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Antiretroviral Therapy may have Anxiogenic Effect on Subjects

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Authors' contributions

This work was carried out in collaboration between all authors. Author EEO designed the study. Author CON did the experimental protocol. Author IOA managed the literature searches and wrote the first draft of the manuscript. Authors MO and SAB managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To observe the effects of the antiretroviral drugs, Lamivudine, Zidovudine, and Efavirenz individually and in combination on locomotor behavior and anxiety.
Methods: Fifty (50) mice were divided into five (5) groups of ten (10) mice each. Group one served as control and was given normal saline. The next three groups received oral solutions of Lamivudine, Zidovudine, and Efavirenz respectively at doses of 2 mg/kg, 4 mg/kg and 8 mg/kg at volumes not exceeding 10 ml/kg body weight (i.e. 0.1 ml/10 g). The fifth group received a combined oral solution of the three antiretroviral drugs.
Results: In the Open field test, the frequency of line crossing, rearing frequency, and centre square entry are significantly (P=0.05) lower in the antiretroviral treated groups compared to the control, with a significantly higher centre square entry in the combined treated group than in the individual

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treated groups. In the light/dark transition box, it was observed that the duration in the light chamber was significantly (p=0.05) lower in the lamivudine and Efavirenz treated groups compared to control. The duration in the Zidovudine and combined treatment groups, though lowered, was not significantly different. There was an increase in grooming duration in the Efavirenz and the combined treatment groups, and a significant decrease in transitions in all the treatment groups compared to control. In the elevated plus maze, the frequency of open arm entry is significantly lower in the Efavirenz treated group compared to control. It is also significantly higher in the combined treatment group compared to all three individual treatment groups. The open arm duration is significantly lower in the three individual treatment groups compared to control. It is significantly lower in the Efavirenz treated group compared to the lamivudine and Zidovudine treated groups. The frequency of head dip did not differ significantly in the lamivudine and Zidovudine treated groups compared to control. It is significantly lower in the Efavirenz treated group compared to control. However it is significantly higher in the combined treatment group compared to control.

Conclusion: Taken together, our findings indicate an anxiogenic effect of antiretroviral therapy. However our results do not show a conclusive evidence that combined antiretroviral therapy has a greater anxiogenic effect on the subjects than single antiretroviral therapy.

Keywords: Lamivudine; Zidovudine; Efavirenz; anxiety; locomotor behavior.

1. INTRODUCTION

Antiretroviral drugs have been instrumental in the reduction of mortality and morbidity associated with the Human Immunodeficiency virus (HIV) infection. These drugs are of various classes – nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors and fusion inhibitors. These drugs interfere with specific steps in the HIV replication cycle. The reverse transcriptase convert viral RNA into pro-viral DNA before its incorporation into the host cell chromosome. The nucleoside reverse transcriptase inhibitors prevent this step by acting as substrate for reverse transcriptase. However they have to be phosphorylated by host cell enzymes in the cytoplasm [1]. These include Lamivudine and Zidovudine. The non-nucleoside reverse transcriptase inhibitors block reverse transcriptase activity by binding adjacent to the enzyme active site, inducing conformational changes in this site. Unlike nucleoside analogues these do not undergo phosphorylation. Efavirenz belongs to that group. When several of these drugs are taken in combination, the combination is known as Highly Active Antiretroviral therapy (HAART). The combination usually comprises two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor or protease inhibitor. One of the common combinations recommended is Zidovudine + Lamivudine + Efavirenz. Due to the current practice of combination therapy, there is a large potential for drug interaction and subsequent toxicity [2].

Antiretroviral drugs have the capacity to induce psychiatric disorders [2,3]. It has been estimated that nearly half the patients receiving treatment for HIV have psychiatric disorders and mood disorders, with depression having an overall prevalence of 20-30% [4]. The mechanism by which these antiretroviral drugs induce neuropsychiatric disturbances is poorly understood.

This research set out to observe the effect of chronic administration of some antiretroviral drugs (Zidovudine, Lamivudine, and Efavirenz) individually and their combined therapy on locomotor/exploratory behaviour, fear and anxiety using the open field apparatus, elevated plus maze, and the light/dark transition box.

The open field test was introduced in the early 1900s. It is used to measure locomotion, exploration and anxiety. This experimental protocol attained popularity due to its simplicity, ease of quantification and wide applicability [5]. The Light/dark transition box, on the other hand is a test of unconditioned anxiety and exploratory behaviour. It is based on the natural aversion of rodents to bright light in novel environments [6]. The elevated plus maze exploits the conflict between the natural tendency of mice to explore novel areas and fear of open spaces. The apparatus consists of two “open” arms and two “closed” arms in the shape of a +. The open

arms are aversive to mice because they are open and the maze is elevated [7]. The closed arms provide a sense of safety because they are enclosed.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Adult Swiss albino mice (18-32 g) obtained from the Department of Physiology, University of Calabar were used for the study. The mice were housed singly at room temperature of 25°C and kept in a reversed 12 hour light/dark cycle. The mice had free access to rodent chow and clean tap water. The animals were allowed one week to acclimatize before drug administration.

2.2 Drug Preparation and Treatment

Lamivudine, Zidovudine and Efavirenz tablets were each dissolved in 0.9% saline to form 150 mg/20 ml of Lamivudine, 300 mg/20 ml of Zidovudine, and 600 mg/20 ml of Efavirenz solution which were administered orally at doses of 2 mg/kg, 4 mg/kg and 8 mg/kg at volumes not exceeding 10 ml/kg body weight (i.e. 0.1 ml/10 g). The dose and frequency of administration were same as in humans.

2.3 Determination of Anxiety and Exploratory Behavior

This was done using the open field apparatus, light/dark transition box and the elevated plus maze.

2.3.1 The open field

The open field test was used to measure locomotion, exploration and anxiety (Walsh & Cummins, 1976). The open field apparatus was designed by Calvin Hall [8,9]. However the method has seen a couple of reviews since its birth [5,10]. The open field apparatus was constructed of white plywood and measured 72 x 72 cm with 36 cm walls. One of the walls was clear Plexiglas, so mice could be visible in the apparatus. Black lines were drawn on the floor with a marker and were visible through the clear Plexiglas floor. The lines divided the floor into sixteen 18 x 18 cm squares. A central square (18 cm x 18 cm) was drawn in the middle of the open field [11].

2.3.2 The light/dark box

The Light/dark transition box is used to test unconditioned anxiety and exploratory behaviour. It is based on the natural aversion of rodents to bright light in novel environments [6]. The experimental model for light/dark box was initially described by Crawley & Goodwin in 1980 [12]. However, many authors have used it with several structural modifications [13-18]. The light/dark box we used (45 x 27 x 27 cm) is made of plywood and consists of two compartments of unequal size as described by Costall et al. [19]. 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 centre 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 centre of the white compartment provides bright illumination of white light. The apparatus is located in a 2 x 5 m laboratory room. The light/dark transition test is limited by its ability to yield false-positive results. As with many experimental protocols, drugs that affect general motor function will affect light/dark performance. Preliminary screening of locomotor activity (such as an open field) appears to be necessary and sufficient for eliminating false-positive result [6].

2.3.3 The elevated plus maze

The Elevated Plus maze was built according to the description of Lister [20]. The maze has two open arms (30 x 5 cm) and two closed arms (30 x 5 x 15 cm high walls) radiating from a central square (5 x 5 cm). The floor of the maze is made of black (or gray) Plexiglas and the walls of clear Plexiglas. The open arms contain a slight ledge (4 mm high) to prevent the mice from slipping and falling off the edge [21]. The apparatus is located in a laboratory room (2 x 5 m) and lit by a 60-watt red lamp for background lighting. To eliminate any lingering olfactory cues, the apparatus is cleaned between each mouse using 70% ethyl alcohol. Tests are videotaped for further analysis.

2.4 Statistical Analysis

Values for the results were expressed as mean ± SEM. The statistical analyses were done using the analysis of variance (ANOVA) and the post/hoc Newmann Keul's test. The computer softwares used were Microsoft excel 2007 edition and SPSS 10.0 for windows. Differences

between means was considered significant at P= 0.05.

3. RESULTS AND DISCUSSION

The frequency of line crossing in the open field is shown in Fig. 1. The frequency of line cross for the control group of mice was 70.5 ± 13.14. The frequency of line cross following administration with Lamivudine (2 mg/kg), Zidovudine (4 mg/kg) and Efavirenz (8 mg/kg) was 34.1 ± 8.89, 31.7 ± 6.63 and 30.4 ± 10.67 respectively and these were significantly lower when compared to control ($p<0.05$). The frequency of line cross for the combined treatment of all the antiretroviral drugs was 57.0 ± 5.40 and this was also significantly lower compared to control ($p<0.05$).

Rearing frequency is shown in Fig. 2. The frequency of rearing for the control group of mice was 6.1 ± 0.80. The frequencies of rearing following administration with Lamivudine (2 mg/kg), Zidovudine (4 mg/kg) and Efavirenz (8 mg/kg) were 1.6 ± 0.70, 1.7 ± 0.56 and 2.2 ± 0.68 respectively and these were lower when compared to control ($p<0.01$). The frequency of rearing for the combined treatment of all the antiretroviral drugs was 2.2 ± 0.86 and this was also lower compared to control.

The frequencies of centre square entry following administration with Lamivudine (2 mg/kg), Zidovudine (4 mg/kg) and Efavirenz (8 mg/kg) is shown in Fig. 3. The values were 0.2 ± 0.13, 0.3 ± 0.21 and 0.1 ± 0.10 respectively and these were lower when compared to control (4.3 ± 1.32; $p<0.01$). The frequency of centre square entry for the combined treatment of all the antiretroviral drugs was 2.4 ± 0.81 and this was also lower compared to control ($p<0.01$) but higher compared to Lamivudine, Zidovudine and Efavirenz ($p<0.05$).

The frequency of open arms entry in the elevated plus maze test (as shown in Fig. 4) was not significant in Lamivudine (1.30 ± 0.26) and Zidovudine (1.50 ± 0.27) treated mice compared with control mice (1.50 ± 0.27), but was significantly lower (0.40 ± 0.22, $P<0.01$) in Efavirenz treated mice and higher in the combined treatment group (7.40 ± 1.80, $P<0.001$) compared with control.

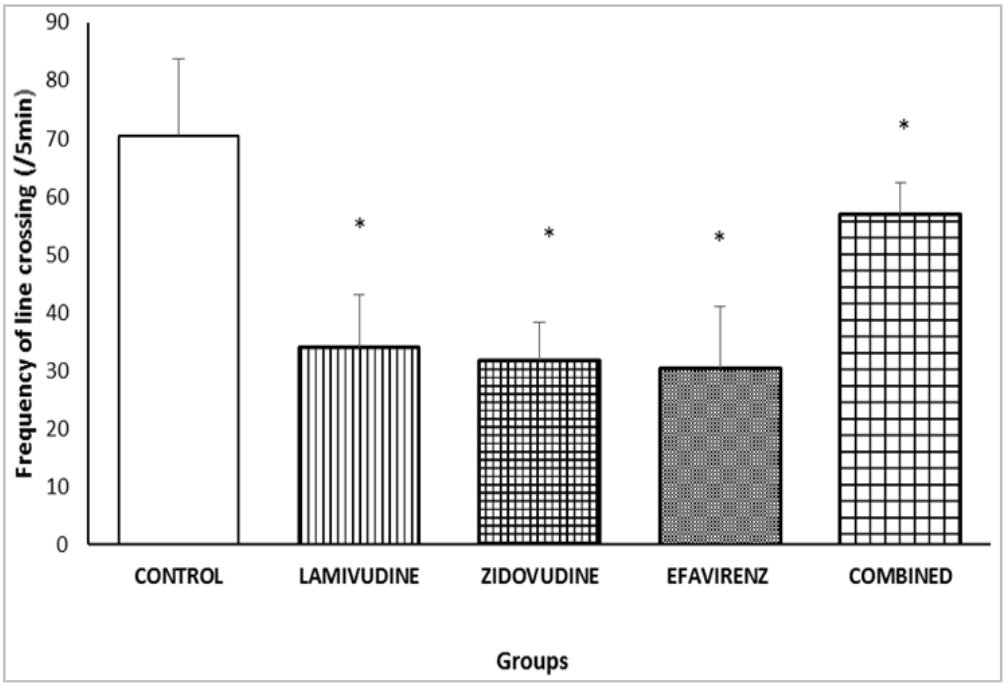

Fig. 1. Comparison of the frequency of line crossing in the open field in mice treated with the anti-retroviral drugs lamivudine (2 mg/kg), zidovudine (4 mg/kg), efavirenz (8 mg/kg) and combined treatment

*n=10 *-p<0.05.vs. Control*

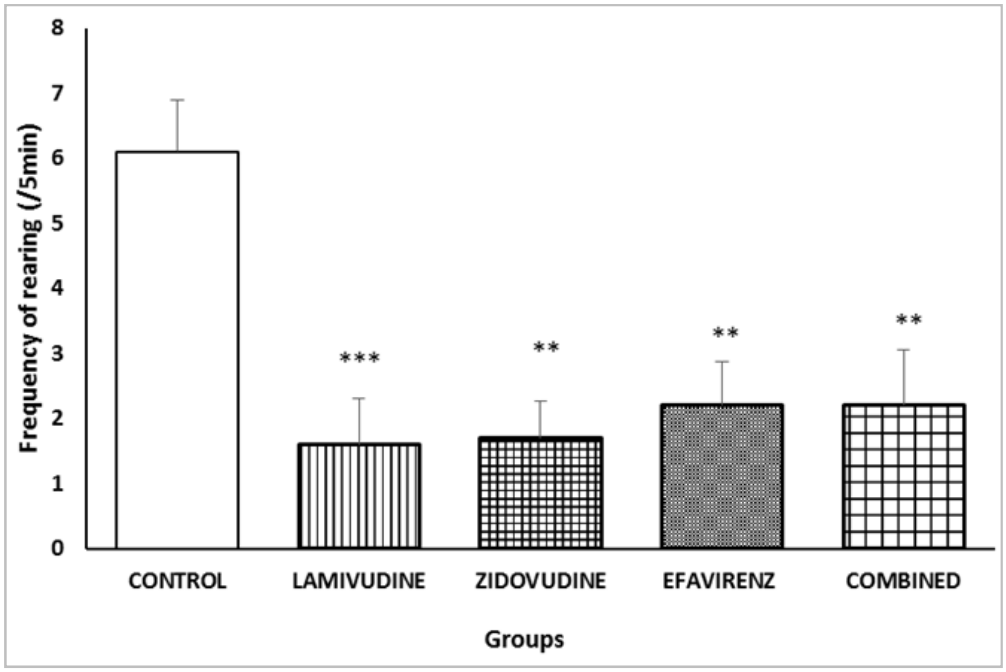

Fig. 2. Comparison of the frequency of rearing in the open field in mice treated with the anti-retroviral drugs Lamivudine (2 mg/kg), Zidovudine (4 mg/kg), Efavirenz (8 mg/kg) and combined treatment

*n=10 **-p<0.01. ***-p<0.001*

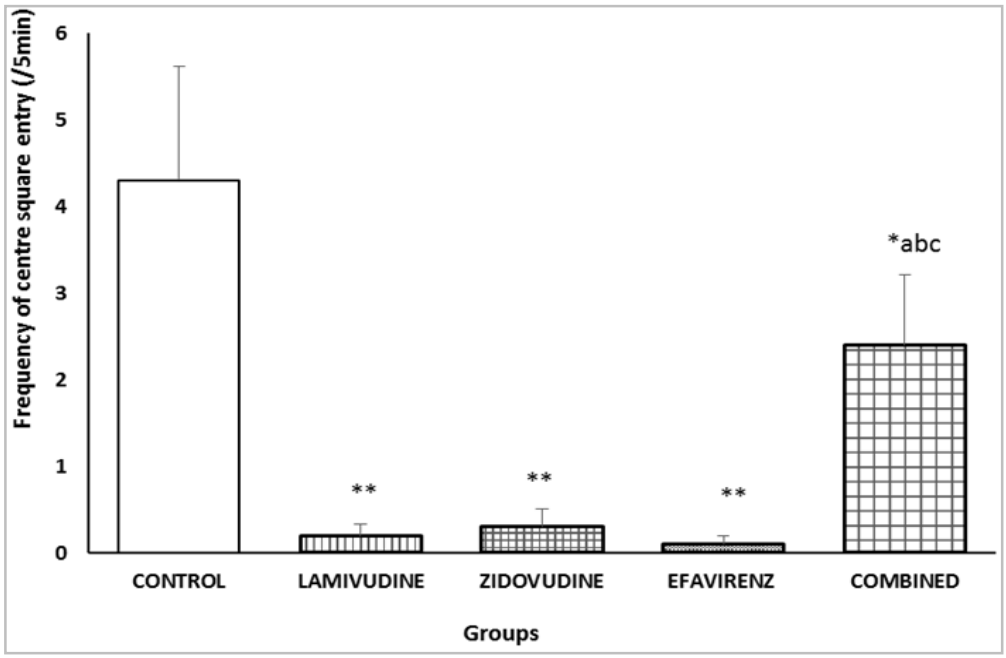

Fig. 3. Comparison of the frequency of open field centre square entry in mice treated with the anti-retroviral drugs lamivudine (2 mg/kg), zidovudine (4 mg/kg), efavirenz (8 mg/kg) and combined treatment

*n=10 *-P<0.05, **-P<0.01 vs control; a – P<0.05 vs lamivudine*

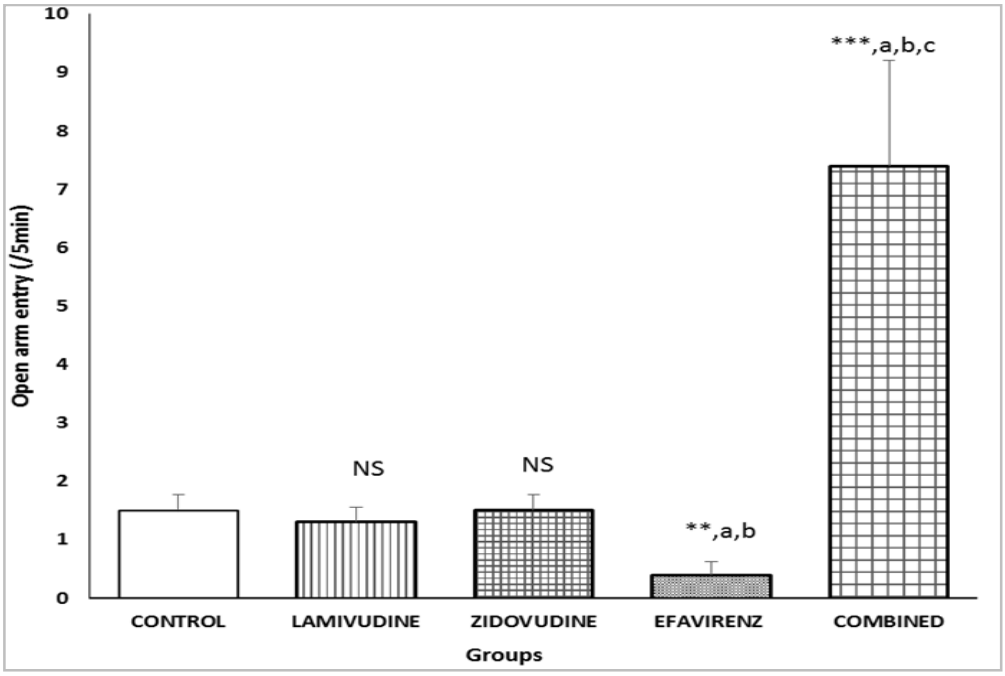

Fig. 4. Comparison of the frequency of open arms entry in the elevated plus maze test in mice treated with the anti-retroviral drugs lamivudine (2 mg/kg), zidovudine (4 mg/kg), efavirenz (8 mg/kg) and combined treatment

*n=10 NS = not significant, *-P<0.05, **-P<0.01 vs control; a – P<0.05 vs lamivudine, b-P<0.05 vs Zidovudine, c-P<0.05 vs Efavirenz*

The open arm duration for the lamivudine (2 mg/kg), zidovudine (4 mg/kg) and efavirenz (8 mg/kg) treated groups of mice were 13.62 ± 5.28, 14.72 ± 2.869 and 3.75 ± 1.34 respectively. These were significantly lower compared to control, 28.66 ± 5.28 ($p<0.05$). The open arms duration for the combined treatment of all the three antiretroviral drugs, 15.8 ± 4.54 did not differ from control and the single treatments. This is shown in Fig. 5.

Fig. 6 shows the comparison of the frequency of head dips in the elevated plus maze test in mice treated with the anti-retroviral drugs Lamivudine (2 mg/kg), Zidovudine (4 mg/kg), Efavirenz (8 mg/kg) and combined treatment groups. The frequency of head dips in the elevated plus maze test in the control, lamivudine, Zidovudine, Efavirenz and combined treatment groups were 4.50 ± 1.15, 5.2 ± 1.59, 5.3 ± 1.89, 2.2 ± 0.53 and 13.2 ± 3.12 respectively. No significant differences were observed between Lamivudine and Zidovudine treated mice when compared with control, but it was significantly lower ($P<0.05$) in Efavirenz treated mice and higher ($P<0.01$) in combined treated group compared with control.

Treatment with the anti-retroviral drugs lamivudine (2 mg/kg), zidovudine (4 mg/kg), efavirenz (8 mg/kg) and combined treatment significantly ($P<0.05$) altered the duration in the light chamber of light/dark transition box in mice (shown in Fig. 7). In the controls, the duration in the light chamber was 116.23 ± 26.52, while in lamivudine, zidovudine, efavirenz and combined treatment groups it was 33.39 ± 6.14, 65.61 ± 11.45, 3706 ± 9.87 and 95.26 ± 26.29 respectively.

The comparison of the duration of grooming in the light/dark transition box test in mice treated with the anti-retroviral drugs lamivudine (2 mg/kg), zidovudine (4 mg/kg), efavirenz, (8 mg/kg) and combined treatment group is shown in Fig. 8. The duration of grooming in the light/dark transition box in lamivudine (27.81 ± 8.36) and zidovudine (11.99 ± 3.43) treated mice was not significantly different from control mice (14.85 ± 3.61) but it was significantly higher in efavirenz (30.45 ± 2.91, $P<0.01$) and combined treatment group (95.16 ± 17.56, $P<0.001$) compared with control.

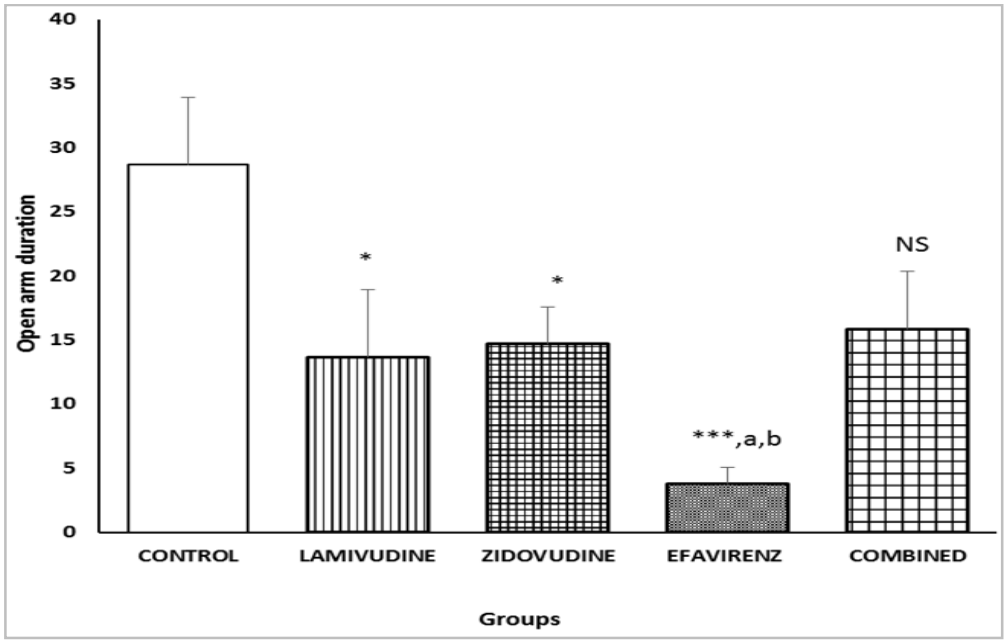

Fig. 5. Comparison of the duration in open arms in the elevated plus maze test in mice treated with the anti-retroviral drugs lamivudine (2 mg/kg), zidovudine (4 mg/kg), efavirenz (8 mg/kg) and combined treatment

*n=10 NS = not significant, *P< 0.05 vs control, **-P<0.01 vs control; ***-P<0.001 vs control, a – P<0.05 vs lamivudine, b-P<0.05 vs Zidovudine*

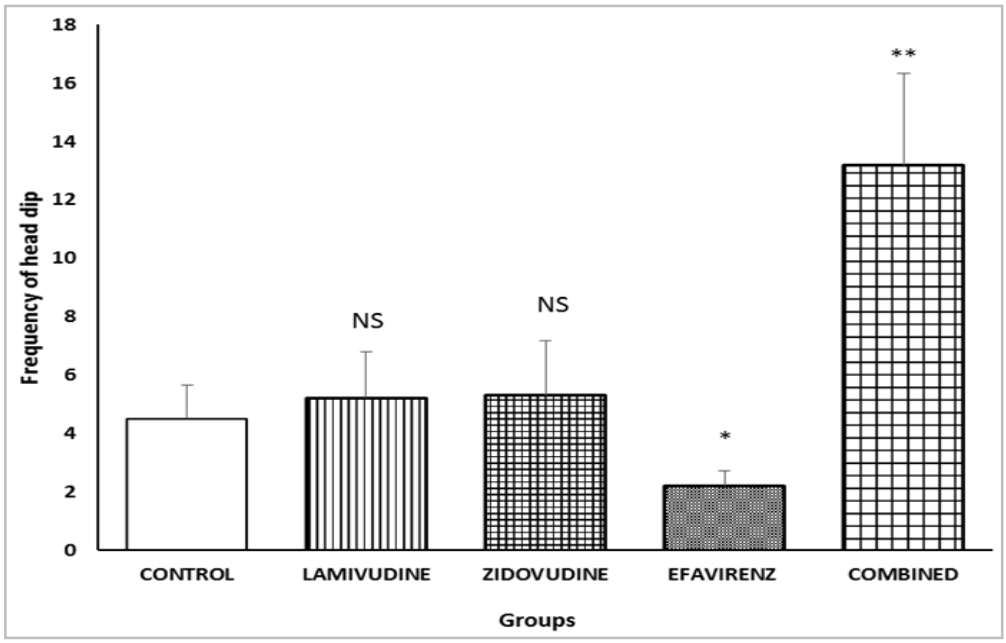

Fig. 6. Comparison of the frequency of head dips in the elevated plus maze test in mice treated with the anti-retroviral drugs lamivudine (2 mg/kg), Zidovudine (4 mg/kg), Efavirenz (8 mg/kg) and combined treatment

*n=10 *-p<0.05 vs control;**P<0.01 vs control*

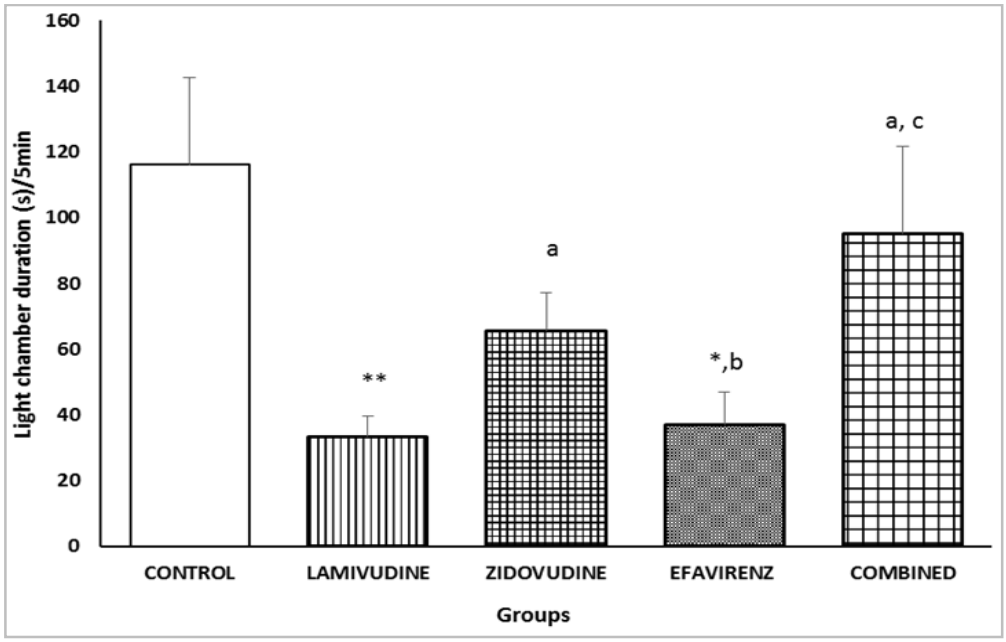

Fig. 7. Comparison of the duration in the light chamber of light/dark transition box in mice treated with the anti-retroviral drugs lamivudine (2 mg/kg), Zidovudine (4 mg/kg), Efavirenz (8 mg/kg) and combined treatment

*n=10 *p<0.05, **P<0.01 vs control; a – P<0.05 vs lamivudine, b-P<0.05 vs Zidovudine, c-P<0.05 vs Efavirenz*

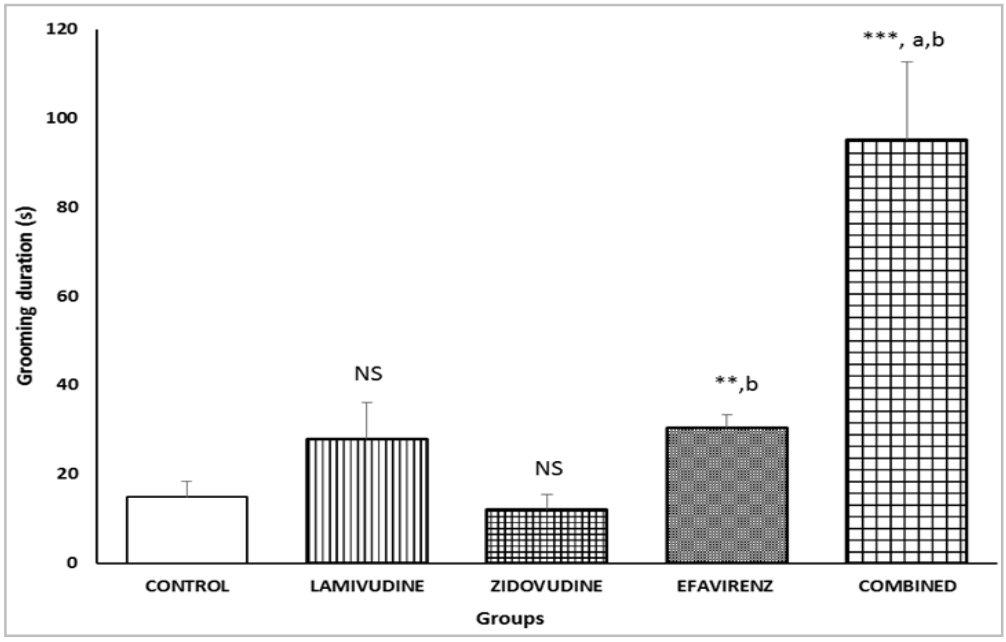

Fig. 8. Comparison of the duration of grooming in the light/dark transition box test in mice treated with the anti-retroviral drugs Lamivudine (2 mg/kg), Zidovudine (4 mg/kg), Efavirenz (8 mg/kg) and combined treatment

*n=10, NS – not significant. **P<0.01, ***P<0.001 vs control; a – P<0.05 vs lamivudine, b-P<0.05 vs Zidovudine, c-P<0.05 vs Efavirenz*

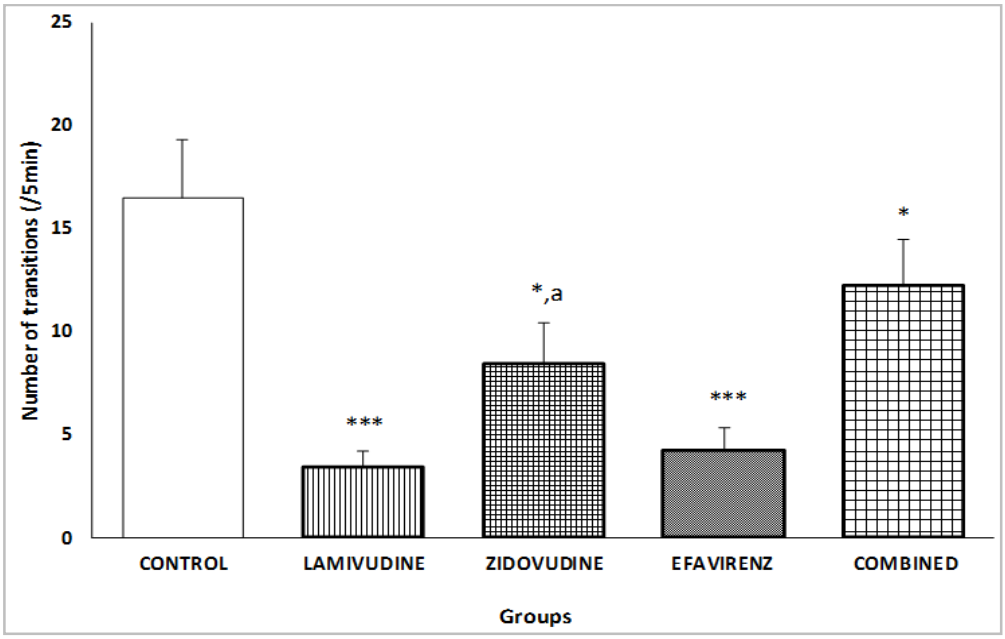

Fig. 9. Comparison of the frequency of light/dark transitions in the light/dark transition box test in mice treated with the anti-retroviral drugs lamivudine (2 mg/kg), zidovudine (4 mg/kg), efavirenz (8 mg/kg) and combined treatment

*n=10 *P<0.05, **P<0.01, ***P<0.001 vs control; a – P<0.05 vs lamivudine*

Fig. 9 illustrates the comparison of the frequency of light/dark transitions in the light/dark transition box test in mice treated with the anti-retroviral drugs Lamivudine (2 mg/kg), Zidovudine (4 mg/kg), Efavirenz (8 mg/kg) and combined treatment. The frequency of light/dark transitions was significantly lower (P<0.001) in Lamivudine (3.40 ± 0.76) and Efavirenz (4.20 ± 1.10) treated mice compared with control (16.50 ± 2.80) and higher (P<0.05) in Zidovudine (8.40 ± 2.01) and combined treatment groups (12.20 ± 2.24) than in control.

In the open field test, the frequency of line crossing, rearing frequency, and centre square entry are significantly lower in the antiretroviral treated groups compared to the control (Figs.1-3). In addition, the centre square entry is significantly higher in the combined treatment group compared to the individual treatment groups. Line crossing, rearing frequency and centre square entry are indices of exploratory behaviour in mice [5]. A reduction in these indices indicates that the antiretroviral drugs, Lamivudine, Zidovudine and Efavirenz reduce exploratory behaviour. This would mean that the drugs have anxiogenic effects. A significantly higher centre square entry in the combined treatment group compared to the individual treatment groups indicates that there might be a higher anxiogenic effect in the individual treated groups than in the combined treatment group.

In the elevated plus maze (Figs. 4-6), the frequency of open arm entry is significantly lower in the Efavirenz treated group compared to control. It is also significantly higher in the combined treatment group compared to all three individual treatment groups. The open arm duration is significantly lower in the three individual treatment groups compared to control. However, it did not differ significantly in the combined treatment group compared to control. It is significantly lower in the Efavirenz treated group compared to the lamivudine and Zidovudine treated groups. Behaviours such as open arm activity and head dipping are considered exploratory, and a greater frequency of these measures shows a greater level of exploration [11]. A reduced open arm entry and duration in the treated groups indicates that the antiretroviral therapy has anxiogenic effects. The higher open arm entry in the combined treatment is in contrast with the trend in the open arm duration where there is consistency in the reduction in the individual treated groups. The open arm duration is not significantly different in the combined treatment group compared to

control. The frequency of head dip did not differ significantly in the lamivudine and Zidovudine treated groups compared to control. It is significantly lower in the Efavirenz treated group compared to control. However it is significantly higher in the combined treatment group compared to control. The results in the elevated plus maze indicates that antiretroviral therapy has anxiogenic effects.

In the light/dark transition box (Figs.7-9), it was observed that the duration in the light chamber was significantly lower in the lamivudine and Efavirenz treated groups compared to control. The duration in the Zidovudine and combined treatment groups, though lowered, was not significantly different. The light chamber is aversive to mice [6]. A decrease in the light chamber duration in the treated groups indicates that antiretroviral therapy may have caused anxiety in the treated rodents. The same trend is observed in the grooming duration and frequency of transitions. There was an increase in grooming duration in the Efavirenz and the combined treatment groups. An increase in grooming duration indicates an increase in anxiety. There was a consistent decrease in transitions in all the treatment groups compared to control. An increase transitions between the light and dark chambers is associated with non-anxious behaviour [22,6,23]. The reverse was observed in this study, indicating an anxiogenic effect of antiretroviral therapy in the mice.

4. CONCLUSION

Taken together, our findings indicate an anxiogenic effect of antiretroviral therapy. However our results do not show a conclusive evidence that combined antiretroviral therapy has a greater anxiogenic effect on the subjects than single antiretroviral therapy.

CONSENT

It is not applicable

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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