

Long-Term Administration of *Cannabis sativa* on Locomotor and Exploratory Behavior in Mice

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Abstract The effect of long term administration of *Cannabis sativa* on locomotion and exploratory activity in mice was studied. A total of 27 albino mice (male and female) were used for the experiment. They were assigned randomly into three groups (control, low dose and high dose) containing nine (9) mice each for the tests on neurobehavior. Group I (the control group) was given 10ml/kg of normal saline. Group II (The low dose group) was administered 10mg/kg/day of *Cannabis sativa*; Group III (high dose group) was administered 20mg/kg/day *Cannabis sativa*. Oral route of administration was used for all groups for 28 days. All three groups were allowed free access to food and water. After 28 days of administration of *Cannabis sativa*, the experiments on neurobehavior were carried out. The open field maze was used to access locomotor/exploratory behavior, the elevated plus maze for fear and anxiety, the light/ dark box for anxiety, fear and exploratory behavior. Locomotor behavior differed in the three groups. The low dose-treated group had lower locomotion and exploratory activity with high anxiety compared to control, as seen in the line crosses, and rearing activities. This effect was observed to be more when high dose of *Cannabis sativa* was administered ($P < 0.001$). This trend was repeated when the animals were exposed to the elevated plus maze and light/dark box apparatus. In conclusion, long term administration of *Cannabis sativa* decreases locomotor and exploratory activity, and also increases fear and anxiety in mice.

Keywords Locomotor activity, Exploratory activity, *Cannabis sativa*, Mice, Open field maze, Light/dark box, Elevated plus maze

1. Introduction

1.1. Background of the Study

Pot, weed, grass, ganja and skunk, are some of the common words used to describe the dried leaves of the plant known as Cannabis. Cannabis is a genus of flowering plant that includes three putative species; *Cannabis sativa*, *Cannabis indica*, *Cannabis ruderalis* (Elsohly, 2007). Each specie has its own unique characteristics, though for the purpose of this research, we will be more concerned with the specie, *Cannabis sativa* due to its psychoactive effect as compared to others.

Cannabis sativa is an annual herbaceous plant in the Cannabaceae family. It has long been used for religious and medicinal purposes, and as a recreational drug (due to its "psychoactive", or mind-altering effects).

Some therapeutic uses of *Cannabis sativa* include; treatment of spasticity, movement disorders, asthma and glaucoma. It is also used in the treatment of allergies, inflammation and infection (Grotenhermen and Russo,

2002). Marijuana is used in the treatment of cancer (Elsohly et al, 1985), multiple sclerosis, menstrual pain and chronic pain (Noyes et al, 1975). It reduces nausea and is used as a muscle relaxant (Matsuda et al, 1990; Hollister, 1971).

Although it has positive effects, *Cannabis sativa* has been documented in several studies to have a link with symptoms of schizophrenia (Hall and Solowij, 1998).

Cannabis has also been known to increase focus and concentration. This may be the reason why students use it during their studies. It has also been said to increase one's level of excitement and sleep (Hollister, 1971).

More than 61 chemicals called cannabinoids have been identified as specific to the Cannabis plant. Its constituent, tetrahydrocannabinol (THC) is the main psychoactive cannabinoid, most responsible for the 'high' associated with marijuana use (Pertwee, 2006). The pharmacological actions of THC result from its partial agonist activity at the cannabinoid receptor CB1, located mainly in the central nervous system (Pertwee, 2006). The psychoactive effects of THC have been said to be primarily mediated by its activation of the CB1 G-protein coupled receptors which results in decrease of the second messenger molecules cAMP through inhibition of adenylate cyclase (Elphick and Egertova, 2001).

Research into the effects of *Cannabis sativa* on the different systems including the nervous system have been

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Published online at <http://journal.sapub.org/neuroscience>

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investigated but there is still paucity of information as regards its effect on neurobehavioral parameters such as locomotor/ exploratory behavior.

2. Materials and Methods

2.1. Preparation of *Cannabis sativa* Extract

The leaves of the plant were dried to evaporate its water content and blended using a manual blender into snuff-like particles, and its weight taken. A solution of 80% ethanol and 20% of distilled water was prepared. The particles were then added into the solution and kept overnight (18 hours). At the expiration of 18 hours, the mixture was filtered using Whatman's No. 1 filter paper into a conical flask. The filtrate obtained was dried using Astell Hearson oven at 45°C. After all the water content in the filtrate had evaporated, the extract was scrapped off and put into an air tight container. Its weight was determined and recorded. The National Drug and Law Enforcement Agency in Cross River State approved the carrying out of the experiment.

2.2. Animal Care

Twenty-seven adult albino mice were housed singly in metabolic cages under standard laboratory conditions in Physiology Department, University Of Calabar, Calabar with room temperature of $25 \pm 2^\circ\text{C}$, and where they could observe the dark/light cycle throughout the duration of the experiment. They were fed with normal rat chow and given water freely for one week to allow for acclimatization before the commencement of the experiment.

2.3. Animal Treatment

Twenty seven albino mice were randomly separated into 3 groups. Group I (the control group) was given 10ml/kg of normal saline. Group II (The low dose group) was administered 10mg/kg/day of *Cannabis sativa*; Group III (high dose group) was administered 20mg/kg/day *Cannabis sativa*. Oral route of administration using an oropharyngeal cannula inserted into a 1ml syringe with detachable needle was used. A small bead was attached to the end of the cannula to avoid injuring the animal's mouth during the process of administration. This was done for a period of 28 days.

Ethical approval

All authors hereby declare that "Principles of laboratory animal care" were followed. All experiments have been examined and approved by the appropriate ethics committee.

2.4. Open Field Maze (OFM)

The open field maze is a box measuring 72 x 72cm with 36cm high wall constructed with plywood located in a 2 x 5m (L x B) neurobehaviour laboratory with a 60-watt lamp for background lighting. The walls and floor are both painted

white. The floor is divided into sixteen 18 x18 cm squares with a black marker thereby forming lines. A central square of equal size is drawn in the middle of the open field (18 x18cm) and the floor is covered with a 72 x 72cm piece of clear Plexiglas.

2.4.1. Experimental Procedure in Open Field Maze

Mice were carried to the test room in their home cages and tested one at a time. Each mouse was exposed to the open field maze by scooping it from its home cage using a small plastic container and placing it at the centre square of the maze and allowed to explore the apparatus for 5minutes. The mouse behavior was scored and the mouse returned to its home cage. The open field was then cleaned with 70% ethyl alcohol and allowed to dry before the introduction of another mouse. This was to eliminate olfactory stimuli. The open field maze is used to measure locomotory, exploratory and anxiety behavior in mice due to its large centre arena.

Behavioral scores

1. Line crosses: This refers to the number of times the animal crossed a line drawn on the floor with the four limbs.
2. Rearing: Frequency with which the animal stands on hind legs or leans against the walls of the box with front paws.
3. Centre square duration: Duration of time the animal spent in the centre square.
4. Stretch attends posture: Frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position. It is a "risk-assessment" behavior which indicates that the animal is hesitant to move from its present position to a new position. A high frequency of this behavior indicates a higher level of anxiety.

2.5. The Light and Dark Transition Box

The light and dark transition box is a test of unconditioned anxiety and exploratory behavior. It is based on the conflict between exploring in a novel environment and avoidance of bright light. The box is divided into two compartments: the light compartment and the dark compartment. Increased activities such as line crosses, rearing and transition between the light and dark chambers are associated with non-anxious behavior.

The light dark transition box measures 45 x 27 x 27cm, made of plywood and consists of two compartments of unequal size. The small (18 x27cm) compartment is painted black (2/5 of the box), while the larger compartment (27 x 27cm) 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 9cm squares and is covered with Plexiglas. The apparatus was located in a 2 x 5m neurophysiology laboratory in the Department of Physiology, University of Calabar.

2.5.1. Experimental Procedure in the Light and Dark Box

Mice were carried to the test room in their home cages and tested one at a time. Each mouse was exposed to the open field maze by scooping it from its home cage using a small plastic container and placed in the centre of the white compartment facing the door and allowed to explore the apparatus for 5 minutes. After 5 minutes, it was removed and returned to its cage after which the floor of the box is cleaned using cotton wool dabbed in a solution of 70% ethyl alcohol and permitted to dry between tests.

Behavioural scores for the light and dark transition box

1. Transitions: Number of times the animal crosses into the opposite Compartment with all four paws.
2. Line crosses: Number of times the animal crossed a line drawn on the floor.
3. Rearing: Frequency with which the animal stands on hind legs or leans against the walls of the box with front paws.
4. Stretch attends posture: Frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position.
5. Dark box duration: Amount of time the animal spent in the dark Compartment of the box.
6. Light box duration: Amount of time the animal spent in the light Compartment of the box.

2.6. The Elevated Plus Maze

The elevated plus maze was built according to the description by Lister (1990). The floor of the maze is made of wood and the walls, of black Plexiglas. The maze structurally, consists of two open arms and two closed arms (30 x 5 x 15cm high walls). The open arm contains a slight ledge (4mm high) to prevent the mice from slipping and falling off the edge. Avoidance of this arm of the maze gives a measure of anxiety (Trullas & Skolnick, 1993). The closed arms provide a sense of safety because they are enclosed like most tests of anxiety. This task exploits the conflict between the natural tendency of mice to explore novel areas and fear of open spaces.

2.6.1. Experimental Procedure for the Elevated Plus Maze

Mice were carried to the test room in their home cages and tested one at a time. Each mouse was exposed to the open field maze by scooping it from its home cage using a small plastic container and placed at the centre square of the maze located among the four arms.

A greater frequency of behaviors such as open arm activity and head dipping indicates a greater level of exploration (Brown *et al*, 1999). Risk assessment behaviors such as stretch attend postures, head dips are an index levels of anxiety (Blanchard *et al*, 2001). A greater number of these measures imply a greater level of emotionality or fear (Lister, 1990). Risk assessment behaviors such as head dips and stretch-attend postures are an index of levels of anxiety (Blanchard *et al*, 2001). The index of open arm avoidance

also gives a measure of anxiety (Trullas & Skolnick, 1993). Behavioural scores for elevated plus maze include:

1. Open arm duration: Duration of time the animal spent in the open arms.
2. Open arm entries: Frequency with which the animal entered the open arms with all four paws.
3. Head dipping: Frequency with which the animal lowered the head over the sides of the open arm towards the floor.
4. Stretch attends posture: Frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position.
5. Rearing: Frequency with which the animal stands on hind legs or leans against the walls of the box with front paws.

2.7. Statistical Analysis

Statistical analysis was completed using SPSS for Windows. For all the neurobehavioral parameters, the dependant variables were analyzed using ANOVA for repeated measures. Post hoc comparisons are made using the Student-Newman-Keuls design among high dose, low dose administered *Cannabis sativa* and the control groups.

3. Results

3.1. Open Field Maze

The frequency of line crossing in open field maze was significantly lower ($P < 0.001$) for the high dose treated group compared to control and lower ($P < 0.05$) compared to LD; LD was also significantly lower ($P < 0.05$) compared to control. The frequency of rearing in the open field maze showed that the treated dose groups were significantly lower ($P < 0.001$) compared to control. Centre square duration in the open field showed that the high dose and low dose treated groups were significantly lower ($P < 0.01$) and ($P < 0.05$) respectively compared to control. However, there was no significant difference between the treated groups. The frequency of stretched attend posture (SAP) in the open field maze for the high dose and low dose were significantly higher ($P \leq 0.001$) and ($P \leq 0.01$) respectively compared to control.

3.2. The Light/Dark Transition Box

Frequency of line crosses in the light/dark transition box in the dark chamber of the light/dark box showed that the low dose ($P < 0.05$) and high dose ($P < 0.001$) were significantly lower compared to control. The frequency of rearing in the light/dark transition box showed that the treated groups were significantly ($P < 0.05$) lower compared to control. Mean duration of time spent in the light chamber was significantly lower for the low dose ($P < 0.05$) and high dose ($P < 0.01$) when compared to control while the mean duration of time spent in the dark chamber was significantly higher for low dose ($P < 0.001$) and high dose ($P < 0.05$) when compared to

control. The results of mean frequency of transitions were significantly lower ($P < 0.05$) for the treated groups compared to control. The results for the mean frequency of SAP for the treated groups in the light chamber of the light dark box were

significantly higher ($P < 0.05$) when compared to control. However, high dose group showed a significantly higher ($P < 0.05$) when compared to control.

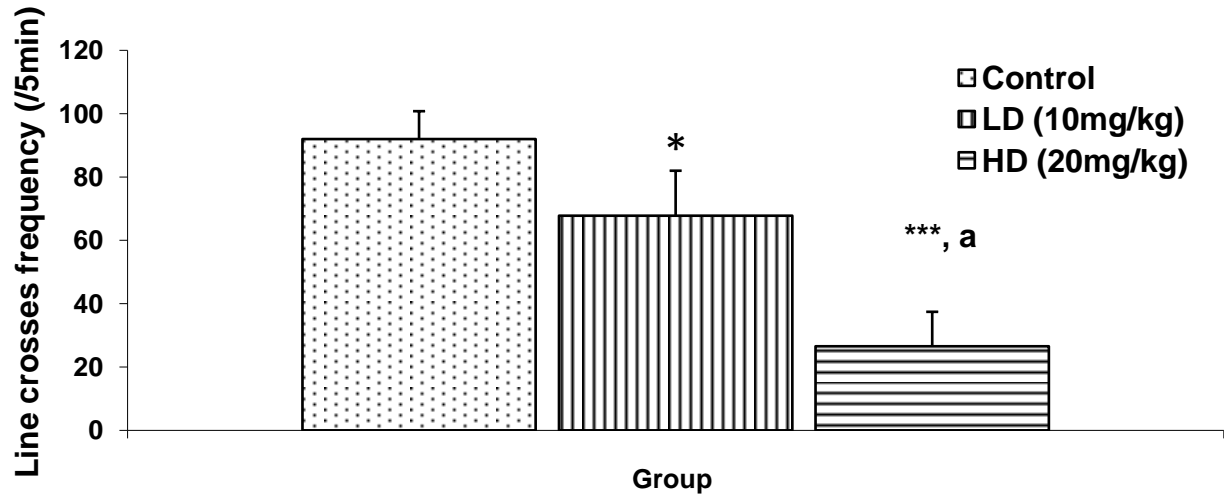


Figure 1. Comparison of frequency of line crosses in the open field maze in control and cannabis-treated groups. Values are mean + SEM, $n = 9$. * $p < 0.05$, *** $p < 0.001$ vs control; a = $p < 0.05$ vs LD

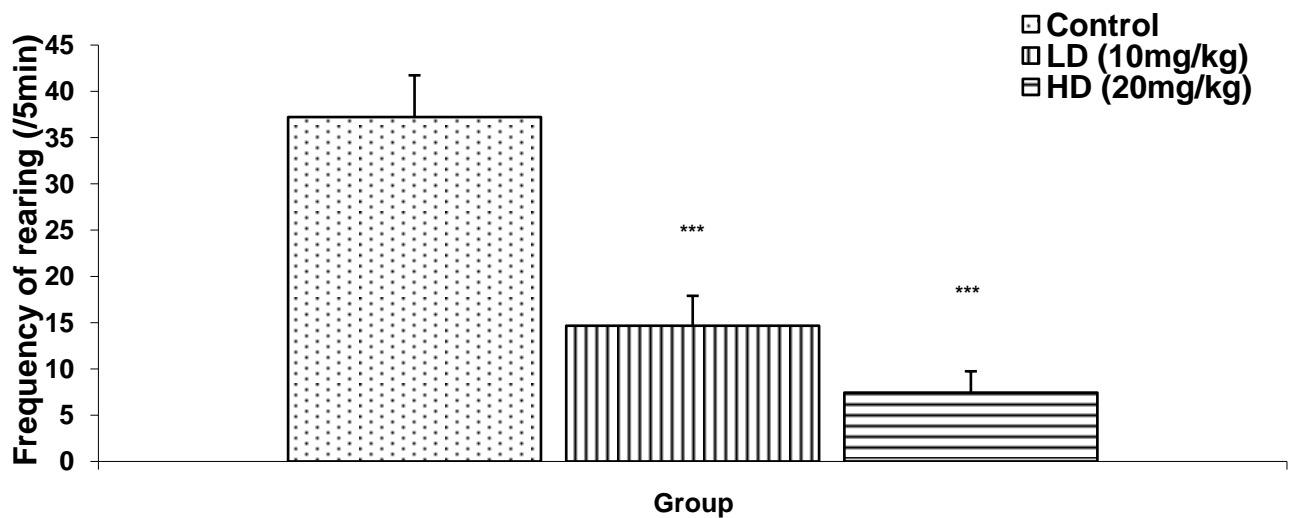


Figure 2. Comparison of rearing frequency in the open field maze in control and cannabis-treated groups. Values are mean + SEM, $n = 9$. *** $p < 0.001$ vs control

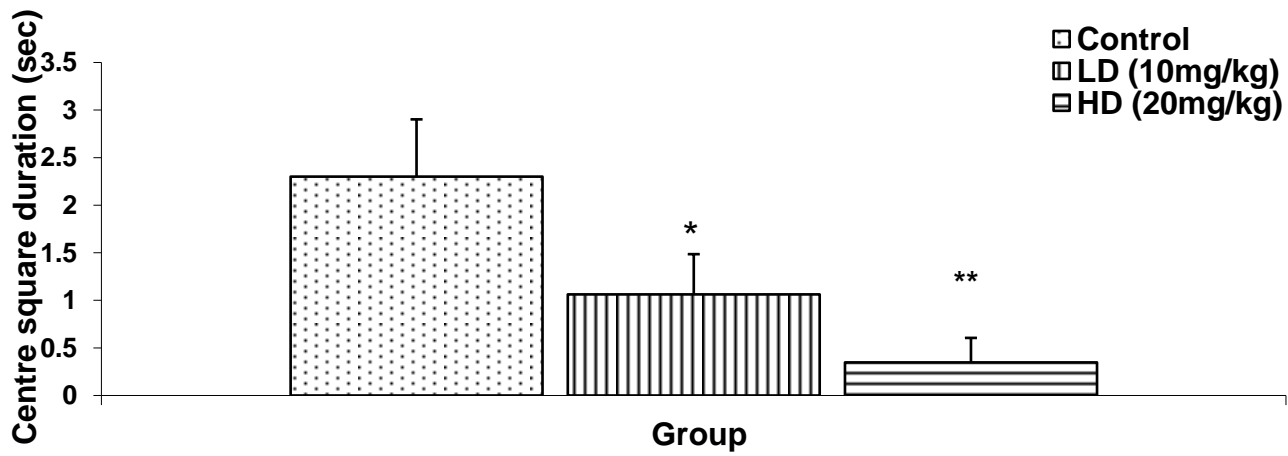


Figure 3. Comparison of duration in centre square in the open field maze in control and cannabis-treated groups. Values are mean + SEM, n = 9. **p<0.01 vs control

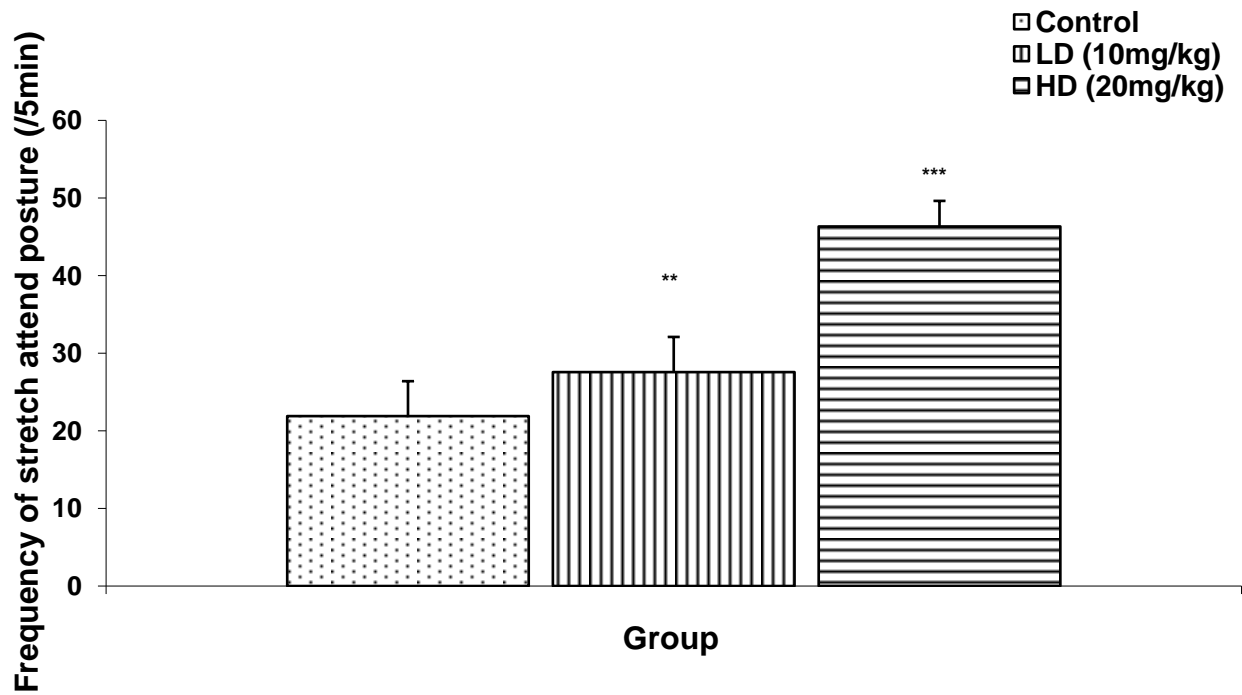


Figure 4. Comparison of frequency of stretch attend posture in the open field maze in control and cannabis-treated groups. Values are mean + SEM, n = 9. **p<0.01, ***p<0.001 vs control

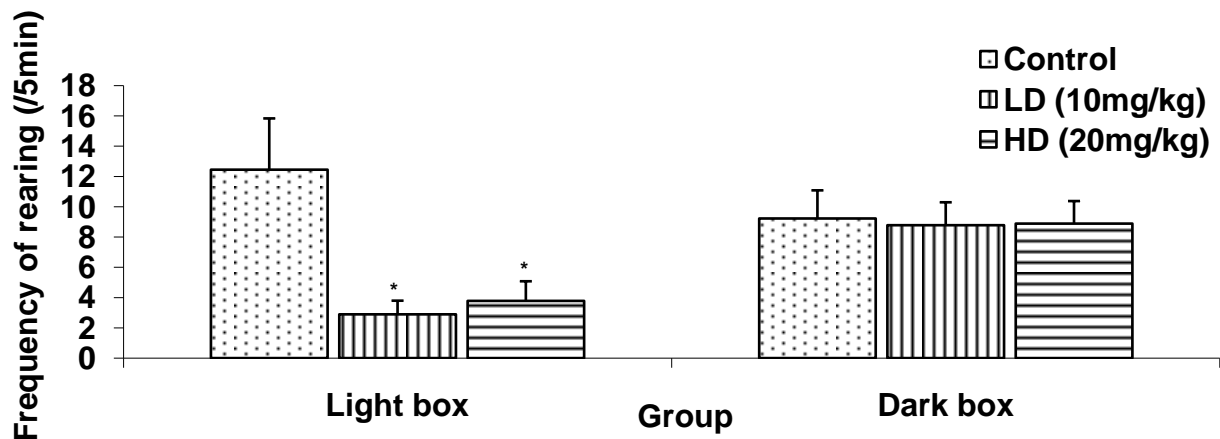


Figure 5. Comparison of frequency of rearing in the light and dark transition box in control and Cannabis-treated groups. Values are mean + SEM, n = 9. *p<0.05 vs control

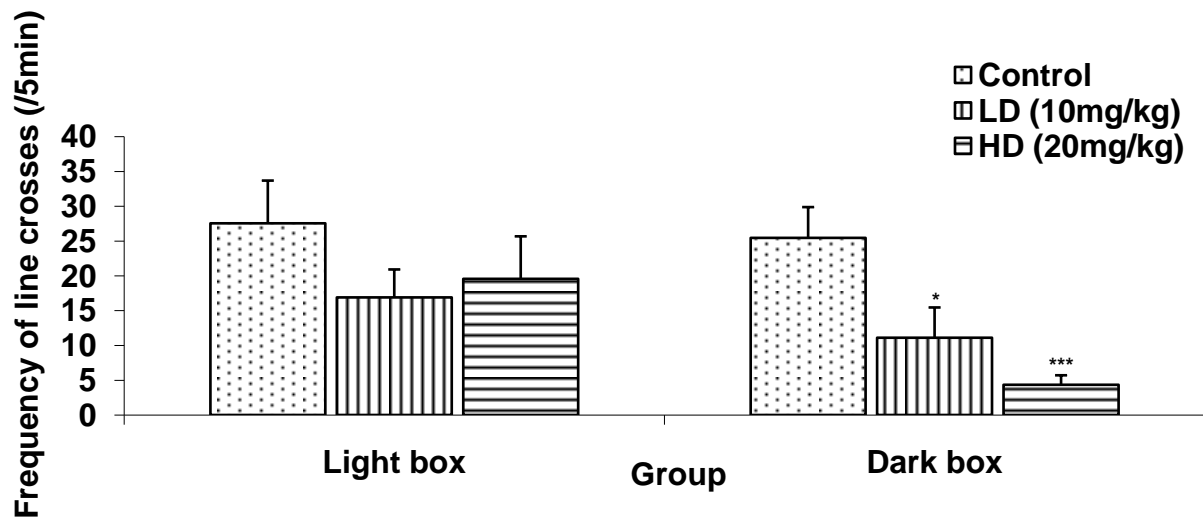


Figure 6. Comparison of frequency of line crosses in the light and dark transition box in control and Cannabis-treated groups. Values are mean + SEM, n = 9. *p<0.05, ***p<0.001 vs control

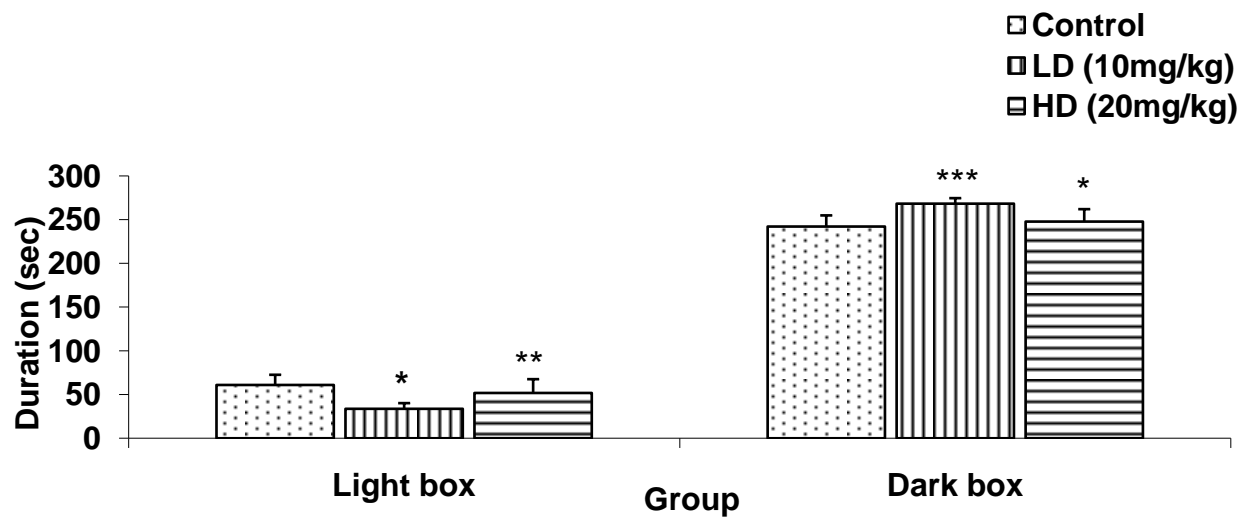


Figure 7. Comparison of duration of time spent in the light and dark transition box in control and Cannabis-treated groups. Values are mean + SEM, n = 9. *p<0.05, **p<0.01, ***p<0.001 vs control

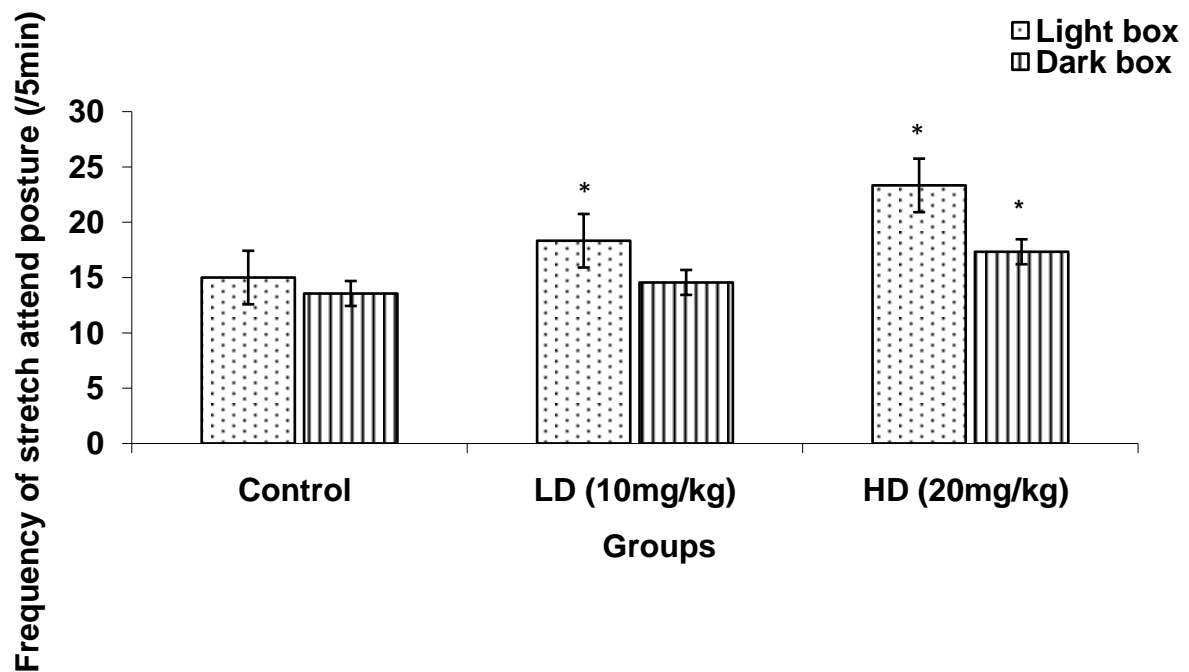


Figure 8. Comparison of frequency of stretch attend posture in the light and dark transition box in control and Cannabis treated groups. Values are mean + SEM, n = 9. *p<0.05 vs control

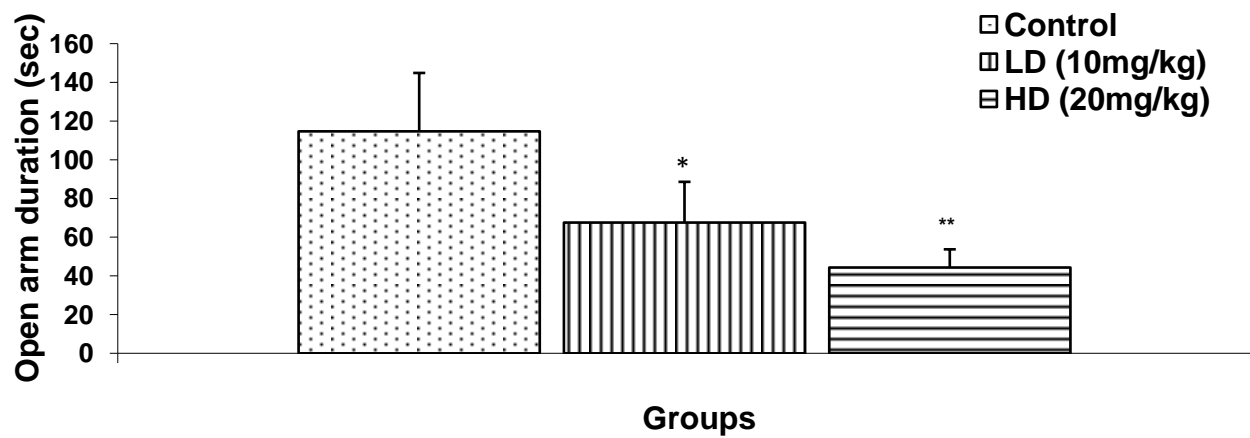


Figure 9. Comparison of duration of open arm in the elevated plus maze in the control and cannabis treated groups. Values are mean + SEM, n = 9. * $p < 0.05$ vs control

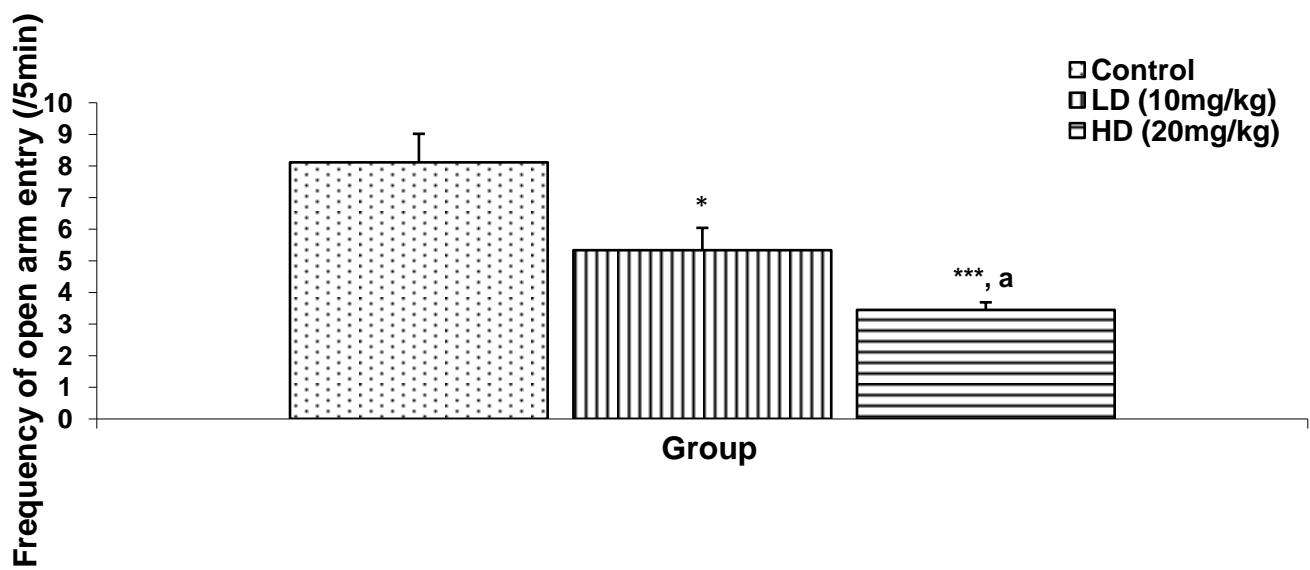


Figure 10. Comparison of frequency of open arm entry in the elevated plus maze in the control and cannabis treated groups. Values are mean + SEM, n = 9. * $p < 0.05$; *** $p < 0.001$ vs control; a = $p < 0.05$ vs LD

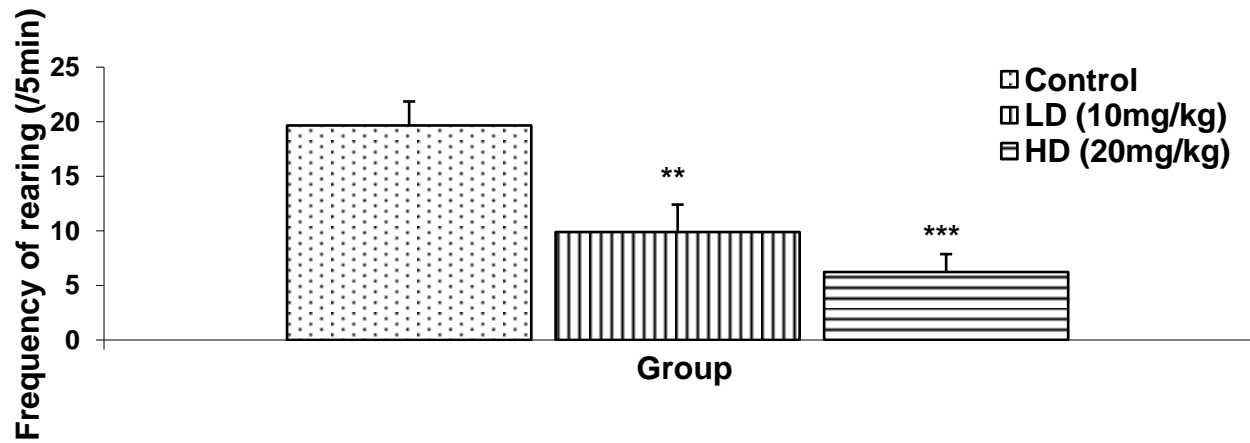


Figure 11. Comparison of frequency of rearing in the elevated plus maze in the control and cannabis-treated groups. Values are mean + SEM, n = 9. **p<0.01, ***p<0.001 vs control

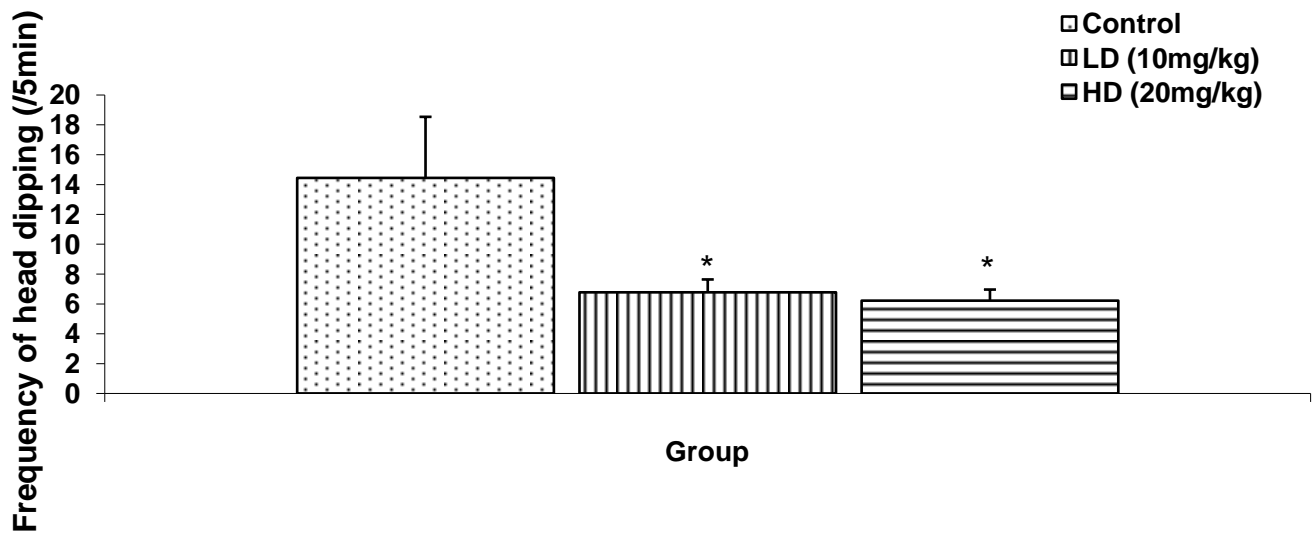


Figure 12. Comparison of frequency of head dipping in the elevated plus maze in the control and cannabis-treated groups. Values are mean + SEM, n = 9

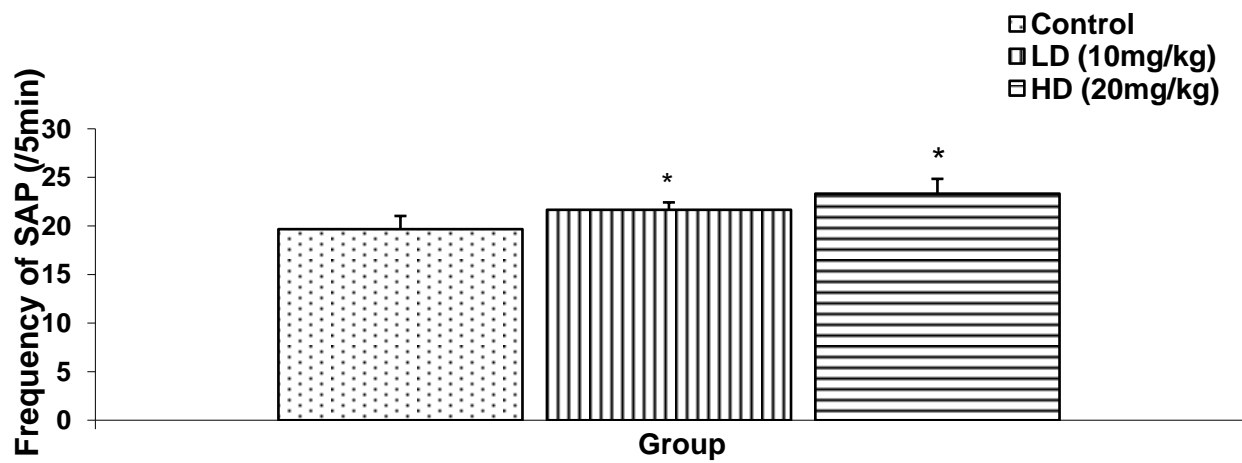


Figure 13. Comparison of frequency of stretch attend posture in the elevated plus maze in the control and cannabis-treated groups. Values are mean \pm SEM, n = 9. *p<0.05 vs control

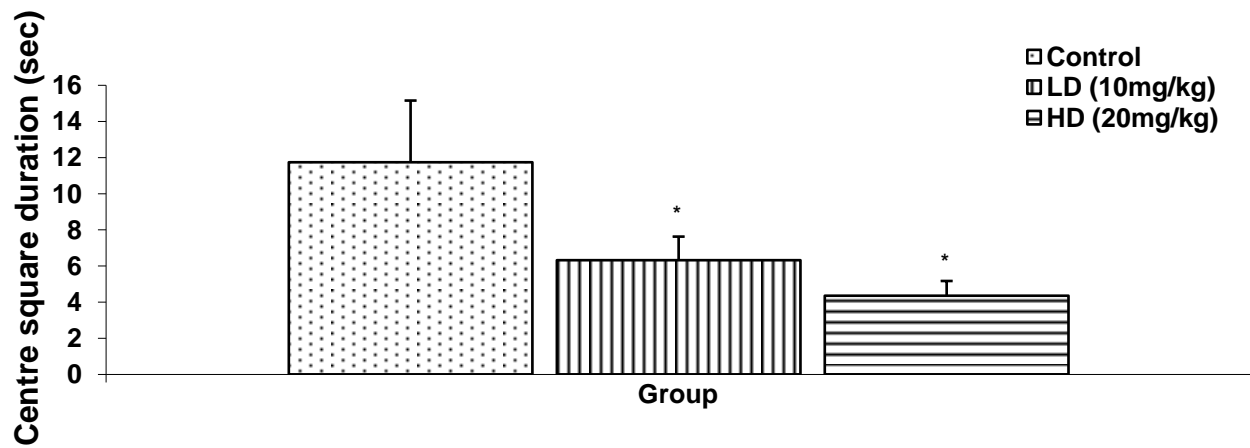


Figure 14. Comparison of duration of centre square in the elevated plus maze in the control and cannabis-treated groups. Values are mean \pm SEM, n = 9. *p<0.05 vs control

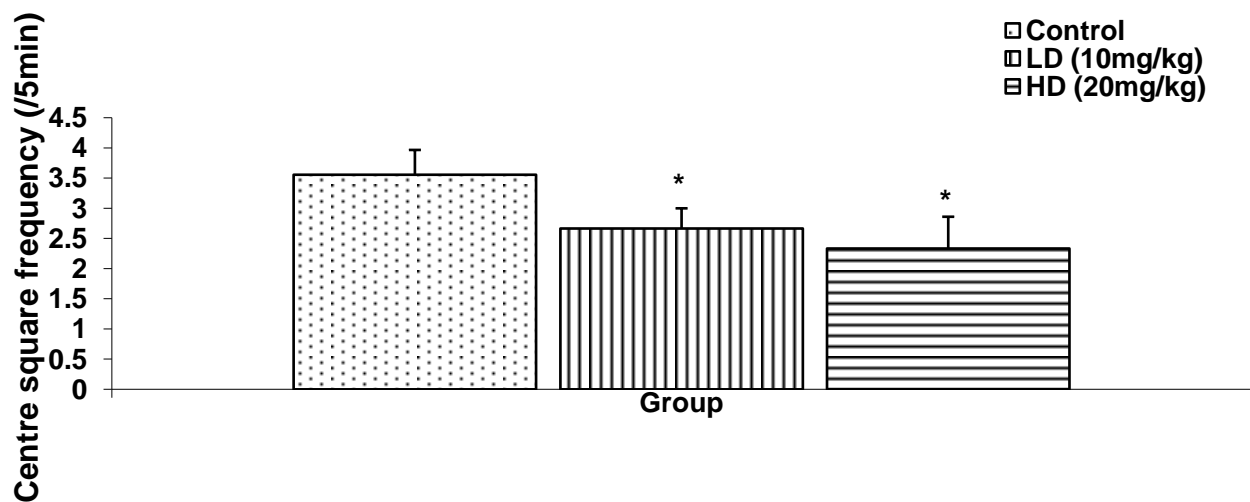


Figure 15. Comparison of frequency of centre square in the elevated plus maze in the control and cannabis-treated groups. Values are mean + SEM, n = 9. *p<0.05 vs control

3.3. The Elevated Plus Maze

The mean duration of time spent in the open arms showed that high dose and low dose groups had significantly lower ($P<0.01$) and ($P<0.05$) respectively compared to control. For the frequency of open arm entries in the elevated plus maze, the low dose group was significantly lower ($P<0.05$) when compared to control. The high dose group on the other hand was significantly lower compared to control ($P<0.001$) and low dose ($P<0.05$). The mean frequency of rearing for the low dose and high dose groups were each significantly ($P<0.01$) and ($P<0.001$) lower compared to control group. The Cannabis-treated groups showed a significantly lower ($P<0.05$) compared to control in the mean frequency of head dips in the elevated plus maze. The low and high dose groups showed a significantly higher ($p<0.05$) compared to control in the frequency of Stretch attend posture (SAP) in the elevated plus maze.

Centre square duration in the elevated plus maze were significantly ($P<0.05$) lower for the cannabis treated groups when compared to control.

4. Discussion, Summary and Conclusions

4.1. Discussion

In order to investigate the effects *Cannabis sativa* (marijuana) might have on locomotor and exploratory behaviour, the open field maze for locomotion and exploratory behavior, the elevated plus maze for fear and anxiety, the light/ dark box for anxiety, fear, and exploratory behavior was done. The open field test provides simultaneous

measures of locomotion, exploration and anxiety (Walsh & Cummins, 1976). Behaviors such as the number of line crosses and the frequency of rearing are not only used as measures of locomotor activity, but as well as measures of exploration and anxiety. A high frequency of these behaviors indicates increased locomotion/exploration and low anxiety (Walsh & Cummins, 1976). In this study, the low dose-treated group had lower locomotion/exploratory activity with high anxiety compared to control, as seen in the line crosses, and rearing activities. This effect was observed to be more when high dose was administered. This result agrees with a study reported by Harte-Hargrove et al, (2012), that tetrahydrocannabinol (THC), a psychoactive constituent of *Cannabis sativa* caused significant dose-dependent locomotor depression during drug administration. The low level of exploratory activity observed in the Cannabis-treated groups could be as a result of the inhibition of transmission of neural signals through the basal ganglia and cerebellum (Joy et al, 1999).

In this study, the low dose group had low exploratory behavior and showed high anxiety which worsened in high dose administration. This is further supported by the frequency of stretched attend postures experiments. A high frequency of the stretched attend posture indicates a higher level of anxiety. In this study, the Cannabis-treated groups had higher levels of anxiety compared to control. This high level of anxiety was observed to be more in high dose administration of *Cannabis sativa*. D'Souza et al, 2004; Genn et al, 2004 had earlier on reported in their studies that tetrahydrocannabinol (THC) increased anxiety. Anxiety induced by THC is facilitated by exposure to novel or stressful environment that appears to be mediated by the amygdala (Patel et al, 2005; Phan et al, 2008). At high doses,

it activates the hypothalamo-pituitary-adrenocortical axis (Manzanares *et al*, 1999; Giuliani *et al*, 2000; Berrendero and Maldonado, 2002; Viveros *et al*, 2005). This contradicts the work of Grotenhermen & Russo (2002).

The results in the light/dark transition box and elevated plus maze followed a similar trend.

In this study, the Cannabis-treated mice had low exploratory activity with increased anxiety and fear compared to control. This increase was more observed for the high dose group compared to low dose and control, as seen in the results of line crosses, rearing, stretched attend postures and duration of time spent in the light and dark compartments of the light/dark box. These results agree with the results of the test using the open field maze. The open arms are aversive to mice because they are open and the maze is elevated (Lister, 1990). The closed arms provide a sense of safety because they are enclosed. Like most tests of anxiety (the light/dark box and the open field), this task exploits the conflict between the natural tendency of mice to explore novel areas and fear of open spaces.

In this study, the Cannabis-treated animals showed a lower level of exploratory activity with increased fear and anxiety compared to control as shown in the results of rearing, open-arm duration, open arm frequency, head dipping, centre square frequency and duration in the elevated plus maze. This result was more observed in high dose administration.

Also, the frequency of stretch attends posture showed that the Cannabis-treated doses had high level of anxiety compared to control. Onaivi *et al* (1990) and Navarro *et al* (1993) reported that low doses of cannabinoid receptor (CB1 agonists) including THC, attenuate anxiety responses in animal models of fear and anxiety, including the elevated plus-maze and social interaction test. Also, THC causes stronger aversion to the open arms of the elevated plus maze, similar to the effect of anxiogenic agents (Moreira and Lutz, 2008; Onaivi *et al*, 1990). THC appears to increase anxiety (Viveros *et al*, 2005; D'Souza *et al*, 2004; Genn *et al*, 2004). In high doses [(>5 mg oral D9-tetrahydrocannabinol (D9-THC) for a man of average weight], cannabis can cause intense fear and anxiety. With higher doses, panic and phobic attacks may occur (Hall and Solowij, 1998; Roy-Byrne and Uhde, 1988; Thomas, 1993; Tournier *et al*, 2003; Tunving, 1987). This probably may be due to the fact that THC binds to cannabinoid receptors thereby increasing hypothalamic dopamine and serotonin levels. These increases might be involved in the activation of the hypothalamo-pituitary-adrenal axis described for cannabinoids. In experimental animals, administration of delta-9-THC increases dopaminergic neuronal firing and striatal dopamine release (Iversen, 2003). Dopamine plays a critical role in the reward system. This is why users of the drug have a feeling of ecstasy after it is used and hence related to the drug's ability to cause dependence.

The results of this study differ from previous work by other researchers who discovered the biphasic effects of cannabinoids in locomotion (Katsidoni *et al*, 2013) and this

could be because of the species of cannabis used and the duration of use. Even the environment it is grown (virgin land is said to be more potent) influences chemical content and hereditary determinants (Kirkham, 2006 & Pate, 1994). In addition, THC has been found to be largely concentrated around the flowering parts of the female plant. The leaves (which were used in this study) and male plants have less THC (Mahlberg *et al*, 2001).

5. Conclusions

Cannabis sativa may lead to decreased locomotion/exploratory behavior, increased fear and anxiety. If these results are applicable to man, then long term administration of *Cannabis sativa* may be dangerous.

ACKNOWLEDGEMENTS

We acknowledge the contribution of Dr. Daniel Ikpi and Mr. Umoh Ededet for assisting in the preparation of the extract that was used for this research.

Competing Interests

Authors have declared that no competing interests exist.

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