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Evaluation of Sedative Activity of *Cannabis Sativa* in Mice

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Abstract
Background: Cannabis sativa Linn. (Family: Cannabaceae) is commonly cultivated in Bangladesh in moist areas such as riverbanks, lakes, canals, and roadsides. The leaves of this plant are traditionally used to treat insomnia, depression, and neurodegenerative diseases.
Objective: The study's objective is to investigate the sedative effects and phytochemical composition of the Cannabis sativa species in Nigeria.
Methods: The elevated plus maze was used to assess the sedative effect of various fractions of aqueous methanol extracts of Cannabis sativa at a dosage of 100 mg/kg in mice. Comparisons were made between the sedative action of the extract and diazepam, which was used as the positive control. Additionally, acute toxicity was also evaluated during the assessment.
Results: During the elevated plus-maze test, various parameters such as the number of entries in open arms and the number of entries in closed arms were recorded. The administration of different fractions of aqueous methanol extracts of Cannabis sativa resulted in a significant increase ($p < 0.001$) in the time spent in and the number of entries into open arms compared to the control group.
Conclusion: The findings suggest that various concentrations of water-methanol extracts from Cannabis sativa exhibit sedative effects that are superior to those of the control (diazepam), supporting the use of this plant in traditional medicine. Further research should be conducted to investigate the active components of Cannabis sativa and understand the mechanism of its effects before proceeding to clinical trials.

Keywords: *Cannabis sativa* Linn, *Cannabaceae*, Sedative, Elevated plus-maze test, -phytochemical, Neuropharmacological

Introduction

Plants have become an important source for meeting the increasing health needs of humans, leading to a greater focus on maximizing the use of these medicinal resources. While medicinal plants were traditionally relied upon in under-developed nations, there is now a growing interest in developed countries due to their minimal or non-existent side effects (1). As a result, these plants are being grown and processed using appropriate methods to isolate the specific components responsible for their medicinal properties. Extracts derived from various plants have been utilized as a form of treatment for a wide range of conditions such as diabetes, cancer, hypertension, and malaria.

The utilization of Cannabis sativa in various aspects of human society has been supported by previous studies dating back to 4000 B.C (2). Its medicinal properties are documented in ancient Chinese pharmacopeia, making it one of the earliest known medications. Cannabis sativa exhibits a wide range of

pharmacological effects, including antimicrobial, appetite-stimulating, and anti-inflammatory properties, as well as effects on the central nervous system (2,3).

Throughout the years, significant attention has been focused on the central nervous system (CNS) impacts of Cannabis sativa, evident in the multitude of countries and regulations governing its consumption and usage. These CNS effects encompass a wide range, including sedation, anxiolytic properties, and tranquilizing effects.

Statistics from developed nations indicate a growing acknowledgment of Cannabis sativa for both medical and recreational purposes (4,5). This acknowledgment, combined with extensive historical evidence supporting its medicinal use, particularly for sedation, and the reduced toxicity associated with herbal medicine compared to conventional synthetic drugs in promoting overall health (6,7), particularly for extended periods, has sparked renewed interest in alternative options such as cannabis.

The sedative effects of Cannabis sativa can be attributed to several factors, with polarity being one of the significant factors. This study provides valuable information about how the sedative properties of different fractions with different polarities compare to a standard and the level of sedation they induce.

The study provides us with information about whether Cannabis sativa mediates sedation and also identifies the fraction that has the strongest effect compared to the synthetic standards commonly used in patients. This helps us understand the composition of the compounds responsible for sedation in the various fractions of the extract from Nigerian hemp plants by examining their polarity.

The solvent used for extraction in this study is methanol, which has been shown in previous research (8) to result in a higher yield compared to other solvents because of its high polarity.

Materials And Methods

Collection of the plants

On April 2, 2021, samples of Cannabis sativa, also known as "indian hemp" or by other names such as "Igbo" (Yoruba), "Nwonkaka" (Ibo), and Gum, including its leaves, roots, and stems, were gathered from the office of the National Drug Law Enforcement Agency in Nsukka, Enugu State, Nigeria. Prior permission to conduct the research using C. sativa was obtained from the National Drug Law Enforcement Agency (NDLEA) due to its classification as a controlled substance in Nigeria. The seeds were authenticated by local residents and a botanist at the University of Nigeria, Nsukka.

Extraction and fractionation of the Plant material

The plants' seeds were dried in the shade and then sifted to remove any additional materials, ensuring the seeds' purity. Approximately 350g of the seeds underwent thorough extraction with multiple portions of aqueous methanol (95%) at 25oC through cold maceration over three consecutive days. The resulting aqueous methanol extract was filtered and subsequently evaporated to dryness at 40oC and reduced pressure using a rotary evaporator. The extraction of the seeds yielded 18.57%. The crude seed extract was then carefully washed with n-hexane, ethyl acetate, and absolute methanol to obtain n-hexane-soluble, ethyl acetate-soluble, and absolute methanol-soluble fractions. Following this, animal screening for sedative activity was conducted on the crude seed extract and the various fractions.

Chemicals

At the University of Nigeria's Model Pharmacy in Nsukka, I purchased diazepam tablets and 0.9% sodium chloride solution (Normal saline). The analytical grade Bangladesh and other reagents were bought from Lavans chemical laboratory ltd in Nsukka.

Animals

The experiment involved acquiring White albino mice of either gender, aged 4–5 weeks, and weighing between 24–32 grams from the animal house of the Department of Pharmacology and Toxicology at the University of Nigeria, Nsukka. These mice were kept in standard environmental conditions, including a temperature of 24.0 ± 1.0 °C, a relative humidity of 55-65%, and a 12-hour light/12-hour dark cycle. The animals had free access to food and water. They were given two weeks to acclimate to the laboratory environment before the start of the experiment. Approval for the use of laboratory animals in the experiments was obtained from the Ethics Committee of the faculty of Pharmaceutical Science at the University of Nigeria, Nsukka, Nigeria (FPSRA/UNN/22/0029).

Drugs and Treatment

The plant extracts were administered to the mice after being reconstituted in 3% tween 80, with a dosage of 100 mg/kg given orally through gavage. The control group received 10 ml/kg of distilled water via gavage. The drugs used as the standard were dissolved in distilled water and then administered intraperitoneally (i.p.) to the animals. Diazepam (5 mg/kg i.p.) was used as the standard CNS depressant drug.

Acute toxicity study

The participants were separated into control and test groups (n = 6). The test groups received an oral dose of the extract at 100mg/kg. Following this, the animals were individually housed in cages and provided with food and water ad libitum. Only the control group was given water. Over a 72-hour period, the animals were monitored for any alterations in behavior, physical changes, allergic reactions, and mortality (10).

Phytochemical screening

Preliminary phytochemical screening of the different fractions of the aqueous methanol extracts was qualitatively done through the standard procedures to detect alkaloids, flavonoids, carbohydrates, glycosides, tannins, Saponins and steroids(11,12).

Sedative Activity Test

Elevated plus-maze test

The plus maze apparatus is constructed with two open arms (16×5×12 cm) and two closed arms (16×5×12 cm), along with an open roof positioned 50 cm above the floor. It is designed to evaluate anxiolytic behavior in animals (13). After 30 minutes of dose administration, each group of mice is placed on the elevated plus-maze apparatus. The mice are positioned with their heads facing the open arms in the central area. Observations of the behavioral effects of the mice are conducted for 5 minutes using various parameters such as time spent in open arms, time spent in closed arms, number of entries in open arms, and number of entries in closed arms.

Statistical Analysis

Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons to compare the results with the control group. Statistical significance was defined as $P < 0.05$ and $P < 0.001$.

Results

Acute Toxicity

The administration of various fractions of Cannabis sativa extracts in aqueous methanol orally at 100 mg/kg did not result in any deaths or noticeable alterations in the mice's behavior during the 72-hour observation period. As a result, it can be concluded that the different fractions of Cannabis sativa extracts in aqueous methanol have a low toxicity profile, with an LD50 exceeding 100 mg/kg.

Results

Phytochemical screening

The aqueous methanol extracts of Cannabis sativa were subjected to a preliminary phytochemical screening, which revealed the existence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, saponins, and steroids (Table 1).

Table 1: Preliminary qualitative phytochemical screening of the different fraction of aqueous methanol extracts of *Cannabis sativa*

Phytochemical constituents	Test	Inference			
		Abs. methanol fraction	Ethyl acetate fraction	n-Hexane fraction	Crude Extract
Alkaloids	Mayer's test	+	-	-	+
	Dragendorff's test	+	-	-	+
Flavonoids	Lead acetate test	++	+	-	++

Carbohydrates	Molisch's test	++	+	-	++
	Fehling's test	++	+		++
Glycosides	Modified borntrager's test	-	-	-	-
Tannins	Gelatin test	++	+	-	++
Saponins	Frothing test	++	+	-	++
	Foam test	++	+	-	++
Steroids	Libermann burchard's test	-	+	++	++
Terpenes	Copper acetate test	-	+	++	++
	Salkowski's test	-	+	++	++
Reducing sugars	Benedict's test	-	-	-	-
	Fehling's test	-	-	-	-
Phenolic compounds	Iodine test	+	++	-	+
	Ferric chloride test	+	++	-	+
	Lead acetate test	+	++	-	+
Fats and Oil	Spot test/stain test	-	+	++	++
	Saponification test	-	+	++	++
Acidic Compounds	Effervescence test	+	-	-	+
Resins	Acetic anhydride test	++	+	-	++
	Turbidity test	++	+	-	++
Proteins	Millon's test	-	-	-	-
	Biuret test	-	-	-	-

Key: + present
- absent

Sedative Activity Test

Elevated plus-maze test

In the elevated plus-maze, mice that received 100 mg/kg doses of the ethyl acetate, absolute methanol, and n-hexane fractions of aqueous methanol extracts from Cannabis sativa showed a preference for the closed arms of the plus-maze and did not exhibit avoidance of the open arms (Tab. 3). The administration of the different fractions of aqueous methanol extracts from Cannabis sativa at 100 mg/kg doses led to increased movement in the mice (statistically significant, $p < 0.001$), resulting in a higher frequency of open arm entries and more time spent in the open arms compared to the control group. The positive control group (diazepam 1 mg/kg) demonstrated a similar effect to the different fractions of aqueous methanol extracts from Cannabis sativa (Table 6).

Table 2: The effect of the different fractions of aqueous methanol extracts of *Cannabis sativa* on elevated plus-maze test in mice

Drug Sample	Doses (mg/kg)	Open Movement (number of movements)	Close movement (number of movements)
Crude Extracts	100	2 ± 0.0707***	2.4 ± 0.0447***
n-Hexane Fraction	10	3 ± 0.044**	3.2 ± 0.0316***
ETOA Fraction	100	1.4 ± 0.0707***	1.4 ± 0.0447***
Absolute MEOH	100	2.1 ± 0.0316***	2.2 ± 0.0447***
Diazepam	5	3.6 ± 0.0400***	4.4 ± 0.0447***
Negative Control (Distilled Water)	10ml/kg	2.8 ± 0.0400	3.6 ± 0.000

Each value is presented as the mean ± SEM (n = 5), MeOH = absolute Methanol fraction of 95% aqueous methanol extract of *Cannabis sativa*, ETOA = Ethyl acetate fraction of 95% aqueous methanol extract of *Cannabis sativa*.

"***p < 0.001, **p < 0.01, *p < 0.05 vs control group (Dunnett's test)"

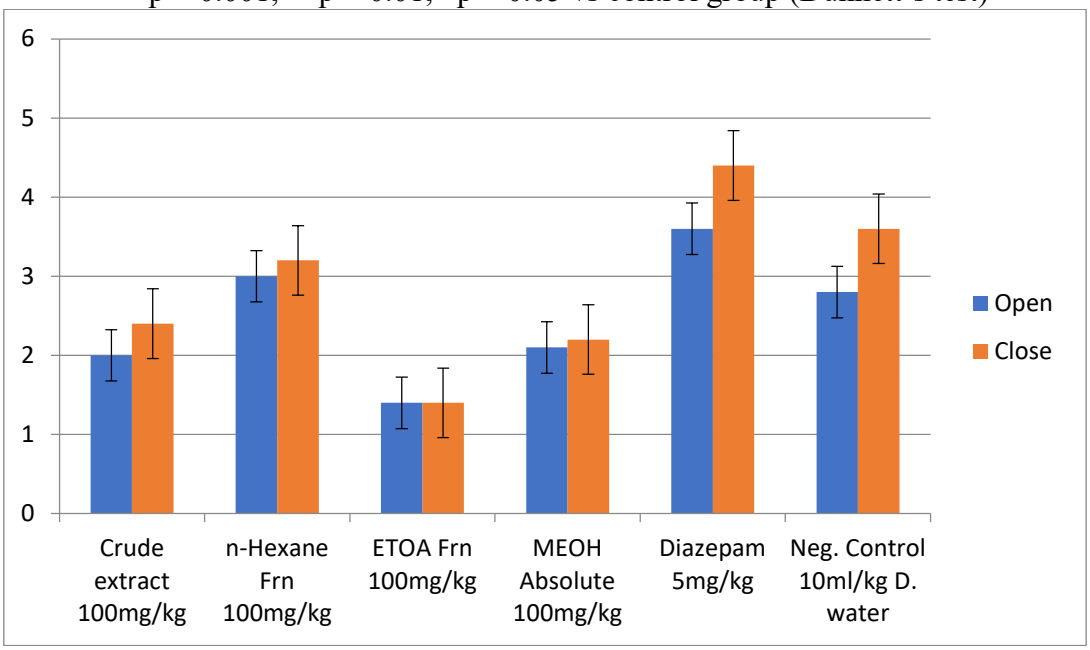

Fig 1: The effect of the different fractions of aqueous methanol extracts of *Cannabis sativa* on elevated plus-maze test in mice on the elevated plus-maze test (Number of entries in open arms; Number of entries in closed arms) in mice

Discussion

Since ancient times, medicinal plants have been a readily available, affordable, and effective source of medicine (14). Numerous traditional medicinal plants have been observed to impact the neurobehavioral state and serve as an alternative to modern medicine. Our research focused on examining the central nervous system (CNS) effects of various fractions of Cannabis sativa's aqueous methanol extracts. The findings indicated that the different fractions of these extracts have a sedative effect on the CNS. In the acute toxicity test, it was noted that the oral administration of the different fractions of Cannabis sativa's aqueous methanol extracts did not produce any noticeable signs of delayed toxicity, behavioral changes, allergic reactions (such as skin rash or itching), or mortality during the 72-hour observation period. Therefore, it was observed that the different fractions of these extracts are non-toxic at our experimental doses of 100 mg/kg. Our initial phytochemical screening revealed the presence of alkaloids, flavonoids, carbohydrates, tannins, saponins, and steroids in the different fractions of Cannabis sativa's aqueous methanol extracts (Table 1).

Several research studies have shown that plant extracts rich in alkaloids and flavonoids have sedative effects by interacting with the benzodiazepine site of the GABAergic complex system, either directly or indirectly (15,16). Tannins may also have an impact on non-specific CNS depression. Therefore, the phytochemical constituents found in various fractions of aqueous methanol extracts of Cannabis sativa could potentially affect sedative effects on the central nervous system.

The anxiolytic activity of Cannabis sativa extracts was assessed using the elevated plus-maze test. Mice treated with various fractions of aqueous methanol extracts exhibited reduced mobility and entries in both the open and closed arms of the maze. These results were consistent with those obtained using the standard anxiolytic agent diazepam, which significantly increased the number of entries into both types of arms (Table 2).

The anxiolytic and sedative effects of benzodiazepines, such as diazepam, are primarily attributed to their ability to enhance the activity of gamma-aminobutyric acid (GABA). Structural modifications of the GABA receptor are thought to increase receptor activity. Benzodiazepines bind to the alpha subunit, leading to the opening of chloride ion channels, increased conductance, and inhibition of action potentials. The pharmacological effects of Cannabis sativa extracts are believed to be mediated by the GABAA receptor, similar to benzodiazepines and non-benzodiazepine agents, which produce sedative and anxiolytic effects by stimulating this receptor. Benzodiazepines' sedative and anxiolytic effects may also be attributed to the

direct activation of glycine synapses in the brain. The mechanism of action of the tested extract may yield similar results to diazepam (17).

The physicochemical properties of central nervous system (CNS) drugs are closely associated with their ability to penetrate the blood-brain barrier, exhibit affinity, and demonstrate CNS activity. Factors such as lipophilicity and molecular weight have been observed to influence the actions of CNS drugs in various reviews. A drug molecule's solubility is influenced by several physicochemical properties, which we will further discuss in relation to blood-brain barrier penetration. The placement of the polar and nonpolar regions of the molecule and their impact on inter- and intramolecular forces in the crystalline state are relevant to solubility and blood-brain barrier penetration, as water must overcome these interactions to solubilize the molecule. There appears to be an interaction between polarity and non-polarity, as evidenced by the ethyl acetate fraction, which exhibits the most significant sedative effect among all compounds. Solubility is generally categorized as moderate (around 10–60 mg/ml) or high (60 mg/ml). CNS drugs bind reversibly to proteins such as albumin and acidic glycoprotein in the blood, and the extent of protein binding is crucial. CNS drugs typically exhibit a high protein binding rate (18). An important question is whether the interaction of these proteins, carrying drug molecules, with the polar surface of the blood-brain barrier leads to the release of the bound drug (15).

Lipophilicity influences various other pharmacological characteristics. Compounds with high lipophilicity are often associated with rapid metabolic turnover, low solubility, and poor absorption. Increased lipophilicity (Log P) raises the likelihood of binding to hydrophobic protein targets other than the intended one, increasing the risk of toxicity (19).

Conclusion:

The investigation findings indicate that different parts of aqueous methanol extracts of Cannabis sativa possess strong sedative properties. Sedation is primarily mediated by the GABA receptor complex in the CNS, which also plays a role in various physiological processes related to behavior, as well as in numerous psychological and neurological disorders. These study results may potentially advocate for the use of C. sativa in traditional medicine for treating depression, insomnia, and neurodegenerative diseases. Research on acute toxicity offers a promising prospect for addressing the toxicity associated with synthetic sedative medications used over the years. Hence, the existing regulations that restrict the use of Cannabis sativa in many countries need reconsideration to allow access while preventing abuse. However, further research is necessary to identify the bioactive substance(s) and elucidate the specific molecular mechanisms underlying the pharmacological effects of the different parts of aqueous methanol extracts of Cannabis sativa. More research is needed to compare variations found in tropical regions like Nigeria with those found elsewhere to determine the most impactful species and avoid wastage of cultivated plants.

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Conflicts of Interest:

The authors declare that there are no conflicts of interest.

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