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RESEARCH ARTICLE

Evaluation of Antibacterial, Antioxidant, and Anticataleptic Activity on Methanol Seed Extract of *Cannabis sativa:* An *In Vitro* and *In Vivo* Study

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Abstract

Background: *Cannabis sativa* (CS) has been traditionally used for its medicinal properties, including antioxidant, antibacterial, and neuroprotective effects. However, its potential anticataleptic activity remains unexplored. **Aim:** This study aimed to investigate the qualitative phytochemical analysis, antioxidant, antibacterial, and anticataleptic activity of methanolic seed extract of *Cannabis sativa* (CSCME). **Methods:** The antioxidant activity of CSCME was evaluated using the DPPH radical scavenging assay. Antibacterial properties were assessed using the agar well diffusion method. For the anticataleptic study, Wistar albino rats (n=30) were divided into five groups: control, disease control (haloperidol 1mg/kg), standard treatment (haloperidol 1mg/kg + trihexyphenidyl 1mg/kg), and two treatment groups receiving haloperidol 1mg/kg with CSCME (10mg/kg and 20mg/kg). Treatments were administered daily for 15 days, and catalepsy severity was assessed using the block and metal bar methods. **Results:** CSCME exhibited significant antioxidant and antibacterial activity comparable to commercial drugs. In vivo, results showed a significant reduction in cataleptic scores in treatment groups ($P<0.05$) compared to disease control. Notably, the 20mg/kg CSCME group demonstrated similar reductions in cataleptic scores as the trihexyphenidyl group ($P>0.05$). **Conclusion:** This study demonstrates that CSCME possesses antibacterial, antioxidant, and anticataleptic activities, suggesting its potential as a therapeutic agent for managing catalepsy and other neurological disorders. The findings warrant further investigation into the clinical applications of CSCME.

Keywords: *Cannabis sativa,* Catalepsy, Medicinal Plants, Neuroprotective, Trihexyphenidyl

1. Introduction

India has a rich tradition of using medicinal plants for various health purposes, with an extensive history of traditional herbal therapies such as Ayurveda, Siddha, and Unani[1]. CS has an extensive history of traditional utilization in India, related to cultural, religious, and therapeutic activities over several decades[2]. CS has been utilized to treat a variety of conditions in Ayurveda, such as pain management, sleeplessness, and digestive problems. Researching the potential medical benefits of CS has drawn more attention in recent years. CS

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belongs to the Cannabaceae family which contains 80 different naturally occurring compounds called "Cannabinoids"[3]. This plant is referred to by many names like hemp, bhang, ganja, charas, and marijuana in India[4]. Many Phyto cannabinoid compounds are found in the CS such as cannabinoids, phenolic compounds, terpenes, etc[5]. FDA has approved only cannabidiol (CBD) and Tetrahydrocannabinol (THC) for the treatment of various medical illnesses[6]. Cannabinoids bind to cannabinoid receptors such as CB1 and CB2 on the surface of cells. In neurodegenerative diseases, Cannabinoids exert their action by blocking glutamate activity, preventing oxidative damage to dopaminergic neurons, and inhibiting the effects of the reactive oxygen species and tumour necrosis factor leading to a neuroprotective effect[7].

Neurodegeneration is the progressive loss of neuronal function that is the primary cause of neurodegenerative disorders. Such neuronal injury may lead to cell death. The most prevalent neurodegenerative diseases are Parkinson's and Alzheimer's[8]. One common sign of Parkinson's illness is catalepsy. The characteristics of catalepsy are muscular rigidity, lack of response to outside stimuli, and limbs remaining in their immobile postures[9]. Catalepsy can be caused by neurological disorders such as Parkinson's disease, schizophrenia, epilepsy, or nervous system medication toxicity. Catalepsy is more common in older people and usually affects those above 65 years of age and older. This is one of the most common neurological illnesses affecting the ageing population. Parkinson's illness impacts around 7-10 million people worldwide. It affects 1% of people over 60, and the prevalence increases with age. It is more prevalent in men than in women. In India, according to research 10 lakh people over the age of 60 are affected by the disease. The disease's pathogenesis is rooted in the depletion and demise of dopaminergic neurons in the substantia nigra, leading to a decrease in striatal dopamine levels[10]. Currently, available treatments for this illness are Trihexyphenidyl, levodopa, and carbidopa. It provides temporary improvement and reverses the symptoms of catalepsy. But it also shows various adverse effects on taking long-term treatment.

Haloperidol, a first-generation typical antipsychotic medication, is widely utilized to treat schizophrenia, delusions, and hallucinations. It works by blocking dopamine D2 receptors in the brain and exerts its antipsychotic action. The primary side effects of typical antipsychotic medication are extrapyramidal symptoms caused by blocking D2 receptors in the nigrostriatal pathway. Extrapyramidal symptoms include dystonia, akathisia, and parkinsonism, which is characterized by rigidity, bradykinesia, tremor, and tardive dyskinesia[11]. Haloperidol-induced catalepsy is a common animal model for studying the neurological deficits seen in Parkinsonian conditions and a common animal model for screening potential compounds for antiparkinsonian[12]. Currently, pharmacotherapy is the primary treatment for Parkinson's disease. However, in chronic conditions, these symptomatic medications have major limitations. There is also evidence supporting that the medicinal properties of CS have anti-neurodegenerative and anti-oxidant properties. There is insufficient data available in the literature analysing the value of CS in catalepsy. With this background, we planned to assess the effectiveness of the anticataleptic, antioxidant, and antibacterial effects in the methanolic seed CSCME of CS.

2. Materials and Methodology

2.1 Preparation of Plant CSCME

CS seeds were obtained from a botanical survey of India, Himachal Pradesh (BSI/HAWHRC/Tech./2023-24/406). The CS seeds (500 g) were acquired, cleansed, dried in the shade for 3 days, and then pulverised into a fine powder using a grinder. The pulverised CS seeds were put into a glass container and subsequently combined with the solvent methanol. Allow the mixture to soak for 72 hours at room temp. After being macerated, Whatman filter paper was used to filter the CSCME. The resulting CSCME was then stored in a refrigerator for further use.

2.2 Chemicals

Trihexyphenidyl and haloperidol have been purchased from a nearby chemist. Methanol was obtained from Merck Life Science Private Limited, Mumbai, and used for the preparation of CS seed extract.

2.3 Material Characterization

The Fourier Transformation Infrared (FTIR) spectroscopy was conducted using a PerkinElmer 400

FT-IR/FIR spectrometer from the United States. The spectroscopic measurements ranged from 400 cm^{-1} to 4000 cm^{-1} [13].

2.4 DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging activity of the CS seed methanolic CSCME (CSCME) was evaluated using a standard method with some modifications[14]. To summarise, 100 microliters of CS seed CSCME were diluted sequentially on a 96-well plate, with concentrations varying from 6.75 to 100 mg/ml. Afterwards, a 0.2 M solution of DPPH was added to each well, with a volume of 100 ml. Afterwards, the assembled well plate was put in a lightless setting and kept at a constant temperature for 30 min. A spectrophotometer was used to measure the Abs at 517 nm. The experiment was performed three times using a methanolic CSCME of CS seed CSCME. DPPH and ascorbic acid have been employed as negative and positive controls, respectively. Methanol served as the control for the experiment. The activity of radical scavenging was measured using the following methodology:

$$Free\ Radical\ Scavening\ Activity(\%) = \left(\frac{Control(Abs) - Test\ Sample(Abs)}{Control(Abs)}\right) \times 100$$

2.5 Qualitative Phytochemical Analysis

The CSCME underwent testing to determine the existence of bioactive chemicals using established standard procedures[15].

2.6 Antibacterial Activity

2.6.1 Preparation of Bacterial Culture

Preparing a bacterial culture for cultivating bacteria involved several steps to ensure the growth and maintenance of the desired organisms. The process began with selecting the medium Luria-Bertani (LB) broth for Bacterial strains, which were procured *Escherichia coli, (E. coli* ATCC 25922*) Staphylococcus aureus (S. aureus* ATCC 25923*), Shigella sonnei (S. sonnei* ATCC 25931*), Salmonella enterica* serotype typhi *(S. typhi* ATCC 6539, and *Pseudomonas aeruginosa* (*P. aeruginosa* ATCC 27853) are the five pathogenic bacterial strains that were received from HiMedia Laboratories Pvt. Ltd. in Mumbai, India. To prepare the bacterial culture, an inoculate loopful of bacteria from a freshly grown culture is inoculated into the medium, typically in a sterile tube or flask. The culture is then incubated with the bacteria at 37°C.

2.6.2 Well Diffusion Method

The good diffusion assay is a method utilized to investigate the antimicrobial property by measuring the ZOI of CS seed CSCME against bacterial strains[16]. The growth of the bacteria is monitored by observing changes in the medium, including turbidity and by measuring Colony-Forming Units (CFUs) by using a UV-spectrophotometer. The bacterial culture is typically grown overnight or until the desired growth phase is reached, such as the exponential (log) phase for bacterial strains. The samples were prepared by dissolving in distilled water at a concentration of 100mg/ml. Ampicillin was used as a positive control, while distilled water served as the negative control for this study. This experimental setting allows the assessment of the antibacterial activity of the CS seed CSCME against the bacterial strains under investigation.

2.7 *In Vivo* Studies

2.7.1 Experimental Animals

The in vivo experiments were carried out at the Central Animal House and the Department of Pharmacology, Chettinad Hospital and Research Institute, Chengalpattu, Tamil Nadu. The investigation was commenced with the endorsement of the Institutional Animal Ethics Committee (IAEC 3/Proposal:93/A. Lr:66/Dt:22.08.2022). For this investigation, a total of 30 Wistar albino rats, of both male and female genders, weighing between 150 and 250 grams, were obtained from THANUVAS, Madhavaram, Chennai. The subjects were housed in hygienic polypropylene enclosures, kept at a temperature that ranges from 23-25 °C and a humidity level of 50-60 %. The subjects were exposed to a 12-hour light/dark cycle, alternating between periods of illumination and darkness. Their diet consisted of unlimited access to water and pellets. The animals that were received underwent a one-week quarantine period to ensure their physiological, psychological, and dietary stability.

2.7.2 Experimental Design

30 Wistar albino rats were separated into 5 categories, each consisting of 6 rats (3 males and 3 females). Category 1 didn't receive any treatment (Control), Category 2 received haloperidol 1mg/kg intraperitoneal injection alone (Disease control), Category 3 received haloperidol 1mg/kg intraperitoneal injection and trihexyphenidyl 2mg/kg orally (Standard treatment), Category 4 received haloperidol 1mg/kg intraperitoneal injection and methanolic seed CSCME of CS 10 mg/kg SC, Category-5 received haloperidol 1mg/kg intraperitoneal injection and methanolic seed CSCME of CS 20 mg/kg SC.

2.7.3 Induction of Catalepsy

Haloperidol, 5 milligrams per ml, has been diluted in a normal saline solution at a concentration of 0.9%. This solution was produced before the commencement of the experiment to achieve the desired concentration of 1mg/kg. An intraperitoneal injection of haloperidol at a dose of 1 mg per kg caused catalepsy, 30 min. after the administration of the study medicines, which included Trihexyphenidyl and the methanolic seed CSCME of CS. The process was replicated for 15 days. The CSCME's anticataleptic activity was evaluated on the 16th day utilising the block and metal bar method. The treatment was conducted in a tranquil environment, free from any external disturbances, at the ambient temperature. The intensity of catalepsy was assessed at 30-minute intervals over 4 hours.

2.7.4 Catalepsy Assessments

Measurement of catalepsy by block method:

Table 1 depicts the highest attainable score is 3.5, which signifies complete catalepsy. A score over 2.5 indicates the existence of catalepsy. The intensity of catalepsy was evaluated at 0, 60, 120, 180, and 240 min. After the injection of haloperidol.

2.7.5 Measurement of Catalepsy by Metal Bar Test

The rat's front paws were positioned on a metal horizontal bar with a diameter ranging from 2 to 5 mm. The bar was suspended 6 cm over the tabletop. The extent of catalepsy was quantified by measuring the duration, in sec., it took for the rat to lower both of its forepaws onto the tabletop. The bar test had a maximum time limit of 180 sec. To compare the results, scores were collected at several time intervals (0, 60, 120, 180, and 240 min.) following the administration of haloperidol.

2.7.6 Safety Assessment

After completing the study, we assessed the safety of the CSCME by examining various factors. These included the total WBC count, Hb levels, RFT such as urea and creatinine levels, LFT such as AST, ALT, and ALP levels, as well the histopathological examination of the brain. Each animal was euthanized by administering 3-5 mL of halothane. Following the act of sacrifice, the organs were carefully removed, gathered, and preserved in a solution of buffered formalin (10%).

Table 1. The highest possible score is 3.5, suggesting total catalepsy

Stages	Behaviour	Score
Stage- I	When the rat is put on the table, it moves as usual.	0
Stage- II	Only when handled or prodded does the rat move.	0.5
Stage –III	Rat on the table with front paws alternately on a 3cm block unable to adjust stance in 15 sec., scores 0.5 for each paw and 1 for this level.	1
Stage –IV	Rat fails to get rid of while front paws are alternately put on a 9cm block, scoring 1 for each paw and 2 for this level.	2

Subsequently, they were submitted for examination under a microscope to study their tissue structure and identify any abnormalities.

3. Statistical Analysis

GraphPad Prism software from San Diego, CA, USA, was utilized for statistical analyses in this study. A two-way ANOVA test was employed to evaluate the caulescence, with the results presented as the mean ± standard deviation. The statistical analysis conducted on CSCME revealed a highly significant variation with a p-value< 0.05 (*), p-value< 0. 01(**), p-value< 0. 001(***), p-value< 0. 0001(****) were contemplating.

4. Results

4.1 Characterization

4.1.1 FTIR

The FTIR approach was utilized to get information about the emission spectrum by analysing the vibrational mode characteristics of the CSCME. The FTIR spectrum analysis reveals distinct peak locations for the samples, as seen in Figure 1. The band at 1650 cm^{-1} indicates the existence of C=N stretching existence of class imine/oxime, the band at 1411 cm^{-1}, and 1313 cm^{-1}, indicates the existence of S=O stretching existence of class sulphate, the band at 1071 cm^{-1} indicates the existence of CO-O-CO stretching existence of class anhydride and band at 946 cm^{-1}, 612 cm^{-1} indicates the existence of C-Br stretching existence of class halo compound[17].

4.2 Antioxidant Assay

4.2.1 DPPH Free Radical Scavenging Assay

Figure 2 displays the DPPH free radical inhibition activity of various compounds at different dosages. The inhibitory impact identified with these compounds exhibited a significantly higher magnitude compared to the other compounds and, to some extent, was comparable to the std., specifically vitamin C. Figure 2 illustrates the impact of varying amounts of plant CSCME on the antioxidant activity of DPPH radicals. Our analysis revealed that the plant CSCME displayed antioxidant activity that was similar to ascorbic acid. The antioxidant activity in the DPPH test depends on

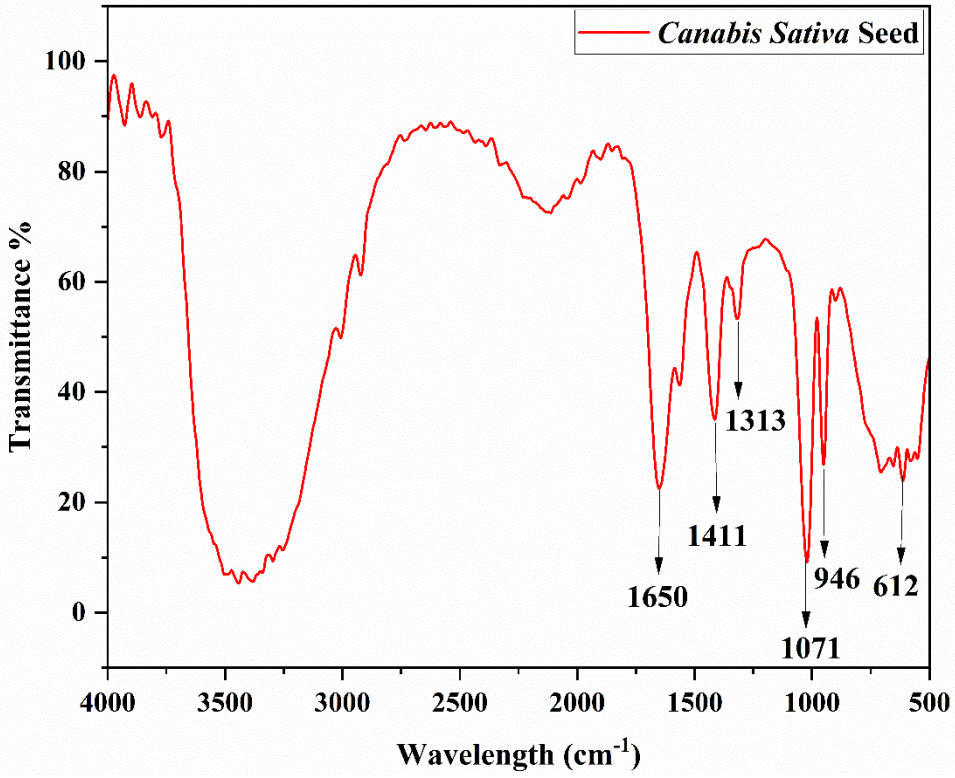


Figure 1. FTIR spectrum of CSCME.

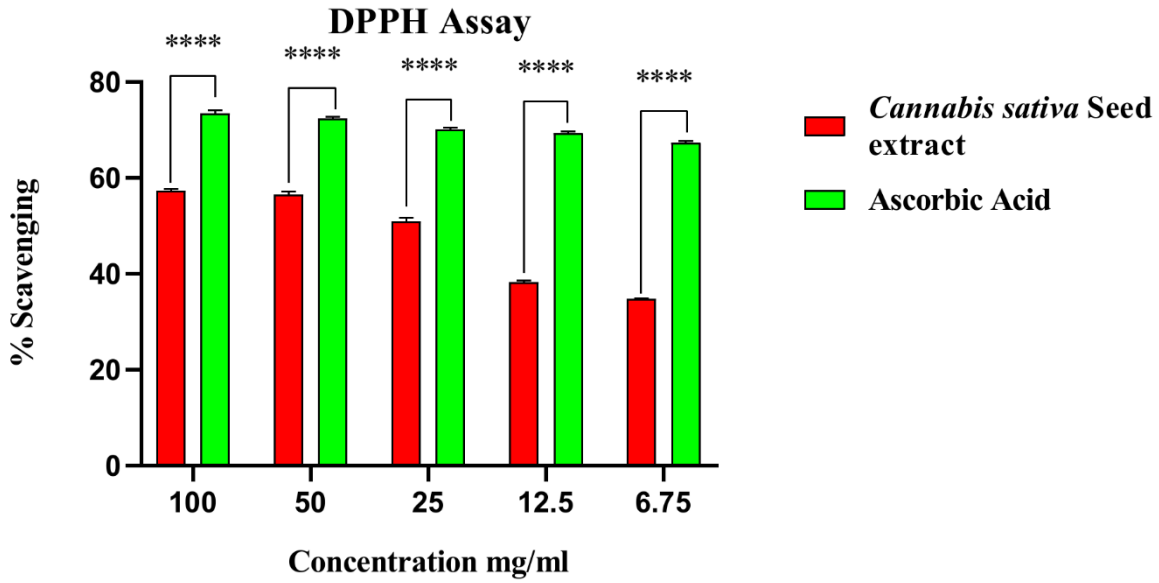


Figure 2. DPPH free radical inhibition at various concentrations. Mean ± SD (n=3). ****p<0.0001.

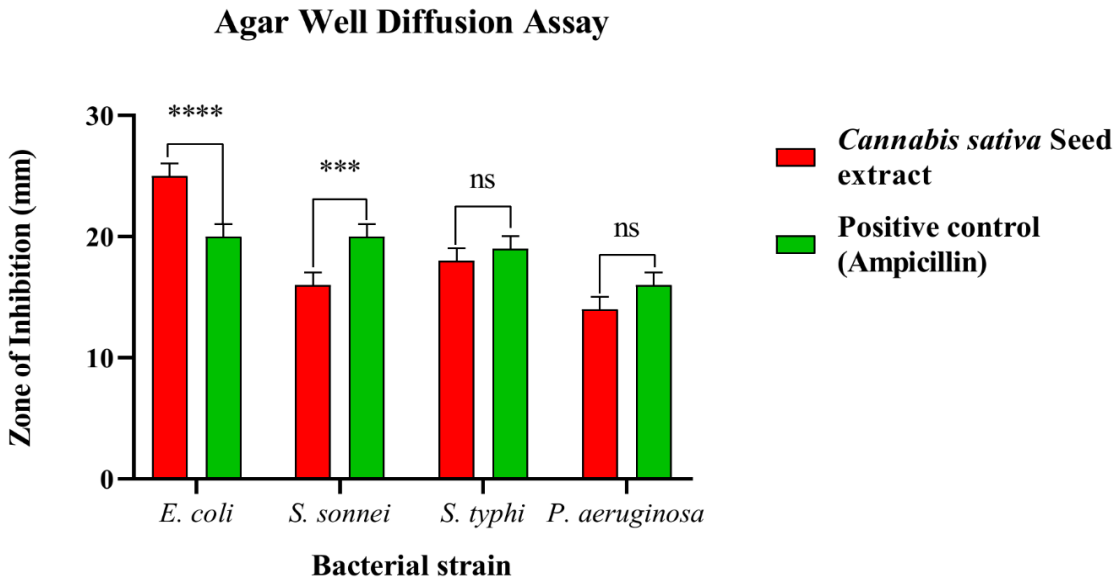


Figure 3. Comparison of Zone of inhibition in mm for CS seed CSCME against different bacterial strains.

the plant CSCME's ability to transfer electrons to the unpaired electron in the DPPH sol. Consequently, the intensity of the n → π* transition at a wavelength of 517 nm diminishes, leading to a shift in colour from a dark purple hue to a pale yellow shade[18]. The observed alteration in colour signifies the existence of antioxidant activity.

4.3 Antibacterial Activity

The antibacterial efficacy of CSCME was evaluated using good diffusion techniques. The existence of an inhibitory zone demonstrated the antibacterial activity of CSCME shown in Figure 3, Each strain was marked accordingly, and 15µl of CSCME was added to the designated wells. The green synthesis of CSCME has demonstrated significant antibacterial activity, in a concentration of 100ml/mg as evidenced by a zone of inhibition in well diffusion assays shown in (Figure 3)[19]. The CSCME synthesised through environment-friendly methods, has demonstrated promising antimicrobial effects against multiple pathogenic bacterial strains. Figure 4 shows that comparison of

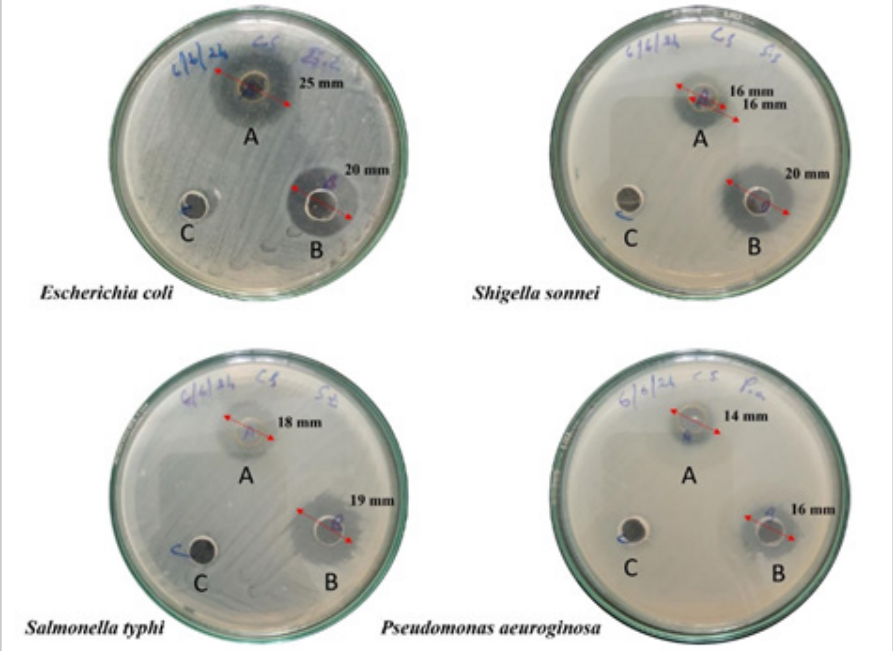


Figure 4. Comparison of antibacterial activity of CS seed CSCME against Bacterial strains. In the Petri plate, streaking was performed for five bacterial strains, denoted as A- CS seed CSCME, B- Positive control, and D- negative control.

the Zone of inhibition in mm for CSCME in different bacterial strains with the variation of *p-value* <0.0001 respectively.

4.4 *In vivo* Studies

4.4.1 Block Method

The haloperidol-induced catalepsy category was compared with the standard drug (Trihexyphenidyl) and test drug (CSCME -10 mg/kg and 20mg/kg). All the rats in the control category exhibited normal locomotion when placed on the table. The administration of Haloperidol at a dosage of 1mg/kg through intraperitoneal injection resulted in a gradual and time-dependent escalation of the cataleptic condition in the categories. The catalepsy score exhibited a significantly greater increase in the category that received haloperidol alone than in the control category. The highest catalepsy score was recorded after 180 min. Categories 3, 4, and 5 showed a substantial decrease in cataleptic scores compared to category 2 (disease control) after 15 days ($p<0.05$). Category 3 exhibited a noteworthy reduction in cataleptic score compared to the CS 10 mg/kg category (4) at all time intervals. Category 5 exhibited a more pronounced decrease in catalepsy score compared to Category 4 across all time intervals (Figure 5). It was observed that the decrease in cataleptic score in Trihexyphenidyl (category 3) and 20 mg/kg CSCSCME (category 5) was similar($P>0.05$) and there was no significant difference between both categories. The results of the cataleptic scores assessment are shown in Table 2 (Block method) and Table 3 (Metal bar method).

4.4.2 Metal Bar Test

The scores obtained from the metal bar test, which measures catalepsy, are presented in (Table 3), Within a few sec., all the rats in the control category lowered their forepaws from the bar. Categories 3, 4, and 5 showed a notable decrease in cataleptic ratings compared to category 2, which was treated with haloperidol alone, after 15 days at all time intervals ($p<0.05$). Category 3 exhibited a notable decrease in the catalepsy score compared to the CS 10 mg/kg and 20 mg/kg category (4,5) across all time intervals ($p<0.05$). There is a notable disparity in the decrease of the catalepsy score between the CS *category* receiving 20 mg/kg (5) and the CS category receiving 10 mg/kg (4) at all time intervals ($p<0.05$) (Figure 6). The category treated with trihexyphenidyl and CS - 20mg/kg showed a greater decrease in catalepsy score at 240 min.

Table 2. Effects of CSCME on haloperidol-induced catalepsy by block method at various time intervals values are demonstrated in mean ± SD (n = 6). P<0.05*

	Degree of catalepsy (Block Method)				
	0 min	**60 min**	**120 min**	**180 min**	**240 min**
Category 1 control	0.00	0.00	0.00	0.00	0.00
Category 2 Catalepsy induction	1.00±0.55	2.08±0.38	2.33±0.52	3.08±0.38	2.25±0.42
Category 3 Standard treatment	0.25±0.27*	0.42±0.20*	0.33±0.26*	0.25±0.27*	0.17±0.26*
Category 4 CS 10mg	0.42±0.49*	0.75±0.69*	0.67±0.52*	0.50±0.55*	0.33±0.41*
Category 5 CS 20mg	0.33±0.41*	0.67±0.41*	0.58±0.58*	0.33±0.32*	0.17±0.26*

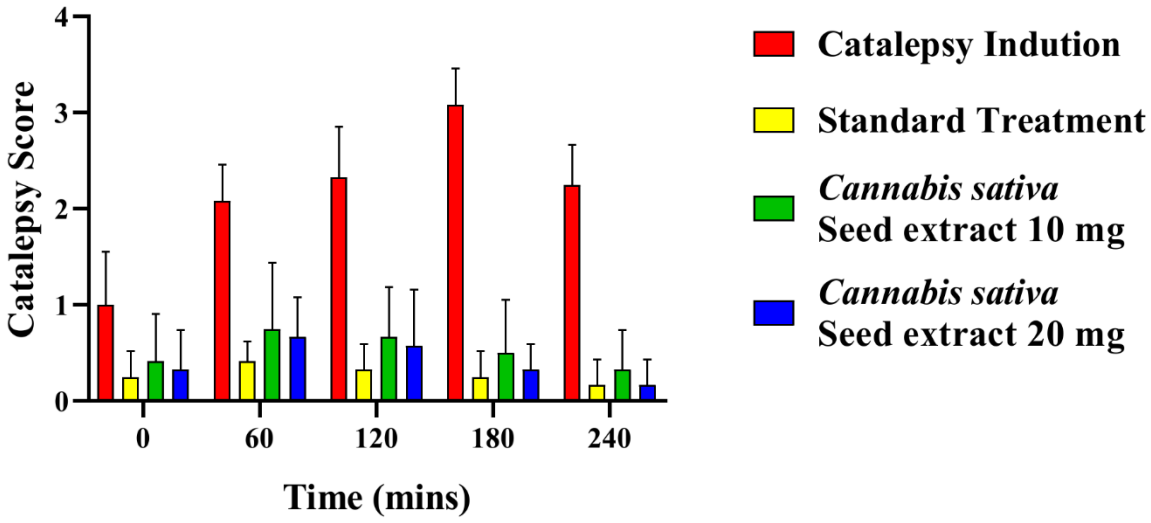


Figure 5. Effect of CSCME on haloperidol-induced catalepsy by block method.

Table 3. Effects of CSCME on haloperidol-induced catalepsy by metal bar test at various time intervals, \mean ± SD (n = 6). P<0.05*, P<0.001** statistically significant.

	Degree of catalepsy (Metal Bar Method)				
	0 min	**60 min**	**120 min**	**180 min**	**240 min**
Category 1 control	0.00	0.00	0.00	0.00	0.00
Category 2 Catalepsy induction	9.42±3.69	90.67±13.38	120.17±13.60	130.33±22.47	124.67±21.97
Category 3 Standard treatment	6.42±1.16*	50.42±17.32*	36.08±12.28*	28.00±3.48*	18.00±3.30*
Category 4 CS 10mg	7.00±1.61*	77.67±11.54	68.00±10.04*	57.33±5.75*	37.00±3.35*
Category 5 CS 20mg	6.58±0.97**	71.08±7.26**	54.33±6.06*	36.83±5.46*	20.58±1.88*

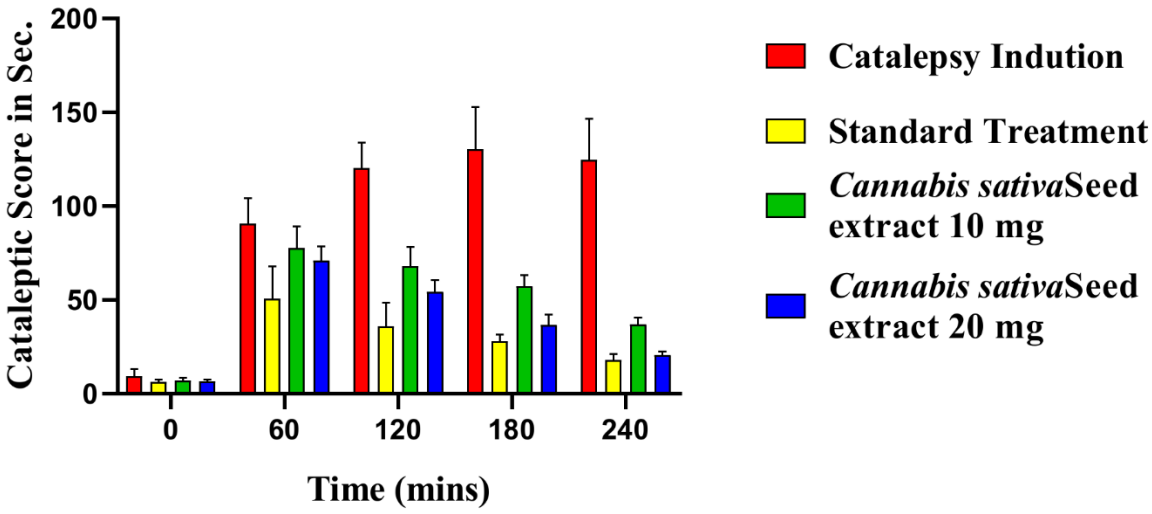


Figure 6. Effect of CSCME on haloperidol-induced catalepsy by metal bar method.

Table 4. Haematological parameters of CSCME

		Category 1	Category 2	Category 3	Category 4	Category 5
Body weight	Baseline	203.33±16.13	204.5±18.72	207.±24.34	203.83±25.97	203.33±28.13
	End	220.33±19.60	217.33±18.66	225.00±24.90	225.00±29.02	222.00±29.44
WBC count	Baseline	9593.33±1015.53	9683.33±1097.08	9586.67±682.08	9687.67±1376.70	9509.67±926.51
	End	9716.66±1755.19	9698.33±1025.58	9394.17±1278.20	9949±2589.57	9977±1829.05
Hb	Baseline	13.33±1.08	11.72±1.15	12.13±1.50	13.23±1.04	13.07±1.06
	End	13.17±1.36	9.97±0.45	13.08±1.40	12.95±1.56	11.02±1.09
Urea	Baseline	28.5±3.45	31.17±3.60	30.83±4.07	29.17±3.76	30.83±3.76
	End	30.83±5.85	29±4.65	29±4.86	29.67±4.08	29.67±4.13
Creatinine	Baseline	0.87±0.18	0.75±0.14	0.94±0.11	0.61±0.14	0.97±0.24
	End	0.74±0.11	0.80±0.16	0.72±0.11	0.65±0.15	0.94±0.20
ALP	Baseline	218.33±20.18	262.67±34.05	280.33±26.96	269.83±23.67	257.79±34.40
	End	222.67±28.49	276.5±36.67	304.33±29.13	275±49.36	269.625±37.37
AST	Baseline	78.67±13.31	82.67±19.53	80.33±16.66	85.33±16.77	74.83±18.18
	End	84.67±17.37	102±5.80	87.67±14.67	80.17±19.72	84±20.32
ALT	Baseline	47.67±7.31	48±7.48	51.5±5.43	45.5±4.93	49.5±6.54
	End	55.33±7.15	54.17±5.67	52.67±9.35	54.17±6.34	54.08±6.59

4.5 Safety Assessment

At the end of the research, the b.wt of the rats increased in all categories from baseline. There were no statistically significant variations among or among the categories. Additionally, no significant changes in other parameters (WBC count, haemoglobin, urea, creatinine, and liver function tests) were observed when comparing baseline values to those at the end of the treatment (15th day). Table 4 represents the Haematological parameters of CSCME.

4.6 Histopathological Examination

The histopathological changes observed in the brains of rats treated with CSCSCMEs revealed a range of structural and cellular alterations. These changes suggest both neurodegenerative and potentially reactive processes. Fibrillar degeneration and hydronic degeneration suggest neuronal distress and damage, while the increase in neuronal cells may reflect a reactive attempt to compensate for this damage. Microcystic

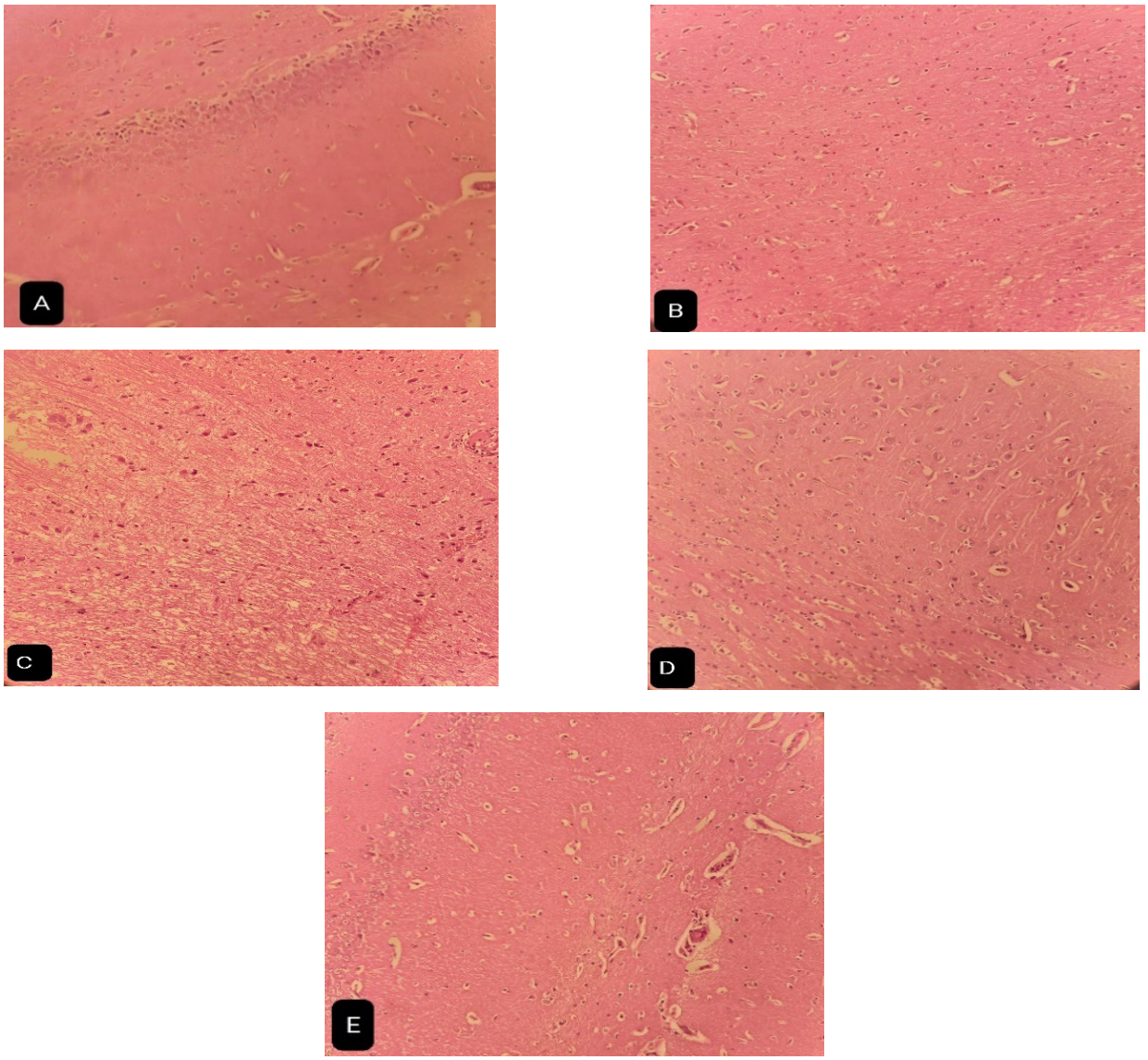


Figure 7. **(A).** Brain G1 Control, **(B).** Brain G2 Catalepsy Induction, **(C).** Brain G2 Standard Treatment, **(D).** Brain G4 CS 10mg/kg, **(E).** Brain G5 CS 20mg/kg.

changes and oedema indicate the extent of cellular and tissue damage produced by CSCSCME treatment.

Figure 7(A) illustrates the normal state of Brain G1 Control. Figure (B) depicts Brain G2 Catalepsy Induction, which exhibits the existence of oedema, enlarged neurons, reactive glial cells, increased thin capillaries, a few lymphocytes, and Fibrillar degeneration. Figure (C) depicts Brain G2 Standard Treatment, which exhibits fibrillar degeneration accompanied by a rise in neuronal cells and hydropic degeneration. Additionally, there are microcystic alterations and oedema present. Figure (D) depicts the effects of administering 10mg/kg of CS on Brain G4, which include oedema, fibrillar degeneration with an increase in neuronal cells, hydropic degeneration, and microcystic alterations. The figure depicts the effects of administering 20mg/kg of CS on the brain, which include the existence of oedema and a rise in neuronal cells.

5. Discussion

The progressive loss and degeneration of neurons in the central nervous system are hallmarks of

neurodegenerative diseases, frequently leading to catastrophic consequences. Parkinson's disease is the most prevalent neurological condition. It is defined by symptoms like catalepsy, which is characterized by prolonged muscle rigidity and immobile posture. Conventional anti-cataleptic drugs frequently have unfavourable side effects. Cannabinoids, specifically THC (tetrahydrocannabinol) and CBD (cannabidiol) include different phytochemicals that could have neuroprotective advantages.

In this study, we have examined the antioxidant and antibacterial activity of CSCME. The results show prominent antioxidant activity and similar antibacterial properties when compared to the commercial drug against the pathogenic bacterial strains.

Haddou *et al.,*[20] examined the antioxidant and antibacterial properties of CSCMEs. The ethanol-based extract from the seeds of CS showed significant effectiveness, with the least IC_{50} value of 0.36mg/mL±0.01. This was better than the reference substance ascorbic acid, which had an IC_{50} value of 0.68mg/mL±0.02. The ethanolic CSCME exhibited the largest inhibition zone width (IZ = 23 mm) against *Penicillium* sp., whereas the hexane CSCME showed the lowest IZ (07 mm) against *Listeria monocytogenes*.

In this research, we assessed the impact of the CSCME on haloperidol-induced catalepsy in Wistar albino rats. Catalepsy was induced by administering 1 mg/kg of haloperidol intraperitoneally, half an hour after the test drugs were given. The results demonstrate a prominent elevation in the catalepsy score in the disease induction category (Category 2) evaluated to the std. Category after 15 days. Categories 3, 4, and 5 exhibited a prominent decrease in catalepsy scores compared to the disease control category ($p<0.05$). Notably, categories 4 (10 mg/kg of CSCSCME) and 5 (20 mg/kg of CSCSCME) demonstrated a substantial decrease in catalepsy scores compared to the disease control category. Furthermore, the reduction in catalepsy scores in the 20 mg/kg CS CSCME category (Category 5) was comparable to that in the trihexyphenidyl category (Category 3) at 240 min. ($P>0.05$). These findings suggest that CS has anticataleptic activity, aligning with other studies that have shown CS to be protective against haloperidol-induced catalepsy.

The present study's findings support earlier research by Abdel-Salam *et al.,*[21], which demonstrated that CS CSCME can lessen catalepsy and oxidative stress brought on by haloperidol in mice. It was observed that the use of CS CSCME decreased catalepsy. Felipe *et al.,*[22] suggested that CS could have potential benefits in treating striatal illnesses by decreasing catalepsy through different methods, such as blocking D2 receptors, inhibiting NOS, activating CB1 receptors, and stimulating the 5-HT1A receptor. Andreza *et al.,*[21] conducted additional research to determine if CBD may mitigate the impact of haloperidol-induced catalepsy and c-Fos expression in the dorsolateral striatum. The researchers noted that CBD effectively counteracted the cataleptic effects caused by haloperidol when administered 2.5 hours later. Additionally, CBD enhanced the activity of the protein c-Fos. In addition, Venderova *et al.,*[23] noted that CS exhibited positive effects on a significant number of individuals with Parkinson's disease, indicating that cannabinoids could potentially be used as a treatment for neurodegenerative illnesses. CS exerts its effects on neurotransmitter systems, including the dopamine, serotonin, and endocannabinoid systems, to alleviate catalepsy. This is especially pertinent when haloperidol is employed to induce catalepsy. The effects of CS compounds, including THC, are partially achieved by influencing the transmission of dopamine in the brain and acting as a partial activator at cannabinoid CB1 receptors. Various research has examined the capacity of cannabinoids, specifically CBD and THC, to regulate haloperidol-induced catalepsy in rats. CBD functions by binding to serotonin receptors (5-HT1A) and regulating the endocannabinoid system.

Research has explored the possible synergistic effects of mixing CBD with THC or other cannabinoids in modifying haloperidol-induced catalepsy. Combining multiple therapies may offer advantages in terms of enhanced effectiveness and tolerability, as well as a broader spectrum of pharmacological actions. Cannabinoids have the potential to offer therapeutic advantages for certain individuals suffering from Parkinson's disease. However, it is important to take into account the potential dangers and side effects, such as cognitive impairment, psychomotor effects, cardiovascular effects, and possible combinations with other medications.

Ultimately, the current work suggests that the CSCME can prevent or alleviate catalepsy.

Nevertheless, additional research is necessary to have a more comprehensive understanding of the precise mechanisms underlying the anti-cataleptic effects of CS. This knowledge is essential for effectively applying these findings in clinical settings to treat movement problems and mitigate the motor adverse effects caused by antipsychotic medications.

6. Conclusion

The research found that the methanolic CSCME of CS seed had anticataleptic efficacy at dosages of 10 mg per kg and 20 mg per kg. The data indicate that the methanolic CSCME exhibits significant antioxidant and antibacterial activity, comparable to that of commercial drugs. The impact of CS was equivalent to that of trihexyphenidyl, and it was shown that the dosages of CS (20 mg/kg) and trihexyphenidyl (1mg/kg) had identical outcomes. There were no deaths seen in any of the categories. Therefore, it may be inferred that the methanolic CSCME of CS seed has an impact on the prevention of catalepsy. Additional study is necessary to thoroughly examine the effectiveness and safety of CS, as well as to get a deeper understanding of how various cannabinoids, dosages, and routes of administration impact the symptoms of catalepsy.

7. Authors Contribution:

Dasa Prakash Annadurai - Investigation, experimentation, writing the original manuscript; Kawsalliya Raj - Data curation; Methun Francis - Visualization; Rajesh T Vinayakam -Visualization; Vijayashree Ragavan - Formal analysis; Satheesh Selvaraj - Reviewing and editing; Susmitha Kalaichelvan - Reviewing and editing; Arunkumar Radhakrishnan - Conceptualization and supervision.

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