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Effect of Aqueous Extract of *Cannabis sativa* Leaf on the Oxidative Stress Markers in the Brain of 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 A received distilled water for 28 days. Group B, C, D and E served as the low, high, low dose recovery and high dose recovery group respectively. Group B were administered with 10mg/kg body weight of *Cannabis sativa* leave aqueous extract for 28 days. Group C were administered with 20mg/kg body weight of *Cannabis sativa* leave aqueous extract for 28 days. Group D was

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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 E received 20mg/kg body weight of *Cannabis sativa* for 28 days and were allowed for further 28days without administration. Groups D and E represent the recovery groups. Group A, B and C were sacrificed a day after their last intubation. The result of the study showed that administration of *Cannabis sativa* led to a non-significant increase in MDA and a corresponding significant reduction in SOD and CAT levels in the experimental groups compared to the control group A. This is a pointer to the presence oxidative stress. It can therefore be concluded that there were dose and time dependent toxic effects of *Cannabis sativa* in the model animals.

Keywords: Cannabis sativa; oxidative stress; malondialdehyde; superoxide dismutase; catalase.

1. INTRODUCTION

The high content of psychoactive compounds in *Cannabis sativa* leads to its common abuse [1]. The medical use of cannabis is attributed to its antioxidant, anticonvulsant, anti-inflammatory, and neuroprotective properties, but its adverse effects should not be taken lightly [2] da Silva et al., 2018).

Cannabis sativa is an annual herbaceous flowering plant that originated in Eastern Asia and now has a worldwide distribution due to its widespread cultivation. It has been grown throughout the course of history, utilized for industrial fiber, seed oil, food, recreation, religious and spiritual practices, and medicinal purposes. Harvesting each part of the plant is done differently, depending on the purpose of its use. *Cannabis sativa* has flowers that bloom during short daylight hours, with staminate (male) plants being generally taller and less sturdy than pistillate (female) plants (United Cannabis Seeds 2021). The flowers of the female plant are grouped into racemes and can yield several seeds. The pollen from male plants is shed and dies a few weeks before the seeds ripen on female plants [27-34]. Heritable X and Y chromosomes ensure that both sexes are produced in equal numbers when light is present for 12 to 14 hours under typical conditions (Clark and Merlin, 2013). Even though genetic factors are the main factor in determining whether a plant becomes male or female, environmental factors, like the diurnal light cycle, can have an impact on sexual expression [3].

Safety considerations hinge on comprehending potential toxicity, particularly when the plant extract is utilized in traditional medicine or as a dietary supplement. The use of cannabis is prevalent among teens and young adults, but the long-term ramifications of doing so are a matter of contention. The onset of cannabis consumption generally occurs during early adolescence and increases in the mid-20s [4]. According to Azofeifa 2016, a survey conducted in the United States found that 7.4% of teenagers had reported using cannabis in the past month and 13.1% had done so in the past year. The use of cannabis can lead to negative health impacts, such as increased chances of developing lung, cardiovascular, and periodontal diseases (Gordon et al. 2013; [5]. There has been a lack of conclusive evidence about its influence on the development of cognitive and affective dysfunction. An initial study indicated that cannabis usage, particularly while in adolescence, leads to a lasting decline in neurocognition, which can result in an 8-point drop in IQ between childhood and adulthood [6]. However, this conclusion is not supported by recent studies. Cannabis users, for instance, exhibit a lower performance on cognitive tests than non-users, but their scores are comparable to their non-using twins [7,35-40]. The brain is home to receptors for THC and other cannabinoid compounds, with concentrations in the frontal cortex, basal ganglia, cerebellum, and limbic regions. Cannabinoid activity in the basal ganglia and cerebellum is believed to be responsible for the influence on psychomotor control (John, 2003). Monitoring and refining ongoing movements can be achieved through sensorymotor signals, while changes in behavioral state, such as arousal and locomotor activity, have an impact on sensory processing and perception (McGinley, Schneider, and Mooney, 2015; Vinck, et al., 2015; Pakan, et al., 2016). Locomotor activity and arousal are implicated in modulating delayed eyeblink conditioning, which is a form of associative learning that involves the cerebellum [8].

Behavioral state across species is profoundly influenced by cannabinoids (Mackie, 2007; [9,10]. In a short period of time, cannabis and THC have a variety of effects on various

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] reported that D9-tetrahydrocannabinol, which is responsible for the psychoactive properties of cannabis, causes autophagy to be disrupted specifically in the striatum, which is responsible for controlling motor behavior, both in vitro and in vivo. In mice, D 9-tetrahydrocannabinol-related impairment of motor coordination can be rehabilitated by increasing autophagy by either pharmacologically (with temsirolimus) or dietary intervention (with trehalose). These findings indicate that inhibition of autophagy is a unique mechanistic link between cannabinoid use and motor performance. Additionally, activators of autophagy could be utilized as potential therapeutic tools to address specific behavioral changes caused by cannabinoid use [41-46].

The influence of cannabis use on decision-making, particularly when it comes to taking risks, is a matter of concern. Differentiation between cannabis users and non-users has been observed by self-report questionnaires and laboratory risk-taking tasks [13,75-78]. Neurocognitive performance, macrostructural and microstructural brain development, and alterations in brain functioning are frequently exhibited by adolescents and teens who engage in heavy marijuana use. It is unclear if these disadvantages are due to differences that have already been present, leading to an increase in substance use and more changes in brain architecture and behavioral [14,47-56]. 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; [15]. The development of cognitive functions in memory and executive functioning, particularly in specialized functions such as cognitive control, is not only closely linked to adolescence and neocortical tissue maturation, but it also has potential to impact school performance and participation in risk/reward behaviors (Casey et al,2008).

Schwartz et al. (1989) conducted a study that first assessed the effects of marijuana on adolescent neurocognitive development and evaluated verbal and nonverbal memory performance of cannabis-dependent adolescents (ages 14 to 16) compared to controls. Schwartz and colleagues found that monitored abstinence for six weeks did not prevent short-term memory impairment. Teichner and colleagues [16] found no correlation between the severity of marijuana use and cognitive performance among adolescents with and without cognitive impairment referred for drug treatment.

According to Takagi and colleagues, cannabis users (ages 13-24) did not perform as well on measures of immediate and delayed verbal memory compared to community controls. No discrepancies were observed between cannabis users and community controls on measures of executive functioning in a study conducted by this team of investigators [17-18,57-66]. Similarly, Gonzalez and colleagues (2012) found differences in immediate and delayed recall among young adult cannabis users (approximately age 20) compared to non-using controls, but no differences were observed in measures of impulsivity. Even though there were no group differences in impulsivity, the authors found that poor performance on a decision-making task was linked to increased symptoms of cannabis use disorder. The study by Solowij and colleagues examined 181 adolescents (ages 16–20) and discovered that cannabis users performed worse on learning and recall, and the worsening performance was linked to the 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 [19-22,67-74]. A group of researchers has suggested that a lifelong history of cannabis use disorders decreases the likelihood of neurocognitive impairment in patients with Parkinson's disease [23,79-89] and may even lead to more youthful and resilient neurocognitive abilities among adults aging with HIV [24].

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.

2.1 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.

2.2 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.

2.3 Lethal Dose (LD50) of *Cannabis sativa* Determination

Lethal Dose (LD50) of *Cannabis sativa* was carried out according to Lorke's method.

2.4 Experimental Design

A total of 25 adult Wistar Rats weighing between 180g-200g was used for this study. The rats were separated into 5 groups (A, B, C, D & E) with 5 rats in each 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

2.5 Animal Sacrifice and Tissue Collection Technique

At the end of the administration period, the rats were anesthetized and sacrificed by cervical dislocation. The brain tissues were carefully removed from the skull and homogenized in phosphate buffer solution at 10,000rpm. It was later centrifuge to separate the supernatant from the residue. The supernatant was used for the oxidative stress parameters analysis.

2.6 Oxidative Stress Analysis

Malondialdehyde (MDA) was evaluated by colorimetric method of Gutteridge and Wilkins, (1982). Catalase was determined by colorimetric method of Sinha [25]. Superoxide dismutase (SOD) was determined by the colorimetric method of Friedewald and Fredovich (1972).

2.7 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

Results are presented as Mean ± SD of 5 rate in each group $p < 0.05$ is considered statistically significant. The result presented in Table 1 below shows no statistically significant difference in serum malondialdehyde (MDA) levels of rat on the experimental groups B, C, D and E compared to control group A.

Table 1. Result of serum malondialdehyde

Group	MDA	P-value
A	2.05 ± 0.26	
B	2.28 ± 0.28	0.211
C	2.32 ± 0.58	0.380
D	2.16 ± 028	0.537
E	2.09 ± 0.23	0.803

Table 2. Result of superoxide dismutase (SOD)

Group	SOD	P-value
A	25.03 ± 1.64	
B	22.92 ± 1.12	0.045
C	22.13 ± 2.42	0.057
D	22.10 ± 2.15	0.042
E	20.40 ± 2.34	0.007

The result of SOD presented in Table 3 below shows significant reduction in SOD levels in the experimental groups B, D and E compared to the control group A.

Table 3. Result of serum catalase LEVEL

Group	CAT	P-value
A	34.46 ± 1.90	
B	30.19 ± 1.80	0.006
C	28.17 ± 3.01	0.004
D	29.75 ± 2.50	0.010
E	29.67 ± 1.10	0.001

F – Value F (4,20) = 6.00 P < 0.0024

The result of serum catalase level shows that catalase levels were significantly reduced in the experimental groups B, C, D and E compared to the control group A.

The result of serum catalase level shows that catalase levels were significantly reduced in the experimental groups B, C, D and E compared to the control group A.

This study shows MDA present no significant difference on serum level on the model groups when compared to control group but CAT and SOD present significant reduction in serum level reason. Abdulrahim et.al (2021) in their study observed that *Cannabis sativa* had no effect on MDA which is in consistent with this study. This data supports the contention that *Cannabis sativa* elicits an anti-oxidative effect on the brain. In this study, we observed no significant difference in MDA, which is similar with what Abdul-Salam and colleagues reported. Similarly, Bloomer et.al, [26] in their study on young and physically active human subjects found no significant difference in serum malondialdehyde or advanced oxidation protein produces between marijuana smokers and non-smokers.

In this study there was a significant reduction in CAT and SOD, this is consistent with Abdulrahim et.al (2021) who also observed reduced SOD levels in the brain of rats exposed to *Cannabis sativa*. They speculated that the increases in the G6PD (which is a second line anti-oxidant) in the brain of rats that received CS was a reactive response to the depletion in the first line anti-oxidant (SOD). Kubiliene et.al (2021) observed no significant changes in serum MDA level of experimental mice after exposure to *Cannabis Sativa* leave extract. This corroborates the findings of the present study.

CAT activity is considered to be a sensitive biomarker of oxidative stress. This study reports a significant decrease in CAT which is in line with the report of Atli et.al (2006). A decrease in catalase activity in cells indicate a state of oxidative stress subject to indications from other parameters.

4. CONCLUSION

It could be deduced from the result of this study that there were dose and time dependent toxic effects of *Cannabis sativa* in the exposed animals. There significant weight gain, attesting that endocannabinoid may activate cannabinoid receptors that are responsible for increasing food intake, thereby increasing body weight in rats. *Cannabis sativa* was shown to cause marked neuronal alterations in the cerebellum of Wistar rats. This finding may also infer that exposure to delta – 9 THC, the psychoactive ingredient of *Cannabis sativa* at the doses used in this study can produce cytoarchitectural distortion in the cerebellum of Wistar rats.

5. RECOMMENDATION

It is recommended that more studies can be done comparing the impact of cannabis on the investigated parameters for shorter and longer durations and also with lower and higher doses.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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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