Cannabis Attenuates Stress Induced Disturbance of Visuo-Spatial Memory and Cognitive Activities in Albino Wistar Rats

*Olorunfemi O.J., Umana K.T., Okosun A.O., Ifedi I.C.
Department of Human Physiology, Faculty of Basic Medical Sciences,
College of Health Sciences, University of Port Harcourt, Choba, Port Harcourt, Nigeria.
olorunfemi.oluwadare@uniport.edu.ng, +2348035760596

ABSTRACT - The research was carried out to investigate the effect of cannabis on stress induced disturbance of visuo-spatial memory and cognitive activities using Albino wistar rat. The experimental procedure for visuo-spatial memory was done using a visual cues cage with objects of different shapes like triangular, round, cylindrical, square and rectangular-shape objects. The cognitive activities were carried out using the navigational maze task, beam walking, inverted screen test and hand grip test while the pre-pulse inhibition test was done using the acoustic bell. Twenty male albino wistar rats were acquired, and divided into four groups (group 1, 2, 3 and 4) of five rats in each cage. While group 1 received only feed and water serving as the control, group 2 were given 0.4g/kg of cannabis orally, group 3 were given 0.8g/kg cannabis orally. Group 4 (valium) were administered intraperitonally. The findings showed a significant (P<0.05) before and after exposure to stressful conditions in the test groups, during the acquisition training there was no significant difference in cognitive activities, spatial and pre-pulse inhibition test between the test and control group (P<0.05) but in reverse training (stressful phase)the cognitive, spatial and pre-pulse inhibition test for group 2 and 3 (cannabis groups) was found to be significantly (P<0.05) higher, compared to the control, while group 4 (valium group) was significantly (P<0.05) lower compared to the first two test and control. From the findings carried out, cannabis tend to increase cognitive activities, pre-pulse inhibition and spatial memory.

Keywords: Cannabis, cognitive activities, navigational maze task, beam walking, acoustic bell.

Introduction

In humans, stress typically describes a negative or positive condition that can have an impact on a person's mental and physical well-being (Baddeley, 1966). Globally about 4.4 million people suffer disturbances of spatial memory and cognitive activities due to stress WHO (2010). When the stress response is initiated, immediate and powerful physiological changes are elicited because of the activation of a particular branch of the nervous system called the Autonomic Nervous System (Bauer et al, 2003; Bauer & Patricia, 2002).

Cannabis appears naturally in many tropical and humid parts of the world, its use as a mind-altering drug has been documented by Archaeological findings in prehistoric societies in Euro- Asia and Africa (Scoville &Milner, 1957). Cannabis is often used for its mental and physical effect such as "high" or "stone" feeling, a general change in perception, euphoria (heightened mood), and an increase in appetite. Its usage has been reported to have some beneficial effects including: improving cognitive properties, memory, locomotive skills etc. (Crippa et *al,* 2009). Cannabis is also known as marijuana among other names, it is prepared from the cannabis plant which was intended to be used as a psychoactive drug or medicine. The main psychoactive part of cannabis is tetrahydrocannabinol (THC), (Schneiderman*et al,* 2005).

Recent studies conducted by the University of Wisconsin-Madison and other places have shown other ameliorative properties of cannabis in neuronal functions. For example, when humans are under chronic stress, permanent changes in their physiological, emotional, and behavioural responses are most likely to occur (Khansari & Murgo, 1990).

Series of research works done have shown that cannabis also possess negative properties including: a decrease in short term memory, dry mouth, impaired motor skill, red eyes and feelings of paranoia or anxiety (Crippa *et al,* 2009). Others are addiction, decreased mental ability in those who started as teenagers and behavioural problems in children whose mother's used cannabis during pregnancy (Conrad, 1964;Ellenbogen et al, 2006; Kaufman et al, 2000).

Materials and Method

The experimental animals were handled in line with the guideline of the care and use of animals in research/teaching (NIH Publication No. 8593, revised 1985) also the present study was carried out in the animal house (pre-clinical) of the University of Port Harcourt, Choba, Rivers state, Nigeria.

Experimental Protocol

Twenty (20) Albino wistar rats were obtained from the animal house of Physiology Department, University of Port Harcourt, Choba. They were fed with rat feed and given water freely for two weeks before the experiment began in order to be acclimatized to the environment. The rats were randomly separated into four groups. Group one was the control group consisting of five animals, group two, three and four were test groups consisting of five animals each. The animals were fed daily for 14 days ad- libitum before the commencement of the experiment. During the 14 days of feeding, water was also given to them.

Administration of Cannabis and Task Evaluation

Cannabis extract was obtained and stored in a small container, a calibrated beaker was used to measure 0.4g/kg into a different container using the weighing balance, and another 0.8g was measuredinto another container using the weighing balance.The 0.4g/kg concentration of cannabis was given to group two (2) animals whereas the 0.8g/kg concentration was given to group three (3) animals. These animals were fed orally with the cannabis concentration for three (3) weeks and in the process the effect of cannabis on them was recorded while carrying out some of the test exercise such as:

1. Cognitive activities test
2. Spatial memory test
3. Pre-pulse inhibition test

1. Cognitive Activities Test

This test comprised of;

Navigation test: In this test the animal was placed at the entrance in a maze and was allowed to move to and fro in the maze and was then observed to know if the animal would be able to remember the entrance of the maze, and the time taken for the animal to return to the entrance was recorded.

Walking on the beam test: In this test, the animal was placed at the edge of a cylindrical pavement and was observed to see if the animal would be able to move from the edge it was placed to the other edge without being scared of falling down, and the time taken for the animal to move was recorded.

Hand grip test: In this test the animal was made to grip or hold an object only with the first two limbs, and the time the animal spent holding on to the object before falling was recorded.

Inverted screen test: In this test the animal is placed in a net cage with the abdominal region facing upward, and the animal was observed to see if it can be able to hold on to it. And the time it took for the animal to be suspended on it without falling was recorded.

2. Spatial Memory Test

In this test, the animal (after a pre-exposure to the objects)is placed in an iron net cage contains objects with different shapes like; triangular shape object, round object, cylindrical object, square object and rectangular object. The animal was timed for 5minutes to know if the animal would be able to recognize the different object. The most frequent object i.e. the object the animal visit more frequently was recorded.

3. Pre-pulse Inhibition Test

In this test the animals were exposed to sounds using a bell (acoustic bell). The animal was placed in the middle while the bell was placed by the side. After the sound of the bell, the animal was observed to know if the animal ran towards the sound of the bell or away from the sound, and some of the animals did not move. And the time taken for the animal to move towards the sound and away from the sound was recorded.

These three test were also carried out on group one (1) animals (the control), which were not given the cannabis extract and there performance were recorded. Group four (4) animals are the anti-anxiety groups, they were given anti-anxiety drug (Valium) for two (2) weeks and they were made to carry out the three test listed above and their performance were observed and recorded.

Statistical Analysis

The analysis of the result of this study was done with SPSS version 20.0 and the results were expressed as mean ±SEM and relative percent change. One-way Analysis of Variance (ANOVA) and Post Hoc Test were used to compare the mean and P-Value ≤ 0.05 was accepted as statistically significant. Results were presented in tables and bar charts.

RESULTS

Cognitive Activities Result

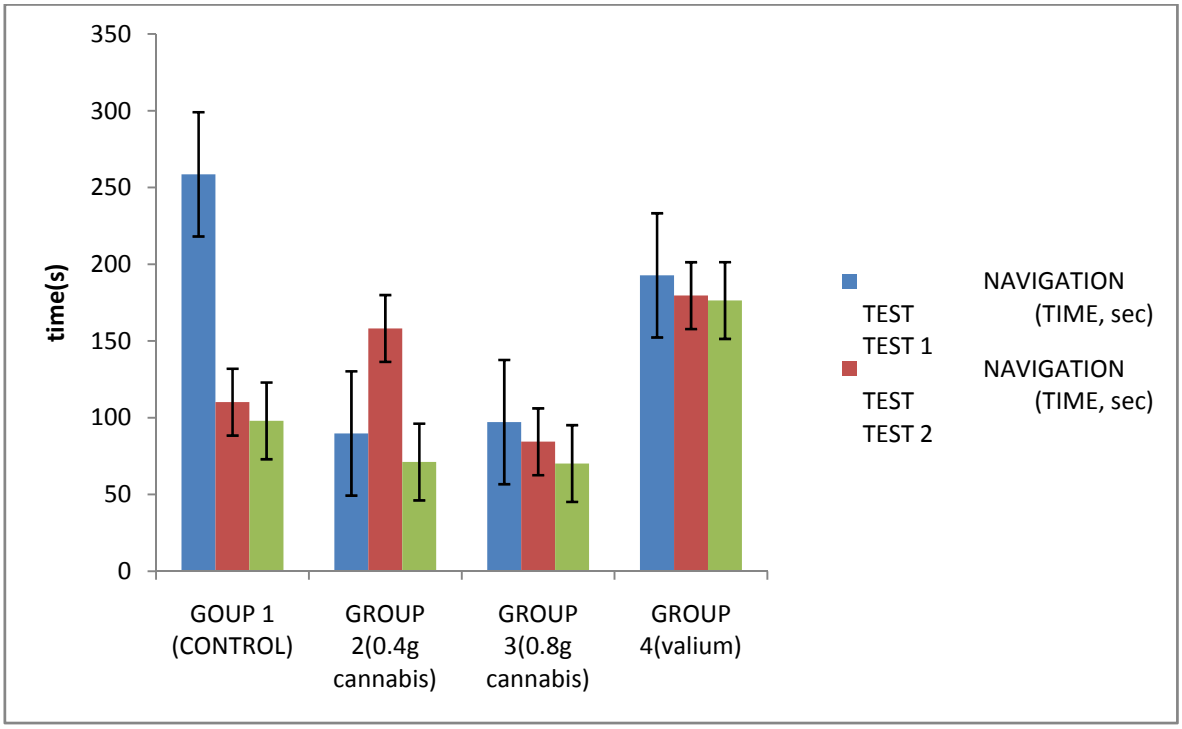

Figure 1. Pattern of navigational test in the control and the test groups

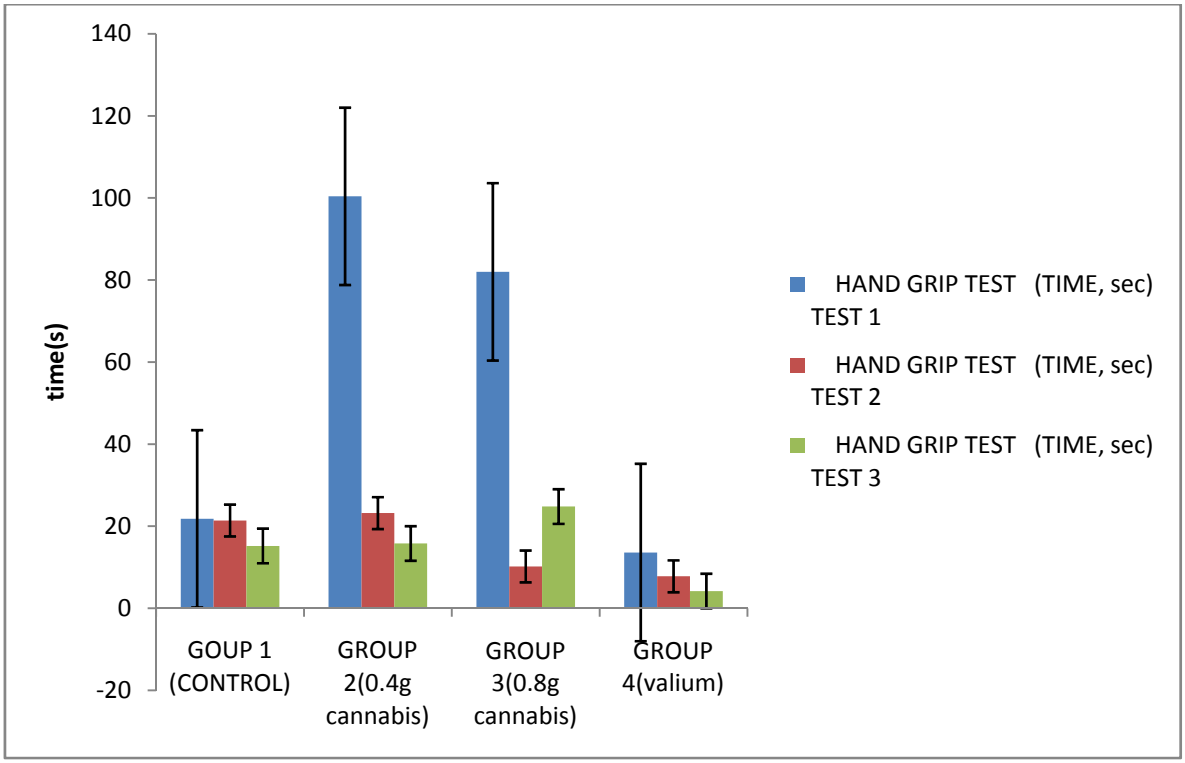

Figure 2:Performance from handgrip test across the test groups and the control

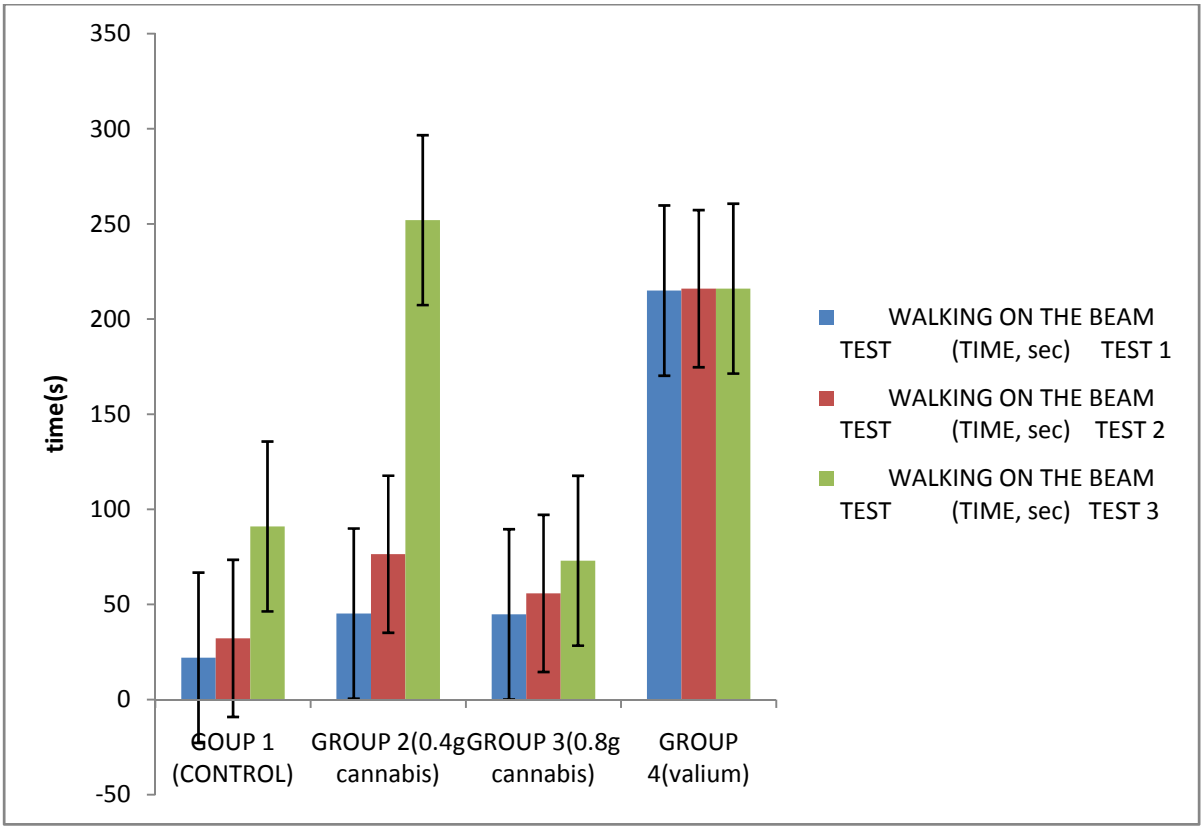

Figure 3. Results from beam walking test across the test groups and the control

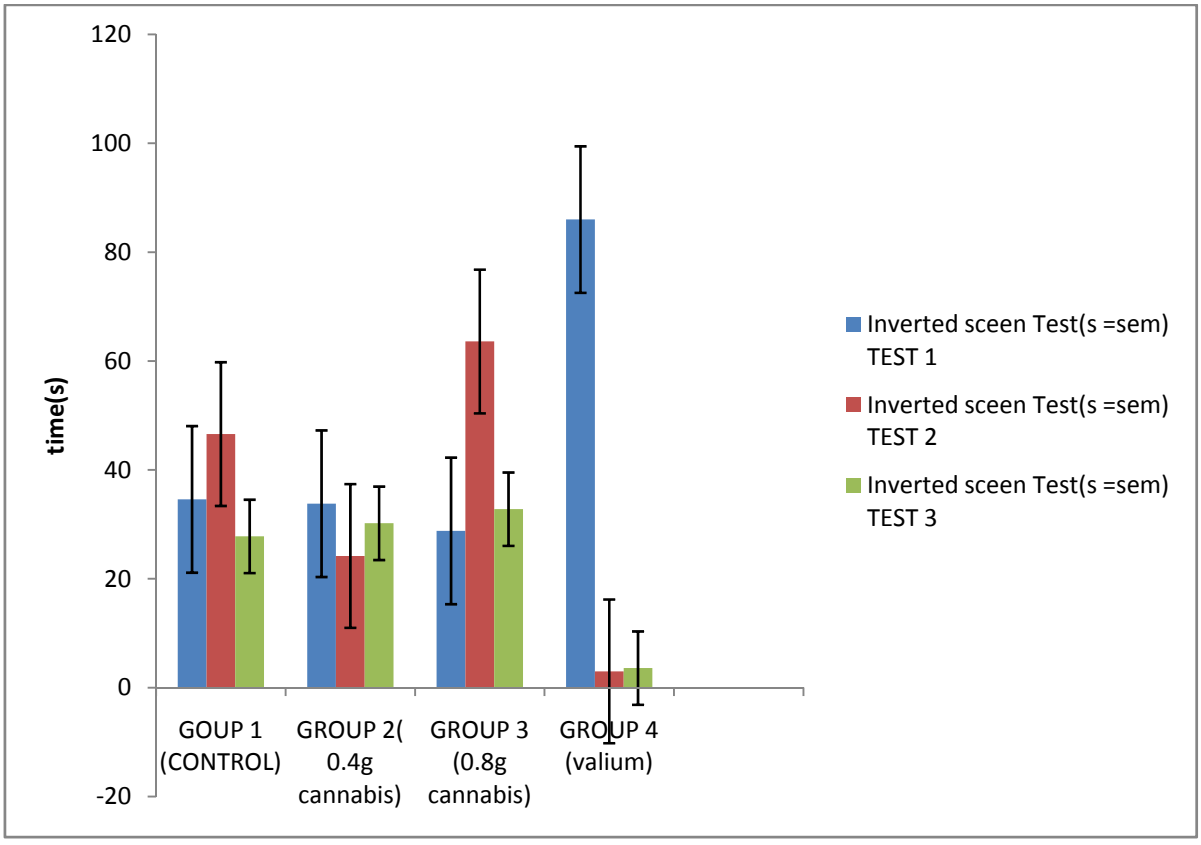

Figure 4: bars showing results from inverted screen test across the test groups and the control

Pre-pulse Inhibition Test

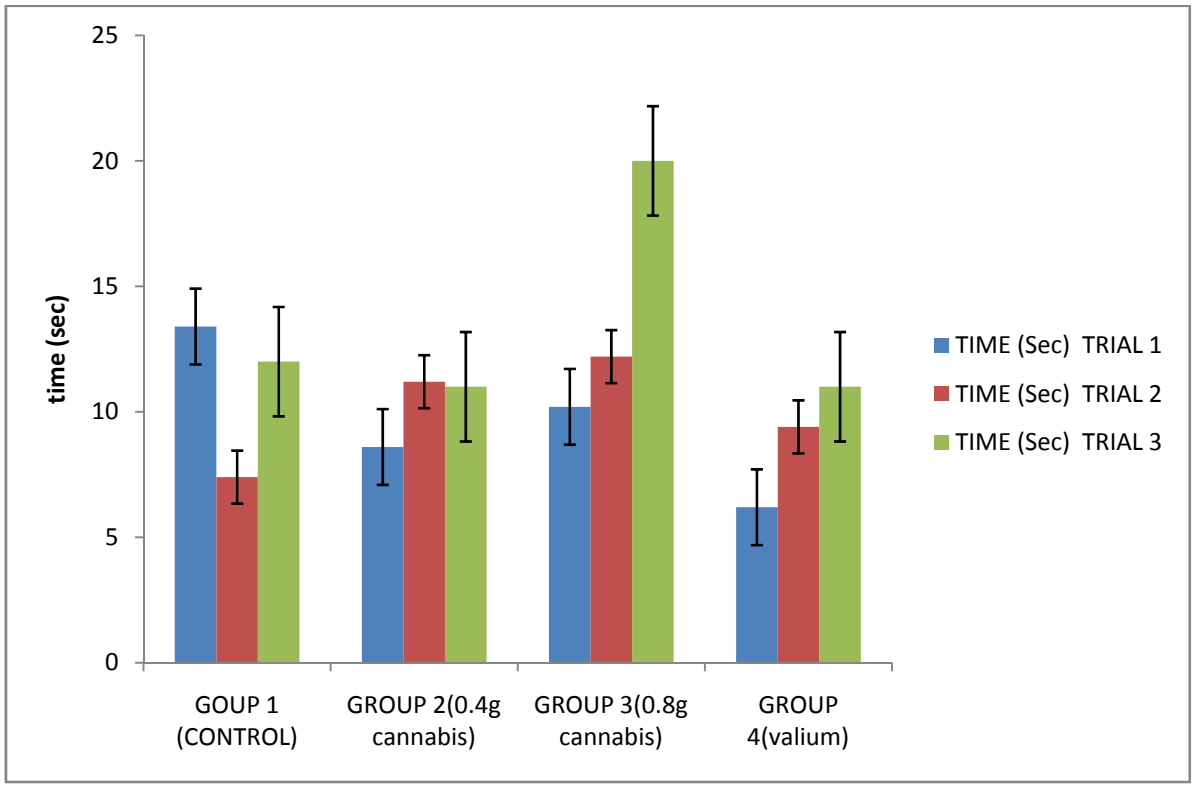

Figure 5: bars showing result for **Threshold Time Test**(Freezing Time) across the test groups and the control

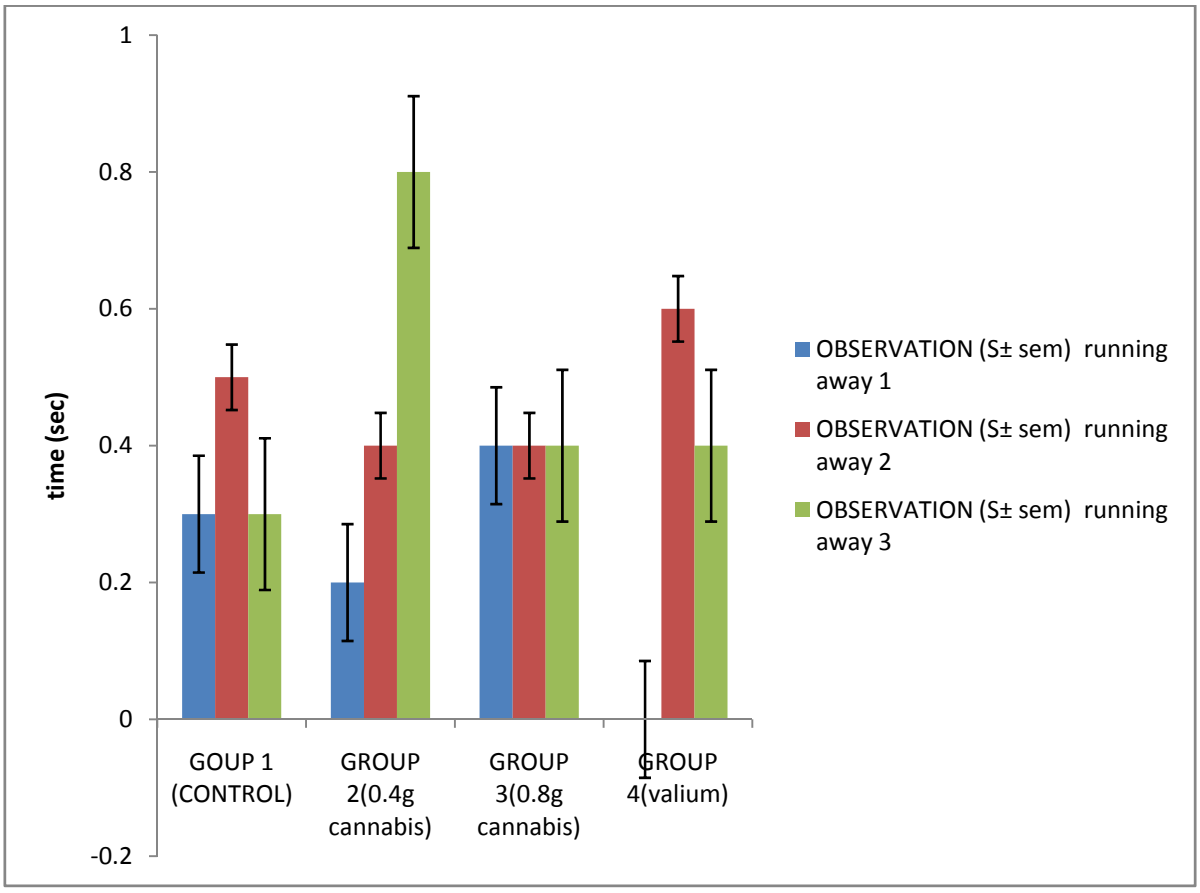

Figure 6: bars showing results for **Positive Reflex Time** (running away from the source) test across the test groups and the control

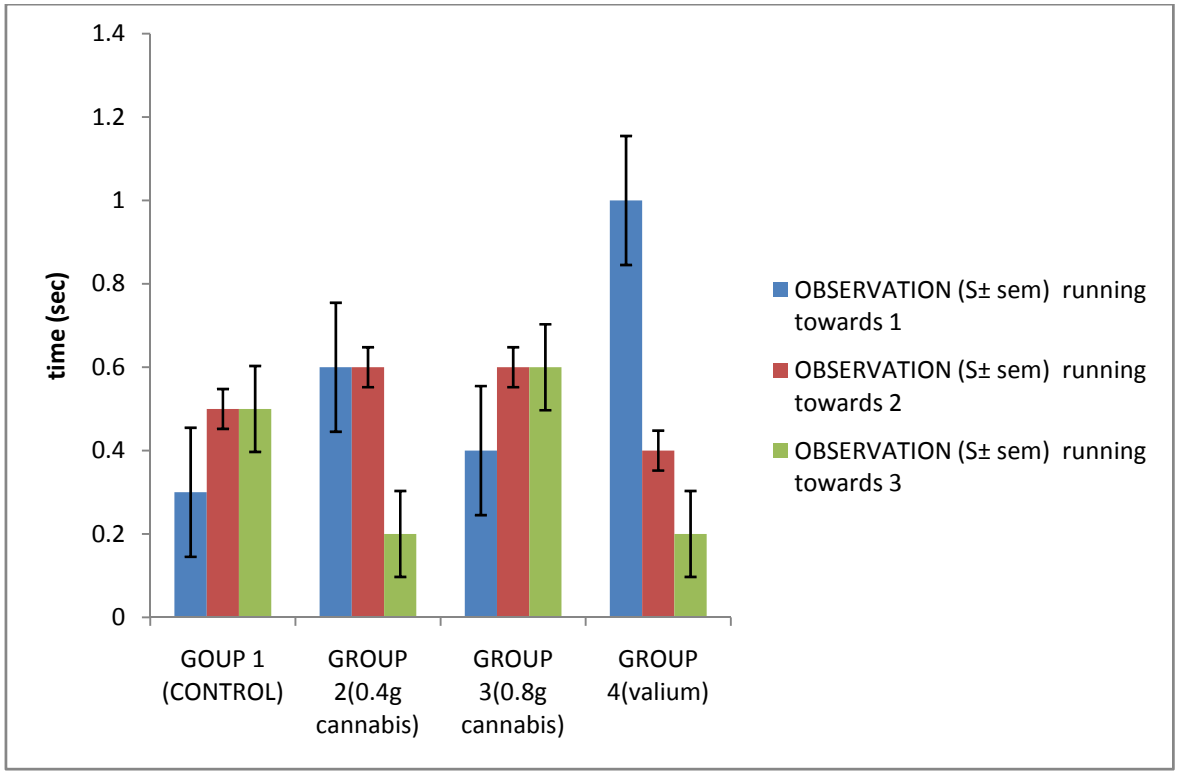

Figure 7: Results for **Negative Reflex Time** (running towards the source) test across the test groups and the control

Table 1 Response patterns from Spatial Memory Testsin the test and control groups

Spatial Memory Test(Explorative Activities) Recognition Of Objects By Shapes Test 1				
GROUPS	Round object % recognition	Cylindrical object % recognition	Rectangular Object % recognition	Squared object % recognition
Group 1(Control)	80	100	40	80
Group2(0.4g/Kg Cannabis)	20	80	80	20
Group3(0.8g/Kg Cannabis)	100	100	20	80
Group4(Valium)	20	20	60	60

Table 2. Response patterns from Spatial Memory Test 2 in the test and control groups

Spatial Memory Test(Explorative Activities) Recognition Of Objects By Shapes Test 2				
GROUPS	Round object % recognition	Cylindrical object % recognition	Rectangular Object % recognition	Squared object % recognition
Group 1(Control)	80	60	40	40
Group2(0.4g/Kg Cannabis)	80	10	20	80
Group3(0.8g/Kg Cannabis)	60	80	80	80
Group4(Valium)	20	20	20	20

Table 3. Response patterns from Spatial Memory Test 3 in the test and control groups

Spatial Memory Test(Explorative Activities) Recognition Of Objects By Shapes Test 3				
GROUPS	Round object % recognition	Cylindrical object % recognition	Rectangular Object % recognition	Squared object % recognition
Group 1(Control)	60	60	60	60
Group2(0.4g/Kg Cannabis)	60	10	40	40
Group3(0.8g/Kg Cannabis)	80	80	80	80
Group4(Valium)	20	20	20	20

Discussion

The study investigated the effect of cannabis on stress induced disturbance of spatial memory and cognitive activities in rat. The patterns of activities recorded in Navigational maze task, Beam walk, Hand grip test, Inverted screen test, spatial memory test and Pre-pulse inhibition were consistently attesting to the fact that pharmacological administration of cannabis can potentially ameliorate cognitive deficits due to stress in rats.

Previous studies have revealed that cannabis is often used for its mental and physical effects, such as a high or stoned feeling, a general change in perception, euphoria (heightened mood) and an increase in appetite; it also has a way of decreasing spatial memory and cognitive activities (Adai.us.edu. 2013).

Results from the Navigational maze task revealed that cannabis in both low and high dose reduced the time spent significantly ($p< 0.05$) to manoeuvre through the matrix in a dose-dependent fashion. The performance of task pattern of group 4 (valium) showed a significantly slowed pace and prolonged time spent in exiting the matrix in comparison with the control and cannabis groups. The outcome of the three trials consistently showed similar patterns in the entire period. The repetitions (tests 1-3) of the tests affirmed the behavioural response and proved that these responses could be both time- and dose-dependent. The outcome of used in behavioural neuroscience to study spatial learning and memory. It can be a very accurate study of learning, memory and spatial working and can also assess damage to cortical regions of the brain (Hooge *et al*., (2001).It is also used as a tool to study drug abuse, neural systems, neurotransmitters and brain development (Wongwitdecha, et al, 1996; McEwen, 2012). Observations from this study showed that the two cannabis group (groups 2&3) were the fastest to traverse the maze, followed by the control group and the last being the fourth group (valium group).

The hand grip test is a simple non- invasive method designed to evaluate mouse muscle force in vivo by taking advantage of the animal's tendency to grasp a horizontal metal bar or grid while suspended by its tail. (Baddeley, 1966; Miller, 1956). From the result of the research it is observe that the two cannabis groups were able to hold on tight to the bar, followed by the control group and the last being the group four (Valium group).

Gait pattern was investigated after an episode of stress exposure across the groups using Beam walk test which accessed their fine motor coordination and balance. This test is used to ascertain the ability of the animal to remain upright and to walk on an elevated and relatively narrow beam. Observation from the beam walk study as shown in Table 4.3 revealed that there was a significant ($P<0.05$) change in the time it took to traverse the beam from 22.0± 4.23 of the first session to 91.0± 35.3 of the third session of the control group, compared to the test groups. Comparatively, the control group traverse the beam faster. It is followed by the second group, the 0.4g cannabis group, then the 0.8g cannabis group which is the third group and the slowest is the fourth group, the anti- anxiety drug (Valium) group. Motor coordination is mainly modulated in the cerebellum. A main player in modulation of cerebellum is GABAergic neurotransmitter in cerebellum.This result was similar to previous report where Beam Walk test was used to detect motor deficit due to age, central nervous system lesion and genetic and pharmacological manipulations in young and old rodent (Tiny *et al*, 2011).

The Inverted Screen test is used to measure motor strength/coordination. The animals are placed individually on top of a square (7.5 cm x 7.5 cm) wire screen which is mounted horizontally on a metal rod. The rod is then rotated 180° so that the rats are on the bottom of the screens. The following behavioural responses are recorded over a 1 min testing session: fell off, did not climb, and climbed up (Baddeley, 1966). Observation from the inverted screen test study as shown in Table 4.4 shows that the third group (0.8g cannabis group) were able to hold on more firmly to the wire screen, compare to the others. This scenario was followed by the second group (0.4g cannabis group), the control group was the third and the weakest is the fourth group (the anti-anxiety drug valium group).

Spatial memory test has to do with the ability of the animals to recognize objects of different shapes after an initial familiarization. From the study, the control and group 3 recognize the round object the same way, followed by group 2 and the last is group 4. For cylindrical object, the two cannabis groups responded more to cylindrical object, followed by control and the fourth group. The third group recognize the square object most, followed by the control, the second group and the fourth comes last. While the control recognize the rectangular object more followed by the two cannabis group and the last being the group four (valium group). The cannabis groups especially at high dose could recall much better most of the shapes alike and could make better judgement at identifying the profoundly. Their cognitive ability of stereo-gnosis was adjudged to be optimal and reinforced.

Prepulse inhibition test is commonly used to evaluate sensorimotor gating as well as attentional processes involved in information selection (www.Panlab .com). The cannabis treated groups showed resilience during the threshold Test time. This proved that the pre-attentive processes that operate outside of conscious awareness was affirmed but this could make the animal vulnerable to danger. The startle response is a brain stem reflex elicited by an unexpected acoustic or tactile stimulus. In the prepulse inhibition test, sensorimotor gating is assessed by evaluating the characteristics of the innate reduction of the startle reflex induced by a weak pre-stimulus as demonstrated in the study and was similar to a previous report (Baddeley, 1966). In this test the

control and the cannabis group, respond more to the sound of the bell (startle response) compare to the fourth group (valium group).

Conclusion

This work demonstrated dose-dependent attenuating effect of cannabis on stress induced disturbance of spatial memory and cognitive activities in rat. In other words, the impact of cannabis on cognitive functions and behaviours is strongly influenced by the concentration of cannabis but this also account for the observed vulnerability of the animal to attendant danger on the long run.

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