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Effect of Aqueous Extract of Cannabis Sativa Leaf on the Motor Coordination Using the Hanging Wire and Open Field Neurobehavioural Tests in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cannabis sativa is a commonly abused drug especially among younger people in society. The cerebellum is located at the back of the brain, immediately inferior to the occipital and temporal lobes within the posterior cranial fossa. The study was designed to show the effect of aqueous leave extract of cannabis sativa on the performance of male Wistar rats in the hanging wire and open field neurobehavioural tests. A total of 40 Wistar rats were used and grouped into five groups. Group I received distilled water for 28 days. Group II, III, IV and V served as the low, high, low dose recovery and high dose recovery group respectively. Group II were administered with 10mg/kg body weight of cannabis sativa leave aqueous extract for 28 days. Group III were administered with 20mg/kg body weight of cannabis sativa leave aqueous extract for 28 days.

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Group IV was administered with 10mg/kg body weight of cannabis sativa leave aqueous extract for 28days and were allowed for further 28 days without any administration while group V received 20mg/kg body weight of cannabis sativa for 28 days and were allowed for further 28days without administration. Group IV and V represent the recovery group. Group I, II and III were sacrificed a day after their last intubation. The result of the study showed that the administration of cannabis Sativa did not significantly change the outcome of the open field test at the final stage compared to the initial but group E showed significant decrease in hanging time following exposure to *cannabis sativa*. We can therefore conclude that administration of cannabis sativa at 10mg/kg and 20mg/kg for 28 days did not adversely affect rats performance in the neurobehavioural tests carried out.

Keywords: Cannabis sativa; hanging wire test; open field test; motor coordination.

1. INTRODUCTION

"Cannabis sativa is a commonly abused plant due to its high content of the psychoactive compound" [1] "Though cannabis has been used for medical purposes due to its antioxidant, anticonvulsant, anti-inflammatory, and neuroprotective properties, its adverse consequences should not be underestimated" [2] (da Silva *et al*., 2018).

"Cannabis sativa is an annual herbaceous flowering plant indigenous to Eastern Asia, but now of cosmopolitan distribution due to widespread cultivation. It has been cultivated throughout recorded history, used as a source of industrial fiber, seed oil, food, recreation, religious and spiritual moods and medicine. Each part of the plant is harvested differently, depending on the purpose of its use. The flowers of *Cannabis sativa* are short-day flowering plants, with staminate (male) plants usually taller and less robust than pistillate (female) plants" (United Cannabis Seeds 2021). "The flowers of the female plant are arranged in racemes and can produce hundreds of seeds. Male plants shed their pollen and die several weeks prior to seed ripening on the female plants. Under typical conditions with a light period of 12 to 14 hours, both sexes are produced in equal numbers because of heritable X and Y chromosomes" (Clark and Merlin, 2013). "Although genetic factors dispose a plant to become male or female, environmental factors including the diurnal light cycle can alter sexual expression" (Schaffner, 2020).

"Understanding potential toxicity is crucial for safety considerations, especially if the plant extract is used in traditional medicine or as a dietary supplement. Cannabis use is common among adolescents and young adults, but the long-term consequences of such use are a topic of debate. Cannabis use typically starts during early adolescence and peaks when users are in their mid-20s" [3]. "In a large US survey, 7.4% of adolescents reported cannabis use during the past month and 13.1% during the past year" [4]. "Cannabis use can have adverse health effects, including increased risks for lung, cardiovascular, and periodontal diseases" (Gordon et al. 2013; [5]. "Its effects on development of cognitive and affective dysfunction, however, have been less conclusive. An initial study reported that cannabis use, particularly during adolescence, contributes to a lasting neurocognitive decline including an 8-point drop in IQ from childhood to adulthood" [6]. "More recent studies, however, do not support this conclusion. For example, cannabis users perform worse on cognitive tests than non-users, but the performance of users is comparable to their non-using twins" [7,32-36].

"Receptors for THC and other cannabinoid compounds are present in the brain, especially in the frontal cortex, basal ganglia, cerebellum, and limbic regions. Cannabinoid action in the basal ganglia and cerebellum probably account for the effect on psychomotor control" (John, 2003). "Sensorimotor signals can be used to monitor and refine ongoing movements, while generalized changes in behavioral state, including arousal and levels of locomotor activity influence sensory processing and perception" (McGinley et al., 2015; Schneider and Mooney, 2015; Vinck et al., 2015; Pakan et al., 2016). "Both locomotor activity and arousal modulate delay eyeblink conditioning, a form of cerebellum-dependent associative learning" [8].

"Cannabinoids are profound modulators of behavioral state, across species" (Mackie, 2007); [9,10]. "Acutely, cannabis and THC produce a range of effects on several neurocognitive and pharmacological systems. These include effects

on executive, emotional, reward and memory processing via direct interactions with the endocannabinoid system and indirect effects on the glutamatergic, GABAergic and dopaminergic systems" [11]. Blázquez *et al*, [12], found that "D 9 -tetrahydrocannabinol, the psychoactive ingredient of cannabis, disrupts autophagy selectively in the striatum, a brain area that controls motor behavior, both in vitro and in vivo". "Boosting autophagy, either pharmacologically (with temsirolimus) or by dietary intervention (with trehalose), rescued the D 9 -tetrahydrocannabinol-induced impairment of motor coordination in mice. Taken together, these findings identify inhibition of autophagy as an unprecedented mechanistic link between cannabinoids and motor performance and suggest that activators of autophagy might be considered as potential therapeutic tools to treat specific cannabinoid-evoked behavioural alterations". [13,37-45].

"Of concern are the effects of cannabis use on decision-making, especially when it involves risk-taking. Self-report questionnaires and laboratory risk-taking tasks have demonstrated differences between cannabis users and non-users" (Burggren *et al* [14]. "Adolescence and teens who engage in heavy marijuana use often show disadvantages in neurocognitive performance, macrostructural and microstructural brain development, and alterations in brain functioning. It remains unclear whether such disadvantages reflect pre-existing differences that lead to increased substances use and further changes in brain architecture and behavioral outcomes" [15,46-51]. "Adult studies of marijuana use often find subtle decreases in performance compared to controls in cognitive domains such as attention, memory, and processing speed; such effects have been discussed as transient in the literature given limited group differences after prolonged abstinence from marijuana" (Grant *et al*, 2003; Pope *et al*, 2001). "Ongoing cognitive development in the domains of memory and executive functioning, and particularly in specialized functions like cognitive control, is not only tightly associated with adolescence and neocortical tissue maturation, but is likely to have implications for school performance and engagement in risk/reward behaviors" (Casey *et al*,2008).

"One of the earliest studies on the effects of marijuana on adolescent neurocognitive development evaluated verbal and nonverbal memory performance in cannabis-dependent adolescents (ages 14 to 16) compared to matched controls" (Schwartz *et al*., 1989). Schwartz and colleagues found that "short term memory impairment persisted after six weeks of monitored abstinence". In contrast, Teichner and colleagues (2000) found "no relationship between marijuana use severity and cognitive performance among cognitively impaired and unimpaired adolescents referred for drug treatment".

"Takagi and colleagues found that cannabis users (ages 13–24) performed worse on measures of immediate and delayed verbal memory compared to community controls. In a similar study by this team of investigators, no differences between cannabis users and community controls were found on measures of executive functioning" [16,17,52-56]. Similarly, Gonzalez and colleagues (2012) found "differences on immediate and delayed recall among young adult cannabis users (approximately age 20) compared to non-using controls, however no differences were observed on measures of impulsivity. Despite no group differences on impulsivity, the authors found that worse performance on a decision-making task was related to more cannabis use disorder symptoms". Solowij and colleagues looked at "181 adolescents (ages 16–20) and found that cannabis users performed worse on learning and recall, and poorer performance was related to severity, frequency, and age of initiation of cannabis use". "Chronic cannabis use has also been associated with reduced gray matter volumes and memory deficits in cohorts comprising both PWH and seronegative controls" [18- 21,57-62]. Recent data from a group suggest that a lifetime history of cannabis use disorders lowers the odds of neurocognitive impairment in PWH [22] and may even promote "youthful" and resilient neurocognitive abilities among adults aging with HIV (Saloner *et al*., 2019b).

"Increased ambulation and exploratory activities as well as decreased immobilisation on paradigms such as open field maze, hole board maze and white and black box indicated intact motor system and low anxiety. While decrease in activities suggest an expression of anxiogenic state" [23]. "Rodents display rearing behaviour by standing on their hind limbs with the forelimbs raised into the air or on the wall of the maze. This behaviour allows the animals to evaluate the potential danger of the immediate space and how to find an escape-route. Increase in this

behaviour indicated fear, agitation and anxiety to leave the maze. Cannabis-diet decreased rearing behaviour in all groups fed except for group fed low quantity cannabis-diet (1 % cannabis-diet). This may suggest that increased cannabis consumption may reduce anxiety, even though the association between rearing behaviour and anxiety remains controvertible" [24] Animal Research International (2019). Cannabis-diet fed groups displayed slightly higher locomotor/exploratory activity which is consistent with low levels of anxiety. Our observation was in contrast with the findings of Okon *et al*. [25] which reported "a dose-dependent decrease in locomotion and exploratory behaviour. Findings in the light/dark transition box showed that cannabis-diet fed mice demonstrated a striking and strong affinity for well illuminated open spaces suggesting that the C. sativa has anxiolytic effect. Rodents naturally associate more with dark spaces as it offers hiding spots and safety. Increased attraction to dark space than light space indicates anxiogenic condition". "In this study, the cannabis-treated mice had exploratory activity not significantly different from the control, which is in contrast with a previous report" [26].

"The neurobehavioral assessments conducted following the administration of *Cannabis sativa* showed that there was no significant difference in freezing and center square entries, while there was a significant difference in the number of lines crossed when groups B and D were compared to the control. Rearing frequency in group C was also relatively different from the control. The changes observed in the decline in the number of lines cross suggests the role of cannabis in influencing locomotor activity and also the increase in rearing frequency directly points to the role of cannabis in initiating anxiety-like behaviors. Y-Maze test was used to test for cognition, spatial learning, and memory assessment in rats exposed to *Cannabis sativa.* There was no significant difference in the total arm entries and spontaneous alteration across all groups, but there was an observable increase in group C when compared with control" [13,63-67].

"Following treatment with Cannabis for 21 days after which behavioral indices were assessed on the 22nd day revealed a significant reduction in the number of lines crossed in groups B (10 mg/kg of *Cannabis sativa*) and D (100 mg/kg of *Cannabis sativa*) when compared to the control and significantly increased rearing frequency in group C (50 mg/kg of *Cannabis sativa*) when compared with the control, while there was no significant difference in freezing and center square entries" [13]. "There is a reduction in locomotor activity (line crossing), strong forelimb support (hanging wire) and slower vestibular response (negative geotaxis value) in the cannabis exposed rats (group B) compared to the control group A, group C values showed a recovery of these deficits, except for the fore limb support (27.81 ± 19.13) where they showed greater strength" [27].

"In the large open field test, there were no main effects or interactions involving smoke exposure condition on total distance traveled, distance traveled in the border zone, distance traveled in the center zone, number of center zone entries, or time spent in the center zone" [28]. "There were no differences in the total number of horizontal or vertical beam breaks between the air-control rats and the cannabis rats before the onset of the smoke exposure sessions.In both groups, there was an *effect of time* on horizontal and vertical beam breaks" [29]. The cannabis rats and air-control rats received vehicle or rimonabant and somatic withdrawal signs were recorded.

Bruijnzeel et al., [28]. reported "an increase in weight across all groups of rats, males weighed more than the females. They noted a significant increase in body weight across smoke exposure days and the magnitude of this increase was significantly greater in males than females". "The rats were exposed to cannabis smoke or air for 8 weeks and during this period both groups gained the same amount of weigh" [29].

The cerebellum is a crucial part of the brain responsible for motor coordination, balance, and cognitive functions. Researching the effects of cannabis leaf extract on the cerebellum contributes to our understanding of neurobiology and can provide insights into how various substances affect brain function [68-73]. If the plant extract is being considered for use in pharmaceuticals or herbal remedies, research on its effects on the cerebellum is essential for regulatory approval and ensuring consumer safety [74-79]. Studying the effects of leaf extract on the cerebellum of rats is significant because it contributes to our understanding of neuroscience, may reveal potential therapeutic benefits or risks associated with the extract, and can inform regulatory decisions regarding its use in medicine or other applications.

2. MATERIALS USED IN THE STUDY

Materials used includes Adult Wistar rats, Cannabis Sativa leaves, distilled water, well-ventilated cages, weighing balance, syringes, dissecting kit, specimen containers, cotton wool, methylated spirit, saw dust which will serve as the animal bedding will be used for the study.

Sourcing and handling of *Cannabis sativa:* Fresh leaves of Cannabis sativa was obtained from the locals and authenticated at botany department, Nnamdi Azikiwe University, Awka.

Sourcing and handling of wistar rats: The rats were obtained from the animal house of Physiology department, Nnamdi Azikiwe University, Nnewi campus. The animals were housed within the standard facilities of a well-ventilated animal house and maintained on a standard of rodent pallets and water ad libitum under standard laboratory conditions of lighting and moderate temperature.

Lethal dose (LD50) of cannabis sativa determination: Lethal Dose (LD50) of *Cannabis Sativa* was carried out according to Lorke's method.

Experimental design: A total of 40 adult Wistar Rats weighing between 180g-200g was used for this study. Fifteen (15 rats) was used for LD50 determination and 25 experimental rats for the study proper with 5 rats per group.

Group I: received distilled water for 28days; Group II: received low dose for 28 days; Group III: received high dose for 28 days; Group IV: received low dose for 28 days and allowed a recovery period of 28 days; Group V: received high dose for 28 days and allowed a recovery period of 28 days.

Animal sacrifice and tissue collection technique: At the end of the administration period, the rats were exposed to open field test and hanging wire tests. Animals were then refereed for other studies.

Statistical analysis: The data were presented as Mean ± SEM of 5 rats in each group, subjected to one-way Anova test using Turkey's post-test to show differences between the mean values of all groups. A value of $p < 0.05$ will be interpreted as statistically significant.

3. RESULTS AND DISCUSSION OF FINDINGS

Result of open field test: The result of open field test presented in Table 1 evaluated 6 different parameters. Summarily, administration of CS did not significantly change the out-come of the open field test at the final stage compared to the initial. On the average rearing was more frequent in the experimental groups compared to the control, although the difference was not statistically significant.

The same goes for grooming, rats had a statistically non-significant grooming tendencies at FINAL following CS administration compared to the initial.

Line arising was non-significantly less frequent at the final state compared to the initial stage. This implies the animals moved less within the chamber. This is corroborated by the higher duration of freezing recorded at the final stage compared to the initial stage.

Urination and defecation were relatively similar for different groups of rats at the final stage compared to the initial, except in the control group A (faeces) and experimental groups B & C (Urination) with significantly higher frequency of defecation and urination respectively.

Amaza et al (2013) observed physical changes which include hyperactivity, increase in appetite as well as increase in weight, this sis due to the fact that endocannabinoids in the hypothalamus activate cannabinoids receptors that are responsible for maintaining food intake and also cannabis Sativa has acute appetite enhancing effects, thereby increasing body weight in experiment model in group III except in group II Wistar rats. "The result for open field test in this study shows cannabis Sativa did not significantly change the outcome of the open field test at the final stage compared to the initial. This is similar to a study conducted on prospective memory evaluation for undergraduate between the ages of 18-24year old, where no difference in self-reported prospective memory was identified, cannabis users did recall fewer location action combination during the video" [30].

Animal moved less within the chamber of line crossing. This is corroborated by the higher duration of freezing recorded at the final stage compared to the initial stage [80-87]. This report is in terms with Rice and Colleague who observed decreased immobilization on

paradigms such as open field maze, hoic board maze and white and black box indicated intact motor system and low anxiety. The decrease in activities suggest an expression of anxiogenic state [23]. Ingestion of whole cannabis plant may not adversely influence neurobehavioral patterns in mice. There were no correlation between the mild change in behavioral pattern and oxidative stress differentials in mice that consumed cannabis within the study period, this report is very consistent generally with the report of this study that the administration of CS did not significantly change the outcome of the open field test and hanging wire at the final stage compared to the initial stage and similarly oxidative stress report (Akinola et al 2019).

Yinka and friends in 2023 accessed behavioral indices of cannabis administration for 21days their results revealed a significant reduction in the number of line crossed in 10mg/kg which is in conformity with our study which we reported line crossing to be non-significantly less at the final stage when compared to the final. Yinka et al [13] Osinubi et.al. [27] also reported reduction in locomotive activity which agrees to this study. In the same study which coincides with this study, Yinka reported an increased rearing frequency in model group, no difference in freezing when compared to control. This study reported more rearing as more frequent in model group when compared to control, and animal move less within the chamber which corroborate high duration of freezing at the final stage [13].

3.1 Result of Hanging Wire Neurobehavioural Test

In this study, the result of hanging wire test shows that rats spent significantly different time in the final stage of the experiment compared to

Table 1. List of parameters used for Urination and defecation

Parameter	Group	Initial	Final	P value
Rearing	A	7.2 ± 1.92	9.8 ± 3.49	0.281
	B	7.2 ± 1.83	9.0 ± 2.23	0.286
	C	6.6 ± 1.14	10.4 ± 4.15	0.149
	D	16.6 ± 7.40	7.0 ± 2.23	0.033
	E	7.2 ± 5.80	7.2 ± 3.83	1.000
Grooming	A	1.0 ± 0.71	1.2 ± 0.45	0.704
	B	0.6 ± 0.55	1.0 ± 1.22	0.477
	C	0.8 ± 0.45	1.0 ± 0.71	0.374
	D	0.2 ± 0.45	1.6 ± 1.14	0.108
	E	0.4 ± 0.55	1.0 ± 0.22	0.208
Line crossing	A	39.2 ± 8.53	24.4 ± 7.63	0.077
	B	27.2 ± 8.58	25.0 ± 11.53	0.727
	C	26.8 ± 6.14	30.4 ± 3.36	0.278
	D	33.4±10.09	29.8 ± 7.56	0.530
	E	32.8 ± 9.47	32.6 ± 8.38	0.976
Faeces	A	1.2 ± 1.30	4.8 ± 1.92	0.041
	B	2.80 ± 2.28	1.80 ± 1.30	0.546
	C	1.80 ± 1.64	2.40 ± 1.95	0.634
	D	4.2 ± 1.64	2.2 ± 0.49	0.389
	E	2.2 ± 2.05	0.4 ± 0.89	0.088
Urine	A	1.8 ± 1.79	1.8 ± 0.84	1.000
	B	4.4 ± 2.07	2.0 ± 1.00	0.024
	C	4.8 ± 1.09	1.4 ± 1.14	0.000
	D	2.6 ± 2.61	4.2 ± 5.21	0.599
	E	1.0 ± 0.41	3.0 ± 1.87	0.089
Freezing	A	41.2 ± 12.83	50.8 ± 12.99	0.311
	B	37.2 ± 17.14	61.2 ± 39.36	0.291
	C	29.6 ± 9.81	20.4 ± 2.60	0.126
	D	37.8 ± 17.37	39.8 ± 9.68	0.867
	E	35.6 ± 16.09	54.8 ± 11.37	0.052

2. Result of hanging wire test

Group	Initial	Final	P value
A	34.80 ± 2.95	38.75 ± 37.51	0.267
B	89.00 ± 34.95	45.60 ± 55.09	0.088
C	72.6 ± 66.13	90.2 ± 59.73	0.335
D	60.00 ± 65.96	39.20 ± 28.54	0.195
E	102.40 ± 30.22	19.60 ± 15.85	0.000

Results are presented as Mean ± SD of 5 rate in each group. P< 0.05 is considered statistically significant.

the initial stage of the experiment, this report is in line with Osinubi and colleagues [31] work where rats have higher hanging wire value compared to the initial.

4. CONCLUSION

The administration of cannabis Sativa did not significantly change the outcome of the open field test at the final stage compared to the initial. Same was observed for the hanging wire test except for group E which showed significant decrease in hanging time following exposure to *cannabis sativa*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance was sought and obtained from the Research Ethics Committee of the Faculty of Basic Health Sciences, Nnamdi Azikiwe University Awka, Anambra State Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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