Caffeine and Cannabis Sativa Ingestion Affected Memory and Anxiety Levels in Juvenile Rat Models: A Potential Indication for Learning and Behavioural Aberrations in Users

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Abstract: *Cognition, memory and behavioural changes associated with the use of psychoactive substances are typically modelled in murine models. This study involved a modelling the use of caffeine and cannabis to provide information potential effects on learning and behavioural aberrations in users [humans]. The natures of memory retention and anxiety levels were observed in juvenile experimental animals after caffeine and cannabis treatment for 21 days. The investigation was conducted to observe the influence of caffeine and cannabis at various dosages on memory and anxiety which are key behavioural and cognition parameters and the regimen was particularly modelled to simulate human conditions of scenarios of these substances uses. Seventy two adult Wistar rats were divided into six groups. Group A was the control group; the Group B received 100mg/kg body weight of caffeine; Group C received 50mg/kg body weight of caffeine; Group D received 500mg/kg body weight of cannabis; Group E received 200mg/kg body weight of cannabis; Group F received 50mg/kg body weight of caffeine and 2000mg/kg body weight of cannabis combined. Animals were taken through the anxiety measurement tests using the elevated plus maze 24 hours after the last administration. The memory tests were conducted using the Barns Maze and this lasted 5 days. Memory and anxiety were generally influenced following different patterns across the treated groups relative to the control. Effects depended largely on substances and dosage.*
Keywords: *Caffeine Cannabis Memory Anxiety Juvenile Rats*

I.   Introduction

Caffeine, a methylxanthine, has been known to have physiological, behavioural and psychological effects in both humans and animals. It acts as both a central and peripheral nervous system stimulant in both humans and animals also. In rats, caffeine has been reported to increase locomotor activity, and induce rotational behaviour with unilateral lesions of the nigrostratial dopamine cells [1]. Caffeine has also been found to improve performance on learning and memory tasks in both rats and non-human primates, as well as improve memory retention in rats [2]. At high doses of >400mg however, one experiences feelings of anxiety, nausea, jitteriness and nervousness [1]. Behavioural effects such as enhanced cognitive performance, auditory vigilance, and reaction time have also been documented in humans [3]. Moderate coffee consumption has been inversely associated with increased risk of cardiovascular diseases (CVD) (Ding *et al.,* 2014) [4]. High levels of caffeine use have reportedly been associated with calcium exertion and bone loss, which may contribute to osteoporosis; however, this reportedly only occurs in individuals with low calcium intake. Caffeine withdrawal was also observed in adolescents, and produces similar effects in them as in adults, including headache, drowsiness and fatigue (Temple, 2009). Regardless of the negative health risks associated with caffeine, a number of health benefits have also been reported. There is evidence showing that caffeine can reduce weight gain [5]. Caffeine also improves sports performance, including perceived exertion and endurance [6]. There is some evidence of an inverse relationship between caffeine and colorectal cancer [7] and Parkinson's disease [8], the mechanism for this however is unknown) [1].

Cannabis is a genus of flowering plants that includes three species or subspecies, cannabis *sativa, indicia,* and *ruderalis.* It however generically refers to the many psychoactive preparation of the subspecies *Cannabis sativa.* The plant is indigenous to Central Asia and Indian subcontinent [9], and has long been used for medical and recreational purposes among other uses. Although cannabis is consumed harmless in some societies, it is considered an illicit drug in most parts of the world and its use is illegal. Cannabis is consumed in many forms, but the most popular form being marijuana, which is prepared from the stems, leaves and dried flower buds of the cannabis plant. Alongside this is hashish, which is a resin gotten from the stem bud of the

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plant, and cannabis oil, an extract from the plant [10]. Marijuana use for recreational purposes has increased markedly worldwide, according to the World Drug Report for 2013 between 128 and 232 million people consumed cannabis for recreational purposes, especially among young people [11].

Few studies have been done on the effects of caffeine on the PFC. However; caffeine has been suggested to impact brain development by impeding the formation of important neural connections that occur during adolescence [12]. Studies have also reported that caffeine affects sleep patterns in children, and this in turn affects brain development [13]. A number of results have been reported on the effects of cannabis on the PFC. A study carried out in 2010 revealed that adolescent cannabis users have decreased right medial orbital prefrontal cortex volume when compared to non-users of cannabis; volume was also found to correlate with the age that consumption begun. A second study however, reported contradictory results, stating that there was no difference in the gray matter macrostructure between adolescent cannabis users and non-users [14]. As regards cognition, a recent study revealed memory deficits in young adult cannabis users, performing worse in immediate and delayed verbal memory when compared to control [14].

This investigation studied the effects of caffeine and *Cannabis sativa* ingestion on the memory and anxiety of experimental animals modelled after human uses of the substances using suitable neurobehavioural apparatus and methods.

II.   Materials And Method

Seventy two [n=72] juvenile Wistar rats were use for the experiment. They were distributed randomly into Groups A-F. Animals were house and handled in compliance with institutional animals care and use protocol standards. All substances were administered using oral gavages daily between 6:00 and 8:00 hours. Animals, based on research design were treated as follows:

Group A animals were the control; animals were only fed *ad libitum* during experiment
Group B animals were administered 100mg/kg body weight [high dose] of caffeine
Group C animals were administered 50mg/kg body weight [low dose] of caffeine
Groups D animals were administered 500 mg/kg body weight [high dose] of cannabis extract
Group E animals were administered 200mg/kg body weight [low dose] of cannabis
Group F animals were administered 50mg/kg body weight of caffeine plus 200mg/kg body weight of cannabis [caffeine and cannabis at low doses]

The neurobehavioural study was conducted after the 21 days of drug administration using the elevated plus maze (EPM) and Barnes maze.

EPM Procedure (Carola *et al*., 2002) [15].

The EPM is useful in measuring anxiety levels in the rats. The maze was placed in an isolated room away from noise, any form of scent and movements. The lighting in the room was also regulated, given that low intensity luminosity reduces open arm avoidance.

The animals were then brought into the room and allowed to acclimatize for about forty- five minutes in other to recover from the stress of being moved. Then, the maze was thoroughly clean with 70% ethanol, to remove any form of smell or dirt. Thereafter, the video camera was turned on and the first rat was placed in the center square of the maze, facing the open arm opposite to the camera. After five minutes of free exploration the rat was moved away and placed back in its home cage. Once again, the maze was cleaned with 70% ethanol removing all the urine and *fecal boli*. These steps were repeated until all the animals were tested The recorded videos were analysed by measuring entries into the closed arms and time spent in the closed arms and entries into the open arm and time spent in the open arm. Animals were subjected to proper handling and treatment to ensure accuracy of results [16, 17]

Barnes Maze Procedure (Sunyer *et al.,* 2007) [18]

Adaptation period**:** The rat was placed in a cylindrical black start chamber in the middle of the maze. The chamber was lifted after 10s, the buzzer was switched on and the rat was gently led to the escape box. Once the rat was inside the box, the buzzer was turned off and the rat was kept in the escape box for 2 minutes.

Spatial acquisition**:** Before the acquisition phase began, the maze was cleaned using 70% ethanol to avoid olfactory cues. The maze was also rotated around its central axis after each trail in order to control for possibly remaining odour cues. The rat was placed in the cylindrical black start chamber in the middle of the maze. After 10 seconds the buzzer was switched on and the mouse was allowed to explore the maze for 3 minutes. During these 3 minutes number of primary errors, total errors and primary latency were measured. The trial ended when the rat entered the goal tunnel or after the 3 minutes ended. The rat was kept in the escape box for 1 minute, and then placed in its cage until the next trail. The steps were repeated until the animals had the desired number of trials for that day.

Probe trial: On day 5, 24 h after the last training day, the probe trial was conducted. The animal was placed under the cylindrical black start chamber and after 10s the chamber was lifted, the buzzer was switched on and the rat was allowed to explore the maze. The rat was removed after a fixed interval of 90s. The probe trial is done in order to determine if the animal remembers where the target goal was located. Number of pokes (errors) in each hole and latency and path length to reach the virtually target hole are measured.

III. Results

Training: Mean Latency

The mean latency for Group A (control group) was 35.8±1.20. The mean latency for Group B (caffeine high dose) was significantly ($p<0.05$) higher than the control at 46.0±1.62. The mean latency for Group C (caffeine low dose) was significantly ($p<0.05$) higher than the control at 43.0±1.49. The mean latency for Group D (cannabis high dose) was significantly ($p<0.05$) higher than the control at 49.1±0.58. The mean latency for Group E (cannabis low dose) was significantly ($p<0.05$) higher than the control at 46.5±1.69. The mean latency for Group F (cannabis low dose + caffeine low dose) was significantly ($p<0.05$) higher than the control at 56.8±1.10.
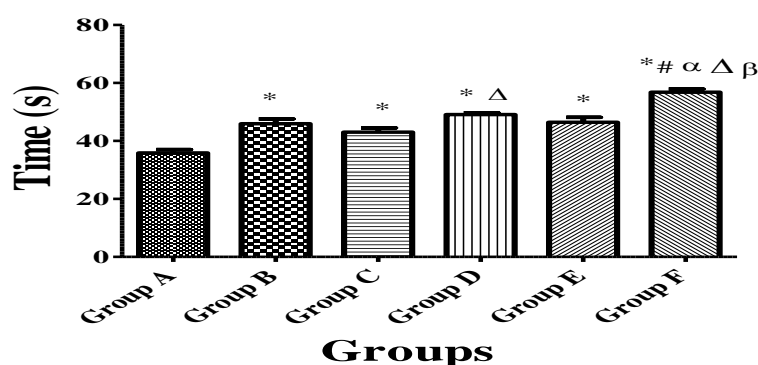
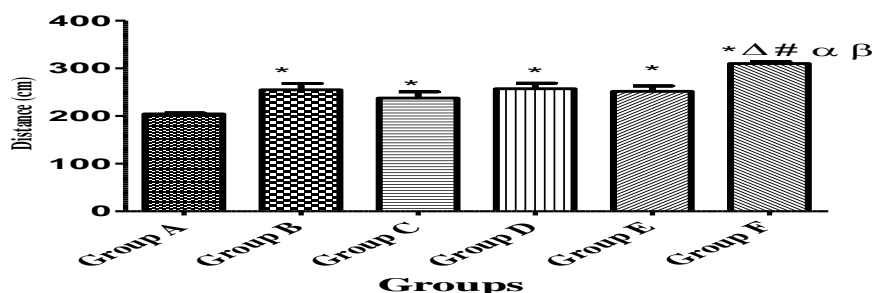**=P<0.05 when compared with Group A*
#=P<0.05 when compared with Group B
Δ=P<0.05 when compared with Group C
β=P<0.05 when compared with Group D
α=P<0.05 when compared with Group E

Figure 1: Bar chart showing the mean latency during training of control and treated groups after 21 days of treatment

Training: Mean Distance Covered

The mean distance for Group A (control group) was 204±1.65. The mean distance for Group B (caffeine high dose) was significantly ($p<0.05$) higher than the control at 253±11.10. The mean distance for Group C (caffeine low dose) was significantly ($p<0.05$) higher than the control at 237±10.60. The mean distance for Group D (cannabis high dose) was significantly ($p<0.05$) higher than the control at 248±9.82. The mean distance for Group E (cannabis low dose) was significantly ($p<0.05$) higher than the control at 248±9.82. The mean distance for Group F (cannabis low dose + caffeine low dose) was significantly ($p<0.05$) higher than the control at 310±3.09.

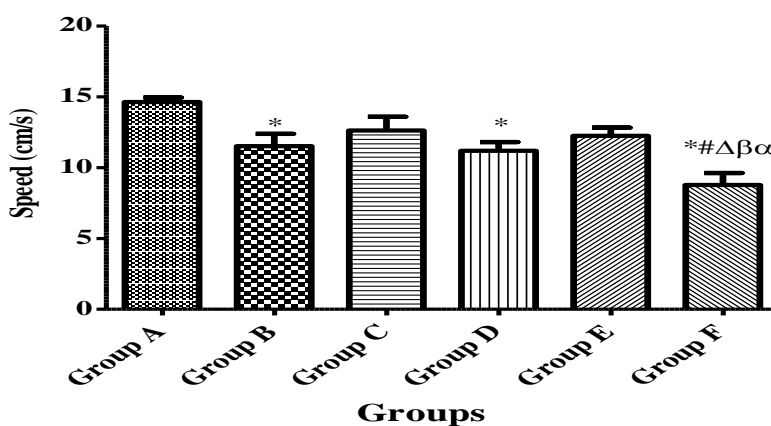**=P<0.05 when compared with Group A*
#=P<0.05 when compared with Group B

Δ=P<0.05 when compared with Group C
β=P<0.05 when compared with Group D
α=P<0.05 when compared with Group E

Figure 2: Bar chart showing the mean distance during training days of control and treated groups after 21 days treatment

Training: Mean Speed

The mean distance for Group A (control group) was 14.6±0.314. The mean distance for Group B (caffeine high dose) was significantly (p<0.05) higher than the control at 11.5±0.867. The mean distance for Group C (caffeine low dose) was significantly (p<0.05) higher than the control at 12.6±0.973. The mean distance for Group D (cannabis high dose) was significantly (p<0.05) higher than the control at 11.2±0.616. The mean distance for Group E (cannabis low dose) was significantly (p<0.05) higher than the control at 12.2±0.579. The mean distance for Group F (cannabis low dose + caffeine low dose) was significantly (p<0.05) higher than the control at 8.78±0.845.
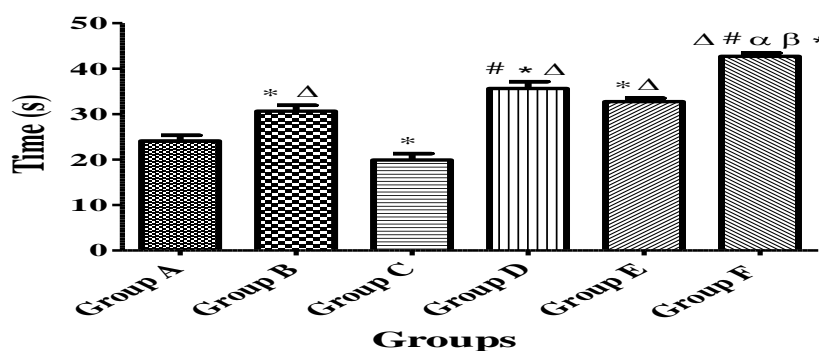

*=P<0.05 when compared with Group A
#=P<0.05 when compared with Group B
Δ=P<0.05 when compared with Group C
β=P<0.05 when compared with Group D
α=P<0.05 when compared with Group E

Figure 3: Bar chart showing the mean speed during training days of control and treated groups after 21 days of treatment

Probe Day: Latency

The latency value of the Group A (control group) was 24.0±1.21. The latency value of Group B (caffeine high dose) was significantly (p<0.05) higher than the control group at 30.7±1.30. The latency value of Group C (caffeine low dose) was significantly (p<0.05) lower than the control group at 19.9±1.39. The latency value of Group D (cannabis high dose) was significantly (p<0.05) higher than the control group at 35.7±1.50. The latency value of Group E (cannabis low dose) was significantly (p<0.05) higher than the control group at 32.8±0.73. The latency value of Group F (cannabis low dose + caffeine low dose) was significantly (p<0.05) higher than the control group at 42.7±0.73.

*=P<0.05 when compared with Group A

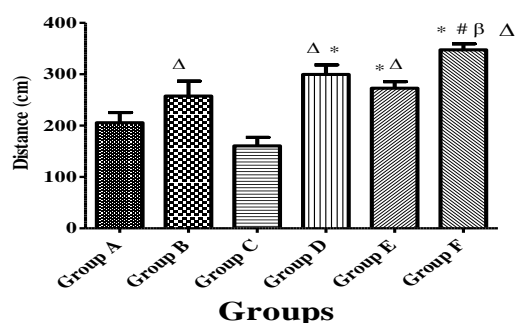*#=P<0.05 when compared with Group B*
Δ=P<0.05 when compared with Group C
β=P<0.05 when compared with Group D
α=P<0.05 when compared with Group E

Figure 4: Bar chart showing the latency during probe day of control and treated groups after 21 days of treatment

Probe Day: Distance

The distance of the Group A (control group) was 206±19.8. The distance of Group B (caffeine high dose) was significantly (p<0.05) higher than the control group at 257±29.1. The distance of Group C (caffeine low dose) was significantly (P<0.05) lower than the control group at 161±16.4. The distance of Group D (cannabis high dose) was significantly (p<0.05) higher than the control group at 300±18.5. The distance of Group E (cannabis low dose) was significantly (P<0.05) higher than the control group at 273±12.8. The distance of Group F (cannabis low dose + caffeine low dose) was significantly (p<0.05) higher than the control group at 347±11.9.

=P<0.05 when compared with Group A
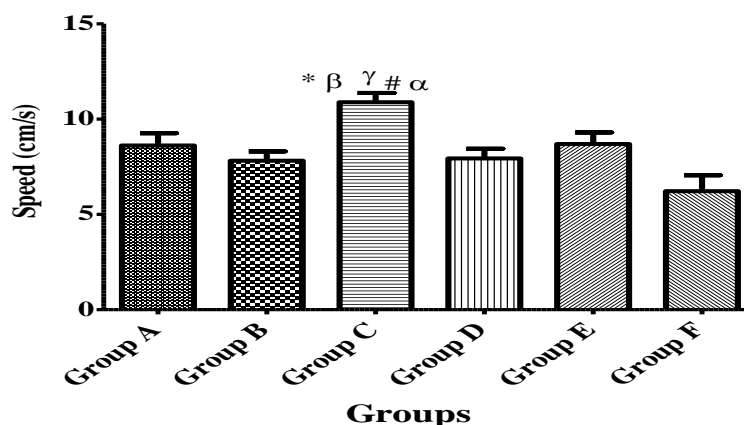*#=P<0.05 when compared with Group B*
Δ=P<0.05 when compared with Group C
β=P<0.05 when compared with Group D
α=P<0.05 when compared with Group E

Figure 5: Bar chart showing the distance covered during probe day of control and treated groups after 21 days of treatment

PROBE DAY: SPEED

The speed of the Group A (control group) was 8.6±0.63. The speed of Group B (caffeine high dose) was slightly lower than the control group at 7.8±0.49. The speed of Group C (caffeine low dose) was z higher than the control group at 10.9±0.49. The speed of Group D (cannabis high dose) was slightly lower than the control group at 7.9±0.51. The speed of Group E (cannabis low dose) was slightly higher than the control group at 8.7±0.61. The speed of Group F (cannabis low dose + caffeine low dose) was significantly (p<0.05) lower than the control group at 6.2±0.84.

=P<0.05 when compared with Group A
#=P<0.05 when compared with Group B
β=P<0.05 when compared with Group D

α=P<0.05 when compared with Group E
γ=P<0.005 when compared with Group F

Figure 6: Bar chart showing the speed during probe day of the control and treated groups after 21 days of treatment

Elevated Plus Maze (Epm)
Time Spent In Open Arm (Tsoa)

TSOA for Group A (control group) was 59.3±15.9. TSOA for Group B (caffeine high dose) was slightly lower than the control group at 42.0±12.0. TSOA for Group C (caffeine low dose) was slightly lower than the control group at 46.0±14.4. TSOA for Group D (cannabis high dose) was slightly lower than the control group at 55.7±26.8. TSOA for Group E (cannabis low dose) was slightly lower than the control group at 36.2±12.0. TSOA for Group F (cannabis low dose + caffeine low dose) was slightly lower than the control group at 23.2±6.24.
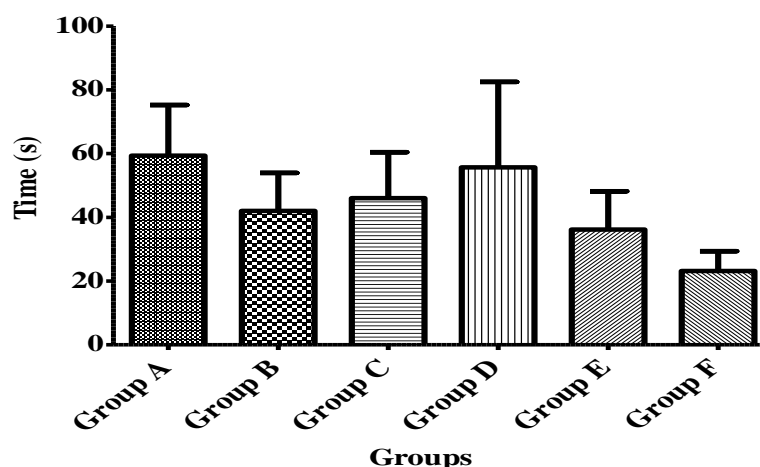

Figure 7: Bar chart showing the TSOA of control and treated groups after 21 days of treatment.

Time Spent In Closed Arm (Tsca)

TSCA for Group A (control group) was 241±15.9. TSCA for Group B (caffeine high dose) was slightly higher than the control group at 258±11.9. TSCA for Group C (caffeine low dose) was slightly higher than the control group at 253±14.0. TSCA for Group D (cannabis high dose) was slightly higher than the control group at 244±26.8. TSCA for Group E (cannabis low dose) was slightly higher than the control group at 264±12.0. TSCA for Group F (cannabis low dose + caffeine low dose) was slightly higher than the control group at 246±11.8.
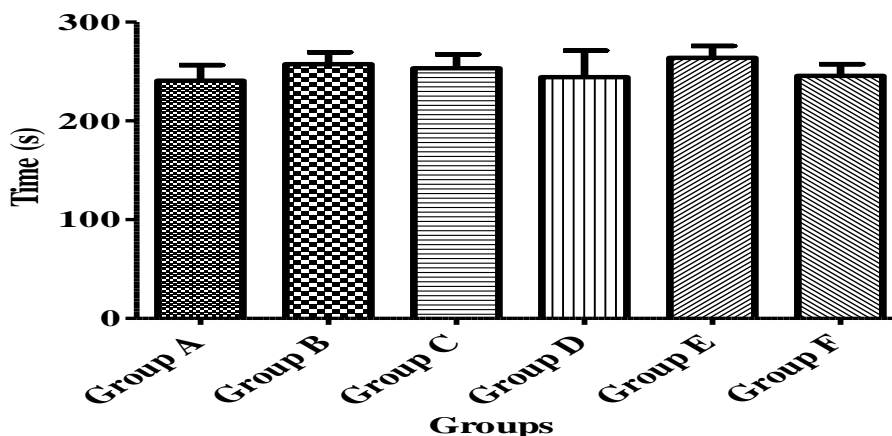

Figure 8: Bar chart showing the TSCA of control and treated groups after 21 days of treatment

Open Arm Entry (Oae)

OAE for Group A (control group) was 3.17±0.75. OAE for Group B (caffeine high dose) was slightly lower than the control group at 2.50±0.86. OAE for Group C (caffeine low dose) was slightly lower than the

control group at 2.17±0.87. OAE for Group D (cannabis high dose) was slightly lower than the control group at 1.33±0.67. OAE for Group E (cannabis low dose) was slightly lower than the control group at 2.17±0.95. OAE for Group F (cannabis low dose + caffeine low dose) was slightly lower than the control group at 0.17±0.17.
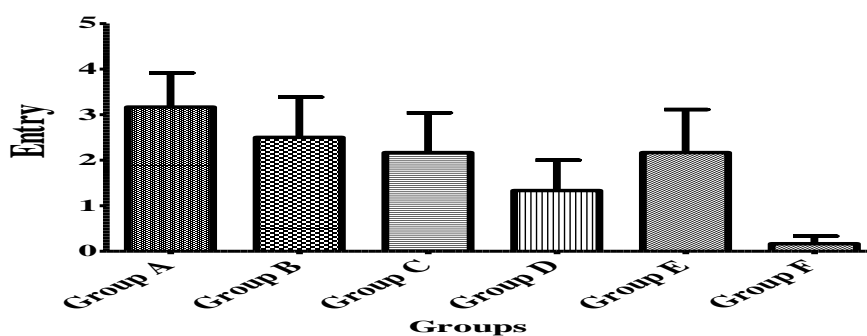

Figure 9: Bar chart showing the OAE of control and treated groups after 21 days of treatment

Closed Arm Entry (Cae)

CAE for Group A (control group) was 3.83±0.70. CAE for Group B (caffeine high dose) was slightly lower than the control group at 3.50±0.89. CAE for Group C (caffeine low dose) was slightly lower than the control group at 3.17±0.87. CAE for Group D (cannabis high dose) was slightly lower than the control group at 2.33±0.67. CAE for Group E (cannabis low dose) was slightly lower than the control group at 3.17±0.95. CAE for Group F (cannabis low dose + caffeine low dose) was slightly lower than the control group at 1.17±0.17.
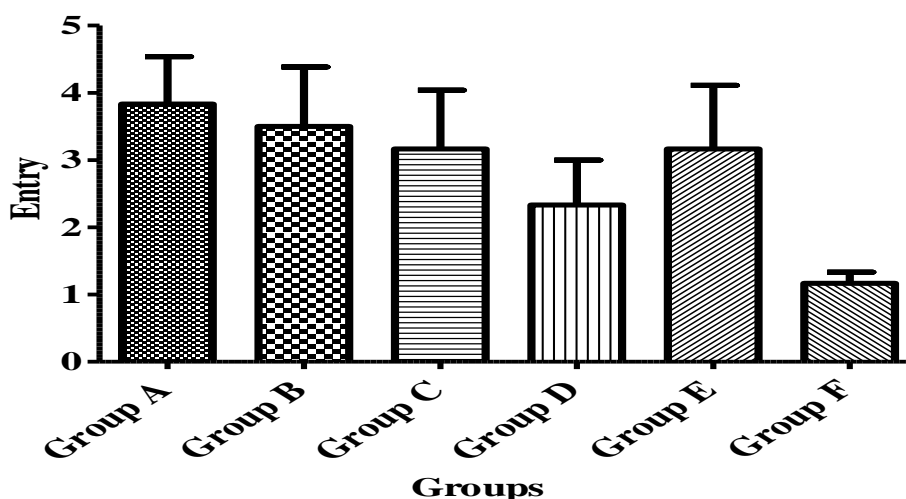

Figure 10: Bar chart showing the CAE of control and treated groups after 21 days of treatment

IV.   Discussion

Animal models, especially rodents are reliably used in neuroscience, pharmacology, psychology and other forms of behavioural and drug testing research. To this end, the results in this study would provide useful information that can serve as potential indication for learning and behavioural aberrations in users of caffeine and cannabis. Latency increased in all the groups of animals that were administered the psychoactive agents. The higher doses- for both caffeine and cannabis also affected values of latency than the low doses. Increased latency generally is associated with reduced memory efficiency. Hence, both agents altered memory efficiency, and the higher doses produced the more significant effects. More specifically, the memory here is more closely associated with the short term memory, hence, these results aggress with a number of reports that suggested that these agents might negatively influence the short term memory [19, 20] . In addition, the combination of both

agents produced aggravated increase in latency, thus indicating that it was grossly negatively influenced relative to the control. Generally, caffeine and cannabis negatively affected memory latency in the animals, with cannabis. Learning or training did not significantly change the trend in the animals' memory recall speed as measured in latency.

Animals generally covered greater distances when they were administered caffeine or cannabis. Higher dose of caffeine and cannabis produced increases in activities levels or movement rates. Combined regimen produced very high movement rates hence, quite high distance covered. These substances no doubt increased activities rates and movements; hence they are often adjudged to elevate performances rates. On the other hand, longer distance travelled before locating the escape hole suggested reduction in learning cum memory and this accompanies the increases in movement rates. In other words, activities increased at the expense of cognition and memory formation and or recall. After the training series, the treated groups of animals still travelled greater distance before locating the escape hole, which implies that the training regimen did not generally alter their learning and recall abilities. It also suggests that caffeine and cannabis effects of the brain functions fundamentally affected learning and recall. Speed generally reduced considerably when the agents were administered. Effects of caffeine and cannabis are seen to be persistent with respect to the speed of movement even after training. Previously, Cannabis use in the short term have been associated with distorted perception while long-term use cause changes in attitude and personality such as ability to carry out long-term plans, a sense of apathy, decreased attention to appearance and behaviour, and decreased ability to concentrate for long periods of time [21]. Caffeine improves performance but influenced short term and working memory largely but not always and absolutely in a negative way [22-24]

The result from the Barnes maze study revealed increased latency in the animals treated with cannabis at both high and low doses. This supports a study done by Battisti *et al.,* [19] which reports that cannabis users had altered memory-related brain activation that resulted in poorer neural efficiency, which is associated with deficits in memory recall. According to the study this was mediated by CB1Rs expression on GABAergic interneurons through glutaminergic mechanism. The report of Shrivasta *et al*., [20] showed a dose related effect on memory and general cognition. This was also observed in the study, as animals treated with high dose of cannabis had a higher latency than those treated with a lower dose, and suggests that cannabis at a higher dose might have a more pronounced effect on cognition. It was also observed that caffeine influenced latency the less at a lower dose, while at a higher dose latency was impaired when compared to the control group. This supports a report by Nehlig, 2010 [25], that caffeine at a low dose can improve learning, and working memory to an extent. At a high dose however, it was reported to impede memory due to an increase in anxiety and nervousness. In combination however it was observed that latency was severely impaired when compared to the control and other treated groups. This suggests that caffeine and cannabis in combination can increase memory impairment.

Relative to the Control the treated groups had higher values for the distance covered, which would suggest increased physical performance or activities. The fact that relatively moderate and lower dose of caffeine increased speed agree with previous reports that caffeine moderate use improve performance. Excessive use however reduces produced negative effects relative to speed or performance. This is also applicable to cannabis as being used.

Substances that produce anxiety in human have been found to significantly reduced the percentage of entries into, and time spent on, the open arms [26]. These were found to include yohimbine, pentylenetetrazole, caffeine and amphetamine [26]. In this research, caffeine at the higher dose reduced the TSOA relative to control and this is being interested as a sign of anxiety in the animals. The TSOA when the lower caffeine dose was used was also lower than the Control Group, however it was higher than when the caffeine higher dose was used. These observations imply that caffeine could produce anxiety tendencies which would increase at the higher dose. It is however important to note that that the differences were not statistically significant relative to the control. Cannabis also lowered the TSOA and the lower dose produced greater effects. The combination of both substances produced much lowering of the TSOA level showing that the combination of both could be synergistic in inducing anxiety. The TSCA was not tangibly influenced in the treated groups relative to the control; there are however very slight increase relative to the Control Group.

Taken together, TSOA and TSCA levels suggest that caffeine and cannabis could produce anxiety tendencies, with caffeine being implicated the more and the effects are dose dependent. Both cannabis and caffeine reduced the OAE values in the Animal Groups relative to the control Group. The difference was greater when both agents were administered to further buttress the fact their combination might make users quite prone

to anxiety tendencies. The CAE values were also reduced relative to the control. Furthermore, there is evidence from these findings that caffeine could influence mood, behaviour and mental performance [27-32]; in addition, this effects are not just acute but could be sustained for a period even after the stoppage of use.

The general effects of caffeine on metal performance has been rated positive in moderate consumers; but could produce behavioural aberrations in cases of abuse or overdose [3]. Caffeine was specifically dubbed a drug that induced anxiety; and it was further suggested to produce lasting effects on neural performances on the brain when there was exposure during pregnancy [27, 28]. It therefore alters basal neural activities [29-33]. The current observations support the above previous reports.

V. Conclusion

Results from neurobehavioural study revealed that at low doses caffeine can improve cognition, while at high doses cognition is impaired. Cannabis however impairs memory and cognition at both high and low doses, and a combination of both substances can significantly impair memory and cognition. The administration of the substances did not reveal significant effects on anxiety, statistically; tendencies were however noted in the trends of values and parameters.

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