in determining subsequent adaptive behaviour. *International Journal of Comparative Psychology. 2:43-56*

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

Van Abeelen, J.H.F. (1970) Genetics of rearing behavior in mice, *Behav. Genet.* **1**, 71-76.

Walsh, R. N. and Cummins, R. A. (1976). The Open Field test. A critical review. *Psychological Bulletin*. 83:482-504