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Pattern of neurobehaviour in albino rats in the open field following oral artesunate administration

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Summary

The neurobehavioural patterns in the open field following oral artesunate administration was studied using 40 albino rats randomly assigned to three Groups, namely A, B and C. Prior to the test, all the animals were acclimatized for 5 minutes in the open field maze. Group A (8 males and 8 females) received therapeutic doses of artesunate (1. 42 mg/kg per oral (p.o.) – using oro-gastric tubes while Group B (8 males and 8 females) received pharmacological doses of 4. 26 mg/kg (p.o.). Group C served as the control and were administered only distilled water (p.o.). Gross behavioural changes were noted following the therapeutic and pharmacological administration of artesunate for five days. Rats in Groups A and B showed marked decrease in loco motor activity (line crossing) and exploratory (rearing and walling) activities in comparison with the control ($P<0.05$). The centre square activity was significantly decreased in Groups A and B in comparison with the control ($P<0.01$). The number of faecal boli and urine puddles did not change significantly in Groups A and B when compared with the control ($P>0.05$). However, the frequency of grooming was significantly lower in Groups A and B rats than in the control ($P<0.01$). The frequency and duration of freezing were significantly higher in Groups A and B rats than in the control rats ($P<0.01$). There were no significant differences between the values for the male and female rats. There was also no dose dependent effect of artesunate on the activities studied. Oral administration of artesunate significantly decreases loco motor and exploratory behaviours in the albino rat.

Keywords: *Artesunate neurobehaviour, rats.*

Résumé

Les fréquences neurologiques et comportementales sur le terrain après l'administration de l'artesunate orale étaient étudiées utilisant 40 rats albinos groupés au hasard en 3 groupes (A, B, C) avant le test. Tous les animaux étaient exposés pour 5 minutes à l'air libre. Le groupe A (8 males et 8 femelles) recevaient un régiment thérapeutique orale d'artesunate de (1.42 mg/kg). Le groupe C (recevaient uniquement de l'eau distillée) et le groupe B recevaient des doses therapautiques de 4.26 mg/kg pendant 5 jours. Les changements du comportement général étaient notés durant l'administration thérapeutique et pharmacologique de l'artesunate. Les rats du groupe A et B montraient une réduction importante de l'activité locomotrice et les activités exploratoires comparées aux groupes contrôles ($P<0.05$). L'activité croisée centrale était significativement bas aux groupes A et B comparé aux groupes de contrôle ($P<0.01$). Le nombre de boule d'excréments et d'urine ne changeait pas significativement aux groupes A et B lorsque comparé au groupe de contrôle ($P>0.05$).Cependant, le fréquence d'accouplement était significativement faible aux groupes A et B qu'aux contrôle ($P<0.01$). La fréquences et la durée de congélation étaient significativement différente entre les valeurs chez les males que chez les femelles. Il n'y avait aucun effet dépendant de l'artesunate sur les activités étudiées. L'administration orale de l'artesunate réduisait significativement les comportements locomoteurs et exploratoires chez les rats albinos.

Introduction

Artesunate is one of the products of the Chinese herbal tea, *ginghaosu* (*Artemisinin annua L. family Asteraceae*). It is a blood *schizonticide* with significant effect on the erythrocytic *schizogony* stage of the life cycle of *plasmodium*. It is believed to act by an iron – catalyzed generation of a carbon centred free radical followed by the *alkylation* of malaria specific proteins [1]. It has been shown to increase the concentration of oxygen and hydrogen peroxide radicals in red blood cells by increasing membrane lipid *peroxidation*. Zhao *et al* [2] showed that artesunate inhibits the activity of *cytochrome* oxidase which is found in the plasma, nuclear membrane and in the mitochondria of the

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trophozoites of *Plasmodium berghei* (species of *plasmodium* found in rats).

Literature is replete with the neurotoxicity of the *artemisinin* products in animal models. Nontprasert *et al* [3] reported multiple neuronal brainstem damage in the mouse model especially involving the neurons in the lower brainstem trapezoid nucleus, the *gigantocellular reticular nucleus* and the inferior *cerebellar peduncle*. A similar effect was observed following intramuscular administration of some *artemisinin* products, *arteether* (AE) and *artelinate*. A histological evaluation of the brains using *theonine* staining showed marked damage to the brain stem nuclei of the AE treated rats, including nuclei rubber, superior olive *trapezoides*, and inferior vestibular nuclei. The damage included chromatolysis, necrosis and *gliosis* [4].

The search for new *antimalaria* drugs against the ever-increasing resistance profile of the *Plasmodium falciparum* species was rewarded by the discovery of *qinghaosu* and its product, including artesunate. Their unique rapid onset of action, improved activity against drug resistant malaria and effective parasite clearance has led to considerable interest in this Group of drugs.

The wide range of neurotoxicity side effects suggest the possibility of *neurobehavioural* alteration especially, in malaria endemic tropics and subtropics where the combined effect of poverty and ignorance exposes the patients to drug abuse. Indeed, *artesunate* is the oral form of *artemisinin* most widely consumed [5]. This study examines the possibility of neurobehavioural alteration following therapeutic and pharmacological oral administration of artesunate for five days.

Materials and method

Forty adult albino Wistar rats (weighing 200 – 250g) bred in the animal house of the Department of Anatomy, University of Calabar, were used for the study. They were kept in plastic cages in a Group of 8 rats per cage and were allowed commercial rat feed (palletized vital feed for growers) and tap water *ad libitum*.

The animals were randomly divided into three Groups A, B and C. Group A (8 males and 8 females) received therapeutic doses of *artesunate* (p.o) at 1.42mg/kg per rat. Group B (8 males and 8 females) received a pharmacological doses of *artesunate* p.o. at 4.26mg/kg. Group C rats (4 male and 4 female rats) served as the control and received distilled water p.o. The National agency for food and drug administration and control (NAFDAC) approved tablets of *artesunate* were bought from a registered pharmacy (*Dominion pharmacy*) in Calabar, Nigeria. Artesunate tablets were crushed to powder and mixed with distilled water to form a stock concentration which was used for administration. The drug was administered orally by force-feeding using oro-gastric tubes attached to a 1ml syringe. The treatment lasted for five (5) days after which the neurobehavioural activities of the animals were observed and scored in the open field maze.

The open field maze

The open field arena was designed as described by Brown *et al* [6] The arena is used to assess the emotionality of in bred animals in a novel environment [7]. The frequency and duration of eight behaviours in the open field were scored during a 5 minutes test for each rat. Timing was begun at the placement of the rat at the centre square. The behaviours were scored by trained individuals, who were blind to the experiments and therefore unbiased, using stopwatches, as described by Archer [8]. The following behaviours were scored.

Line Crossing: When the rat crosses one of the grid lines which separate the squares in the open field with all four feet.

Rearing: Standing on hind legs.

Walling: Standing on hind legs and leaning against the wall of the maze.

Centre square activity: The duration and the number of times the rat enters one of the four centre squares with all four paws.

Grooming: When the rat, while stationary, licks or scratches using front or back paws.

Freezing: When the rat is completely stationary.

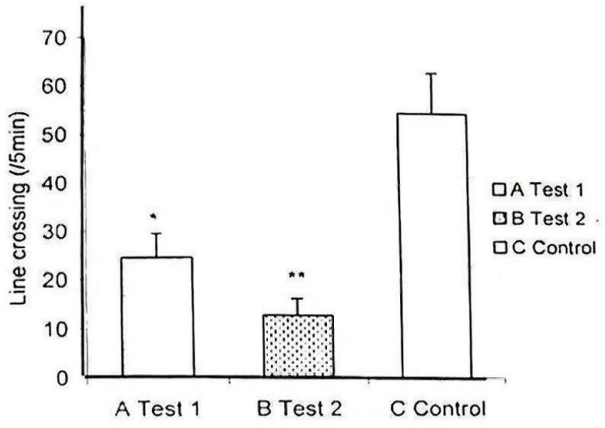
Fecal boli And Urine Spots on the floor were also counted

Statistical analysis

The analysis of variance was used to analyse the data. This was followed by a post hoc student's t– test for differences between two Groups. Values of $p<0.05$ were considered significant.

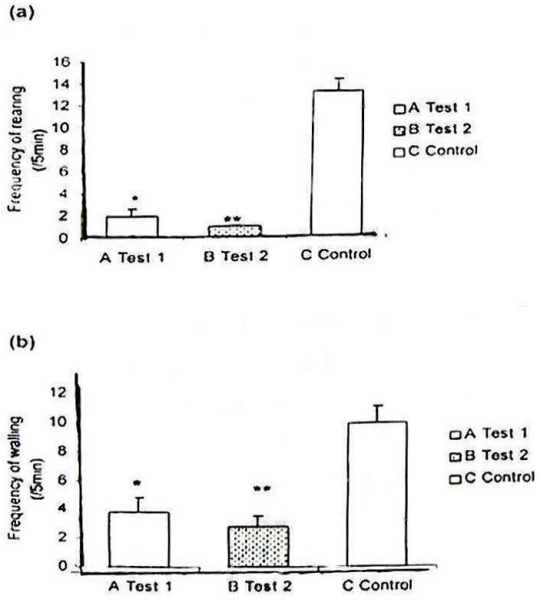
Results

Rats in Groups A and B showed marked decrease in *locomotor* activity (line crossing, rearing and walling) following the administration of the therapeutic and pharmacological doses in comparison with the control ($P< 0.05$) (Figs 1 and 2).



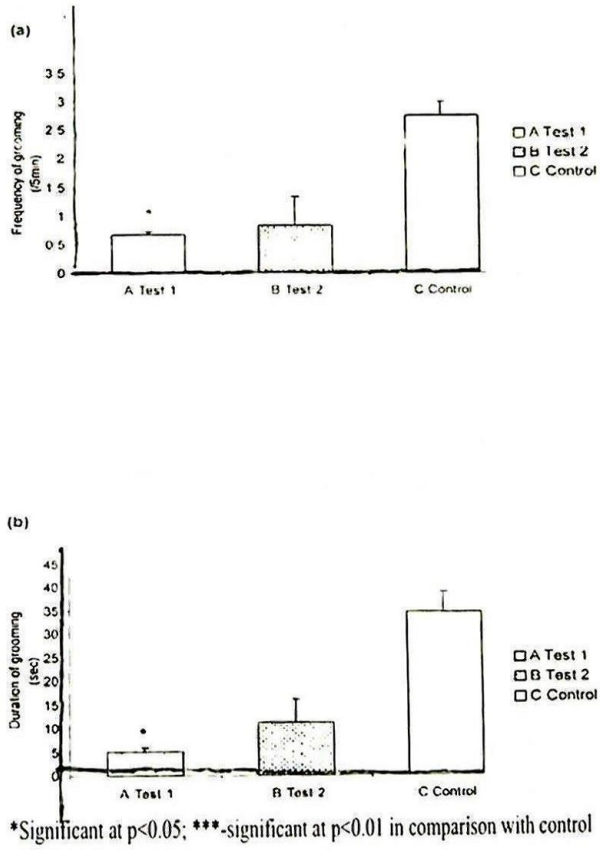
*Significant at p<0.05; ***-significant at p<0.01 in comparison with control

Fig. 1: Locomotor activity of albino Wistar rats administered 1.43mg/kg Artesunate (Test 1), 4.29mg/kg Artesunate (Test 2) and distilled water per oral (Control)



*Significant at p<0.05; ***-significant at p<0.01 in comparison with control

Fig. 2: Exploratory behaviour of albino wistar rats in the open field maze following administration of 1.43mg/kg Artesunate (Test 1), 4.29mg/kg Artesunate (Test 2) and distilled water per oral (Control).

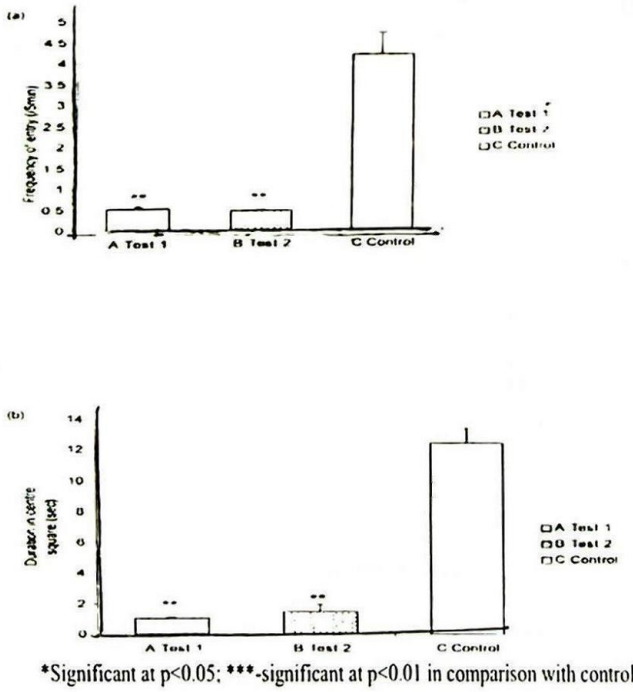


*Significant at p<0.05; ***-significant at p<0.01 in comparison with control

Fig. 3: Frequency and duration of grooming of albino Wistar rats in the open field maze following administration of 1.43mg/kg Artesunate (Test 1), 4.29mg/kg Artesunate (Test 2) and ditalled water per oral (Control).

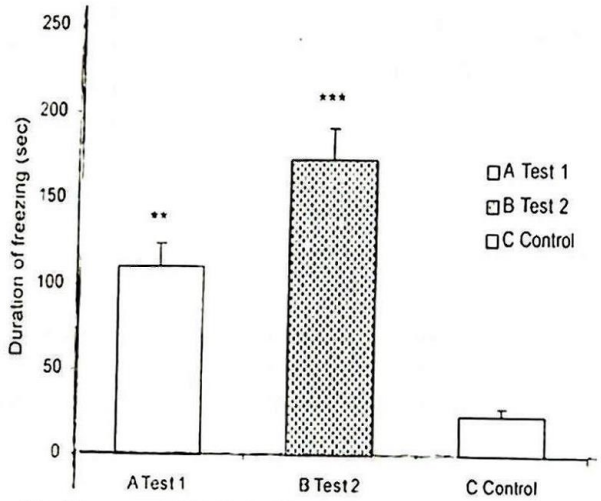
The frequency of grooming was significantly lower in Groups A and B than in control rats ($P < 0.01$) (Fig 3)

The centre square activity was significantly decreased in Groups A and B in comparison with the control ($P < 0.01$)(Fig 4). The number of faecal boli and urine puddles did not change significantly in Groups A and B when compared to the control rats ($P > 0.05$) however, the frequency and duration of freezing were significantly higher in Groups A and B rats than in the control rats ($p<0.01$) (fig 5).



*Significant at p<0.05; ***-significant at p<0.01 in comparison with control

Fig. 4: Centre square activity of albino Wistar rats in the open field maze following administration of 1.43mg/kg Artesunate (Test 1), 4.29mg/kg Artesunate (Test 2) and distilled water per oral (Control).



*Significant at p<0.05; ***-significant at p<0.01 in comparison with control

Fig. 5: Duration of freezing of albino Wistar rats in the open field mase following administration of 1.43mg/kg Artesunate (Test 1), 4.29mg/kg Artesunate (Test 2) and distilled water per oral (Control)

Discussion

The results from the open field maze show that the rats in Groups A and B had marked decrease in line crossing, rearing and walling activities in comparison with Group C. This indicates that the Group C rats performed more locomotor activities than Groups A and B, and in the absence of mechanical injury and organic disease, as was the case with the rats in this experiment, constitute an expression of fear and anxiety [6]. The frequency of grooming was significantly lower in Groups A and B in comparison with Group C. The frequency and duration of freezing was also significantly lower in Groups A and B than in the control ($P<0.01$). This suggests a reduction in the confidence of the animals due to a possible anxiety based increased risk assessment [9]. Blanchard posits that when the nature and location

of the threat source are uncertain, mammals may show orientation to the potential threat and cessation of ongoing activity (freezing). Many marsupials will show flight or freezing as a defensive response to fear stimuli [10]. These findings may be supported by the changes observed by Genovesse *et al* [4] who showed a progressive and severe decline in accuracy, increase in response time and eventually response suppression using the auditory discrimination test (ADT) in rats administered with *artemisinin* derivatives.Though this behavioural changes point to alteration of function in the locomotor and limbic systems, literature is lacking on the possible mechanism behind the effects.

In conclusion, oral administration of artesunate decreases locomotor and exploratory behavior and induces fear and anxiety in rats.

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