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Locomotor Behaviour and Anxiety in the Open Field and Light/Dark Box in CD1 Mice Treated with Aspirin, Cataflam and Ethanolic Extract of *Cannabis sativa*

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Authors' contributions

This work was carried out in collaboration between all authors. Author CON designed the study and author IOA managed the literature searches and wrote the first draft of the manuscript. Both authors IOA and GOO carried out the behavioural experiments. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the effects of cataflam, aspirin and the ethanolic extract of *Cannabis sativa* on nociception in CD1 albino mice of both sexes.
Methods: Twenty (20) albino mice were divided into four (4) groups of five (5) each. The control group (group 1) received normal saline orally. Meanwhile groups 2-4 received p.o. cataflam (1.5 mg/kg), aspirin (13.5mg/kg) and *Cannabis sativa* (10 mg/kg) respectively. All four (4) groups were given access to normal mice chow and water *ad libitum*. The Open field apparatus and the light/dark box were used to measure locomotor/exploratory behaviour and anxiety.
Results: There was a significant (p=0.05) reduction in the frequencies of rearing, walling and line crossing in the Open field test and a reduction in frequency of transition, rearing and line crossing in the light/dark box. There was no significant difference in the chamber durations in the light/dark box test.
Conclusion: Cataflam, Aspirin and the ethanolic extract of *Cannabis sativa* all have anxiogenic

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effect and reduced locomotor behaviour. The ethanolic extract of *Cannabis sativa* seems to have the greatest anxiogenic effect, followed closely by Cataflam and Aspirin.

Keywords: Open field; light/dark box; aspirin; cannabis; cataflam; anxiety; locomotor behaviour.

1. INTRODUCTION

The open field test, introduced in the early 1900s, is used to measure locomotion, exploration and anxiety. This experimental protocol attained popularity due to its simplicity, ease of quantification and wide applicability [1]. 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 [2].

Cannabis sativa, more commonly known as marijuana or Indian hemp is a plant that grows freely throughout the world. Medically, Indian hemp has been used in the treatment of diseases and health problems such as neuropathic pain associated with HIV/AIDS, glaucoma, eye problems, cachexia, muscle spasticity, convulsion, insomnia, asthma, hypertension and depression [3]. The constituent of marijuana that has been found to be responsible for its pharmacologic property is Δ^9-tetrahydrocannabinol (THC) [4]. In humans, THC produces psychological effects that include euphoria, sedation, altered sensory inputs, distortion of time perception and impaired cognitive function [5]. In laboratory animals, THC produces sedation, analgesia, hypothermia, catalepsy, and motor incoordination [6].

Autoradiographic studies show cannabinoid receptors to be present in high quantities in the central nervous system. The highest levels of cannabinoid receptors are found in brain structures that are associated with neurophysiologic functions altered by cannabinoids [7]. The densest binding occurs in the basal ganglia (substantia nigra pars reticularis, globus pallidus, entopeduncular nucleus, and lateral caudate putamen) and the molecular layer of the cerebellum. Intermediate levels of receptor binding were found in the pyramidal cell layers of the hippocampus, the dentate gyrus, and layers I and VI of the cortex. The presence of cannabinoid receptors in these regions is expected, given the effects of cannabinoids on cognitive processes. The hippocampus stores memory and codes sensory information. The presence of cannabinoid receptors in regions associated with mediating brain reward (ventromedial striatum and nucleus accumbens) is consistent with the role that cannabinoids play in the neurobiology of reward. Lower levels are found in the brain stem, hypothalamus, corpus callosum, and deep nuclei of the cerebellum [8,9].

Cataflam (diclofenac potassium) is a non-steroidal anti-inflammatory drug (NSAID). The mechanism by which Cataflam relieves pain is by reducing substances in the body that cause pain and inflammation. Cataflam is used to treat pain and to treat the signs and symptoms of rheumatoid arthritis and osteoarthritis. It is also used to treat cramping pain in the lower abdomen associated with menstruation [10].

Aspirin (acetylsalicylic acid) is also a non-steroidal anti-inflammatory drug (NSAID) that is used as a remedy for pain. The aspirin molecule transfers an acetyl group onto cyclooxygenase enzyme [11]. This inhibits, irreversibly, the enzyme. Thus making it unable to bind arachidonic acid; therefore, the enzyme can no longer convert arachidonic acid to prostaglandins (which mediates inflammation and sensitizes pain receptors) and thromboxane. The blockage of cyclooxygenase has many results including failure in platelet aggregation. The decrease in prostaglandin production causes a reduction in inflammation and oedema [12]. The inhibition of prostaglandin and thromboxane synthesis may not be the only effect that aspirin exerts upon the tissues [13].

Recent research indicates that 'over the counter' analgesics may alleviate depression and anxiety [14]. Our prior research also shows that *Cannabis sativa* has analgesic property [15]. This research is aimed at investigating the effects of Cataflam and Aspirin on locomotor behaviour and anxiety using the open field maze and the light/dark transition box, and comparing these with any observed effects of *Cannabis sativa* on anxiety and locomotion.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Swiss white albino mice (male & female) were obtained from the animal house of the Department of Physiology, University of Calabar and housed in the Neurobehaviour laboratory of the College of Medical Sciences. They were kept at room temperature with alternating 12-hour dark/light cycle. The animals were given free access to food and water. The animals were allowed one week to acclimatize before commencement of extract administration.

2.2 Plant Materials and Extract Preparation

Fresh leaves of *Cannabis sativa* were obtained from Obubra local government area of Cross River state. The leaves were washed to remove debris and oven dried at room temperature. The dried leaves were then grinded to fine powder using a manual blender. The powdered leaves was macerated (cold extraction) in an extraction jar using ethanol for 72 hours. The crude extract was then filtered using Whatmann's filter 1. The filtrate was evaporated in an oven at 40ºC. The pasty concentrate was stored in a refrigerator until required for use.

2.3 Drug Treatment

Cataflam and Aspirin were purchased from Bez Pharmacy, Calabar and administered (p.o.) at the dosage of 1.5 mg/kg and 13.5 mg/kg [16,17]. The ethanolic extract of *Cannabis sativa* was administered at a dose of 10 mg/kg [18].

2.4 Determination of Anxiety and Exploratory Behaviour

The open field test was used to measure locomotion, exploration and anxiety. The Light/dark transition box was also used to test unconditioned anxiety and exploratory behaviour.

2.4.1 The open field

The open field test was used to measure locomotion, exploration and anxiety [1]. The originator of the open field is Calvin Hall [19,20]. However the method has seen a couple of reviews since its birth [21,1]. 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 [22].

2.4.2 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 [2]. The experimental model for light/dark box was initially described by Crawley & Goodwin in 1980 [23]. However, many authors have used it with several structural modifications [24-29]. 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. [30]. 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 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 [2].

2.5 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

Fig. 1 shows the frequency of line crosses in open field test. The frequency of line crosses was significantly lower (p=0.05) in the groups treated with Aspirin (95.2±16.578), Cataflam (70.574±28.377) and Cannabis (67.4±9.963) compared to control (149±21.517).

Fig. 2 shows the rearing frequency in the open field test. Rearing frequency is significantly lower (p=0.05) in the three test groups [Aspirin (11.8±1.934), Cataflam (7.591±3.234) & Cannabis (6.6±0.927)] compared to the control (15.6±1.030). Rearing frequency is also significantly lower (p=0.05) in the Cannabis and Cataflam treated groups compared to the Aspirin treated group.

Fig. 3 shows the walling frequency in the open field test. The frequency of walling is significantly (p=0.05) lower in the groups treated with cataflam (22.629±8.061) and Cannabis (20±2.449) compared to control (44.2±7.372). Walling frequency, though reduced in the group treated with Aspirin (30.8±8.061), is not significant compared to control.

Fig. 4 shows the centre square frequency in the open field test. The centre square frequency is significantly (p=0.05) higher in the group treated with Cannabis (7±0.950) compared to both control (3.8±0.374) and the Cataflam (2.517±1.001) treated groups. However there was no significant difference in the groups treated with Aspirin (5±0.894) and Cataflam compared to control.

Fig. 5 shows the centre square duration in the open field test. There is no significant (p=0.05) difference in centre square duration in all three test groups [Aspirin (5.522±2.095), Cataflam (4.167±1.073) and Cannabis (9.1±2.293)] compared to control (6.86±2.191).

Fig. 6 shows the light chamber duration in the light-dark box. There was no significant (p<0.05) difference between the three test groups [Aspirin (145.34±22.306), Cataflam (106.28±12.479) & Cannabis (142.4±46.470)] and control (140.3±30.583).

Fig. 7 shows the dark chamber duration in the light-dark box. There was no significant (p=0.05) difference in the test groups [Aspirin (181.98±36.949), Cataflam (193.54±12.490) & Cannabis (159.6±45.098)] compared tocontrol (153.22±37.896).

Fig. 8 shows the transition frequency in the light-dark box. Transition frequency was significantly (p=0.05) lower in all three test groups compared to control (11±0.707). It was also significantly lower in the Cataflam (5±0.707) and Cannabis (3.6±0.678) treated group compared to the Aspirin (8±0.949) treated group.

Fig. 9 shows frequency of line crosses in the light-dark box. There was a significantly (p=0.05) lower frequency of line crossing in the Cataflam (65.2±6.851) and Cannabis (64±10.895) treated group compared to control (92±9.803). However, there was no significant difference in the Aspirin (76.6±5.732) treated group compared tocontrol.

Fig. 10 shows the frequency of rearing in the light-dark box. There was a significantly (p=0.05) reduced rearing frequency in all three test groups [Aspirin (23.2±5.472), Cataflam (20.4±1.939) & Cannabis (14.6±3.776)] compared to control (39.8±5.783).

4. DISCUSSION

The results obtained from the Open field test show a significant (p=0.05) decrease in frequencies of rearing, line crosses and walling in the test groups compared to control (Figs. 1-3). A decrease in these parameters indicates low exploration and conversely increased anxiety in the test animals compared to control. The rearing frequency is also significantly (p=0.05) lower in the Cannabis and Cataflam treated groups compared to the Aspirin treated group (Fig. 2). This would indicate that Cannabis and Cataflam caused a more significant reduction in locomotor/exploratory behaviour than Aspirin. This result is consistent with the frequency of walling where there is a significant reduction in frequency of walling in the Cataflam and Cannabis treated group compared to control, but no significant difference in the Aspirin treated group compared to control (Fig. 3). Yakovchyuk et al. [31] found that Aspirin at high doses significantly raised locomotor and exploratory behaviour in rats, which is evidence of its anxiolytic activity. They also observed that this anxiolytic effect was reversed at the upper limit of the therapeutic dose.

The centre square frequency, however, tells a different story. We find a significantly higher centre square frequency in the Cannabis treated

group compared to both control and the Cataflam treated groups (Fig. 4). There is no significant difference in the Cataflam and Aspirin treated groups compared to control. An increase in centre square frequency would indicate a high exploratory behaviour and less anxiety. However, this is inconclusive as there was no significant difference in the centre square duration between the test groups and the control (Fig. 5).

In the light/dark transition box test, there was no significant difference (p=0.05) in both the light chamber duration and the dark chamber duration in all the test groups (Aspirin, Cataflam & Cannabis) compared to control (Figs. 6 and 7). We also find a significant reduction in transition frequency in all three test groups compared to control (Fig. 8). An increase in transition frequency would mean a reduction in anxiety. However, our result shows a reduction in transition frequency. Therefore Aspirin, Cataflam and Cannabis decreased exploration and increased anxiety. There is a significantly reduced transition frequency in the Cannabis and Cataflam treated group compared to control. This is in agreement with the result in the frequency of rearing and walling in the Open field test (Figs. 2 and 3) which point to the fact that Cannabis and Cataflam may have a greater effect in reducing exploratory behaviour and increasing anxiety in mice than Aspirin. However, a study by Berrendero & Maldonado [32] has shown that the administration of a low dose of tetrahydrocannabinol (0.3 mg/kg) produces clear anxiolytic-like responses. Apparently, low doses of cannabinoid receptor (CB1) agonists tend to be anxiolytic and high doses tend to increase aversion and anxiety-related behaviours [33].

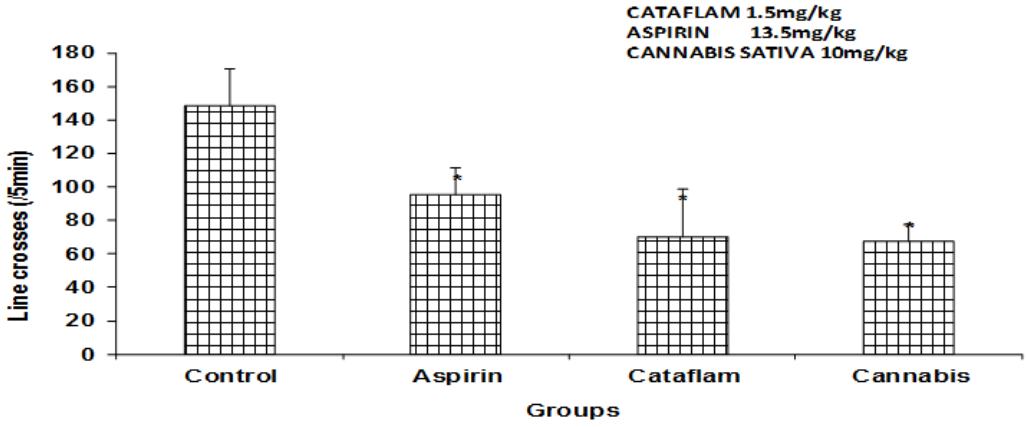

Fig. 1. Frequency of line crosses in control and test groups in the open field test
*Values are mean + SEM, n = 5; *p<0.05 vs control*

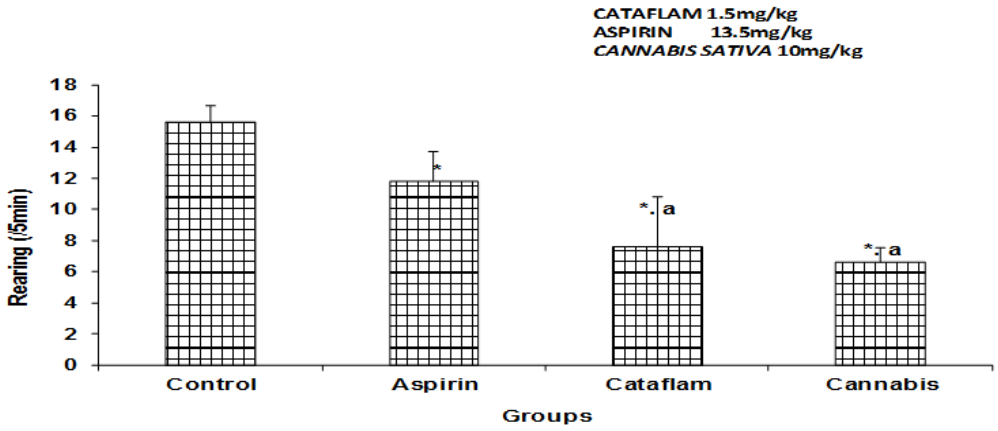

Fig. 2. Frequency of rearing in control and test groups in the open field test
*Values are mean + SEM, n = 5; *p<0.05 vs control; a = p<0.05 vs aspirin*

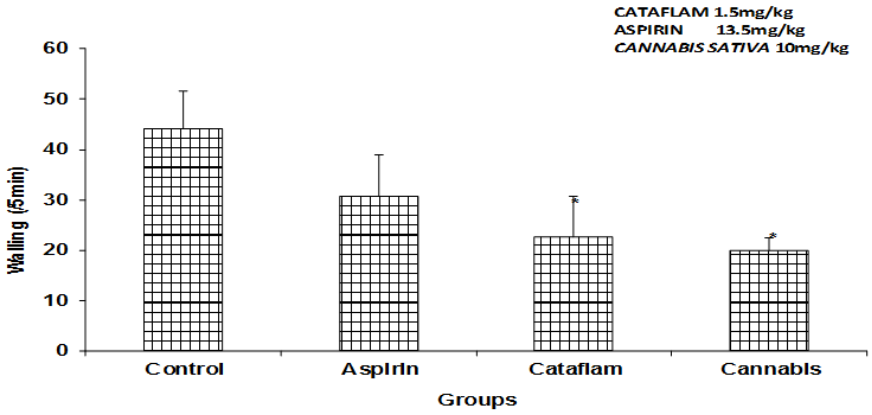

Fig. 3. Frequency of walling in control and test groups in the open field test
Values are mean + SEM, n = 5.
**p<0.05 vs control*

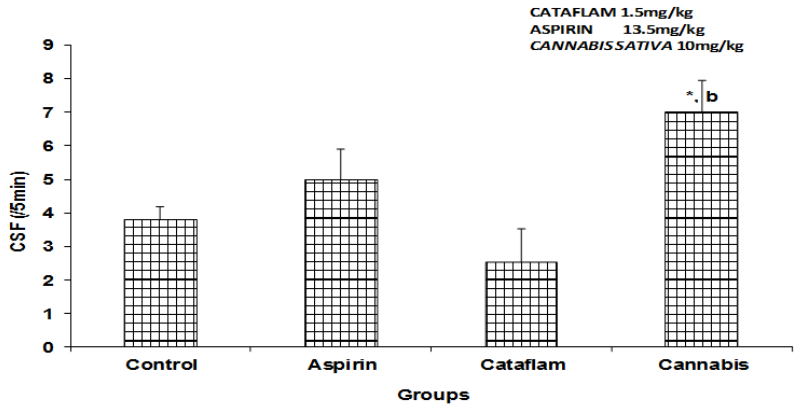

Fig. 4. Centre square frequency in control and test groups in the open field test
Values are mean + SEM, n = 5.
*p<0.05 vs control
b = p<0.05 vs cataflam

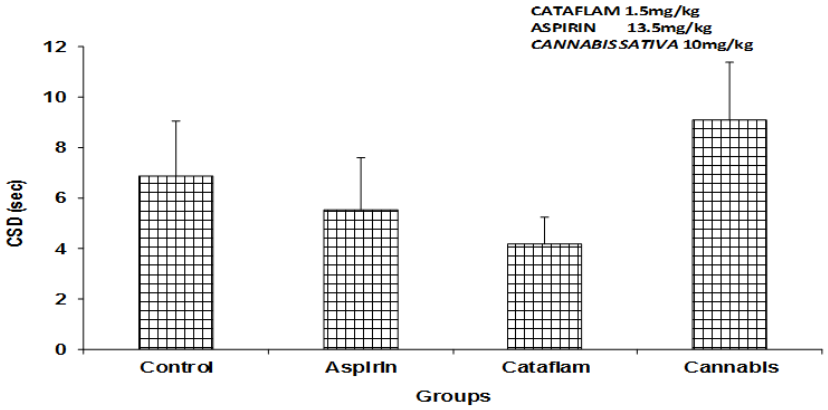

Fig. 5. Centre square duration in control and test groups in the open field test
Values are mean + SEM, n = 5

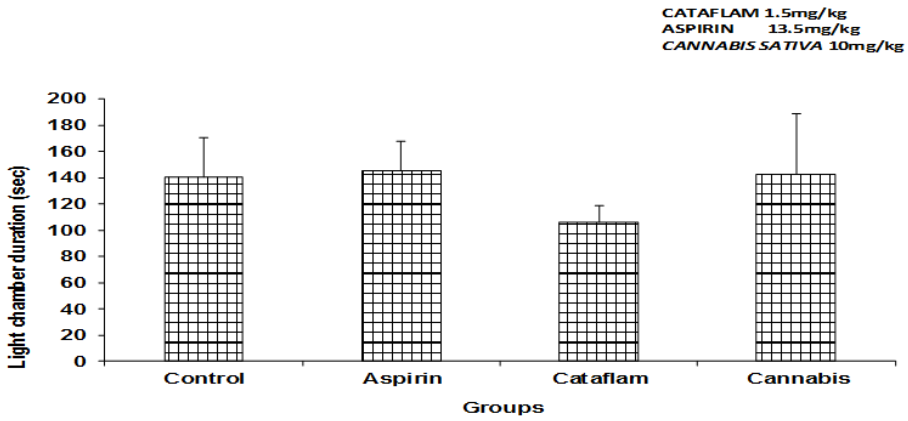

Fig. 6. Light chamber duration in the control and test groups during the light/dark transition box test

Values are mean + SEM, n = 5

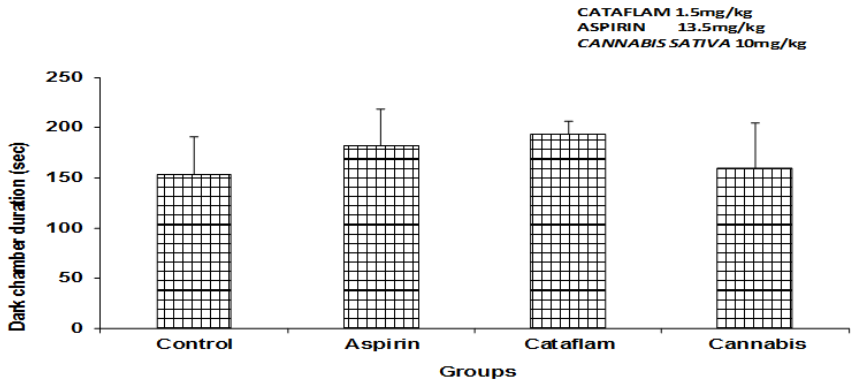

Fig.7. Dark Chamber duration in control and test groups during the light/dark transition box test

Values are mean + SEM, n = 5

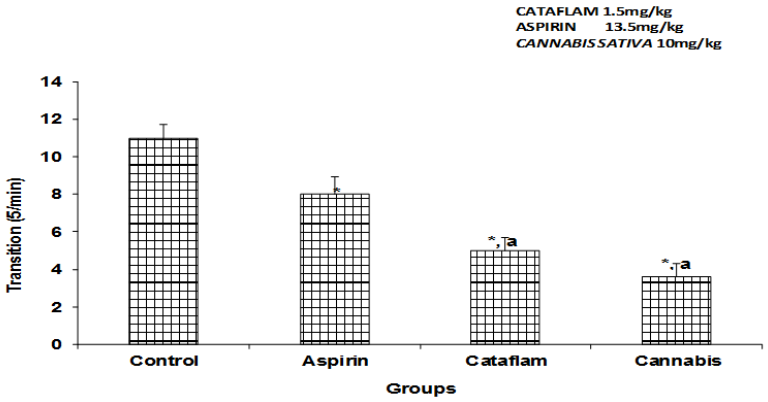

Fig.8. Transition frequency in the control and test groups during the light/dark transition box test

Values are mean + SEM, n = 5.
**p<0.05 vs control*
a = p<0.05 vs aspirin

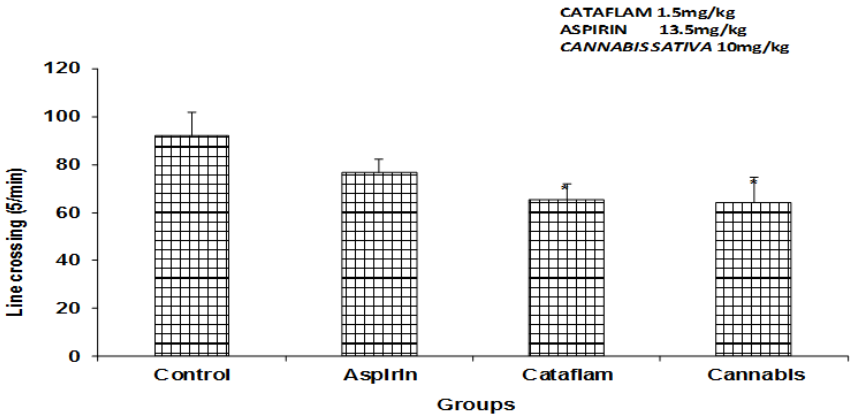

Fig. 9. Frequency of line crossing in control and test groups during the light/dark transition box test

Values are mean + SEM, n = 5.
**p<0.05 vs control*

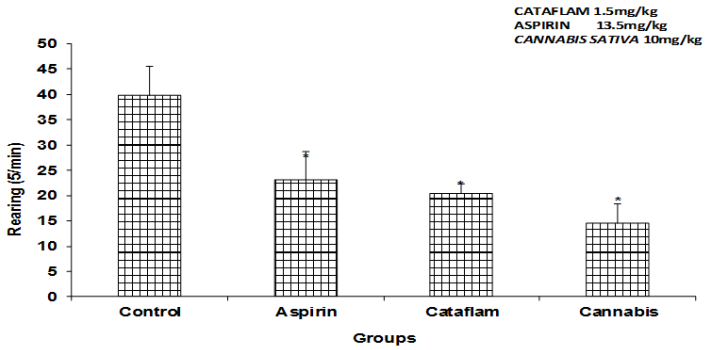

Fig. 10. Frequency of rearing in control and test groups during the light/dark transition box test

*Values are mean ± SEM, n = 5.*p<0.05 vs control*

There is a significantly lower frequency of line crosses in the Cataflam and Cannabis treated groups compared to control during the light dark/box test. There seemed to be some reduction in the Aspirin treated group too. However, this was not significant. This agrees (to a great extent) with the frequency of line crosses during the Open field test. There was a significantly reduced frequency of rearing in the three test groups compared to control during the light/dark box test. This too agrees with the rearing frequency in the Open field test.

Our results indicate that Aspirin, Cataflam and *Cannabis sativa* decrease exploratory/locomotor behaviour and increase anxiety in mice. A clinical trial by DeWall et al. [14] shows that 'over the counter' analgesics may alleviate depression and anxiety. Our results show quite the opposite in mice.

5. CONCLUSION

In conclusion, all three test substances reduced locomotor behaviour and increased anxiety. However the ethanolic extract of *Cannabis sativa* seems to have the greatest anxiogenic effect, followed closely by Cataflam and Aspirin.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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