



Cannabis sativa Boosts Brain Functions and Ameliorate Anxiety, when used Sensibly and not Abusively

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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 an illegal substance with documented analgesic, antipyretic, anti-inflammatory, and anti-diarrheal properties. Numerous other cannabinoids found in cannabis have an impact on brain activity. So, the purpose of this study was to examine how cannabis sativa extract affected the cognito-motor activity in rats. Twenty-four (24) Wistar rats weighing between 80.53 and 100.49g were randomly divided into four groups (A, B, C, and D) of six animals each after a 14-day acclimatization period to laboratory conditions. Only food and water were provided to Group A. Groups B and C received an oral ethanolic extract of Cannabis sativa at doses of 400 mg/kg and 800 mg/kg, respectively. Group D received an intraperitoneal dose of diazepam at a rate of 50

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mg/kg. To ascertain the impact of the cannabis sativa extract on the neuro behavior of the rats, each animal in the four groups is subjected to an experimental test following each administration of the extract (the Barnes maze test, the beam walk test, and the inverted screen tests). The rats in groups B and C that received cannabis sativa extract showed a notable improvement in learning and memory, and the majority of the rats in group C who received cannabis sativa extract walked upright without stumbling. Group A, the control group, exhibited indicators of anxiety and panic but no discernible change in learning or memory. Due to muscle weakness, members of group D (standard drug group) were essentially inactive throughout the experiment, staggering and falling. It can be concluded that cannabis sativa's ethanolic leaf extract is not harmful, boosts brain functions, and promotes learning and memory when used sensibly and not abusively. Thus, it can be inferred that the plant may help with the treatment of several mental health conditions, such as anxiety.

Keywords: *Cannabis sativa*; neuro behavior; anxiety; diazepam.

1. INTRODUCTION

Cannabis sativa, usually referred to as marijuana, is a member of the plant family Cannabinaceae. It contains the chemical THC (delta-9 tetrahydrocannabinol), which is thought to be largely responsible for the psychoactive properties of cannabis that result in the "high" that is felt after consuming it [1].

Cannabis sativa is a dioecious (male and female flowers on separate plants) green, leafy plant with distinctive opposing, typically seven-fingered lance-shaped leaves that may reach heights of over seven meters in dry, sandy, somewhat alkaline soil.

On the female flower, glandular hairs typically grow and produce resin. For commercial purposes, female plants are more significant than male plants because they produce more

nutritious seeds, have stronger fibers, and contain the hallucinogenic compound tetrahydrocannabinol [2].

Cannabis has been the most often used illicit substance across time due to its accessibility and low cost. Additionally, there are several contradicting and disputed findings regarding the psychological and physiological effects of cannabis, making it a highly contentious drug [3,4].

Numerous studies have connected cannabis use to the emergence of psychosis. Additionally, some research contends that cannabis use is just a method of self-medication for those with psychotic symptoms and not the primary cause of the development of psychosis. There seems to be an upsurge in reports in recent years that support cannabis psychosis [3,5].



Plate 1. Cannabis Sativa Leaves (John, 2020)

Cannabinoids affect all facets of short-term memory, particularly short-term episodic and working memory, according to a review of the research regarding their acute effects on memory. Numerous studies have demonstrated that cannabis smoking has major physical impacts on the body in addition to its psychological effects. Cannabis has been shown to cause a 20–50% rise in heart rate. This is the most rapid reaction and happens shortly after using cannabis. Other physical effects of cannabis include reddening of the eyes due to congested conjunctiva blood vessels, lowering of the body temperature, dry mouth, reduced intraocular pressure, and relaxation of the muscles. After cannabis use, a sudden change in posture from lying down to standing up may produce orthostatic hypotension, a feeling of light-headedness, and faintness that is frequently the first indication of intoxication in naive users. The immune system has been demonstrated to be impacted by cannabinoids. Cannabis enhances hunger, according to surveys and clinical trials [6,7]. It has been demonstrated that the primary chemical in cannabis, 9-tetrahydrocannabinol (THC), affects both the activity and release of insulin. This may help to explain why some people with diabetes self-medicate with cannabis. A study by Mobisson *et al.* demonstrated the impact of cannabis aqueous extract on some biochemical indices [8]. Cannabis has also reportedly been used medicinally to alleviate sadness. Additionally, it has been stated that cannabis sativa is utilized to treat a variety of human conditions, including leprosy, leukoderma, scabies, smallpox, allergies, burns, cuts, and wounds [4,9].

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh leaves of Cannabis sativa were procured from Ibusa, Delta State, Nigeria, with the assistance of a botanist. First, the leaves were allowed to dry at room temperature. Ethanol and water serve as the extraction solvents (hydroethanolic extraction). A semi-permeable membrane known as a "thimble" was placed inside the extraction chamber with 40 g of the combined cannabis sativa. The heating mantle was adjusted to 100°C and 150 ml of 70% ethanol was measured and placed inside a flask with a circular bottom. The ethanol boils at 78°C, and the resulting vapor travels through the extraction chamber and onto the condenser. The condenser changes the vapors into liquid. The

thimble containing the sativa leaf combination is then filled with the liquid. The process continues for roughly 4 to 6 hours as the liquid soaks the dried leaf and extracts the bioactive components, which then build in the extracting chamber before being eventually decanted into the round bottom flask. The ethanol and water that are present in the crude extract of sativa are then evaporated by heating the extract in liquid form in a water bath.

2.2 LD₅₀ of Cannabis sativa

Utilizing Dietrich Lork's method, the Department of Human Physiology at Uli's Faculty of Basic Medical Sciences determined the lethal dose (LD₅₀) of an ethanolic leaf extract of Cannabis sativa [10]. Thirteen rats were used in the study. They were given the extract orally, and it was administered in two stages.

2.3 Experimental Animal Groupings

Group A (Control group) was given rat meal and tap water ad libitum without any drugs or extract.

Group B received 400mg/kg of the extract for 14 days.

Group C received 800mg/kg of the extract for 14 days.

Group D received standard drugs daily for 14 days.

2.4 Neurobehavioural Test

Barnes Maze Test: It is a visual-spatial learning and memory exercise that is unique to rats. A raised circular surface with holes all around it makes up the object.

Principles - This behavioral paradigm assesses spatial learning and memory in rodents that live in arid environments. The animal is positioned in the middle of the platform at the beginning of each trial and given a specific amount of time to get to the Target Escape Hole. If an animal enters the Target Escape Hole before the timer expires, the experiment is over. Animals that don't enter the Target Hole in time are led there by the experimenter and given a limited opportunity to dwell within the tube before being sent back to their original cage. Every day, the Target Escape Hole is moved, but the other elements stay the same. The participants receive a total of four trials. The rats use visual cues from outside the labyrinth to locate an exit, which

enables them to leave the open area and brilliant light and enter a dark box beneath the maze. It's important to note how long it takes to locate the exit into the pitch-black box beneath the maze.

Beam Walk Test: This test is designed to evaluate balance as well as motor coordination, especially in the hind limb. The mouse must maintain its balance while crossing a raised, narrow beam to reach a secure box.

Each rat was evaluated individually after being transported from its home cage. The rats were initially allowed to walk over the short beam at least three times after being placed at one end and the other. The 30-second trial is over. To obtain a reliable baseline measurement, this training phase proved helpful. Each trial's number of foot slips and the amount of time it took to cross the beam are noted.

The rats were encouraged to keep moving forward by being prodded, poked, or pushed from behind with gloved fingers when they did stall, smell, or look around without moving (the rats may stall more if they are overtrained and become too accustomed to the task). To keep testing sessions comparable, this was done in all

animals. The rats were given a short period (about 15 seconds) to relax in the box before the subsequent experiment. Before each trial, the beam and box were cleaned with 70% ethyl alcohol. The rats were then immediately taken back to their cages following each training session.

Inverted Screen Test: The Inverted Screen exam is used to measure coordination and motor strength. Individually untrained rats are then placed on top of the wire screen, which is then turned upside down or rotated 180 degrees so that the rats are at the bottom of the glass screen. Throughout a 60-second testing session, the following behavioral responses were noted: falling off, climbing, and not climbing.

2.5 Statistical Analysis of Results

The outcomes are displayed as $M \pm SD$ of measurements in triplicate. The data were examined using SPSS version 23 (Statistical Package for Social Sciences) (IBM Corp., Armonk, New York). Utilizing a one-way analysis of variance, a comparison was made (ANOVA). When $P < 0.05$, values were deemed significant.

3. RESULTS AND DISCUSSION

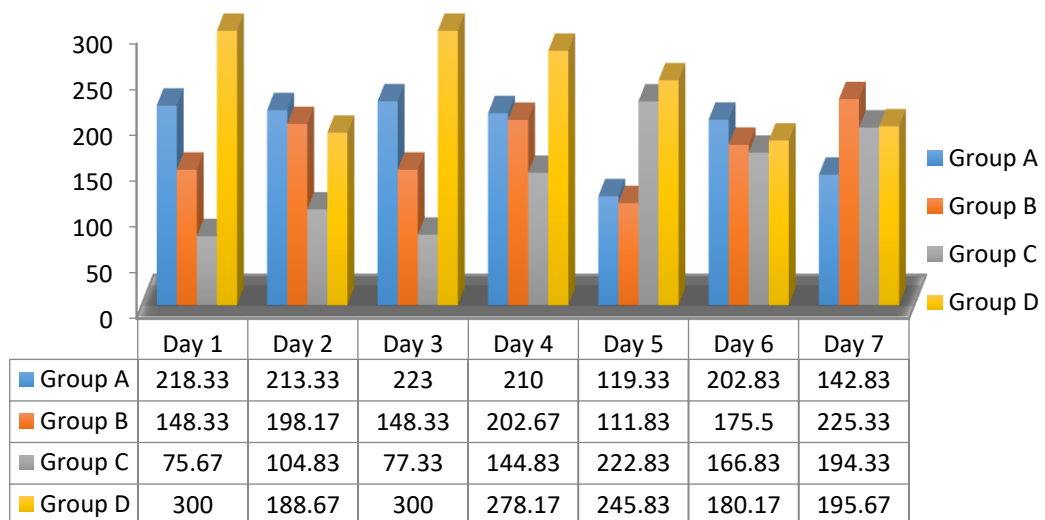


Fig. 1. Values of Bernes maze during first week of administration

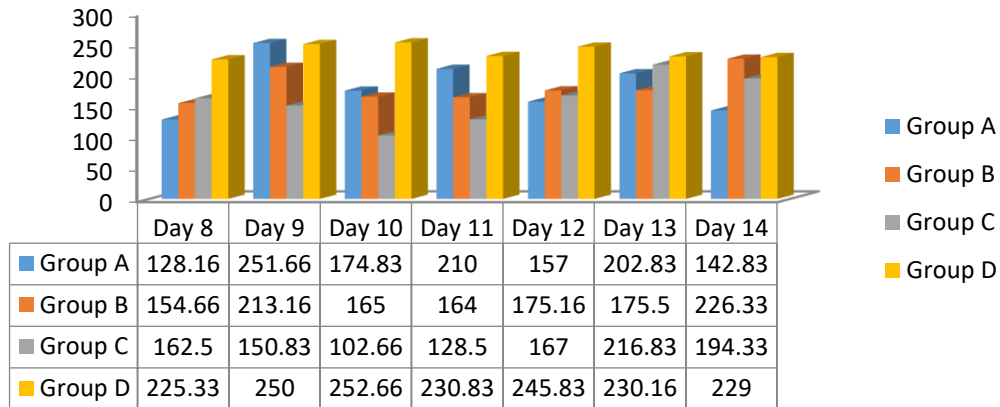


Fig. 2. Values of Bernes maze during second week of administration

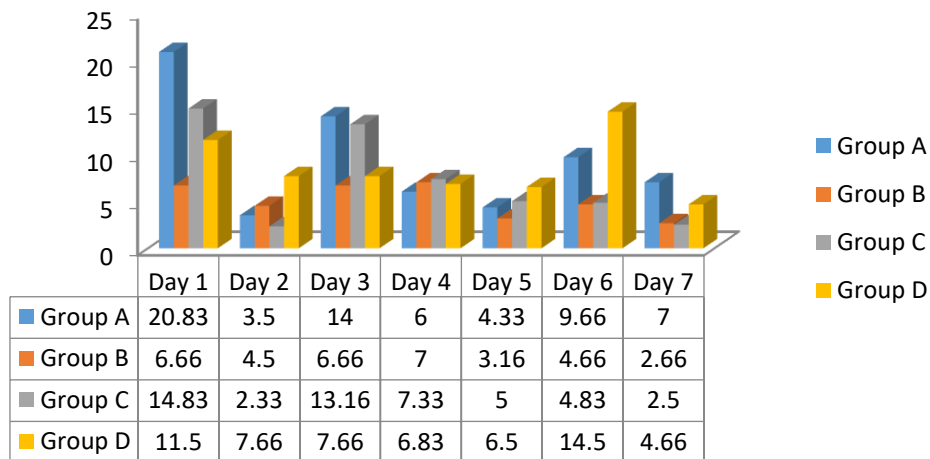


Fig. 3. Values of Beam work among different groups in first week

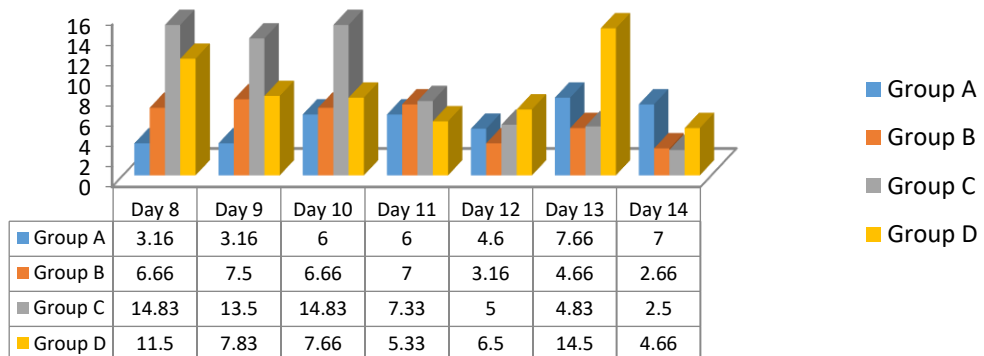


Fig. 4. Values of Beam work among different groups in second week

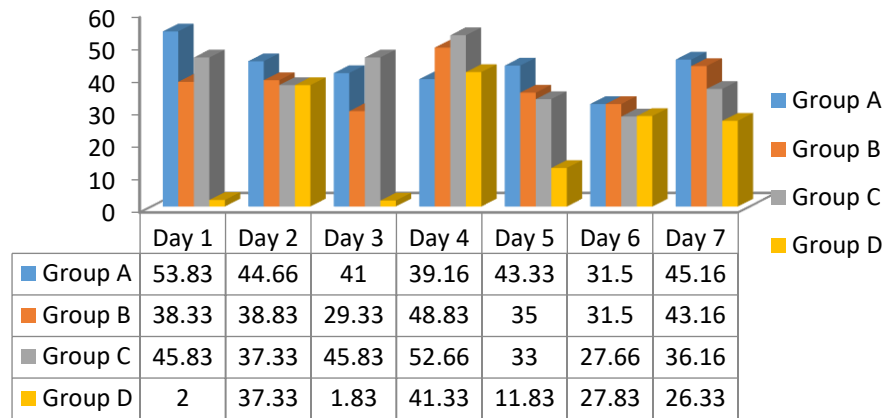


Fig. 5. Values of inverted screen test among different groups in first week

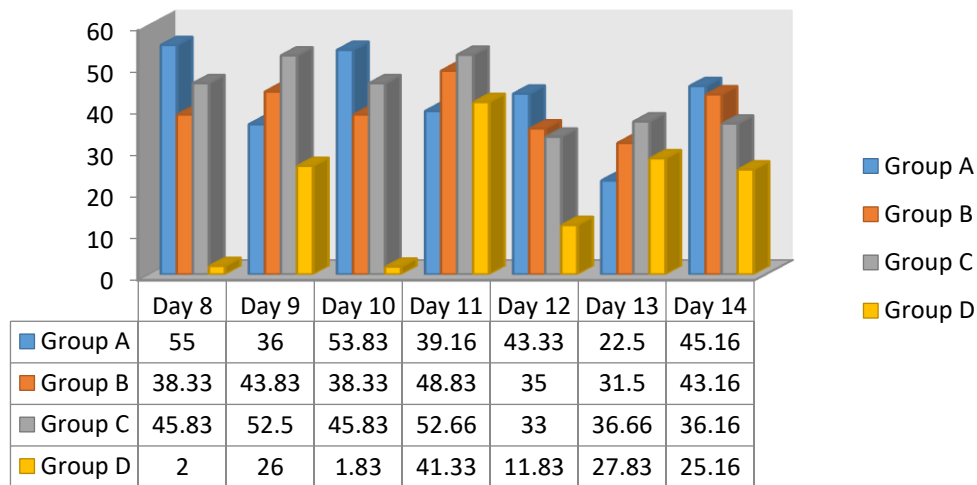


Fig. 6. Values of inverted screen test among different groups in second week

The Barnes maze is a test that looks at an animal's ability to learn and remember spatial relationships (rodents). The acquisition phase and acquisition probe trial are in the first stage of the BM task, which provides for an assessment of spatial learning and spatial memory. While the second portion of the task—the reversal learning trials—allows for the assessment of the cognitive flexibility linked to frontal brain function, this portion is thought to be connected to hippocampus function. This study was conducted to look at how Cannabis Sativa affected the behavior of Wistar rats. Overall, the results indicate that, as compared to Groups A, B, and C, Group D (the diazepam group) exhibited minor deficiencies in working memory and spatial learning.

In these studies of the Barnes maze, it was discovered that Cannabis Sativa increased the likelihood and learning in the test groups (Groups B and C) with an average time of 300 seconds than in Group A (control group) and Group D (Diazepam group). The groups treated with cannabis sativa extract located the escape hole in a shorter period than the control group and diazepam group, respectively; this is consistent with McGrath's findings [11].

A straightforward test of motor coordination and skills is the Balance Beam exercise (more sensitive in evaluating certain types of motor coordination deficits).

The Balance Beam test is used to examine mice's sensory and motor abilities. A longer time

is typically required for subjects who are anxious or have weak limbs to cross the beam. A lack of motor skills can also be brought on by illness and injury. The Balance Beam test is commonly used to measure the following parameters: *Latency to cross the beam, Hind leg, foot slips (right and left), Number of falls during the trial, Total number of steps.*

The test helped assess the effects of Cannabis Sativa on Wistar rats' motor capabilities. In this study, balance and coordination were found to decline faster in group D (the diazepam group), since these rats always stagger and are unable to grip the beam stick due to muscle weakness. The rats treated with cannabis sativa extract (groups B and C) retained their balance without falling and rushing inside the box in agreement with the Naraine report, but the rats in group A (the control group) skipped several steps and took longer to walk through the beam [12].

The Inverted Screen exam is used to measure coordination and motor strength. Individually untrained mice are placed on top of a wire screen that is set horizontally on a metal rod and is square (7.5 cm x 7.5 cm). The rod is then turned 180 degrees so that the mice are on the screens' bottoms. Throughout a 1-minute testing session, the following behavioral responses were noted: falling off, climbing, and not climbing. The diazepam group (group D) in this study exhibits a deficit in motor strength because the rats were unable to hold the wire crest for at least 10 seconds. The control group (group A) also exhibits a deficit in motor strength, whereas the group receiving cannabis sativa extract (groups B and C) exhibits an improvement in motor coordination because the majority of the rats were able to hold the wire crest for an extended period. According to Russo & Ethan's findings, phytocannabinoid constituents may be responsible for the current study's improvement of cognitive functioning [13].

4. CONCLUSION

Cannabis sativa, popularly known as hemp or marijuana, has the potential to develop into an effective and much-needed medicine, but this is gravely hampered by its classification as a deadly narcotic due to its high level of reliance.

According to this study's conclusions, cannabis sativa has a considerable impact on neurobehavioural functions, including improved learning and memory, motor strength and

coordination, and the ability to access motor capacity and balance. Due to the high level of misuse, this particular plant has been classed as an unlawful drug. To reduce abuse, a prescribed dosage should be made mandatory for users.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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