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Research Article

Analgesic Effect of Cannabidiol and Tetrahydrocannabinol on Cold Hypersensitivity in Reserpine Model of Parkinson's Disease

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Abstract

Parkinson's disease is the most common neurodegenerative disease after Alzheimer's disease characterized by early degeneration of dopaminergic neurons in the substantia nigra pars compacta and accumulation of Lewy bodies. Parkinson's disease is associated with non-motor symptoms like pain which may precede motor symptoms by more than a decade. The aim of the study was to investigate the effect of Cannabidiol (CBD) and Tetrahydrocannabinol (THC) on Cold Hypersensitivity in reserpine induced Parkinson's disease in mice. Forty-two (42) mice were randomly divided in to seven groups of six mice each. All administration were carried out for three weeks. Cold pain threshold increased significantly ($P < 0.05$) in reserpine (0.5 mg/kg) + CBD (60 mg/kg) and reserpine (0.5 mg/kg) + THC (6 mg/kg) groups. Malonaldehyde (MDA) concentration decreased significantly in the reserpine (0.5 mg/kg) + CBD (30 mg/kg), reserpine (0.5mg/kg) + THC (4mg/kg) and reserpine (0.5 mg/kg) + CBD (60 mg/kg) +THC (6 mg/kg) treatment groups respectively. Superoxide dismutase (SOD) concentration increased significantly ($P < 0.05$) in reserpine (0.5 mg/kg) + CBD (60 mg/kg), reserpine (0.5 mg/kg) + THC (6 mg/kg) and reserpine (0.5 mg/kg) + CBD (60 mg/kg) +THC (6 mg/kg). Reduced glutathione level (GSH) increased significantly ($p < 0.05$) in reserpine (0.5 mg/kg) + THC (6 mg/kg) and reserpine (0.5 mg/kg) + CBD (60 mg/kg) + THC (6 mg/kg) groups. In conclusion, CBD and THC were able to improve cold pain threshold and at the same time decreasing MDA concentration and increasing SOD activity and GSH concentration.

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Introduction

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder characterized by early prominent death of dopaminergic neurons in the substantia nigra pars compacta and the accumulation of Lewy bodies, abnormal intracellular aggregates containing proteins such as α-synuclein and ubiquitin. PD is also associated with non-motor symptoms, which may precede motor symptoms by more than a decade [1], and which become especially troublesome in the later stages of the disease [2]. Pain is among the most common yet underappreciated non-motor symptoms of PD, reported by between 68% and 85% of patients, and it responds poorly to conventional PD medications [3].

Sensory processing, including thermal perception and nociception, is frequently altered in PD, reflecting dysfunction not only within the nigrostriatal dopaminergic system but also within central pain-modulating circuits, such as descending inhibitory pathways [3], [6]. Reduced thermal thresholds and cold hypersensitivity have been reported in PD and are thought to arise from a combination of central sensitization, impaired descending pain modulation, and dopaminergic dysregulation of nociceptive processing [3], [6]. Evaluating cold hypersensitivity therefore provides a clinically relevant and experimentally tractable endpoint for probing PD-associated pain, distinct from classical models of peripheral neuropathic pain.

The administration of reserpine to rodents has been widely used as a pharmacological model for studying non-motor aspects of PD. Reserpine interferes with the vesicular storage of monoamines, causing monoamine depletion in nerve terminals and, depending on the dose, transient hypolocomotion and muscular rigidity [4]. Importantly, the reserpine model does not reproduce the progressive dopaminergic neurodegeneration or α-synuclein pathology that characterize human PD, and its validity is therefore restricted to specific behavioral and neurochemical endpoints rather than to disease progression [4], [5]. The severe motor impairment produced by higher doses of reserpine also precludes other behavioral evaluations, such as memory and cognitive/emotional assessments. However, a single administration of reserpine at low doses (0.1–0.5 mg/kg), as used in the present study, has been shown to induce non-motor deficits with comparatively limited accompanying motor impairment [5], making this protocol appropriate for isolating and investigating cold hypersensitivity with reduced confounding from gross motor deficits.

Pain in PD is multifactorial, arising from altered dopaminergic modulation of nociception, central sensitization, and abnormalities of descending pain pathways, together with peripheral contributions in some patients [3], [6]. This multifactorial, predominantly central profile distinguishes PD-associated pain from purely peripheral neuropathic pain syndromes. Dopaminergic therapies remain the mainstay of PD management and primarily target motor symptoms; while some agents may also influence selected non-motor features, PD-associated pain often remains inadequately controlled despite conventional treatment [6], [7], underscoring the need to explore alternative pharmacological approaches.

The endocannabinoid system (ECS) has emerged as a promising target for modulating pain in PD. Cannabinoid receptors and endocannabinoid signaling components are present at peripheral nociceptors, spinal dorsal horn neurons, and supraspinal pain-processing centers, where they modulate nociceptive transmission [9], [10]. Within the basal ganglia, cannabinoid signaling is closely associated with dopaminergic circuitry and has been implicated in the modulation of both motor and sensory processing relevant to PD, providing a mechanistic rationale for evaluating cannabinoids against PD-associated pain [10].

Cannabis, in its crude form (dried flowers, stems, seeds, and leaves), is derived from the *Cannabis sativa* plant and contains more than 85 identified phytocannabinoids, distinguished from synthetic cannabinoids and from the structurally distinct endogenous cannabinoids. Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are two of the best-characterized phytocannabinoids, with distinct pharmacological profiles. THC acts primarily as a partial agonist at cannabinoid type 1 (CB1) and type 2 (CB2) receptors and is responsible for the psychoactive effects of cannabis, alongside analgesic and muscle-relaxant properties [7].

CBD, by contrast, has comparatively low affinity for CB1/CB2 receptors and produces its effects through a more complex, largely non-cannabinoid-receptor-mediated pharmacology; it is non-psychoactive and has documented hypnotic, anxiolytic, antipsychotic, and neuroprotective effects [7].

CB1 receptors are abundant throughout the central nervous system, whereas CB2 receptors, though classically associated with cells of the immune system, are also expressed within the nervous system, particularly under inflammatory or neurodegenerative conditions such as PD [9]. Beyond well-recognized effects such as reduced intraocular pressure, appetite stimulation, and modulation of smooth-muscle activity [8], cannabinoids exert analgesic, anti-inflammatory, neuromodulatory, and potentially

neuroprotective actions that are directly relevant to PD-associated pain [8].

The prominent presence of cannabinoid receptors within the basal ganglia has been proposed as a basis for the therapeutic potential of cannabis and cannabinoids in PD and related movement disorders [10].

To our knowledge, the effects of cannabinoids on nociceptive and sensory abnormalities in the reserpine-induced model of PD, and specifically the comparative effects of CBD and THC on cold hypersensitivity in this model, have not been directly investigated, representing a clear gap in the literature. Considering the involvement of monoaminergic and endocannabinoid signaling in nociceptive processing, we hypothesized that CBD and THC would attenuate reserpine-induced cold hypersensitivity. Therefore, the present study aimed to investigate and compare the analgesic effects of CBD and THC on cold hypersensitivity in a low-dose reserpine model of Parkinson's disease.

Materials and Methods:

Ethical approval was obtained from Committee on Animal Use and Care, Ahmadu Bello University, Zaria with approval number (ABUCAUC/2024/057) dated 16th October 2024.

The experiment was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. Reserpine (Batch Number:R817202) (Shanghai Ideal Medical Technology, China), *Cannabis sativa leaves* (National Drug Law Enforcement Agency (NDLEA) Kaduna State Command, Nigeria) Tetrahydrocannabinol (Extracted from the Cannabis Sativa in Department of Pharmacognosy, ABU Zaria, Nigeria) Cannabidiol (Releaf Pharmaceuticals, South Africa), ELISA Kits, distilled water, Tween 80, oral garage, disposable latex hand gloves, saw dust, dissecting kits, electronic weighing balance, syringe (1ml), standard animal feed, plastic cages and plain bottles. All other chemicals used were of analytical grade.

Experimental Animals

A total of forty (42) adult mice of either sex weighing between 20-35g were used for the study. They were sourced from the Animal house, Department of Human Physiology, Ahmadu Bello University Zaria. Standard animal feed made of pellets from growers' mash was fed to the mice and allowed free access to drinking water *ad libitum.*

Experimental Design

After the period of acclimatization, Reserpine, Cannabidiol and Tetrahydrocannabinol were administered intraperitoneally.

Induction of Parkinson's-like symptoms

In this study, induction of Parkinson's-like symptoms was carried out by the administration of reserpine intraperitoneally (0.5 mg/kg i.p) once per day for a period of 10 days [11], after which observation for the non-motor symptoms of Parkinson's disease in mice was made.

Treatment with Cannabidiol and Tetrahydrocannabinol

Cannabidiol (30 mg/kg and 60 mg/kg) and Tetrahydrocannabinol (4 mg/kg and 6 mg/kg) were administered intraperitoneally for a period of fourteen (14) days after the last day of induction of Parkinson's like symptoms with reserpine.

Animal Groupings

Mice were divided randomly into 7 groups of 6 each as follows.

Group 1 (Control): Distilled water (10 ml/kg *i.p*)
Group 2: Reserpine (0.5 mg/kg *i.p*)
Group 3: Reserpine (0.5 mg/kg *i.p*) + Cannabidiol (30 mg/kg *i.p*)
Group 4: Reserpine (0.5 mg/kg *i.p*) + Cannabidiol (60 mg/kg *i.p*)
Group 5: Reserpine (0.5 mg/kg *i.p*) + Tetrahydrocannabinol (4 mg/kg *i.p*)
Group 6: Reserpine (0.5 mg/kg *i.p*) + Tetrahydrocannabinol (6 mg/kg *i.p*)
GROUP 7: Reserpine (0.5 mg/kg *i.p*) + Cannabidiol (60 mg/kg *i.p*) + Tetrahydrocannabinol (6 mg/kg *i.p*)

Neurobehavioural Assessments

After induction of Parkinson's disease and treatment with CBD and THC, the animals underwent the following neurobehavioural assessment:

Assessment of cold hypersensitivity (Tail-Flick)

The cold tail-flick test is a test of the pain response in animals, like the hot plate test except in this instance a cold water was used instead of hot water to detect cold hypersensitivity [12].

This procedure is a modification of the method used by Janssen *et al.* (1963) in which hot water (55°C) served as the noxious stimulus. In our model, the noxious stimulus is cold water (4°C). Cold has also been used in studies of pain in man [13].

Assessment of Cold Hypersensitivity (Cold Tail-Flick Test) (Snapped by Candidate, 2024)

The mice were allowed to acclimatize to the neurobehaviural laboratory before the assessement. The animals were tested once.The mice were held firmly over the opening of a beaker containing cold water and 2.5cm (Immersion depth) of their tails submerged into the cold water. The nociceptive threshold was taken as the latency until the mouse flicked its tail. The time from immersion to attempt of tail removal(flick) was measured to the nearest tenth of a second. To minimize damage to the tail, a 60 s cut-off was utilized; this was considered to be the maximum latency [14].Pre-reserpine assessement was not carried out becuase a pilot study was carried out prior to the main study.

Euthanisation

At the end of the experiment, the mice were anaesthetized using ethyl acetate inhalation, a rapid-acting inhalational method consistent with the protocol approved by the Committee on Animal Use and Care, Ahmadu Bello University, Zaria (ABUCAUC/2024/057), and their brains harvested for further analysis.

Blood Sample Collection and Assay of Oxidative Biomarkers

For the purpose of the biochemical test, blood samples were drawn via heart puncture and placed in plain bottles.

Estimation of lipid peroxidation

Malondialdehyde content, a marker of lipid peroxidation, was assessed in the serum by a method previously described by Willis *et al.,* 2002[16]. A mixture consisting of 0.5 ml of Tris–HCl and serum (0.5 ml) was incubated for 2 hours at 37 °C. To this incubated mixture, 1 ml of 10% trichloroacetic acid was added and centrifuged for 10 min at 1000 × *g*. Thereafter, 1 ml of thiobarbituric acid was added to 1 ml of supernatant obtained after centrifugation and was kept in boiling water for 10 min. Finally, on cooling, 1 ml of distilled water was added, and the absorbance was measured at 532 nm. TBARS was calculated using the molar extinction coefficient of 1.56 × 105 M−1 cm−1 and expressed as nanomole of malondialdehyde per milligram protein.

Estimation of Superoxide Dismutase Activity

Superoxide dismutase activity was assayed by the method described by Kono [17]. Briefly, the assay system consisted of equal volumes of serum and hydroxylamine hydrochloride (pH 6.0) along with 0.1 mM ethylene diamine tetra-acetic acid (EDTA), 50 mM sodium carbonate, and 96 mM of nitro blue tetrazolium (NBT). Change in absorbance, which indicated auto-oxidation of hydroxylamine, was measured for 2 min at 30-s intervals by measuring absorbance at 560 nm.

Estimation of reduced Glutathione Concentration

Reduced glutathione level was assayed using a method previously described by Ellman [18]. A mixture of equal volumes of serum and 5% sulphosalicylic acid was kept at 4 °C for 1 hour followed by centrifugation at 4500 × *g* for 10 min at 4 °C. Thereafter, 450 µl of phosphate buffer and 1.5 ml of 5, 5, dithiobis-(2-nitro benzoic acid) in 0.1 M phosphate buffer (pH 8.0) was added to 50 µl of supernatant obtained earlier. The reaction mixture was then incubated for 10 min at 37 °C, and the color developed was analyzed at 412 nm. GSH levels was calculated using molar extinction coefficient of the chromophore, i.e., 1.36 × 104 M^{-1} cm^{-1} and results be expressed as µg/mg.

Statistical Analysis

Data was analyzed using statistical package for social sciences (SPSS) version 23.0 and GraphPad Prism version 10.2.0 (Windows). The data from each group was analyzed using one-way analysis of variance (ANOVA), followed by Tukey's *post-hoc* test for multiple comparison. Data obtained were expressed as mean ± S.E.M, and $P < 0.05$ was considered statistically significant.

Results

Effect of CBD and THC on Cold Hypersensitivity in Reserpine Model of Parkinson's Disease

Figure 1 shows the effect of CBD and THC on cold hypersensitivity in a reserpine model of Parkinsonian features.

Mice administered with reserpine only showed significant decrease ($P < 0.05$) in Cold (4^0C) tail flick

latency (18.5 ± 2.87s) when compared to the control group. Reserpine + CBD (30mg/kg) treatment group showed a significant decrease ($P < 0.05$) in cold (4^0C) tail flick latency (1.185 ± 0.06s) when compared with the Control group. RSP+CBD60mg/kg treatment group showed a statistically significant increase ($P < 0.05$) in cold (4^0C) tail flick latency (46.75 ± 4.01s) when compared to the reserpine only group (18.5 ± 2.87s). Reserpine + THC 6mg/kg (57.75 ± 10.06s) group significantly increase ($P < 0.05$) tail flick latency when compared to the reserpine only group (18.5 ± 2.87s). Reserpine + THC4mg/kg (44.75 ± 7.94s) and Reserpine + CBD(60mg/kg) + THC(6mg/kg) (40.25 ± 2.14s) groups showed no significant difference when compared to reserpine only group (18.5 ± 2.87s).

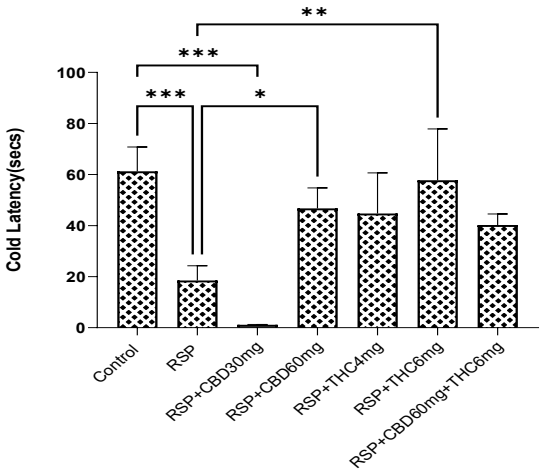

Figure 1: Effect of CBD and THC on cold hypersensitivity in reserpine model of Parkinson's disease**.**

* =P < 0.05, ** =P < 0.01, ***=P < 0.001 CBD; Cannabidiol, THC; Tetrahydrocannabinol, RSP; Reserpine.

MDA Concentration in a Reserpine Model of Parkinsonian Features

Figure 2 shows the effect of CBD and THC on MDA concentration in a reserpine model of Parkinsonian features.

The MDA concentration was significantly lower ($P < 0.05$) in reserpine + THC (4mg/kg) (318.95 ± 11.90 nmol/mg), reserpine + CBD 30mg/kg (346.25 ± 25.54 nmol/mg) and reserpine + CBD (60mg/kg) + THC (6mg/kg) (349.30±3.43 nmol/mg) groups when compared with the reserpine only group (415.65 ± 6.13 nmol/mg) (Figure 2). MDA concentration for the CBD 60 mg/kg(363.58±11.34) and THC 6 mg/kg groups(368.20±15.48) were not statistically significant when compared to the reserpine only group (415.65 ± 6.13 nmol/mg). The MDA concentration for the control group is (369.03±8.83).

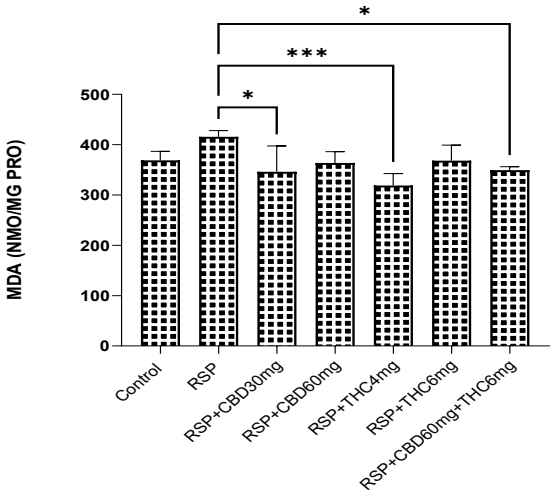

Figure 2: Effect of CBD and THC on malonaldehyde concentration in reserpine model of Parkinson's disease**.**

*** =P < 0.05, ** =P < 0.01, ***=P < 0.001, CBD; Cannabidiol, THC; Tetrahydrocannabinol, RSP; Reserpine.**

Superoxide Dismutase Activity Estimation in a Reserpine Model of Parkinsonian Features

Figure 3 shows the effect of CBD and THC on superoxide dismutase activity estimation in a reserpine model of Parkinsonian features. Superoxide dismutase activity significantly reduced ($P < 0.05$) in reserpine only group (5.35 ± 1.25 U/mg) when compared with the control group (23.35 ± 1.63 U/mg).

Similarly, superoxide dismutase activity significantly increased ($P < 0.05$) in reserpine + CBD 60mg (15.88 ± 0.67 U/mg), reserpine + THC (6mg/kg) (25.18±2.40 U/mg) and reserpine + CBD (60mg/kg) +THC (6mg/kg) group (12.10 ±1.59 U/mg) when compared with the reserpine only group (5.35±1.25 U/mg) (Figure 3).

The reserpine + CBD 30mg/kg group (8.40±0.64 U/mg) and reserpine + THC4mg/kg(10.83±1.21 U/mg) group showed no statistically significance difference when compared to the reserpine only group (5.35±1.25 U/mg).

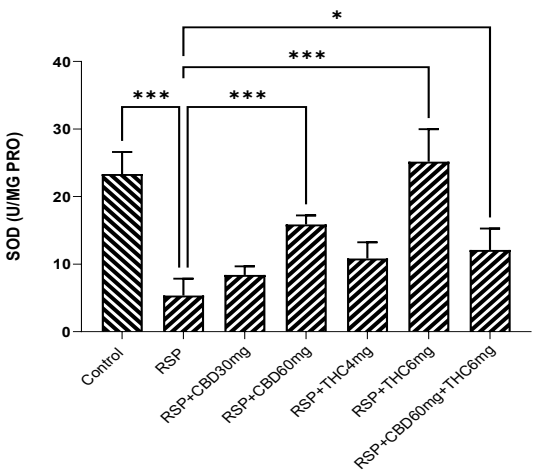

Figure 3: Effect of Cannabidiol and Tetrahydrocannabinol on superoxide dismutase activity in reserpine model of Parkinson's disease**.**

* =P < 0.05, ** =P < 0.01, ***=P < 0.001, CBD; Cannabidiol, THC; Tetrahydrocannabinol, RSP; Reserpine.

Reduced Glutathione Concentration Estimation in a Reserpine Model of Parkinsonian Features

Figure 4 shows the effect of CBD and THC on reduced glutathione concentration estimation in a reserpine model of Parkinsonian features. Reduced Glutathione level was significantly (P < 0.05) lower in reserpine only group (5.83 ± 0.98 µg/mg) when compared with the control group (15.70 ± 2.04 µg/mg). On the other hand, the Reduced Glutathione Concentration was statistically higher in reserpine + THC (6 mg/kg) (16.50 ± 2.33 µg/mg) and Reserpine + CBD (60 mg/kg) + THC (6mg) (18.95 ± 4.01 µg/mg) when compared with the reserpine only group (5.83 ± 0.98 µg/mg) (Figure 4).

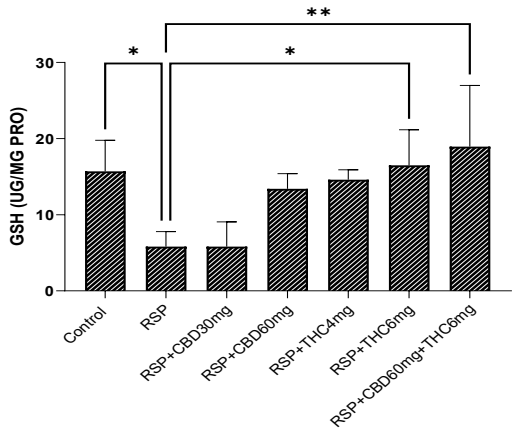

Figure 4: Effect of CBD and THC on reduced glutathione concentration in reserpine model of Parkinson's disease**.**

* =P < 0.05, ** =P < 0.01, ***=P < 0.001, CBD= Cannabidiol, THC= Tetrahydrocannabinol, RSP=Reserpine.

Discussion

The present study demonstrates that reserpine administration reduced cold-withdrawal latency and altered oxidative-stress biomarkers (MDA, SOD activity, and GSH) in mice, consistent with the induction of cold hypersensitivity and oxidative stress in this reserpine model of Parkinsonian features. Selected CBD and THC regimens were associated with partial reversal of these changes. These findings are considered below in relation to previous literature.

The reduction in cold-withdrawal latency following reserpine administration reflects increased cold nociceptive sensitivity (cold hypersensitivity); this behavioral outcome does not, by itself, demonstrate a change in neuronal pain-signal transmission, which was not directly assessed in this study. Cold-withdrawal latency increased in mice treated with the higher dose of CBD following reserpine administration, consistent with reduced cold hypersensitivity. This observation is consistent with that of Russo [19], who reported that CBD can improve cold and thermal nociceptive thresholds.

Similarly, cold-withdrawal latency increased in mice that received the higher dose of THC, consistent with the findings of Mabou Tagne *et al.* 2024 [20], who demonstrated that THC administration reduced cold hypersensitivity in a mouse model. Because THC, and CBD at higher doses, can also produce sedation and alter locomotor activity and motor coordination, an increased withdrawal latency cannot be interpreted purely as improved pain threshold; the possibility that sedative or motor confounds contributed to this outcome cannot be excluded and should be considered when interpreting these findings.

The concentration of MDA (TBARS/MDA equivalents), a marker of lipid peroxidation, increased in mice administered reserpine alone, while it decreased in mice administered THC and in mice administered the CBD/THC combination. This observation is in line with the findings of Abdel-Salam 2022 [21], who reported that THC and CBD improve lipid peroxidation in a rodent model relevant to Parkinson's disease.

The concurrent occurrence of decreased cold-withdrawal latency and increased oxidative stress may be associated with elevated reactive oxygen species (ROS) affecting nociceptor function through sensitization of ion channels such as Transient Receptor Potential (TRP) channels—including TRPV1, TRPA1, and TRPM8—which contribute to thermal, chemical, and cold nociceptive signaling and could thereby increase pain perception [22]. Oxidative stress may also promote inflammatory signaling, including the production of pro-inflammatory cytokines and prostaglandins, which can sensitize nociceptors and increase pain sensitivity [23]. It should be noted that the present study demonstrates a concurrent, rather than a demonstrated causal, relationship between oxidative-stress markers and cold hypersensitivity, as no correlational or mechanistic analyses linking these outcomes were performed.

The increase in cold-withdrawal latency observed in CBD-treated groups may be related to CBD's reported modulation of TRPM8, TRPV1, and TRPV2 channels, which are involved in cold, thermal, and mechanical/noxious signaling, respectively; CBD has been reported to desensitize or modulate these channels, potentially reducing nociceptive signaling [24]. These receptor-level mechanisms were not directly assessed in the present study, and this interpretation should be regarded as a plausible mechanism drawn from previous literature rather than a finding of the present work.

The increase in cold-withdrawal latency observed in THC-treated groups may similarly be related to THC's action at CB1 receptors in the central nervous system, which can modulate nociceptive pathways by inhibiting the release of excitatory neurotransmitters [25]. As with CBD, this mechanism was not directly examined in the present study and is presented as a possible explanation supported by previous literature rather than a direct finding.

A possible mechanism for CBD's effect on oxidative stress is its ability to neutralize free radicals via its phenolic structure. CBD has also been reported to increase SOD activity and GSH concentration, possibly by activating nuclear factor erythroid 2-related factor 2 (Nrf2), which binds the antioxidant response element and increases production of endogenous antioxidants such as SOD and GSH [26]. Nrf2 activation was not directly measured in the present study, and this remains a proposed rather than demonstrated mechanism.

Effects of THC on oxidative stress may be related to regulation of mitochondrial function via CB1 receptor signaling, potentially reducing excessive ROS production [27]. Mitochondrial function, CB1 signaling, and ROS production were not directly measured in this study, and this remains a proposed mechanism supported by the cited literature rather than a direct finding.

Increased oxidative stress, characterized by decreased SOD activity and reduced GSH concentration, was evident in this reserpine model of Parkinsonian features. Increased oxidative stress is well recognized to be associated with the pathophysiology of Parkinson's disease [28]. Conversely, SOD activity and GSH concentration increased in the THC, CBD, and CBD/THC combined-treatment groups relative to reserpine alone. Han *et al.* 2020 [29] demonstrated that CBD and THC are potent antioxidants. Prakash & Carter 2021 [30] also reported that combined CBD/THC treatment in a rotenone model relevant to Parkinson's disease restored pro-oxidant/antioxidant balance. Hacke *et al.* 2019 [31] reported that THC can act as an antioxidant at sub-micromolar concentrations and prevent oxidative cell death. Dawidowicz *et al.* 2021 [32] has also reported that THC exhibits strong antioxidant activity, potentially exceeding that of vitamin C.

MDA (reported here as TBARS/MDA equivalents) reflects lipid peroxidation, SOD activity reflects enzymatic antioxidant defense, and GSH concentration reflects non-enzymatic antioxidant capacity; these biomarkers represent distinct, non-interchangeable aspects of oxidative stress and are interpreted individually here rather than as a single composite measure. As noted above, the combined CBD/THC group did not consistently exceed the higher THC dose alone across these biomarkers, and this should not be interpreted as evidence of a generally superior or synergistic effect of the combination unless supported by a direct statistical comparison.

This study has several limitations. The sample size was relatively small (n = 6/group), which may limit statistical power, particularly for pairwise dose comparisons. Both male and female mice were used, but sex-stratified analysis was not performed, and potential sex differences in response to reserpine, CBD, or THC cannot be excluded. Cannabinoid receptor, TRP-channel, and Nrf2-pathway involvement were not directly measured and are inferred from previous literature rather than demonstrated experimentally. Cold-withdrawal latency may also be influenced by sedative or motor effects of CBD and THC independent of analgesia, which were not separately assessed. Finally, because the reserpine model does not reproduce the progressive dopaminergic neurodegeneration or α-synuclein pathology of human PD, these findings should be interpreted as relevant to reserpine-induced nociceptive and oxidative changes rather than to the full spectrum of PD neuropathology.

In conclusion, reserpine administration was associated with reduced cold-withdrawal latency and increased oxidative stress, characterized by elevated MDA (TBARS/MDA equivalents) and reduced SOD activity and GSH concentration, consistent with previously reported patterns of oxidative stress [33].

This study examined the effects of CBD, THC, and combined CBD/THC treatment on cold-withdrawal latency, a marker of cold nociceptive sensitivity, in a reserpine model of Parkinsonian features, alongside the oxidative-stress biomarkers MDA, SOD activity, and GSH.

Conclusion

Based on the findings of the present study, selected CBD and THC regimens were associated with increased cold-withdrawal latency and favorable changes in oxidative-stress biomarkers (reduced MDA and

increased SOD activity and/or GSH concentration) in reserpine-treated mice, in a pattern that varied by dose, treatment, and biomarker. These data may support the conclusion that selected CBD and THC regimens were associated with changes in cold-withdrawal latency and oxidative-stress biomarkers in reserpine-treated mice; they do not yet establish a therapeutic role for CBD or THC in Parkinson's disease-associated pain. Further studies with complete group-wise reporting, direct statistical comparison of treatment groups against the reserpine-only group, larger sample sizes, and direct mechanistic assessment are needed to confirm and extend these observations.

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Patient informed consent
There is no need for patient informed consent.

Ethics committee approval
Ethical approval was obtained from Committee on Animal Use and Care, Ahmadu Bello University, Zaria with approval number (ABUCAUC/2024/057) dated 16th October 2024.

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Conflict of interest
There is no conflict of interest to declare.

Author contributions subject and rate
Tahir Muhammad Disina (50%): Research Design, Data Collection and analysis, wrote the whole manuscript
Ahmed Sherif Isa (25%): Research Design & Supervise the research
Philemon Paul Mshelia (20%): Supervise the research
Amat Abdoulie Tekanyi (5%): Data Collection and analysis

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