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Full Length Research Paper

Suppression of exploration and locomotion in adult Wistar rats following quinine administration

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The effect of quinine commonly used for the treatment of chloroquine resistant cases and cerebral malaria on exploration in Wistar rats was investigated. Adult male rats (n= 27), weighing between 150 and 190 g were randomly selected into two treatments groups (n=9) and control (n=9). Each control rat in Group I received intramuscularly injection of physiological saline. The rats in Group II were injected intramuscularly with quinine, 20 mg/kg for a starting dose, followed by 10 mg/kg 8 hourly for 7 days. The Group III rats received the same treatment as Group II, but were subjected to a withdrawal period of one week after the treatment. The exploration and locomotion of treated rats were significantly suppressed ($P < 0.05$) by quinine. The frequency of line crossing reduced significantly from 30.8 ± 5.7 in Group I to 2.2 ± 0.93 in Group II and 2.5 ± 1.1 in Group III rats. The frequency of hinding also reduced significantly from 7.4 ± 1.3 in group I to 0.7 ± 0.4 in Group II and 0.9 ± 0.3 in Group III. The marked decrease observed in exploratory activities in the treated groups might be the consequence of degeneration of cerebellar cortex.

Key words: Quinine, locomotion, exploration, suppression, Wistar rats.

INTRODUCTION

Malaria was a global epidemic disease causing great havoc in several countries, claiming a heavy toll of human lives and eluding an effective cure from eminent physicians during the seventeenth century (Elimear et al., 2003) Although a variety of drug formulations have been developed for used in the treatment of malaria over the years, quinine dihydrochloride appears to be among the spectrum of drugs still being preferred for the treatment of malaria especially in poor countries (Editorial., 2003).

The use of quinine in the treatment of malaria is particularly significant because extracts from the bark of 'quinquina' (cinchona) tree were the most widely used preparations for treating malaria between 1600 and 1800s, thus making them the first chemical compounds, used successfully to treat an infectious disease (Elimear et al., 2003). Quinine is used today as chemotherapy for malaria especially in chloroquine resistant cases and cerebral malaria (White and Warrell, 1983). Quinine is said to be more toxic than chloroquine (Kakkilaya, 2002). It was also found to cross the blood brain barrier thus, affecting the central nervous system (Kenmochi and Eggerment, 1997).

The cerebellum is vulnerable to damage from a variety of sources such as developmental defects, degenerative diseases infectious processes, chronic alcoholism, trauma, toxic and metabolic effects (West, 1995).

Ataxia and other gait disturbances had been reported to be due to the effects of such drugs as antibiotics, chloroquinine, quinine and guanidine (Eisenhauer and Murphy, 1998). Quinine sulphate had been observed to cause peripheral ototoxic and central effects on the central nervous system (Kenmochi and Eggermont, 1997). Cerebellar injuries also results from toxins, and autoantibodies, structural lesions and inherited cerebellar degeneration (Timothy, 2003).

The cerebellum is largely involved in co-ordination of equilibrium, posture and muscle tone necessary for smooth, co-ordinated motor activities (West, 1995). Any degeneration or distortion of the cerebellar cortical layers many impair cerebellar functions. This paper reports the exploration and locomotor activities in Wistar rats

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following experimental quinine administration.

In view of several reported cases of cerebellar pathologies following toxins and drugs. It became necessary to appraise the effects of quinine on explorations and locomotion in Wistar rats.

MATERIALS AND METHODS

Twenty-seven mature male albino rats of Wistar strain, weighing between 150 and 190 g were used for this study. The rats were fed daily with normal rats pellets purchased form Ladokun feeds (Ibadan, Nigeria) and water was given to the animals *ad libitum.* All the rats were carefully assessed, screened and confirmed to be healthy during the period of acclimatization.

Rats were randomly selected into three groups in relation to their weights. Each group contained nine rats. Group I served as the control and received intramuscularly injection of physiological saline of the same quantity as contained in the experimental doses. Groups II and III served as the treated groups. 300 mg/ml of the liquid quinine contained in the ampoule was used for the present study. Group II rats were injected intramuscularly with liquid quinine dihydrochloride; 20 mg/kg body weight for a starting dose, followed by 10 mg/kg 8 hourly for 7 days (Kakkilaya, 2002). Group III received the same treatment as Group II. However, they were subjected to post-treatment withdrawal period of one week to determine possible withdrawal effects on them before they were sacrificed by cervical dislocation.

The open field test was conducted following the methods of Brown et al. (1999), during the period of acclimatization and each day of the treatment period and at the end of one week withdrawal period.

Apparatus

The open field apparatus was constructed with plywood and measured 72 × 72 cm with 36 cm walls. The walls and floor were both white. Blue lines were drawn on the floor with a marker and were visible through the clear plexi-glass floor. The lines divided the floor into sixteen 18×18 cm squares. A central square of equal size was drawn in the middle of the open field (18 × 18 cm), (Brown et al., 1999).

Rats were taken from their home cages and placed randomly into one of the four corners of the open field, facing the centre and allowed to explore the apparatus for 5 min. After the 5 min test, the rats were returned to their home cages and the open field was cleaned with 70% ethyl alcohol and permitted to dry between test. The behaviours scored following the methods of Brown et al. (1999) which include:

1. Line crossing: Frequency with which rats crossed one of the grid lines with all four limbs.
2. Rearing: Frequency with which the rats stood on their hind legs in the open field.
3. Rearing against a wall: Frequency with which the rats stood on their hind legs while the forelimbs rested against the wall of the open field.
4. Hinding; the sum of the 2nd and 3rd behaviour.
5. Locomotor activity refers to the movement from one location to another. Locomotion is one of the most important components of exploration.
6. Exploration refers to all activities concerned with gathering information about the environment. Locomotor activity and exploratory behaviors such as rearing and rearing against a wall are components of exploration and are associated with behavioral activities in rats.

The animals were treated in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health (1985).

Statistical analysis of the data obtained was performed using analysis of variance and student's t-test (Araoye, 2003). Experimental data were presented as mean and standard error of mean (Mean ± SEM). Values of probability ($P < 0.05$) were taken to be statistically significant.

RESULTS

Table 1 shows the mean ± SEM of the frequency of line crossing and body weights of the rats at the Acclimatization period and the End of the Study Periods.

Figure 1 similarity, shows the pattern of changes in the frequency of line crossing of treated and control rats during those periods. The frequency of line crossing in control rats did not increase significantly from the beginning of the treatment to the end of the treatment, while the frequency of line crossing decreased significantly from the beginning of the treatment to the end of the treatment ($P < 0.05$) in quinine treated rats. The frequency of line crossing in treated rats was significantly reduced throughout the treatment period compared with control rat (Figure 1).

However, the frequency of line crossing in Group III treated rats did not decrease significantly at the end of one week withdrawal period, while in control rats, the frequency increases significantly during that same period (Table 1 and Figure 1).

Table 2 shows the mean ± SEM of the frequency of hinding of the rats at the Acclimatization Period and the End of the study periods. Figure 2, shows the pattern of changes in frequency of hinding of rats in treated and control rats. The frequency of hinding in control rats did not decrease significantly from the beginning of the treatment to the end of the treatment, while the frequency of hinding decreased significantly from the beginning of the treatment to the end of the treatment ($P < 0.05$). The frequency of hinding in quinine–treated rats was signify-cantly reduced throughout the treatment period compared with control rats (Figure 2). However, the frequency of hinding in control rats and Group III treated rats did not decrease significantly at the end of one week withdrawal period (Table 2 and Figure 2).

Table 3 shows that the population and diameters of Purkinje cells in the cerebellar cortex of the treated rats were significantly reduced ($P < 0.05$) compared with the control.

DISCUSSION

The cerebellum is largely involved in co-ordination, persons whose cerebellum does not work well are generally clumsy and unsteady (Timothy, 2003). The

Table 1. Mean ± SEM of the frequency of line crossing and body weights of the rats at the acclimatization period and the end of the study periods.

Group	n	No of lines crossed in 5 min before the treatment	No of Lines crossed in 5 min during the treatment	n	No of lines crossed after withdrawal period	Initial body weight (g)	Final body weight (g)
I	9	30.0 ± 7.1	30.8 ± 5.7	4	50.5 ± 2.4	148.0 ± 5.5	178.5 ± 8.2
II	9	48.5 ± 5.9	2.2 ± 0.93 *	-	-	149.6 ± 6.5	173.2 ± 4.7
III	9	43.0 ± 6.0	2.5 ± 1.1 *	9	2.1 ± 1.2 *	149.5 ± 4.6	171.3 ± 4.4

*(P < 0.05) Significant difference when compared with control using the t-test.

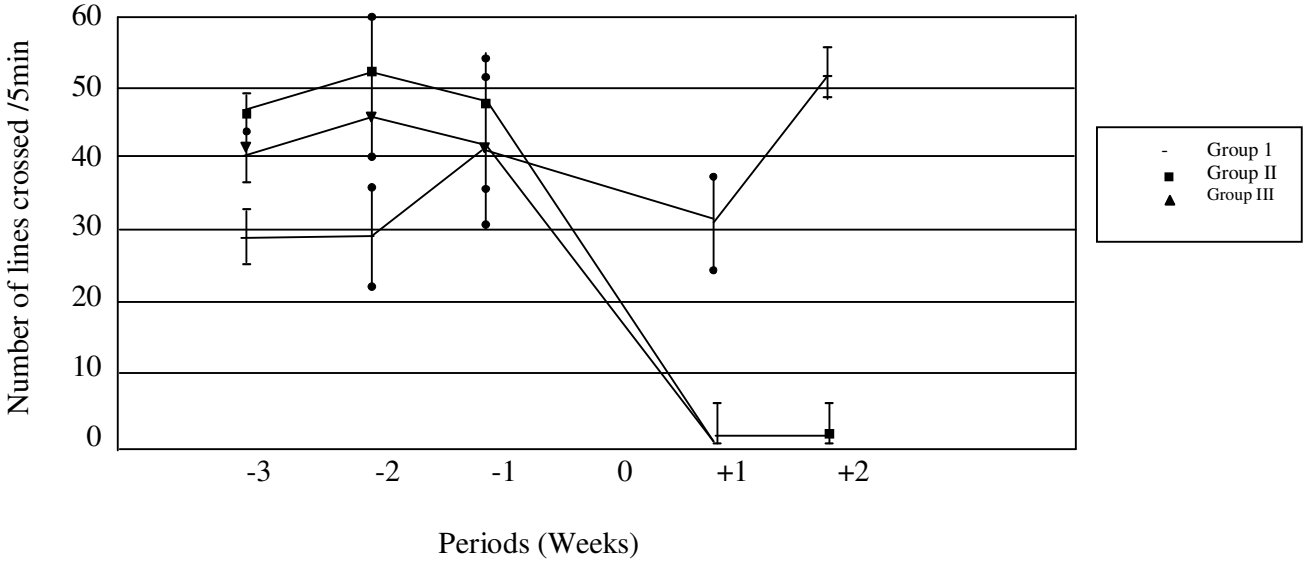

Figure 1. Graphs showing the frequency of line crossing in the animals from the acclimatization period to the end of the study.

cerebellum functions as a comparator and co-ordinator in that, it compares movement intention with performance and co-ordinates equilibrium, posture and muscle tone necessary for smooth, coordinated motor activities (West, 1995).

The open field test provides simultaneous measures of locomotion, exploration and anxiety, the frequency of line crossing and the frequency of rearing are usually used as measures of locomotor activity, these are also measures of exploration and anxiety of these rats (Walsh and Cummins,1976). The open field test (OFT) is a common measure of exploratory behavior and general activity in both mice and rats, where both the quality and quantity of the activity can be measured. The most basic and common outcome of interest is “movement”; however, this can be influenced by motor output, exploratory drive, freezing or other fear-related behavior, sickness, relative time in circadian cycle, among many other variables (Toddy et al., 2008). Distance moved, time spent moving, rearing, and change in activity over time are among many measures that can be tabulated and reported. Some outcomes, particularly defecation, center time and activity within the first 5 min, likely gauge some aspects of emotionality including anxiety. The OFT is also

Table 2. Mean ± SEM of the frequency of hindings of the rats at the acclimatization period and the end of the study periods.

Group	n	Values during acclimatization. No of times in 5 min	Values at the end of treatment. No of times in 5 min	n	During one week possible self cure period
I	9	8.7 ± 2.8	7.4 ± 1.3	4	7.6 ± 1.11
II	9	18.0 ± 3.8	0.7 ± 0.4 *	-	-
III	9	14.6 ± 3.8	0.9 ± 0.3 *	9	0.7 ± 0.2 *

*(P < 0.05) Significant difference when compared with control using the t-test.

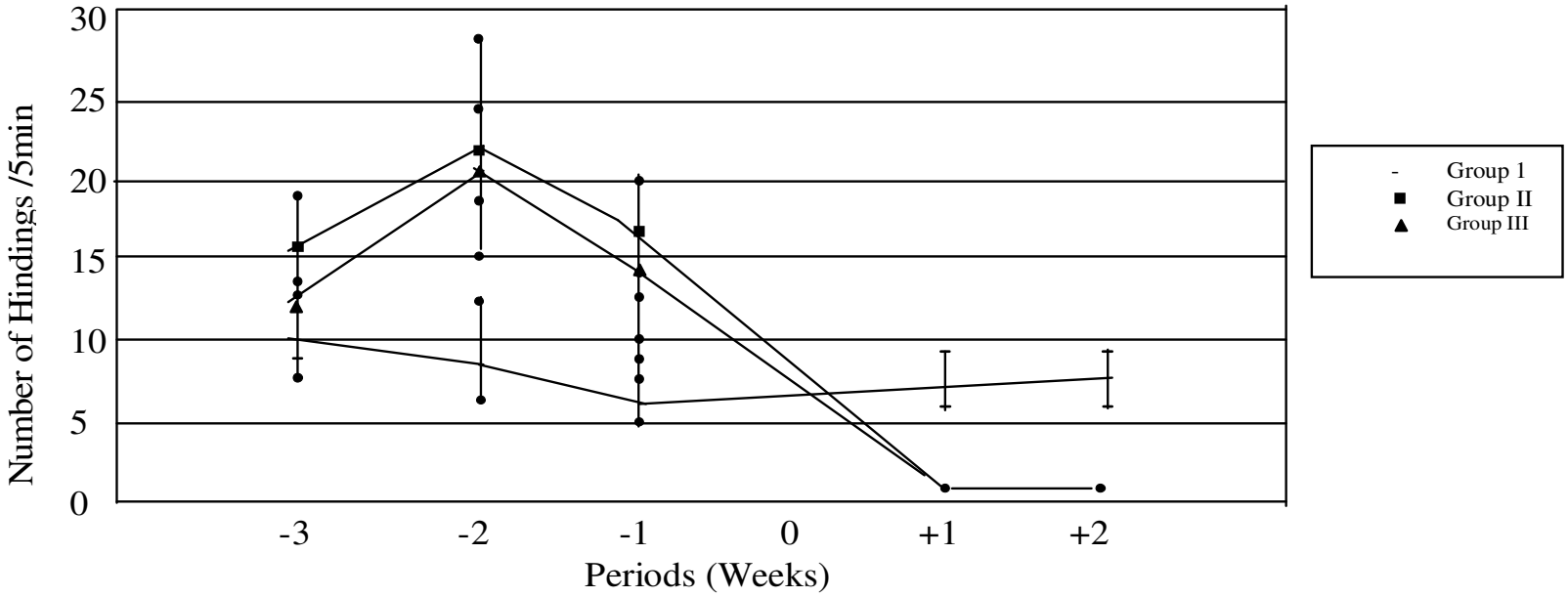

Figure 2. Graphs showing the frequency of hindings in the animals from the acclimatization period to the end of the study.

Table 3. Mean values of Purkinje cell population and transverse diameters of Purkinje cells in the cerebellar cortex of Wistar rats following quinine administration.

Group	Population of Purkinje cells (cells/mm^2) (Mean ± SEM)	Diameters of Purkinje cells (µm) (Mean ± SEM)
I	363 ± 5.2	1.20 ±0.02
II	239 ± 9.5$^+$	1.09 ±0.01$^+$
III	220 ± 6.6$^+$	0.75±0.03$^{+\ a}$

+ Significant difference when compared with control using t-test ($P < 0.05$). [a]Significant difference when compared with group II using t-test ($P < 0.05$).

commonly used as a mechanism to assess the sedative, toxic, or stimulant effects of compounds. Thus, the OFT measures a number of facets of behavior beyond simple locomotion. As such, the test has a number of uses and is included in almost every thorough analysis of rodent behavior (Todd et al., 2008).

A high frequency of these behaviours indicated an increase in locomotion and exploration of the animals. Repeated exposure to the open field apparatus result in time dependent changes in behaviours (Choleris, 2001). The assessment of open field locomotor activities of these rats before and following treatment is very significant in relation to cerebellar functions. Carrey et al. (2000) employed the open field test to assess the locomotor and exploratory behaviour of prepubertal mice following chronic methyl phenidate administration. Increased post weaning open field rearing activity was observed in rats following treatment with a toxic dye, Allura red AC (Vorhees et al., 1983).

Similarly, Benesova et al. (2001) have reported that the pattern of behavioural and neuroendocrine deviations in adult rats was dependent on the ontogenic stage exposed to drug insult. They recorded a depression of open field motor activity (from open field test) and emotional reactivity, as well as higher pituitary weight following treatment with idomethacin.

Effects of some central nervous system stimulants on exploration and locomotive activity have been reported (Agarwal, 1995). CNS stimulants such as Amphetamine, Leptazol, Picrotoxin, strychnine and Nikethamide have been reported to significantly suppressed the open field exploration and locomotor activity in mice treated with these stimulant drugs. Conversely, caffeine was known to increase locomotor activity in mice treated with the drug. Da Cunha et al. (1992), reported a decreased exploratory activity in rats treated with flumazenil, a benzodiazepine receptor antagonist. The open field locomotor activities of adult Wistar rats have been adversely affected following monosodium glutamate administration (Eweka and Om'Iniabohs, 2008).

In this study, quinine significantly decreased line crossing and hinding which constitute exploratory behaviour. Direct deficits in open field exploratory behaviour following quinine administration manifested as a suppression of open field locomotor activity. The decreased locomotor activity of the treated animals compared with control, might be partly due to neurotoxic effect of quinine on neuronal cells of the cerebellar cortex and the associated neurotransmitter substances. The neurotoxic effects of the quinine on the cerebellar cortex of these rats that were subjected to open field which resulted in cerebellar cortical degeneration had already been reported in (Ajibade et al., 2006). The marked suppression of exploration and locomotion is probably a consequence of neuronal degeneration in the cerebellar cortex which might have affected the integrity of the neuronal cells in the cerebellar cortex. Table 3 similarly, shows degeneration and massive loss of purkinje cells which are the principal neurons in the cerebellum were observed in the cerebellar cortex of these rats subjected to open field test, following quinine administration (Ajibade et al., 2008). The significantly reduced population and transverse diameters of the purkinje cells (principal cells of the cerebellum) in the treated rats might have resulted from neuronal degeneration following the neurotoxic effects of quinine. The activities of the various neurotransmitters associated with the neurons in the cerebellar cortex might also be altered by the toxic effect of quinine which may be a contributing factor to a suppressed exploration and locomotion.

The result of the open field locomotor activity in the present study is consistent with the findings of some investigators that recorded suppression of exploration and locomotor activity in the open field following drug administration. The significantly reduced exploratory activities in the treated rats may be the consequence of neuronal degeneration and loss in the cerebellum which might have consequently impaired cerebellar functions that partly manifested as suppressed exploratory activities in the treated rats.

It is possible that quinine had an irreversible effect on the locomotor and exploratory activities in Wistar rats which brought irreversible effects on exploration as observed in rats which were subjected to one week withdrawal period.

CONCLUSION AND RECOMMENDATION

The study concludes that the administration of quinine at

20 mg/kg body weight start followed by 10 mg/kg body weight to adult Wistar rats results in reduced locomotor and exploratory activities in Wistar rats. This study clinically implies the need for consultation and prescription before use and the need for adequate rest while on quinine therapy. The intake of glucose may be beneficial during quinine therapy. It is recommended that further studies aimed at corroborating these findings particularly on the biochemical and haematological parameters be carried out.

This study further revealed that consumption of MSG affects the locomotor activities in the adult Wistar rats. The line crossing, walling, hinding and grooming of the locomotor activities of the treated group in the open field test were significantly affected as compared to the control group.

The open field test (OFT) is a common measure of exploratory behavior and general activity in both mice and rats, where both the quality and quantity of the activity can be measured. The most basic and common outcome of interest is “movement”; however, this can be influenced by motor output, exploratory drive, freezing or other fear-related behavior, sickness, relative time in circadian cycle, among many other variables. Distance moved, time spent moving, rearing, and change in activity over time are among many measures that can be tabulated and reported. Some outcomes, particularly defecation, center time, and activity within the first 5 minutes, likely gauge some aspects of emotionality including anxiety. The OFT is also commonly used as a mechanism to assess the sedative, toxic, or stimulant effects of compounds. Thus, the OFT measures a number of facets of behavior beyond simple locomotion. As such, the test has a number of uses and is included in almost every thorough analysis of rodent behavior.

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