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Research Article

Effect of *Cannabis sativa* on Cognitive Behavior in Wistar Rats

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Abstract

Cannabis is the most widely used illicit drug after nicotine and alcohol and can impair several aspect of cognitive functions. It is the flowering or fruiting top of a cannabis plant from which the resin has not yet been extracted. This study was aimed at assessing the effect of Cannabis Sativa on cognitive function. Haloperidol (an antipsychotic drug) blocks the dopaminergic pathways in the brain. Thus, it is expected that administration of haloperidol cause cognitive decline. A total of forty-six (46) adult Wistar rats of both sexes were used for this study. Animals were housed in a conducive environment as expected and were fed regularly with standard animals feed and allowed to acclimatize for two weeks before commencement of the study which lasted for two weeks. Group I served as normal control and were administered 1 ml distilled water. Group II were administered Haloperidol 2 mg/kg, Group III, IV and V were administered cannabis and haloperidol in the following proportions; 5 mg/kg + 2 mg/kg haloperidol, 10 mg/kg + 2 mg/kg haloperidol and 20 mg/kg + 2 mg/kg haloperidol respectively. Group VI, VII and VIII were administered cannabis only in the following proportion; 5 mg/kg, 10 mg/kg and 20 mg/kg respectively. The result of this studies shows that there was no statistically significant difference ($P > 0.05$) observed in all the treated groups compared to the normal control, there was however an increase in all the groups compared to the control. Based on the result obtained, it may be suggested that cannabis may increase short term memory.

Keywords: Cannabis; Haloperidol; Dopamine; Cognition; Memory

Abbreviation

THC: Tetrahydrocanabinol; CBD: Cannabidiol; CBN Cannabinol; (Δ^9-THCA: Δ^9 –tetrahydrocannabinolic), CBDA: Cannabidiolic Acid; AD: Alzheimer's Disease; CS: Cannabis Sativa; HLPDL: Haloperidol

Introduction

Cannabis is the most globally used addictive drug after nicotine and alcohol [1] and can cause impairment in several aspects of cognition. In humans, the consumption of cannabis or cannabinoids impair both encoding and recall of both verbal and non-verbal information depending on dose and task difficulty [2]. In animal model, cannabinoids causes impairment of memory in a variety of experimental conditions [3].

Cannabis is the preferred name of the plant *Cannabis sativa, Cannabis indica,* and of minor significance*, Cannabis ruderalis* [4]. According to the 1961 United Nations Single Convention on Narcotic Drugs, "it is defined as the flowering or fruiting top of the cannabis plant (excluding the seeds and leaves when not accompanied by the tops) from which the resin has not been extracted, by whatever name they may be designated". Cannabis resin means separated resin, whether crude or purified, obtained from the cannabis plant [4]. The principal cannabinoids in the cannabis plant include delta-9-tetrahydrocannabinol (THC), Cannabidiol (CBD), and Cannabinol (CBN). THC is the primary psychoactive compound, with CBD, a non-psychoactive compound, ranking second. Generally, THC is found at higher concentrations than CBD, unless the ratio is deliberately altered [5].

There are more than 104 cannabinoids which are identified in the cannabis plant [6]. Other compounds identified include flavonoids, terpenoids, nitrous compounds, and more common plant molecule [7]. Among these, Δ^9 - Tetrahydrocannabinoid (Δ^9-THC) has received the most attention for being responsible for the intoxicated state sought after by recreational cannabis users, owing to its ability to act as a partial agonist. Cannabinoid exist mainly in plants as their carboxylic precursor Δ^9-THCA and CBDA (Δ^9-tetrahydrocannabinolic acid and cannabidiolic acid) and are decarboxylated by light or heat while in storage or when combusted [8].

Cannabinoid receptors, found throughout the body, forms part of the endocannabinnoid system, which is involved in a variety of physiological processes including appetite, pain sensation, mood and memory. Cannabinoid receptors belong to the Gprotein coupled receptor superfamily [9]. These receptors are ligand-activated. They are activated by three major groups of ligands; Endocannabinoids, Phytocannabinoids and Synthetic cannabinoids.

The cannabinoid receptors include; CB_1 and CB_2 receptors.

CB_1 are thought to be one of the most widely expressed Gprotein coupled receptor in the brain. They are also found in other parts of the body. For instance, in the liver, activation of CB_1 receptor is known to increase *de novo* lipogenesis. They are also found on peripheral nerve terminals and some extra-neural sites such as the testis, eye, vascular endothelium and spleen. Interestingly, CB_1 receptors are highly enriched at presynaptic and axonal compartments, restricting their function to sites of synaptic activity.

The CB_2 receptor exhibits a more definite pattern of expression in the brain than CB1 receptors, and is found predominantly in cells and tissues of the immune system. In the CNS, CB_2 receptor expression is associated with inflammation and it is primarily localized to microglia, resident macrophages of the CNS. This selective localization together with the modulatory effect of theCB_2 receptor on microglia function is particularly relevant since microglial cells have a significant role in Alzheimer'sdisease (AD) and other diseases associated with the basal ganglia.

Therapeutic effect of *Cannabis Sativa*

Chronic pain

Relief from chronic pain is one of the commonest therapeutic effect of cannabis. It is by far the most common condition cited by patients for the medical use of cannabis. Likewise, [10] reported that 87 percent of participants in their study were seeking medical marijuana for pain relief. In addition, there is evidence that some individuals are replacing the use of conventional pain medications (e.g., opiates) with cannabis.

Similarly, recent analyses of prescription data from Medicare part D enrollees in states with medical access to cannabis suggest a significant reduction in the prescription of conventional pain medications [11].

Cancer

Cancer is a broad term used to describe a wide-range of related diseases that are characterized by an abnormal, unregulated division of cells; it is a biological disorder that often results in tumor growth.

Although the anticancer effect of cannabinoids has been intensively studied in cell cultures (test-tube studies) and in animals with tumours, no firm conclusions has yet been ascertain. It has been confirmed repeatedly that various cannabinoids, binding to both of the known types of cannabinoid receptor, can retard or prevent the growth of cancer cells as well as their ability to invade surrounding normal tissues and metastasize. It has also been suggested that some actions of endocannabinoids (substances found naturally in the body that have actions similar to those of THC) may reduce the risk that mutations will give rise to cancer cells. Only one small, uncontrolled clinical trial has been carried out in humans, where THC was injected directly into the cancers of nine patients when recurrent brain cancers were first detected. Although there was an initial relief of symptoms, the THC treatment was not able to cure the cancer or slow the rate of recurrence.

Glaucoma

Glaucoma is one of the leading causes of blindness globally. This disorder is characterized as a group of eye conditions that can produce damage to the optic nerve and result in a loss of vision. This damage is often caused by abnormally high intraocular pressure. Because high intraocular pressure is a known major risk factor that can be controlled [12], most treatments have been designed to reduce it. Research suggests that cannabinoids may have potential as an effective treatment for reducing ocular pressure.

Anorexia and weight loss

Anorexia and weight loss are common side effects of many diseases, especially cancer. And prior to the availability of highly active antiretroviral therapy, a wasting syndrome was a frequent clinical manifestation in patients with human immunodeficiency virus (HIV) infection and advanced acquired immune deficiency syndrome (AIDS). The labeled indications for dronabinol were expanded in 1992 to include treatment of anorexia associated with weight loss in patients with AIDS [13].

There is some evidence for oral cannabinoids being able to increase weight in patients with the HIV-associated wasting syndrome and anorexia nervosa. No benefit has been demonstrated in cancer-related anorexia/cachexia syndrome. The studies have generally been small and of short duration and may not have investigated the optimal dose of the cannabinoid. In one study in HIV patients, both dronabinol and inhaled cannabis increased weight significantly compared to the placebo dronabinol. Cannabis has long been felt to have an orexigenic effect, increasing food intake [14].

Dementia

Dementia is characterized by a decline in cognition that typically affects multiple cognitive functions such as memory, language, executive function, and perceptual motor function [15]. Alzheimer disease, vascular dementia, and Parkinson's disease with dementia are three prominent dementing disorders. Behavioral and psychological symptoms, including agitation, aggression, and food refusal, are common symptoms in the more advanced stages of dementia. These symptoms causes distress to the patient and caregivers, and may precipitate the patient being placed in institutional care. Current treatments for dementia (e.g., cholinesterase inhibitors) have only modest effects, and treatments for behavioral disturbances such as antipsychotic medications, have both modest benefits and substantial adverse effects [16]. CB_1 receptors are found throughout the central nervous system, and the endogenous cannabinoid system is thought to be important in the regulation of synaptic transmission [17], a process that is disordered in patients with dementia. Accumulating evidence suggest that cannabinoids have the potential for neuroprotective effects [18,19]. This developing understanding of the endogenous cannabinoid system along with cannabinoids anxiolytic and appetite-stimulating effects provides a rationale for its study in patients with dementia.

Adverse effect

There is a lack of research documenting the risks associated with the medical use of cannabis. A recent cross-sectional study examining the effects of inhaled or ingested cannabis on cognitive functioning in patients with multiple sclerosis revealed that cannabis users performed significantly poorer than non-users on measures of information-processing speed, working memory, executive functioning and visuospatial perception.

Studies of recreational cannabis users provide some indication of the health risks that may result from smoking cannabis over the long term, including neuro-cognitive deficits, psychosis, various respiratory ailments and possibly cancer.

This study is designed to evaluate the neurobehavioral effect of acute ingestion (oral) of *C. Sativa L.* extract, using Novel Object Recognition Test (NORT) paradigm. Because of its potential socio-economic, socio-cultural, therapeutic importance, this study attempts to decipher the short term physiological/behavioral effect of cannabis ingestion.

Materials and Method

Syringe, cannula, methylated spirit, handkerchief, NORT box, hand gloves, haloperidol, cannabis extract, white plastic cages (50 x 30 x 20 cm), novel object, two familiar objects.

Drug and reagent

Haloperidol: Haloperidol is an antipsychotic drug which blocks D_2 receptor. It was gotten from Phamib pharmacy, Tudun Jukun Zaria, Kaduna State, Nigeria.

Cannabis extraction method

Dried leaves of *cannabis sativa* was obtained from National Drug Enforcement Agency (NDLEA) in Kaduna state, Nigeria.

All the parts of the drug sample were placed in a conical flask with the extraction solvent, and allowed to stand for three days, with occasional stirring. The mixture was stained and marc pressed to remove retained solution. The liquid was combined and any precipitate solid removed by filtration.

After the filtration, the liquid was put in water bath at 50°C (50 degree Celsius) until all the solvent escaped and the placed in a suitable container.

Animals and groupings

Animals: Adult male wistar albino rats, weighing 130-230 g were used for this study. The animals were housed in an animal house provided with light/dark cycle and free access to food and water ad libitum. The rats were housed in white, plastic cages (50 x 30 x 20 cm) with sawdust, in groups of 6 animals per group, and each group were placed in separate cages. Animals were housed in a conducive environment as expected and were fed regularly with standard animals feed and allowed to acclimatize for two weeks before commencement of the study.

Groups	Treatment	Route
I	Control (1 ml distilled water)	Orally
II	HPDL (2 mg/kg)	Orally
III	Cannabis extract only (5 mg/kg) + HPDL (2 mg/kg)	Orally
IV	Cannabis extract only (10 mg/kg) + HPDL (2 mg/kg)	Orally
V	Cannabis extract only (20 mg/kg) + HPDL (2 mg/kg)	Orally
VI	Cannabis extract only (5 mg/kg)	Orally
VII	Cannabis extract only (10 mg/kg)	Orally
VIII	Cannabis extract only (20 mg/kg)	Orally

Table 1

n: 6 Animals Per Group; HPD: Haloperidol

Grouping: A total of forty six (46) animals were used for this study. Animals were randomly grouped into eight (8) groups, containing six (6) animals per group. All drugs were administered orally for two weeks. The groupings are as follows

Behavioural assessment

The Novel Object Recognition test (NORT) was introduced by Ennanceur and Delacour in 1988 and can be regarded as a spontaneous Delayed-Non-Matching-to-Sample (DNMS) test. The test is based on a spontaneous behavior. The main assumption at the base of this test is that access to novelty (e.g., an object or an environment) can elicit approach behaviors in animals. This apparent 'unconditioned preference' for novelty has been used in the NOR test in order to study memory functions, assessing the ability of animals to recognize a novel object in a familiar environment, because they maintain a representation of those is more familiar stored in memory.

This test was conducted in two trials.

In the first trial (T1) the animals were (each) exposed to two identical objects (sample object) for a period of three minutes. Following the sample object exposure, the animals were returned to his home cage for a retention period.

In the second trial (T2), which follows the retention time, the animals were returned to the environment (arena) and presented with a familiar (sample) and a novel object.

When the animal 'remembers' the previous exposure to the familiar object, it will explore the novel object to a greater degree than that of the familiar one.

Result and Discussion

The figure above shows the result of cannabis sativa, and haloperidol on cognition in adult Wistar rats. Although there was no statistically significant difference ($P > 0.05$) observed in all the treated groups compared to the normal control, there was however an increase in preference score (%) in all the groups compared to the control; HLPDL 2 mg/kg (20.00 ± 2.00 vs 16.67 ± 1.66), CS 5 mg/kg (36.67 ± 1.76 vs 16.67 ± 1.66), CS 10 mg/kg (45.90 ± 1.47 vs 16.67 ± 1.66), CS 20 mg/kg (62.46 ± 2.16 vs 16.67 ± 1.66), CS 5mg/kg + HLPDL 2mg/kg (44.45 ± 1.60 vs 16.67 ± 1.66), CS 15mg/kg + HLPDL 2mg/kg (61.12 ± 1.53 vs 16.67 ± 1.66), CS 20mg/kg + HLPDL 2mg/kg (31.48 ± 1.73 vs 16.67 ± 1.66).

The cannabis sativa treated groups showed increase in preference score (%) when compared to the haloperidol treated group. CS 5 mg/kg (36.67 ± 1.76 vs 20.00 ± 2.00), CS 10 mg/kg (45.90 ± 1.47 vs 20.00 ± 2.00), CS 20 mg/kg (62.46 ± 2.16 vs 20.00 ± 2.00).

The cannabis sativa and haloperidol treated group also showed an increase in preference score (%) when compared with the haloperidol treated group; CS 5 mg/kg + HLPDL 2 mg/kg (44.45 ± 1.60 vs 20.00 ± 2.00), CS 10 mg/kg + HLPDL 2 mg/kg (61.12 ± 1.53 vs 20.00 ± 2.00), CS 20 mg/kg + HLPDL 2 mg/kg (31.48 ± 1.73 vs 20.00 ± 2.00).

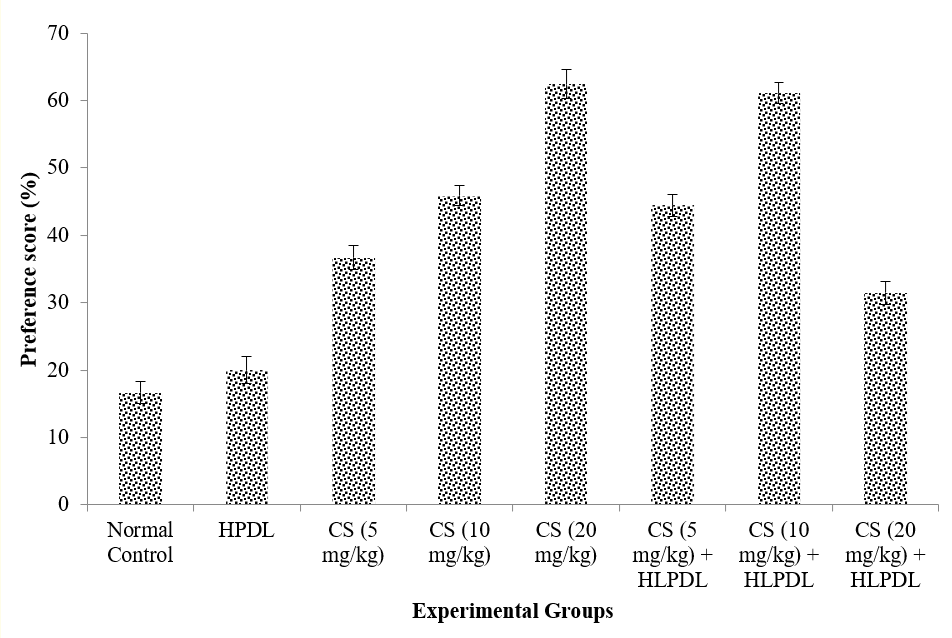

Figure 1: Showing the effect of cannabis sativa on cognition in adult Wistar rats. HLPDL = Haloperidol, CS= Cannabis sativa, CS + HLPDL= Cannabis sativa and Haloperidol.

Studies have shown that the route of administration of cannabis can affect the onset, intensity, and duration of the psychotropic effects, the effects on organ systems, and the addictive potential and negative consequences associated with its use [20]. The consumption of cannabis causes a particular combination of relaxation and euphoria, commonly referred to as a "high". When cannabis is smoked, Δ^9-THC (Δ^9 –tetrahydrocannabinod) quickly diffuses to the brain, eliciting a perceived 'high' within seconds to minutes.

Studies have also shown that cannabis use causes cognitive decline, particularly with long-term usage. Majority of studies have suggested a significant cognitive decline in cannabis abusers compared to non-abusers and healthy controls [21].

The brain consist of many dopaminergic pathways which enhance cognition. Dopamine receptors that enhance cognition are D_2 and D_3 receptors. Haloperidol (an antipsychotic drug) blocks the dopaminergic pathways in the brain. Although mechanism of action of haloperidol is not known, but it blocks D_2 receptor. Thus, it is expected that administration of haloperidol causes cognitive decline.

Conclusion

The result of this study showed that acute intake of cannabis causes increase in cognition and co-administration with haloperidol causes an increase in learning, however the exact mechanism for this is not fully elucidated. However, from figure 1 above, it showed that cannabis (5 mg/kg) co- administered with haloperidol cause a decline in learning.

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Conflict of Interest

There are no conflict of interest during the course of this work.

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