The World Journal of Biological Psychiatry

ISSN: 1562-2975 (Print) 1814-1412 (Online) Journal homepage: https://www.tandfonline.com/loi/iwbp20

Investigation of the effects of cannabidiol on vacuous chewing movements, locomotion, oxidative stress and blood glucose in rats treated with oral haloperidol

Jaiyeola Abiola Kajero, Soraya Seedat, Jude Ohaeri, Abidemi Akindele & Oluwagbemiga Aina

To cite this article: Jaiyeola Abiola Kajero, Soraya Seedat, Jude Ohaeri, Abidemi Akindele & Oluwagbemiga Aina (2020): Investigation of the effects of cannabidiol on vacuous chewing movements, locomotion, oxidative stress and blood glucose in rats treated with oral haloperidol, The World Journal of Biological Psychiatry, DOI: 10.1080/15622975.2020.1752934

To link to this article: https://doi.org/10.1080/15622975.2020.1752934

Accepted author version posted online: 08 Apr 2020.
Published online: 05 May 2020.

THE WORLD JOURNAL OF BIOLOGICAL PSYCHIATRY
https://doi.org/10.1080/15622975.2020.1752934

ORIGINAL INVESTIGATION

Investigation of the effects of cannabidiol on vacuous chewing movements, locomotion, oxidative stress and blood glucose in rats treated with oral haloperidol

Jaiyeola Abiola Kajero[a], Soraya Seedat[b], Jude Ohaeri[c], Abidemi Akindele[d] and Oluwagbemiga Aina[e]

[a]Federal Neuropsychiatric Hospital Yaba, Lagos, Nigeria; [b]Department of Psychiatry, University of Stellenbosch, Cape Town, South Africa; [c]Department of Psychological Medicine, University of Nigeria, Enugu State, Nigeria; [d]Faculty of Basic Medical Sciences, Department of Pharmacology, Therapeutics & Toxicology, College of Medicine, University of Lagos, Lagos, Nigeria; [e]Department of Biochemistry, Nigerian Institute of Medical Research Yaba Lagos, Lagos, Nigeria

ABSTRACT

Objectives: Tardive dyskinesia (TD) unlike acute dystonia may be irreversible. This study investigated the effects of oral cannabidiol (CBD) on haloperidol-induced vacuous chewing movement (VCM) model of TD.

Methods: There were six experimental groups with different combinations of oral cannabidiol with 5 mg/kg of haloperidol given orally. Behavioural assays and FBS were measured. VCMs were assessed after the last dose of medication. Blood for oxidative stress assays was collected on the 8th day after the administration of the last dose of medication.

Results: This study found that CBD co-administration with haloperidol attenuated the VCMs and increased motor tone produced by haloperidol. CBD alone at 5 mg/kg appears to have anxiolytic properties but may not be as effective as haloperidol which exhibited a greater anxiolytic effect at 5 mg/kg. Treatment with CBD alone at 5 mg/kg also appeared to enhance brain DPPH scavenging activity.

Conclusions: We confirmed that CBD can ameliorate motor impairments produced by haloperidol. Our data suggest that CBD can be combined with haloperidol to prevent the emergent of extrapyramidal side effects and long-term movement disorders, such as acute dystonic disorder and TD.

ARTICLE HISTORY
Received 5 November 2019
Revised 20 March 2020
Accepted 31 March 2020

KEYWORDS
Antioxidative systems; antipsychotics; behaviour; tardive dyskinesia; cannabidiol

Introduction

Movement disorders, such as Parkinsonism, anti-Parkinsonism-induced drug dyskinesia, akathisia, tardive dystonia and tardive dyskinesia (TD), are associated with not only the new generations and conventional antipsychotics, they are also seen in some D_2 receptor agonists such as levodopa though they are more common in patients on conventional antipsychotics (e.g. haloperidol) (Rochon et al. 2005; Thanvi et al. 2007; Chouinard and Chouinard 2008). Movement disorders constitute one of the most problematic side effects of conventional antipsychotics because they bind strongly to D2 receptors in the mesolimbic, mesocortical, nigrostrial and tuberoinfundibular pathways to exert their actions; binding to the D_2 receptors in the mesolimbic pathways is associated with the relief of positive symptoms of schizophrenia; mesocortical D_2 blockade is linked to cognitive impairments and secondary negative symptoms; activity at the nigrostrial pathway is associated with extrapyramidal side effects; while blockade of D2 receptors in tuberoinfundibular pathways increases prolactin level by promoting its release in the pituitary gland (King and Voruganti 2002; Seeman 2002; Selemon and Zecevic 2015; Guzman and Farinde 2016; Stahl 2018). Movement disorders and cognitive impairments are, therefore, the consequences of attempts to alleviate symptoms of psychosis (Stahl 2018).

Fortunately, most movement disorders can be reversed with antipsychotic discontinuation or managed with medications such as anti-cholinergic agents (e.g. benzhexol). Tardive dyskinesia (TD), an involuntary movement disorder characterised by repetitive and persistent purposeless movements in the orofacial and limb truncal areas (Berger and Rexroth 1980; Baldessarini 1988; Citrome 2017) is not readily

CONTACT Jaiyeola Abiola Kajero jaiyeolakajero@yahoo.com Federal Neuropsychiatric Hospital Yaba, 8, Harvey Road, Yaba, Lagos, Nigeria.

reversed and unlike acute dystonia may be irreversible (Carney and Sheffield 1976; Ozdemir et al. 2001; Margolese et al. 2005). The first step in the management of TD is removal of the causative drug whenever possible but slow taper is recommended, as sudden withdrawal is more likely to precipitate TD or a withdrawal emergent syndrome (Casey 1990; Janno et al. 2004; Mejia and Jankovic 2010).

Until recently, tetrabenazine (TBZ), approved by the United States Food and Drug Administration (FDA) for the treatment of chorea associated with Huntington's disease, was the only medication proven to be effective in the management of TD unresponsive to other treatment modalities (Ondo et al. 1999; Leung and Breden 2011; Caroff et al. 2018). It is well tolerated but at higher doses, which are often needed to control the symptoms of TD, can cause side effects, such as depression, lethargy, akathisia, and Parkinsonism (Yero and Rey 2008; Chen et al. 2012; Kaur et al. 2016). These side effects may occur because it is not selective for dopamine and equally depletes synaptic serotonin and norepinephrine (Kenney and Jankovic 2006). Recently, the FDA approved two additional medications for TD, Valbenazine and Deutetrabenazine (Factor et al. 2017; Touma and Scarff 2018). Both Valbenazine and Deutetrabenazine are devoid of the side effects of TBZ, though their mechanisms of action are similar (Shao and Hewitt 2010; Josiassen et al. 2017). Side effects and discontinuation rates increase with prolonged use of these medications and the symptoms of TD appear to return after discontinuation, making them less than ideal (Factor et al. 2017; Josiassen et al. 2017; Touma and Scarff 2018). Investigation of other pharmacological agents in the management of TD is, therefore, needed.

Anecdotal evidence suggests that patients who use cannabis are able to tolerate high doses of conventional antipsychotics, sometimes up to 80 mg of haloperidol per day, for relatively long periods with minimal side effects. An earlier study suggested that *Cannabis sativa* may improve Parkinsonian symptoms in a rodent model (Omar et al. 2012). Further, there is evidence that cannabis may enhance dopamine release in the human striatum (Voruganti et al. 2001; Bossong et al. 2009). The most abundant non-psychotomimetic compound in the *Cannabis sativa* plant is cannabidiol (CBD) (Booz 2011). CBD inhibits the degradation of anandamide, an endogenous cannabinoid receptor partial agonist, known to be reduced in patients with schizophrenia (Lewekwe et al. 2012; Steeds et al. 2015); another study also reported that anandamide ameliorates haloperidol induced VCM in rats (Röpke et al. 2014). It is plausible that this compound may be responsible for the tolerance of patients to high dosages of haloperidol by increasing anandamide concentrations in the central nervous system to ameliorate the side effects of conventional antipsychotic agents. Recently, Peres et al. (2016) documented that cannabidiol prevented VCM in a reserpine model of TD.

Though the pathophysiology of TD is not well understood, oxidative stress and neuroinflammatory mechanisms may be involved (Kulkarnin and Naidu 2004; Zai et al. 2010; Cho and Lee 2013; Waln and Jankovic 2013). Several theoretical models have been proposed to explain the role of oxidative stress in TD pathogenesis (Thaakur and Himabindhu 2009; Kovacic and Somanathan 2012; Waln and Jankovic 2013; Zhang and Yao 2013). This involves the generation of reactive oxygen species (ROS) from reduction-oxidation (REDOX) reactions in the mitochondria of neural cells; several neurotransmitters when in excess can produce ROS, through quinone/semiquinone metabolites. The toxic effects at the molecular level include lipid peroxidation, neuronal damage leading to degeneration of the different neurotransmitter systems, neuronal loss and gliosis. Together this can lead to an imbalance between direct and indirect basal ganglia pathways and dysfunction of the sensorimotor cortex producing the clinical symptoms of TD (Gunne et al. 1984; Elkashef and Wyatt 1999; Margolese et al. 2005; Gittis et al. 2011; Teo et al. 2012). Antioxidants that influence the redox system in the brain could be of potential benefit in the management of TD.

CBD is a potent anti-oxidative agent that also protects against glutamate neurotoxicity (Malfait et al. 2000). Investigation of the effects of CBD on TD using a well validated animal model is, therefore, warranted. The vacuous chewing movement (VCM) model has been the most employed animal model of TD (Kulkarni and Naidu 2001; Blanchet et al. 2012; Patil et al. 2012; Sekiguchi et al. 2012; Lister et al. 2014). We sought to determine if treatment with CBD would attenuate VCMs induced by sub-chronic administration of haloperidol in a rat model.

Sub-chronic administration of haloperidol is also associated with cognitive impairment in clinical and laboratory studies (Levin et al. 1996; Addy and Levin 2002; Rezvani and Levin 2004; Gallhofer et al. 2007). An association between cognitive impairments and movement disorders such as Parkinsonism, akathisia and TD has also been reported (Waddington et al. 1985; Waddington and Youssef 1986; Dewolfe et al. 1988; Gureje 1988; Bartzokis et al. 1989), though the

mechanism remains unknown. Oxidative stress-induced damage to the neurons in the right dorsolateral prefrontal cortex, right posterior parietal cortex and the hypocampal formation may be involved and may actually serve as a link between movement disorders and cognitive impairment (Mizoguchi et al. 2000; van Asselen et al. 2006; Wu et al. 2014). There is evidence for the involvement of endogenous cannabinoids in the cognitive symptoms of schizophrenia (Micale et al. 2013; Kucerova et al. 2014) and since CBD inhibits the enzymatic degradation of anandamide an endogenous cannabinoid (Lewekwe et al. 2012; Steeds et al. 2015) it may also influence cognitive deficits in schizophrenia. It is, therefore, important to also investigate the effects of CBD on cognition.

Though published studies on the relationship between anxiety disorders and EPS are unavailable, the anxiolytic and anti-depressant effects of CBD have been explored by some workers and the proposed mechanism is via CB_1, $TRPV_1$ and $5\text{-}HT_{1A}$ receptors (Blessing et al. 2015; Micale et al. 2015; Lee et al. 2017). There is an increased risk of anxiety disorders in Parkinson's disease (PD) and anxiety symptoms sometimes precede onset of PD. The neurobiology of anxiety disorders in Parkinson's disease is unclear and treatment may be challenging (Dissanayaka et al. 2010; Voon et al. 2011; Marques et al. 2018). CBD has also been proposed as a treatment for the management of anxiety disorders in PD (Crippa et al. 2009). We were, therefore, interested in observing the anxiolytic properties of CBD in our study as a secondary objective.

Materials and methods

Animals

Male Wistar adult rats ($n = 43$), used in this study were obtained from the colony of the Nigerian Institute of Medical Research (NIMR) in Yaba, Lagos, Nigeria. The animals, in groups of five, were kept in clean polypropylene cages in well-ventilated and hygienic compartments, maintained under standard environmental conditions and fed with standard rodent pellets (Ladokun Feed Plc, Ibadan, Nigeria) and water *ad libitum*. The animals were acclimatised for a period of 2 weeks before experimental procedures were undertaken in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (National Research Council 2011). The study was one component of a larger study approved by the Institutional Review Board (IRB) of the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria (IRB/16/329) and Stellenbosch University's Health Research Ethics Committee: Animal Care and Use (SU-ACUD16-00137).

Drugs

Cannabidiol [(-)-Cannabidiol, GMP (Cannabidiolum)] (CBD) (VAKOS X, a.s., Permova 28a,186 00 Praha 8, in Czech Republic .Company number: 04801938) was supplied in fine granule form with the amount administered weekly calculated and dissolved in 70% ethanol, as recommended by the manufacturers, and diluted with distilled water. CBD was administered orally. Haloperidol tablets (Janseen Pharmaceuticals Beerse, Belgium) were dissolved in 0.5% acetic acid and distilled water and administered orally.

Experimental design

The experimental groups ($n = 7$), except for the control group with eight animals, yielded a total of 43 and were constituted as follows:

Group A: Haloperidol 5 mg/kg p.o.
Group B: Haloperidol 5 mg/kg p.o. + Cannabidiol 3 mg/kg p.o.
Group C: Haloperidol 5 mg/kg p.o. + Cannabidiol 5 mg/kg p.o.
Group D: Haloperidol 5 mg/kg p.o. + Cannabidiol 10 mg/kg p.o.
Group E: Cannabidiol 5 mg/kg p.o.
Group F: Control (Distilled water 2 ml) p.o.

The interventions in B, C and D were administered concurrently using different oral cannulae. All medications in A, B, C, D and E were administered once daily for 21 d (Sasaki et al. 1995; Naidu and Kulkarni 2001a, 2001b; Bishnoi and Boparai 2012). The dose of 5 mg/kg was used by Bishnoi and Boparai (2012). Guimaraes et al. (1990) stated that effective doses of CBD in rats are 2.5–10 mg/kg.

VCMs were assessed at 8 h, 24 h and 8 d after the last dose of medication. The 8th day assessment was to ensure that the VCM model was established. Side effects were assessed through monitoring of weight and fasting blood sugar (FBS) before drug administration, 2 weeks after administration of drugs and at the end of the study.

Behavioural assays

Open field, elevated plus maze and object recognition tests were used to assess behavioural responses. All behavioural assessments were carried out 24 h after the last dose of medication and distilled water were administered. Blood was collected from the lateral saphenous vein of each animal on the 8th day after the administration of the last dose of medication. Animals were later sacrificed on the same day by cervical dislocation and dissected by opening the abdomen. The brain, liver and kidneys of the rats were isolated and dissected on ice. 10% w/v of organs sample (0.03 M sodium phosphate buffer, pH 7.4) was homogenised. The homogenates generated from processed tissues were then used for oxidative stress, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and nitric oxide (NO) assays.

VCMs were assessed by placing each animal in individual transparent glass plexiform cages. Each animal was allowed to acclimatise for 5 min before counting started. The number of VCMs (mouth openings in the vertical plane not directed towards physical material) was counted for 10 min (Crowley et al. 2012).

Elevated plus-maze test: Anxiety was monitored using this test (Chopin et al. 1986). The elevated plus-maze consisted of two open arms (30 × 5 cm) and two closed arms (30 × 5 × 15 cm) that extended from the central platform (5 × 5 cm). The entire maze was elevated to 40 cm above the floor. During the first 5 min of free exploration, the number of entries into and the time spent in the open and closed arms were recorded. An entry was defined as the point at which the animal placed all four paws onto the arm.

Open-field test: This test was used to assess locomotion (Walsh and Cummins 1976; Eweka and Om'Iniabohs 2007). The number of line crosses and the frequency of rearing are usually used as a measure of locomotor activity, exploration and anxiety. A high frequency of these behaviours indicates increased locomotion and exploration and/or a lower level of anxiety (Walsh and Cummins 1976). The open-field box is a rectangular area composed of a hard floor measuring 36 cm × 36 cm × 26 cm and made of a white painted wood. The floor was divided by permanent read markings into 16 equal squares at the bottom. Each rat was introduced singly into one corner of the field and after each session the area was cleaned with 70% alcohol to eliminate olfactory bias and the area allowed to dry before introducing a new animal.

Object recognition test: This test by Ennanceur and Delacour (1988) was used to study drug-induced cognitive effects. Each animal was placed in a plexiform glass transparent cage and was allowed to acclimatise for 5 min before two similar spherical objects (Balls) were introduced. The animal was allowed 10 more min in the cage with the objects and the time the animal spent exploring the objects was measured with a stop watch by an observer before the animal was removed. The animal was re-introduced into the cage after an hour, with a different object (square shaped) added before the animal was introduced and one of the previous objects removed. Time spent exploring both new and old objects was measured for 10 min by two different observers using stop watches. The time spent by the animal using its nose to touch the object was measured while the time spent using other parts of the body were ignored.

Rota-rod test: The method used for the assessment of locomotor (forced motor) activity in rodents was described by Ozturk et al. (1996). A rota-rod treadmill device (Ugo Basile No. 7600 Varese, Italy) was used for this purpose. Rats were trained to remain on slowly moving (16 revolutions min^{-1}) rods of 5 cm in diameter for 150 s by walking. The animals were then placed on the treadmill after training and the time spent on the treadmill before falling was measured for each animal.

Antioxidant indices assays

The following antioxidant indices were determined spectrometrically:

Malondialdehyde (MDA) is an index of lipid peroxidation which was determined using the method of Buege and Aust (1978)

1. Rats were decapitated and the brain or any organ tissue was removed carefully, immediately weighed and homogenised with cold ice 1.15% KCl to make 10% homogenate.
2. 1 ml of tissue homogenate was combined with 2ml of tri-carboxylic acid (TCA)- thiobarbituric acid (TBA)- hydrochloric acid (HCl) reagent and mixed thoroughly.
3. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 revolution per minutes for 10 min.
4. The absorbance of the supernatant was measured at 532 nm against a blank that contains all the reagents minus the homogenate. The MDA concentration of the sample was calculated using an extinction coefficient of 1.56×10^5 $M^{-1}CM^{-1}$ MDA

concentration (M) = Absorbance at 532 nm/1.56×10^5.

The reduced glutathione (GSH) content of the tissue as non-protein sulfhydryl was estimated according to the method described by Sedlak and Lindsay (1968). To the homogenate, 10% TCA was added and then centrifuged. 1.0 ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5, 5-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nanometre (nm), $\Sigma = 1.34 \times 104\ M^{-1}$ centimetre $(cm)^{-1}$

Catalase activity was determined according to the method of Sinha (1972). It was assayed colorimetrically at 620 nm and expressed as micromoles (μmol) of H_2O_2 consumed/min/mg protein at 25 °C. The reaction mixture (1.5 ml) contained 1.0 ml of 0.01Mole phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate and 0.4 ml of 2 Mole (M), H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1:3 ratio). $\Sigma = 40\ M^{-1}cm^{-1}$.

Superoxide dismutase activity was determined as described by Sun and Zigman (1978). The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH (10.2), 0.2 ml of tissue homogenate and 0.03 ml of epinephrine in 0.005 normal(N) HCl was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min $\Sigma = 4020\ M^{-1}cm^{-1}$.

Nitric oxide (NO) scavenging activity

A volume of 2 ml of sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of the tissue homogenate at various concentrations (0.2–1.0 mg/mL). The mixture was incubated at 25 °C for 180 min. An aliquot of 0.5 ml of the solution was added to 0.5 ml of Griess reagents [(1.0 ml of sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5 min with 1 ml of Naphthyethylenediamine chloride (0.1% weight/volume (w/v))]. The mixture was incubated at room temperature for 30 min. The absorbance (Abs) was then measured at 540 nm. The amount of NO radical was calculated using the equation:

NO radical scavenging activity = [(Abs control–Abs sample)/(Abs control)]/100, where, Abs control is the absorbance of NO radical + methanol; Abs sample is the absorbance of NO radical + sample tissue homogenate or standard.

DPPH scavenging assay

A portion of 0.135 mM DPPH was prepared in methanol containing 0.5 mg of the tissue homogenate and standard drug (Butylated hyroxytoulene (BHT) and Rutin). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the tissue homogenate on DPPH was calculated using the equation:

DPPH scavenging activity (%) = [(Abs control – Abs sample)]/(Abs control)]/100,

where, Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + tissue homogenate or standard. IC_{50} = tissue homogenate concentration that yields half maximum free radical scavenging activity.

Statistical analysis

Data on weight in grams and FBS were analysed with paired *t*-tests which were used to compare data within groups, while one-way analysis of variance (ANOVA) followed by Bonferroni's *post-hoc* tests were used to compare differences across groups. Data from the behavioural experiments, including the VCM, were analysed with one-way ANOVA and where there was a significant difference, Bonferroni's *post-hoc* tests were done. One-way ANOVA and Bonferroni's *post-hoc* tests were also used for the biochemical analysis. Statistical analysis was performed using the Stata software package version 15.

Results

Weight gain

For all groups, including the control group, there was a significant change in weight from pre- administration of medication until the end of the experiment. This indicates that rats gained weight normally as expected, with Group E (Cannabidiol alone at 5 mg/kg) experiencing greater weight gain compared to the other five groups ($p = .000$), as determined on *post-hoc* testing.

Fasting blood sugar

Using paired *t*-tests, for three of the groups there was a significant increase in FBS. In Group A (oral 5 mg/kg Haloperidol alone), there was a significant increase in FBS between week 0 (before administration of haloperidol) and week 3 (after the administration of last dose of haloperidol) ($p = .0200$). There was also a significant increase in FBS in Group B ($p = .0163$) (oral Cannabidiol 3 mg/kg + oral Haloperidol 5 mg/kg) and Group F (Control, Distilled water 2 mls) ($p = .0072$) between weeks 0 and 3. For Group C (oral Cannabidiol 5 mg/kg + oral Haloperidol 5 mg/kg), the change in FBS between weeks 0 and 3 was not statistically significant ($p = .9404$); similarly for Groups D (oral Cannabidiol 10 mg/kg + oral Haloperidol 5 mg/kg) ($p = .1497$) and E (oral Cannabidiol 5 mg/kg alone) ($p = .1090$) (Table 1). There was no statistically significant difference in mean FBS across the groups in week 2 ($p = .0751$) and week 3 ($p = .3020$) on *post-hoc* analysis.

Vacuous chewing movements

The results showed that there was a significant difference between Group A and the other groups on *post-hoc* analysis, ($p = .0001$) with Group A (haloperidol alone) exhibiting more VCM compared to the other groups (Figure 1) (haloperidol combined with different doses of cannabidiol, cannabidiol alone and control). On *post-hoc* analysis, differences were significant between Group B and A ($p = .005$), Group D and A ($p = .003$), Group E and A ($p = .0001$), and Group F and A ($p = .0001$).

Behavioural assays

Elevated plus maze test

Rats in groups A, B, C, D and E spent more time in the open arms compared to rats in Group F (the control) (Figure 2). It was also observed that rats in the haloperidol alone group spent more time in the open arms compared with the groups that received haloperidol co-administered with CBD or the group that received cannabidiol alone (Group D) (Figure 2). *Post-hoc* analysis revealed significant differences between Group B and Group A ($p = .018$), Group D and Group B ($p = .026$), Group E and Group A ($p = .038$), Group F and Group A ($p = .011$), and Group F and Group D ($p = .016$). There was also a statistically significant difference in the mean time spent in closed arms. *Post-hoc* analysis revealed significant differences between

Table 1. Comparison of fasting blood sugar by group and week.

	Fasting blood sugar (mean ± SD)								
Group	Week 0 vs Week 2	*p*-Value	95% CI	Week 0 vs Week 3	95% Conf. interval	*p*-Value	Week 2 vs Week 3	*p*-Value	95% CI
A	70.86 ± 18.92 vs 92.43 ± 3.78	.0290*	(−40.06, −3.08)	70.86 ± 18.92 vs 89.71 ± 5.56	(−33.53, −4.18)	.0200*	92.43 ± 3.78 vs 89.71 ± 5.56	.3752	(−4.22, 9.65)
B	80.00 ± 11.45 vs 75 ± 17.02	.5155	(−12.71, 22.71)	80.00 ± 11.45 vs 97.14 ± 3.29	(−29.83, −4.46)	.0163*	75.00 ± 17.02 vs 97.14 ± 3.29	.0173*	(−38.79, −5.50)
C	88.57 ± 14.28 vs 83.86 ± 5.37	.4739	(−10.39, 19.81)	88.57 ± 14.28 vs 89.14 ± 8.63	(−18.52, 17.38)	.9404	83.86 ± 5.37 vs 89.14 ± 8.63	.1333	(−12.74, 2.17)
D	94.71 ± 6.45 vs 84.57 ± 15.01	.1426	(−4.57, 24.86)	94.71 ± 6.45 vs 87.43 ± 8.81	(−3.51, 18.08)	.1497	84.57 ± 15.01 vs 87.43 ± 8.81	.4498	(−11.51, 5.79)
E	80.57 ± 19.82 vs 79.43 ± 18.64	.8776	(−16.26, 18.55)	80.57 ± 19.82 vs 91.86 ± 13.78	(−25.96, 3.39)	.1090	79.43 ± 18.64 vs 91.86 ± 13.78	.2064	(−33.90, 9.04)
F							72.86 ± 10.62 vs 88.86 ± 4.30	.0072*	(−25.80, −6.20)

*Statistically significant.
CI: confidence interval.
Group A: Haloperidol 5 mg/kg p.o.; Group B: Haloperidol 5 mg/kg p.o. + Cannabidiol 3 mg/kg p.o.; Group C: Haloperidol 5 mg/kg p.o. + Cannabidiol 5 mg/kg p.o; Group D: Haloperidol 5 mg/kg p.o. + Cannabidiol 10 mg/kg p.o.; Group E: Cannabidiol 5 mg/kg p.o.; Group F: Control (Distilled water 2 ml) p.o.

groups B and A ($p = .003$), E and A (0.006), F and A ($p = .001$), F and D ($p = .027$).

Rota rod test

Group A spent the least time on the treadmill compared to the other groups, suggesting that high dose haloperidol reduced motor activity and may have increased motor tone in the rats. Group D, comprising the highest dose of CBD combined with haloperidol, spent the most time on the treadmill, suggesting an amelioration of the motor side effects of haloperidol by 10 mg/kg of cannabidiol. *Post-hoc* analysis revealed that the difference between the time spent on the treadmill was significant between Groups D and A, D and B, D and E, and D and F ($p = .0000$). This also suggests that CBD was effective in improving motor symptoms at 10 mg/kg (Figure 3). There was a statistically significant difference in the mean time on the treadmill across the groups ($p = .0001$), with differences observed between Groups D and A, D and C, E and D, and F and D.

Object recognition test

There were no significant differences across the six groups ($p = .7591$) suggesting that CBD did not enhance cognitive impairment in our study; at the same time none of the pharmacological agents had deleterious effects on object recognition.

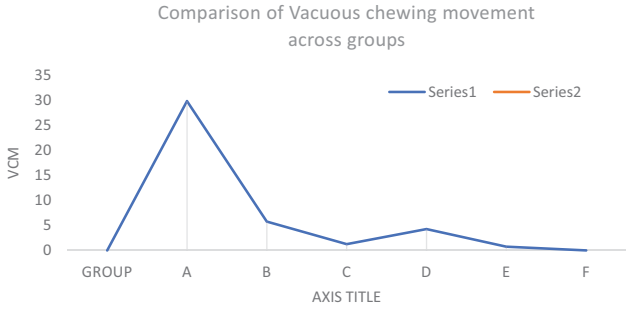

Figure 1. Group A: Haloperidol 5 mg/kg p.o.; Group B: Haloperidol 5 mg/kg p.o. + Cannabidiol 3 mg/kg p.o.; Group C: Haloperidol 5 mg/kg p.o. + Cannabidiol 5 mg/kg p.o.; Group D: Haloperidol 5 mg/kg p.o. + Cannabidiol 10 mg/kg p.o.; Group E: Cannabidiol 5 mg/kg p.o.; Group F: Control (Distilled water 2 ml) p.o.

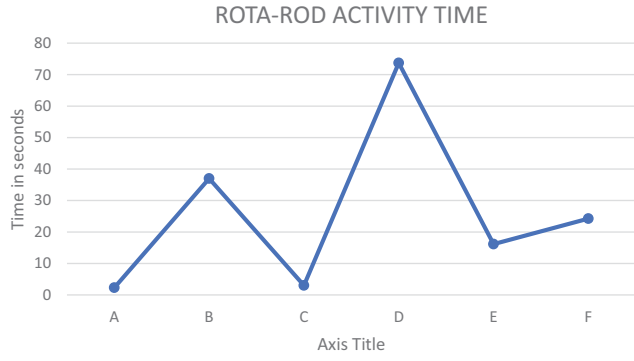

Figure 3. Comparison of time spent on the treadmill in ROTA ROD activity time.

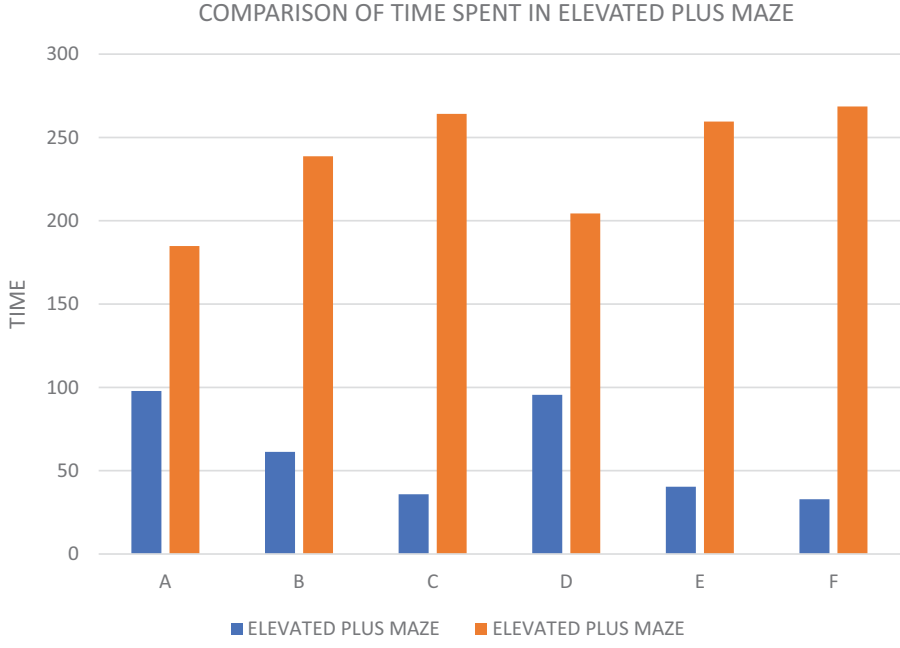

Figure 2. Comparison of time spent in open arms and close arms.

Open-field test

Group A had a reduced frequency of line crossing compared to the other groups, but this was only statistically significant between Groups A and B ($p = .030$) on *post-hoc* analysis (Figure 4). Overall, cannabidiol and haloperidol did not adversely affect locomotor activity in these animals. There was a statistically significant difference in the mean line crossing frequency across the groups ($p = .0193$), with a significant difference observed between groups B and A ($p = .030$).

Antioxidant assays

Antioxidant indices were assayed in the brain, blood, kidney and liver but no significant group differences were detected. In brain tissue, there were no significant differences in MDA ($p = .3783$), catalase ($p = .1646$), SOD ($p = .2914$), and GSH ($p = .3950$) measurements across the groups.

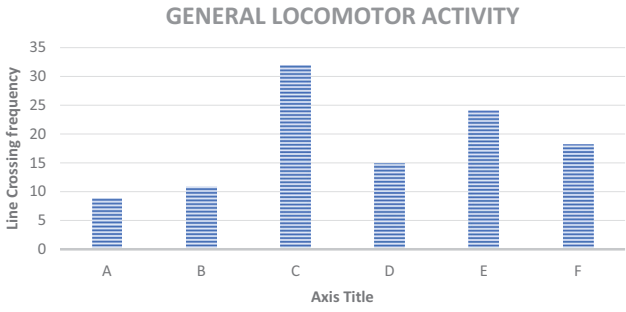

Figure 4. Comparison of line crossing frequency in open-field test.

Nitric oxide and DPPH assays

There was no difference in NO scavenging activities across the groups for brain ($p = .3643$), kidney ($p = .1201$), or liver ($p = .7978$). However, in brain samples, there were significant group differences in DPPH ($p = .0145$). *Post-hoc* tests revealed that Group E had increased scavenging activities compared to the other groups, with significant differences between Groups E and A, E and B, E and D, E and F. However, no significant group differences in DPPH scavenging activities was found in the liver ($p = .3915$) and the kidney ($p = .5668$) (Figure 5).

Discussion

This study found that CBD co-administration with haloperidol attenuated VCMs and increased motor tone produced by haloperidol. CBD also ameliorated haloperidol-induced increased blood glucose levels. CBD alone at 5 mg/kg appears to have anxiolytic properties but may not be as effective as haloperidol which exhibited greater anxiolytic effects at 5 mg/kg. Treatment with CBD alone at 5 mg/kg also appeared to enhance brain DPPH scavenging activity.

CBD alone at 5 mg/kg was associated with a greater increase in weight when compared with other interventions. This is in contrast to the work of Ignatowska-Jankowska et al. (2011), who reported that CBD decreased body weight gain in rats. Osborne et al. (2017), did not observe a change in body weight gain. Carvalho et al. (2018) also reported body weight gain occurred as expected in animals on CBD. These

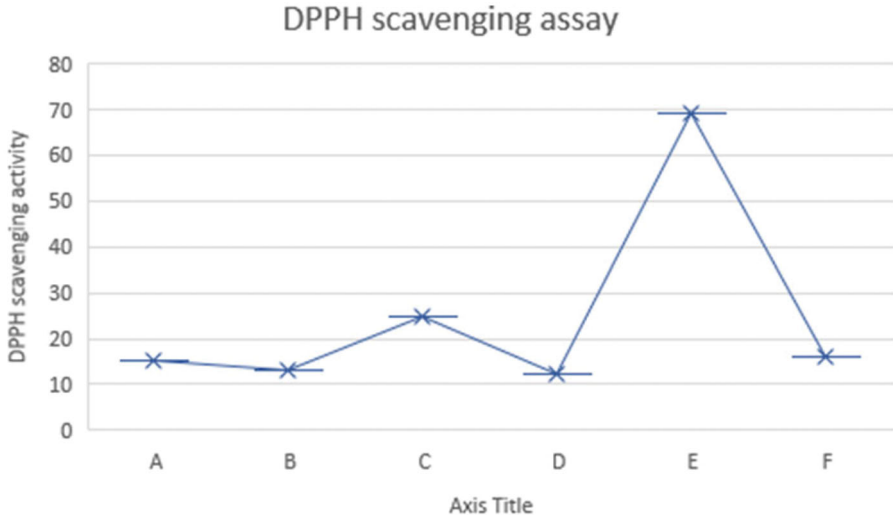

Figure 5. Comparison of DPPH scavenging assay across groups. ANOVA indicated that there was a statistically significant difference in mean DPPH scavenging activities across the six groups in the brain, with observed differences between animals in Groups E and A, E and D, and F and E.

differences may be due to differences in experimental design and route of administration. Clinical studies are needed to clarify weight changes in humans, although the interaction with serotonergic receptors may underlie the weight increase (Pacher et al. 2006).

Haloperidol's mechanism of action is mainly through blockage of dopamine receptors. This can, however, lead to an increase in dopamine turnover which is associated with increased free radical formation by monoamine oxidase and auto-oxidation of dopamine molecules into free radicals and quinines (Elkashef and Wyatt 1999; Cho and Lee 2013). Increased production of free radicals, coupled with deregulation of the antioxidant system and leading to increase oxidative stress, has been reported with chronic neuroleptic administration (Cadet and Perumal 1990). There is a strong association between free radical formation and movement disorders in psychotic patients, especially TD, and the level of oxidative stress enzyme activity in these patients correlates with clinical symptoms and severity (Kiriakakis et al. 1998; Zhang et al. 2003; Cho and Lee 2013).

In our study, sub-chronic oral administration of haloperidol over 21 d induced VCMs in a group of rats while co-administration of haloperidol and CBD at different doses resulted in far less frequent VCMs. This may be related to the antioxidant, anti-inflammatory and neuroprotective effects of CBD (Formukong et al. 1988; Watzl et al. 1991; Malifa et al. 2000). Peres et al. (2016) observed that cannabidiol prevents VCMs in a reserpine model of TD. We also observed that increased muscle tone or rigidity, as assessed by the Rota-rod test, was prevented when haloperidol was combined with cannabidiol as compared to haloperidol alone. This may be related to cannabidiol's ability to decrease symptoms of Parkinson's disease (Zuardi et al. 2009; Gomes et al. 2013). Peres et al. (2016) also reported attenuation of cataleptic behaviours in their study. Three decades earlier, Sandyk et al. (1986) reported a reduction in symptoms of dystonia in humans.

In our model of anxiety, haloperidol significantly altered anxiety-like behaviour when compared with the control. CBD at 5 mg/kg also altered anxiety-like behaviour when compared with the control but this was not statistically significant. The anxiolytic effect of haloperidol and CBD combination was only significant at 10 mg/kg. Haloperidol is known to have anxiolytic properties (Pich and Samanin 1986). Pickens (1981) documented sedative and anxiolytic effects of CBD in a comparison of tetrahydrocannabinol (THC) and CBD with chlorpromazine, while Zuardi et al. (1982) claimed that a high dose of CBD was needed to produce anxiolysis. Peres et al. (2016) did not observe any modification of anxiolysis by CBD in their study. In the present study, the high dose of cannabidiol may have been responsible for the anxiolytic effects exhibited when administered in combination with haloperidol. However, we are not able to explain why the lower dose of Cannabidiol did not produce more obvious anxiolytic effects as suggested by other authors (Guimaraes et al. 1990; Levin et al. 2012; Nazario et al. 2015) who found that cannabidiol's effects on anxiety follows an inverted U-shaped pattern.

The haloperidol-alone group had the least locomotor activity as measured by line crossing in the open-field test, though this was only statistically significant when compared with Group B which interestingly received the lowest dose of Cannabidiol (3 mg/kg) combined with haloperidol. Studies from other groups have found the lowest effective dose of cannabidiol (1 mg/kg) to be effective against social interaction and contextual fear deficits in animal models of schizophrenia (Levin et al. 2012; Almeida et al. 2013; Peres et al. 2016) and at this dose may, therefore, improve locomotion and exploration (Walsh and Cummins 1976; Ramos et al. 1997).

We can only conclude from our study, based on the object recognition test, that the combination of haloperidol and cannabidiol did not adversely affect the animals' cognitive functioning. Cannabidiol alone at 5 mg/kg did not result in any appreciable difference in cognitive functioning when compared with other groups; there was also no appreciable improvement in cognition when the cannabidiol with haloperidol groups were compared with the haloperidol-alone group. This finding contrasts with most preclinical studies of cannabidiol but is supported by clinical studies. Osborne et al. (2017), in a systematic review of 18 preclinical and 9 clinical studies, concluded that there was no clinical evidence to support the efficacy of CBD in improving cognition despite the positive results from some animal studies of impairment. Peres et al. (2016) reported an improvement in cognition in rats using the Plus-Maze Discriminative Avoidance Task. The difference in findings may relate to the difference in tasks employed across studies. We used the object recognition test to rule out cognitive side effects as cognitive impairments may affect motor responses - the focus of the present study.

Several workers have reported a link between oxidative stress and movement disorders in neuropsychiatry (Sagara 2002; Parikh et al. 2003; Pillai et al. 2007).

The increased dopamine metabolism caused by dopamine receptor blockade can lead to increase free radical production which can induce lipid peroxidation, of the neuronal phospholipid membrane. This in turn can decrease glutathione (GSH), superoxide dismutase (SOD) and catalase activities in cells triggering a cascade reaction leading to more lipolysis, more free radical production and eventually apoptosis and neuronal death (especially GABA neurons). The end result may be movement disorders, such as TD (Sagara 2002; Evans et al. 2003; Pandya et al. 2015; Shireen 2016) Neuroleptics can also stimulate the release of arachidonic acid and this can lead to increased prostaglandin release which can lead to increased free radical production and the cascade described above (Shireen 2016).

CBD ameliorates the reaction described above by suppressing fatty acid amide hydrolase (FAAH) that degrades anandamide, an endocannabinoid that can modulate free radical production and cellular migration. It also acts on the PPARs to reduce the inflammatory cascade and promote neurogenesis (Booz 2011; Valvassori et al. 2011; Rosales-Corral et al. 2015) which may be important for its beneficial effects on TD. CBD also downregulates the expression of vascular cell adhesion molecule-1 (VCAM-1), chemokines (CCL2 and CCL5) and the proinflammatory cytokine IL-1β, leading to decreased blood leukocytes migration (Mecha et al. 2013). The blockade of the phosphorylated form of p38 MAP kinase and the transcription factor nuclear factor-κB activation by CBD have been associated with inhibition of nitric oxide synthase production and nitric oxide production. This is one of the mechanisms by which CBD demonstrates its antioxidant effects (Esposito et al. 2006).

However, in our study, CBD did not modify oxidative stress enzymes in contrast to findings by Sonego et al. (2018), who reported that cannabidiol modified oxidative stress enzymes. So far, only two published studies have investigated the effects of CBD on haloperidol induced movement disorders (Gomes et al. 2013; Sonego et al. 2016) and only one, Sonego et al. (2018), investigated the influence of CBD on biochemical parameters but the lack of methodological detail provided in the aforementioned precludes us from commenting on the reasons for the difference in findings. Though the mechanism by which CBD produces its motor effects is still open to debate, pathways other than the endocannabinoid system may be involved.

Ion channels are known to be involved in the pathophysiology of movement disorders. There is evidence that CBD changes the conductance of voltage-dependent anion channels (VDAC1) (Rimmerman et al. 2013). VDAC is required for the degradation of mitochondria which causes the loss of dopaminergic neurons seen in Parkinson's disease (PD), VDAC may therefore be involved in the effect of CBD on movement disorders (Geisler et al. 2010; Ibeas Bih et al. 2015). CBD has also been shown to be involved in the activation and upregulation of Peroxisome Proliferator-activated Receptors ($PPAR_{\gamma}$), a receptor known to be involved in oral dyskinesia (O'Sullivan et al. 2009; Stone et al. 2009; Ramer et al. 2013). Voltage-gated calcium channels (VGCC) may also be involved in the pathophysiology of tremors and they are also blocked by CBD at low doses (Bourin and Nic Dhonnchadha 2005; Ross et al. 2008; Ibeas Bih et al. 2015). The receptors targeted by CBD to improve movement disorders includes $5HT_{1A}$ and $5HT_{2A}$ subtypes in the basal ganglia (Russo et al. 2005), CBD is believed to interact with these subtypes to ameliorate the dysfunction of the dopaminergic system seen in TD (Russo et al. 2005; Gomes et al. 2013; Ibeas Bih et al. 2015). CBD is also a strong antagonist at cholinergic receptors A4β2and α7 nAchRs (Mahgoub et al. 2013). These receptors are implicated in acute dyskinesia because their rapid desensitisation reduces dyskinesia (Henry et al. 2001). The above may also contribute to the effects of CBD on movement disorders in our study.

We found an increase in DPPH scavenging activity in the cannabidiol-only group in our study, a finding that to our knowledge has not previously been reported. The increase in DPPH scavenging activity is indicative of cannabidiol's potent antioxidant properties. We observed a stabilisation of fasting blood glucose in our study, Lehmann et al. (2017) had earlier suggested that CBD could prevent the development of type 1 diabetes by ameliorating the effects of various cytokines secreted by macrophages and splenocytes, a closer look at our results revealed an inverted U curve effect pattern in which haloperidol 5 mg/kg p.o. + Cannabidiol 5 mg/kg p.o was more effective than haloperidol 5 mg/kg p.o. + Cannabidiol 3 mg/kg p.o and haloperidol 5 mg/kg p.o. + Cannabidiol 10 mg/kg p.o. These observations will require further investigations.

In summary, we confirmed that CBD can ameliorate motor impairments produced by haloperidol. Our data suggest that CBD can be combined with haloperidol to prevent the emergent of extrapyramidal side effects and long-term movement disorders, such as acute dystonic disorder and TD. CBD has also been documented to have antipsychotic properties in animal studies (Zuardi et al. 1991; Moreira and Guimaraes 2005), however, the results of clinical trials have been

inconclusive (Osborne et al. 2017). A pharmaceutical formulation that combines cannabidiol and haloperidol may have reduced propensity to side effects, such as TD, and provide effective antipsychotic cover at relatively low cost. Further studies in this direction are needed.

Acknowledgements

None.

Disclosure statement

The company however did not contribute towards the development of the protocol, the experiments or to the analysis or interpretation of the data.

Funding

This research is supported by the South African Research Chair in PTSD hosted by the Stellenbosch University, funded by the Department of Science and Technology South Africa and administered by the National Research Foundation. This work was supported by Cannabis Science Inc.

ORCID

Jaiyeola Abiola Kajero http://orcid.org/0000-0003-3228-168X
Soraya Seedat http://orcid.org/0000-0002-5118-786X
Abidemi Akindele http://orcid.org/0000-0002-9007-0437

References

Addy N, Levin ED. 2002. Nicotine interactions with haloperidol, clozapine and risperidone and working memory function in rats. Neuropsychopharmacology. 27(4):534–541.

Almeida V, Levin R, Peres FF, Niigaki ST, Calzavara MB, Zuardi AW, Hallak JE, Crippa JA, Abílio VC. 2013. Cannabidiol exhibits anxiolytic but not antipsychotic property evaluated in the social interaction test. Prog Neuropsychopharmacol Biol Psychiatr. 41:30–35.

Baldessarini RJ. 1988. A summary of current knowledge of tardive dyskinesia. L'Encéphale. 14(Spec Issue):263–268.

Bartzokis G, Hill MA, Altshuler L, Cummings JL, Wirshing W, May PRA. 1989. Tardive dyskinesia in schizophrenic patients: correlation with negative symptoms. Psychiatr Res. 28(2):145–151.

Berger PA, Rexroth K. 1980. Tardive dyskinesia: clinical, biological, and pharmacological perspectives. Schizophrenia Bull. 6(1):102–116.

Bishnoi M, Boparai RK. 2012. An animal model to study the molecular basis of tardive dyskinesia. Methods Mol Biol. 829:193–201.

Blanchet PJ, Parent MT, Rompre PH, Levesque D. 2012. Relevance of animal models to human tardive dyskinesia. Behav Brain Funct. 8(1):12.

Blessing EM, Steenkamp MM, Manzanares J, Marmar CR. 2015. Cannabidiol as a potential treatment for anxiety disorders. Neurotherapeutics. 12(4):825–836.

Booz GW. 2011. Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. Free Radical Biol Med. 51(5):1054–1061.

Bossong MG, van Berckel BN, Boellaard R, Zuurman L, Schuit RC, Windhorst AD, van Gerven JMA, Ramsey NF, Lammertsma AA, Kahn RS, et al. 2009. Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacol. 34(3):759–766.

Bourin M, Nic Dhonnchadha BA. 2005. 5-HT2 receptors and anxiety. Drug Dev Res. 65(3):133–140.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. Meth Enzymol. 52:302–310.

Cadet JL, Perumal AS. 1990. Chronic treatment with prolixin causes oxidative stress in rat brain. Biol Psychiatr. 28(8):738–740.

Carney M, Sheffield B. 1976. Comparison of antipsychotic depot injections in the maintenance treatment of schizophrenia. Br J Psychiatr. 129(5):476–481.

Caroff S, Aggarwal S, Yonan C. 2018. Treatment of tardive dyskinesia with tetrabenazine or valbenazine: a systematic review. J Comprehensive Effect Res. 7(2):135–148.

Carvalho RK, Souza MR, Santos ML, Guimarães FS, Pobbe RLH, Andersen ML, Mazaro-Costa R. 2018. Chronic cannabidiol exposure promotes functional impairment in sexual behavior and fertility of male mice. Reprod Toxicol. 81:34–40.

Casey D. 1990. Tardive dyskinesia. West J Med. 153(5):535–541.

Chen JJ, Ondo WG, Dashtipour K, Swope DM. 2012. Tetrabenazine for the treatment of hyperkinetic movement disorders: a review of the literature. Clin Ther. 34(7):1487–1504.

Cho C, Lee H. 2013. Oxidative stress and tardive dyskinesia: pharmacogenetic evidence. Prog Neuropsychopharmacol Biol Psychiatry. 46:207–213.

Chopin P, Pellow S, File SE. 1986. The effects of yohimbine on exploratory and locomotor behaviour are attributable to its effects at noradrenaline and not at benzodiazepine receptors. Neuropharmacology. 25(1):53–57.

Chouinard G, Chouinard V. 2008. Atypical antipsychotics: CATIE study, drug-induced movement disorder and resulting iatrogenic psychiatric-like symptoms, super sensitivity rebound psychosis and withdrawal discontinuation syndromes. Psychother Psychosom. 77(2):69–77.

Citrome L. 2017. Clinical management of tardive dyskinesia: five steps to success. J Neurol Sci. 383:199–204.

Crippa JA, Zuardi AW, Martín-Santos R, Bhattacharyya S, Atakan Z, McGuire P, Fusar-Poli P. 2009. Cannabis and anxiety: a critical review of the evidence. Hum Psychopharmacol Clin Exp. 24(7):515–523.

Crowley JJ, Adkins DE, Pratt AL, Quackenbush CR, van den Oord EJ, Moy SS, Wilhelmsen KC, Cooper TB, Bogue MA, McLeod HL, et al. 2012. Antipsychotic-induced vacuous chewing movements and extrapyramidal side-effects are highly heritable in mice. Pharmacogenomics J. 12(2):147–155.

Dewolfe AS, Ryan JJ, Wolf ME. 1988. Cognitive sequelae of tardive dyskinesia. J Nerv Mental Dis. 176(5):270–274. doi: 10.1097/00005053-198805000-00003

Dissanayaka NNW, Sellbach A, Matheson S, O'Sullivan JD, Silburn PA, Byrne GJ, Marsh R, Mellick GD. 2010. Anxiety disorders in Parkinson's disease: prevalence and risk factors. Mov Disord. 25(7):838–845.

Elkashef AM, Wyatt RJ. 1999. Tardive dyskinesia: possible involvement of free radicals and treatment with vitamin E. Schizophr Bull. 25(4):731–740.

Ennaceur A, Delacour J. 1988. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res. 31(1):47–59.

Esposito G, De Filippis D, Maiuri MC, De Stefano D, Carnuccio R, Iuvone T. 2006. Cannabidiol inhibits inducible nitric oxide synthase protein expression and nitric oxide production in beta-amyloid stimulated PC12 neurons through p38 MAP kinase and NF-kappaB involvement. Neurosci Lett. 399(1–2):91–95.

Evans DR, Parikh VV, Khan MM, Coussons C, Buckley PF, Mahadik SP. 2003. Red blood cell membrane essential fatty acid metabolism in early psychotic patients following antipsychotic drug treatment. Prostaglandins Leukot Essent Fatty Acids. 69(6):393–399.

Eweka A, Om'Iniabohs F. 2007. The effects of monosodium glutamate on the open field locomotor activities in adult wistar rats. The Internet J of Nutr and Wellness, 6(2):1–6. doi: 10.5580/129e

Factor SA, Remington G, Comella CL, Correl CU, Burke J, Jimenez R, Liang GS, O'Brien CF. 2017. The effects of Valbenazine in participants with tardive dyskinesia: results of the 1-year kinetic 3 extension study. J Clin Psychiatr. 78(9):1344–1350.

Formukong EA, Evans AT, Evans FJ. 1988. Analgesic and anti-inflammatory activity of constituents of *Cannabis sativa* L. Inflammation. 12(4):361–371.

Gallhofer B, Jaanson P, Mittoux A, Tanghøj P, Lis S, Krieger S. 2007. Course of recovery of cognitive impairment in patients with schizophrenia: a randomised double-blind study comparing sertindole and haloperidol. Pharmacopsychiatry. 40(6):275–286.

Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W. 2010. PINK1/Parkin- mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol. 12(2):119–131.

Gittis AH, Leventhal DK, Fensterheim BA, Pettibone JR, Berke JD, Kreitzer AC. 2011. Selective inhibition of striatal fast-spiking interneurons causes dyskinesia. J Neurosci. 31(44): 15727–15731.

Gomes FV, Del Bel EA, Guimarães FS. 2013. Cannabidiol attenuates catalepsy induced by distinct pharmacological mechanisms via 5-HT1A receptor activation in mice. Prog Neuropsychopharmacol Biol Psychiatr. 46:43–47.

Guimaraes FS, Chiaretti TM, Graeff FG, Zuardi AW. 1990. Anti-anxiety effect of cannabidiol in the elevated plus-maze. Psychopharmacology (Berl). 100(4):558–559.

Gunne L, Häggström J, Sjöquist B. 1984. Association with persistent neuroleptic-induced dyskinesia of regional changes in brain GABA synthesis. Nature. 309(5966): 347–349.

Gureje O. 1988. Topographic subtypes of tardive dyskinesia in schizophrenic patients aged less than 60 years: relationship to demographic, clinical, treatment, and neuropsychological variables. J Neurol Neurosurg Psychiatr. 51(12):1525–1530.

Guzman F, Farinde A. 2016. First-generation antipsychotics: an introduction. Psychopharmacology Institute. [Accessed 2020 April 20]. https://psychopharmacologyinstitute.com/antipsychotics/first-generation-antipsychotics/.

Henry B, Fox SH, Crossman AR, Brotchie JM. 2001. Mu- and delta- opioid receptor antagonists reduce levodopa-induced dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. Exp Neurol. 171(1):139–146.

Ibeas Bih C, Chen T, Nunn AVW, Bazelot M, Dallas M, Whalley BJ. 2015. Molecular targets of cannabidiol in neurological disorders. Neurotherapeutics. 12(4):699–730.

Ignatowska-Jankowska B, Jankowski MM, Swiergiel AH. 2011. Cannabidiol decreases body weight gain in rats: involvement of CB2 receptors. Neurosci Lett. 490(1):82–84.

Janno S, Holi M, Tuisku K, Wahlbeck K. 2004. Prevalence of neuroleptic-induced movement disorders in chronic schizophrenia inpatients. Am J Psychiatr. 161(1):160–163.

Josiassen RC, Filmyer DM, Gillean J, Shah SS, Dietterich TE, Shaughnessy RA. 2017. Successful treatment of severe tardive dyskinesia with valbenazine, including a patient's perspective. Am J Case Rep. 18:1185–1189.

Kaur N, Kumar P, Jamwal S, Deshmukh R, Gauttam V. 2016. Tetrabenazine: spotlight on drug review. Ann Neurosci. 23(3):176–185.

Kenney C, Jankovic J. 2006. Tetrabenazine in the treatment of hyperkinetic movement disorders. Expert Rev Neurother. 6(1):7–17.

King C, Voruganti LN. 2002. What's in a name? The evolution of the nomenclature of antipsychotic drugs. J Psychiatr Neurosci. 27(3):168–175.

Kiriakakis V, Bhatia KP, Guinn NP, Marsden CD. 1998. The natural history of tardive dystonia. A long-term follow-up study of 107 cases. Brain. 121(11):2053–2066.

Kovacic P, Somanathan R. 2012. Redox processes in neurodegenerative disease involving reactive oxygen species. Curr Neuropharmacol. 10(4):289–302.

Kucerova J, Tabiova K, Drago F, Micale V. 2014. Therapeutic potential of cannabinoids in schizophrenia. Recent Pat CNS Drug Discov. 9(1):13–25.

Kulkarni KS, Singh A, Naidu PS. 2002. Carvedilol attenuates neuroleptic-induced orofacial dyskinesia: possible antioxidant mechanisms. Br J Pharmacol. 136(2):193–200.

Kulkarni SK, Naidu PS. 2001. Animal models of tardive dyskinesia–a review. Indian J Physiol Pharmacol. 45(2): 148–160.

Kulkarnin SK, Naidu PS. 2004. Oxidative stress and tardive dyskinesia: role of natural antioxidants. Iran J Pharm Res. 3(1):11–11.

Lee JLC, Bertoglio LJ, Guimaraes FS, Stevenson CW. 2017. Cannabidiol regulation of emotion and emotional memory processing: relevance for treating anxiety-related and substance abuse disorders. Br. J. Pharmacol. 174(19): 3242–3256.

Lehmann C, Fisher NB, Tugwell B, Szczesniak A, Kelly M, Zhou J. 2017. Experimental cannabidiol treatment reduces early pancreatic inflammation in type 1 diabetes. Clin Hemorheol Microcirc. 64(4):655–662.

Leung J, Breden E. 2011. Tetrabenazine for the treatment of tardive dyskinesia. Ann Pharmacother. 45(4):525–531.

Levin ED, Wilson W, Rose JE, McEvoy J. 1996. Nicotine-haloperidol interactions and cognitive performance in

schizophrenics. Neuropsychopharmacology. 15(5):429–436. (96)00018-8

Levin R, Almeida V, Fiel Peres F, Bendlin Calzavara M, Derci da Silva N, Akimi Suiama M, Tamie Niigaki S, Waldo Zuardi A, Eduardo Cecilio Hallak J, Alexandre Crippa J, et al. 2012. Antipsychotic profile of cannabidiol and rimonabant in an animal model of emotional context processing in schizophrenia. Curr Pharmacol Des. 18(32): 4960–4965.

Lewekwe FM, Piomelli D, Pahlisch F, Muhl D, Gerth CW, Hoyer C, Klosterkotter J, Hellmich J, Koethe D. 2012. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl Psychiatry. 2(3):e94–e94.

Lister J, Nobrega JN, Fletcher PJ, Remington G. 2014. Oxidative stress and the antipsychotic-induced vacuous chewing movement model of tardive dyskinesia: evidence for antioxidant-based prevention strategies. Psychopharmacology (Berl.). 231(11):2237–2249.

Mahgoub M, Keun-Hang SY, Sydorenko V, Ashoor A, Kabbani N, Al Kury L, Sadek B, Howarth CF, Isaev D, Galadari S, et al. 2013. Effects of cannabidiol on the function of α7-nicotinic acetylcholine receptors. Eur J Pharmacol. 720(1–3):310–319.

Malfait AM, Gallily R, Sumariwalla PF, Malik AS, Andreakos E, Mechoulam R, Feldman M. 2000. The non-psychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. Proc Natl Acad Sci USA. 97(17):9561–9566.

Margolese HC, Chouinard G, Kolivakis TT, Beauclair L, Miller R, Annable L. 2005. Tardive dyskinesia in the era of typical and atypical antipsychotics. Part 2: incidence and management strategies in patients with schizophrenia. Can J Psychiatr. 50(11):703–714.

Marques A, Durif F, Fernagut PO. 2018. Impulse control disorders in Parkinson's disease. J Neural Transm. 125(8): 1299–1312.

Mecha M, Feliú A, Iñigo PM, Mestre L, Carrillo-Salinas FJ, Guaza C. 2013. Cannabidiol provides long-lasting protection against the deleterious effects of inflammation in a viral model of multiple sclerosis: a role for A2A receptors. Neurobiol Dis. 59:141–150.

Mejia NI, Jankovic J. 2010. Tardive dyskinesia and withdrawal emergent syndrome in children. Exp Rev Neurotherapy. 10(6):893–901.

Mentzel TQ, Lieverse R, Bloemen O, Viechtbauer W, Van Harten PN; Genetic Risk and Outcome of Psychosis (GROUP) Investigators. 2017. High incidence and prevalence of drug-related movement disorders in young patients with psychotic disorders. J Clin Psychopharmacol. 37(2):231–238.

Micale V, Di Marzo V, Sulcova A, Wotjak CT, Drago F. 2013. Endocannabinoid system and mood disorders: priming a target for new therapies. Pharmacol Ther. 138(1):18–37. doi:10.1016/j.pharmthera.2012.12.002

Micale V, Tabiova K, Kucerova J, Drago F. 2015. Role of the endocannabinoid system in depression: from preclinical to clinical evidence. In: Campolongo P., Fattore L., editors. Cannabinoid modulation of emotion, memory, and motivation. New York, NY: Springer; p. 97–129.

Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui DH, Tabira T. 2000. Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. J Neurosci. 20(4):1568–1574.

Moreira FA, Guimarães FS. 2005. Cannabidiol inhibits the hyperlocomotion induced by psychotomimetic drugs in mice. Eur J Pharmacol. 512(2-3):199–205.

Naidu PS, Kulkarni SK. 2001a. Effect of 5-HT1A and 5- HT2A/2C receptor modulations on neuroleptic-induced vacuous chewing movements. Eur J Pharmacol. 428(1):81–86.

Naidu PS, Kulkarni SK. 2001b. Possible involvement of prostaglandins in haloperidol-induced orofacial dyskinesia in rats. Eur J Pharmacol. 430(2–3):295–298.

National Research Council 2011. Guide for the Care and Use of Laboratory Animals: Eighth Edition. Washington, DC: The National Academies Press.

Nazario LR, Antonioli R, Capiotti KM, Hallak JEC, Zuardi AW, Crippa JAS, Bonan CD, da Silva RS. 2015. Caffeine protects against memory loss induced by high and non-anxiolytic dose of cannabidiol in adult zebra fish *(Daniorerio)*. Pharmacol Biochem Behav. 135:210–216.

Omar ME, Marawa EE, Neveen AS, Alaa EM. 2012. Effects of *Cannabis sativa* extract on haloperidol-induced catalepsy and oxidative stress in the mice. EXCLI J. 11:45–58.

Ondo W, Hanna P, Jankovic J. 1999. Tetrabenazine treatment for tardive dyskinesia: assessment by randomized videotape protocol. Am J Psychiatr. 156(8):1279–1281.

Osborne AL, Solowij N, Weston-Green K. 2017. A systematic review of the effect of cannabidiol on cognitive function: relevance to schizophrenia. Neurosci Biobehav Rev. 72: 310–324.

O'Sullivan SE, Sun Y, Bennett AJ, Randall MD, Kendall DA. 2009. Time-dependent vascular actions of cannabidiol in the rat aorta. Eur J Pharmacol. 612(1–3):61–68.

Ozdemir V, Basile V, Masellis M, Kennedy J. 2001. Pharmacogenetic assessment of antipsychotic-induced movement disorders: contribution of the dopamine D3 receptor and cytochrome P450 1A2 genes. J Biochem Bioph Methods. 47(1–2):151–157.

Oztürk Y, Aydin S, Beis R, Başer KH, Berberoğlu H. 1996. Effects of Hypericum perforatum L. and Hypericum calycinum L. extracts on the central nervous system in mice. Phytomedicine. 3(2):139–146.

Pacher P, Bátkai S, Kunos G. 2006. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev. 58(3):389–462.

Pandya CD, Howell KR, Pillai A. 2013. Antioxidants as potential therapeutics for neuropsychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry. 46:214–223.

Parikh V, Mohammad MK, Sahebarao PM. 2003. Differential effects of antipsychotics on expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. J Psychiatr Res. 37(1):43–51.

Patil R, Kiran D, Gound H, Gadakh R. 2012. Protective effect of leaves of *Murraya koenigii* on reserpine-induced orofacial dyskinesia. Iran J Pharm Res. 11(2):635–641.

Peres F, Levin R, Mayra A, Suiama M, Diana M, Gouvêa D, Almeida V, Santos C, Lungato L, Zuardi A, et al. 2016. Cannabidiol prevents motor and cognitive impairments induced by reserpine in rats. Front Pharmacol. 28(7):1–10.

Pich M, Samanin R. 1986. Disinhibitory effects of buspirone and low doses of sulpiride and haloperidol in two experimental anxiety models in rats: possible role of dopamine. Psychopharmacology. 89(1):125–130.

Pickens JT. 1981. Sedative activity of cannabis in relation to its Delta-1-trans-tetrahydrocannabinol and cannabidiol content. Br J Pharmacol. 72(4):649–656.

Pillai A, Parikh V, Terry AV, Mahadik SP. 2007. Long-term antipsychotic treat- ments and crossover studies in rats: differential effects of typical and atypical agents on the expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. J Psychiatr Res. 41(5):372–386.

Ramer R, Heinemann K, Merkord J, Rohde H, Salamon A, Linnebacher M, Hinz B. 2013. COX-2 and PPAR-γ confer cannabidiol-induced apoptosis of human lung cancer cells. Mol Cancer Ther. 12(1):69–82.

Ramos A, Berton O, Mormede P, Chaouloff F. 1997. A multiple-test study of anxiety-related behaviours in six inbred rat strains. Behav Brain Res. 85(1):57–69.

Rezvani AH, Levin ED. 2004. Nicotine-antipsychotic drug interactions and attentional performance in female rats. Eur J Pharmacol. 486(2):175–182.

Rimmerman N, Ben-Hail D, Porat Z, Juknat A, Kozela E, Daniels MP, Connelly PS, Leishman E, Bradshaw HB, Shoshan-Barmatz V, et al. 2013. Direct modulation of the outer mitochondrial membrane channel, voltage-dependent anion channel 1 (VDAC1) by cannabidiol: a novel mechanism for cannabinoid-induced cell death. Cell Death Dis. 4(12):e949–e949.

Rochon PA, Stukel TA, Sykora K, Gill S, Garfinkel S, Anderson GM, Normand S-LT, Mamdani M, Lee PE, Li P, et al. 2005. Atypical antipsychotics and Parkinsonism. Arch Intern Med. 165(16):1882–1888.

Röpke J, Busanello A, Leal CQ, de Moraes Reis E, de Freitas CM, Villarinho JG, Figueira FH, Mello CF, Ferreira J, Fachinetto R. 2014. Anandamide attenuates haloperidol-induced vacuous chewing movements in rats. Progr Neuropsychopharmacol Biol Psychiatr. 54:195–199.

Rosales-Corral S, Hernández L, Gallegos M. 2015. Cannabinoids in neuroinflammation, oxidative stress and neuro excitotoxicity. Pharmaceutical Analytica Acta. 6:346.

Ross HR, Napier I, Connor M. 2008. Inhibition of recombinant human T-type calcium channels by Delta9-tetrahydrocannabinol and cannabidiol. J Biol Chem. 283(23): 16124–16134.

Russo EB, Burnett A, Hall B, Parker KK. 2005. Agonistic properties of cannabidiol at 5-HT1a receptors. Neurochem Res. 30(8):1037–1043.

Sagara Y. 2002. Induction of reactive oxygen species in neurons by haloperidol. J Neurochem. 71(3):1002–1012.

Stahl SM. 2018. Beyond the dopamine hypothesis of schizophrenia to three neural networks of psychosis: dopamine, serotonin, and glutamate. CNS Spectr. 23(3):187–191.

Sandyk R, Snider SR, Consroe P, Elias SM. 1986. Cannabidiol in dystonic movement disorders. Psychiatry Res. 18(3):291.

Sasaki H, Hashimoto K, Maeda Y, Inada T, Kitao Y, Fukui S, Iyo M. 1995. Rolipram, a selective c-AMP phosphodiesterase inhibitor suppresses oro-facial dyskinetic movements in rats. Life Sci. 56:443–447.

Sedlak J, Lindsay RH. 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 25(1):192–205.

Seeman P. 2002. Atypical antipsychotics: mechanism of action. Can J Psychiatr. 47(1):27–38.

Sekiguchi K, Kanno H, Yamaguchi T, Ikarashi Y, Kase Y. 2012. Ameliorative effect of Yokukan-san on vacuous chewing movement in haloperidol-induced rat tardive dyskinesia model and involvement of glutamatergic system. Brain Res Bull. 89(5–6):151–158.

Selemon LD, Zecevic N. 2015. Schizophrenia: a tale of two critical periods for prefrontal cortical development. Transl Psychiatr. 5(8):e623–e623.

Shao L, Hewitt MC. 2010. The kinetic isotope effect in the search for deuterated drugs. Drug News Perspect. 23(6): 398–404.

Shireen E. 2016. Experimental treatment of antipsychotic-induced movement disorders. J Exp Pharmacol. 8:1–10.

Sinha AK. 1972. Colorimetric assay of catalase. Anal Biochem. 47(2):389–394.

Sonego AB, Gomes FV, Del Bel EA, Guimaraes FS. 2016. Cannabidiol attenuates haloperidol-induced catalepsy and c-Fos protein expression in the dorsolateral striatum via 5-HT1A receptors in mice. Behav Brain Res. 309:22–28.

Sonego AB, Prado DS, Vale GT, Sepulveda-Diaz JE, Cunha TM, Tirapelli CR, Del Bel EA, Raisman-Vozari R, Guimarães FS. 2018. Cannabidiol prevents haloperidol-induced vacuos chewing movements and inflammatory changes in mice via PPARγ receptors. Brain Behav Immun. 74: 241–251.

Steeds H, Carhart-Harris RL, Stone JM. 2015. Drug models of schizophrenia. Therap Adv Psychopharmacol. 5(1):43–58.

Stone JM, Day F, Tsagaraki H, Valli I, McLean MA, Lythgoe DJ, O'Gorman RL, Barker GJ, McGuire PK. 2009. Glutamate dysfunction in people with prodromal symptoms of psychosis: relationship to gray matter volume. Biol Psychiatry. 66(6):533–539.

Sun M, Zigman S. 1978. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Anal Biochem. 90(1):81–89.

Teo JT, Edwards MJ, Bhatia K. 2012. Tardive dyskinesia is caused by maladaptive synaptic plasticity: a hypothesis. Mov Disord. 27(10):1205–1215.

Thaakur S, Himabindhu G. 2009. Effect of alpha lipoic acid on the tardive dyskinesia and oxidative stress induced by haloperidol in rats. J Neural Transm. 116(7):807–814.

Thanvi B, Lo N, Robinson T. 2007. Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. Postgrad Med J. 83(980): 384–388.

Touma KTB, Scarff JR. 2018. Valbenazine and deutetrabenazine for tardive dyskinesia. Innov Clin Neurosci. 15(5–6): 13–16.

Valvassori SS, Elias G, de Souza B, Petronilho F, Dal-Pizzol F, Kapczinski F, Trzesniak C, Tumas V, Dursun S, Nisihara Chagas MH. 2011. Effects of cannabidiol on amphetamine-induced oxidative stress generation in an animal model of mania. J Psychopharmacol. 25(2):274–279.

van Asselen M, Kessels RPC, Neggers SFW, Kappelle LJ, Frijns CJM, Postma A. 2006. Brain areas involved in spatial working memory. Neuropsychologia. 44(7):1185–1194.

Voruganti LN, Slomka P, Zabel P, Mattar A, Awad AG. 2001. Cannabis induced dopamine release: an in-vivo SPECT study. Psychiatr Res Neuroimaging. 107(3):173–117.

Voon V, Mehta AR, Hallett M. 2011. Impulse control disorders in Parkinson's disease: recent advances. Curr Opin Neurol. 24(4):324–330.

Waddington JL, Youssef HA, Molloy AG, O'Boyle KM, Pugh MT. 1985. Association of intellectual impairment, negative

symptoms, and aging with tardive dyskinesia: clinical and animal studies. J Clin Psychiatr. 46(4 Pt 2):29–33.
Waddington JL, Youssef HA. 1986. Late onset involuntary movements in chronic schizophrenia: relationship of "tardive" dyskinesia to intellectual impairment and negative symptoms. Br J Psychiatry. 149(5):616–620.
Waln O, Jankovic J. 2013. An update on tardive dyskinesia: from phenomenology to treatment. Tremor Other Hyperkinet Mov (N Y). 3:1–11.
Walsh RN, Cummins RA. 1976. The Open-Field Test: a critical review. Psychol Bull. 83(3):482–504.
Watzl B, Scuderi P, Watson RR. 1991. Marijuana components stimulate human peripheral blood mononuclear cell secretion of interferongamma and suppress interleukin-1 alpha in vitro. Int J Immunopharmacol. 13(8):1091–1097.
Wu JQ, Chen DC, Tan YL, Tan SP, Wang ZR, Xiu MH, Yang FD, Zhang XY. 2014. Cognition impairment in schizophrenia patients with tardive dyskinesia: association with plasma superoxide dismutase activity. Schizophrenia Res. 152(1):210–216.
Yero T, Rey JA. 2008. Tetrabenazine (xenazine), an FDA-approved treatment option for Huntington's disease-related chorea. Pharmacy Therapeut. 33(12):690–694.
Zai C, Tiwari A, Basile V, de Luca V, Müller D, Voineskos A, Remington G, Meltzer H, Lieberman J, Potkin S, et al. 2010. Oxidative stress in tardive dyskinesia: genetic association study and meta-analysis of NADPH quinine oxidoreductase 1 (NQO1) and Superoxide dismutase 2 (SOD2, MnSOD) genes. Prog Neuropsychopharmacol Biol Psychiatr. 34(1):50–56.
Zhang XY, Yao JK. 2013. Oxidative stress and therapeutic implications in psychiatric disorders. Prog Neuro-Psychopharmacol Biol Psychiatr. 46:197–199.
Zhang ZJ, Zhang XB, Hou G, Yao H, Reynolds GP. 2003. Interaction between polymorphisms of the dopamine D3 receptor and manganese superoxide dismutase genes in susceptibility to tardive dyskinesia. Psychiatr Genet. 13(3): 187–192.
Zuardi AW, Crippa J, Hallak JEC, Pinto JP, Chagas MHN, Rodrigues GGR, Dursun SM, Tumas V. 2009. Cannabidiol for the treatment of psychosis in Parkinson's disease. J Psychopharmacol. 23(8):979–983.
Zuardi AW, Rodrigues JA, Cunha JM. 1991. Effects of cannabidiol in animal models predictive of antipsychotic activity. Psychopharmacology (Berl). 104(2):260–264.
Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG. 1982. Action of cannabidiol on the anxiety and other effects produced by Delta-9-THC in normal subjects. Psychopharmacology. 76(3):245–250.