**Effects of Cannabidiol on Oxidative Stress Parameters in the Liver and Kidney Induced by Prolonged Haloperidol Administration**

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**Running title: Influence of Cannabidiol on Adverse Effects Induced by Haloperidol in end-organs.**

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Abstract

We hypothesized that cannabidiol (CBD), a potent antioxidant, would protect the liver and the kidney by alleviating oxidative stress induced by prolonged administration of haloperidol. Haloperidol was administered sub-chronically via the intraperitoneal (IP) route for twenty-one days in a group. In another group, haloperidol was administered for twenty-one days before the administration of CBD. Haloperidol alone was also administered chronically for 90 days via the intramuscular (IM) route to a group of rats while another group had haloperidol for 90 days via the IM route before the administration of CBD. Oxidative stress parameters were assessed after the last dose of medication. In the subchronic haloperidol alone group, the organs revealed elevated (superoxide dismutase) SOD, catalase (CAT) activity, and reduced glutathione (GSH) levels. Chronic intramuscular (IM) haloperidol administration however did not increase the activity of SOD and CAT or reduced GSH levels. CBD reduced nitric oxide (NO) levels in the liver and kidney when administered after subchronic (IP) haloperidol. Both subchronic and chronic haloperidol resulted in high Malondialdehyde (MDA) activity in the kidney, and this was ameliorated by CBD. There was increased activity in 2,2-diphenyl-1-picrylhydrazyl (DPPH) in the liver in the subchronic (IP) haloperidol alone group but low activity in DPPH in the liver in the chronic (IM) haloperidol alone group.

**Introduction**

Haloperidol is widely used in the treatment of patients with psychosis, it acts mainly by blockade of D2 and D3 receptors in the mesolimbic and mesocortical pathways. Chronic receptor blockade, however, is associated with increased dopamine turnover and auto-oxidation of dopamine molecules leading to increased free radical production (Cho & Lee, 2013), increased lipid peroxidation, damage to the phospholipid membrane of neurons, and induction of oxidative stress in the brain (Kiriakakis et al., 1998). Chronic receptor blockade may also disrupt the activity of endogenous antioxidant enzymes such as SOD, catalase, GSH, and glutathione peroxidase (GPx) in the striatum (Kulkarnin & Naidu, 2004; Zhang &Yao, 2013).

Increased oxidative stress in the brain can interrupt normal physiological processes leading to an influx of extracellular Ca2+  intracellularly and consequently cellular death (Cho and Lee 2012; Yin et al.,2016). This can lead to conditions such as tardive dyskinesia (TD), an abnormal involuntary purposeless movement disorder linked to chronic exposure to dopamine receptor-blocking agents (DRBA), such as haloperidol (Lerner et al., 2015; Umar, 2018). Prolonged administration of haloperidol has also been shown to induce oxidative stress in end organs such as the liver and the kidneys in animal studies (Halici et al.,2009; Uyanik et al.,2009; Andreazza et al., 2015; Roversi et al.,2015).

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Lipid peroxidation was observed in the liver of adult rats exposed to haloperidol for 28 days (Andreazza et al.,2015). In another study, Haloperidol was also linked to decreased volume fractions of the cortex in relation to the whole kidney (Uyanik et al.,2009). However, there are few studies examining the effects of antioxidants on oxidative stress-induced changes in the end organs. Sucrose and grape seed extract were shown to reduce haloperidol-induced oxidative stress in the brain and liver in a study (El-Awdan et al.,2015). Coenzyme Q10 (CoQ10) and N-acetyl cysteine (NAC) also reduced oxidative stress in the liver (Eftekhari et al.,2016).

CBD is a potent antioxidant and neuroprotective compound being investigated by several workers in the management of movement disorders produced by antipsychotics (Peres et al., 2018; Sonego et al.,2018; Kajero et al., 2020). CBD has been shown to reduce inflammation and oxidative stress in a mouse model of hepatic ischaemic/ reperfusion injury (Mukhopadhyay et al., 2012) but the effects of CBD on oxidative stress induced by antipsychotics in the liver and the kidney have not been studied.

Some researchers have reported CBD is not free of side effects and may interact with the enzymes responsible for the metabolism and elimination of certain antipsychotics (Iffland & Grotenhermen 2017; Brown & Winterstein 2019). CBD is metabolized in the liver and the intestine by cytochrome (CYP)2C19, CYP3A4, and 5'-diphosphoglucuronosyltransferase (UGT) to produce hydroxylated and carboxylated metabolites. CBD also inhibits CYP3A4 and CYP2C19 microsomal enzymes and these pharmacokinetic actions can affect the interactions of CBD with other medications (Devinsky et al.,2014; Greenwich Biosciences 2019). This is important because if CBD is in future administered with antipsychotics in real-world clinical settings, its complex pharmacokinetics could affect the plasma concentration of various medications (Samara et al., 1991) with consequences for oxidative stress parameters in key organs. It is therefore pertinent to study the synergistic effects of CBD and antipsychotics on oxidative stress parameters in the liver, and kidney using haloperidol as the prototype antipsychotic. We hypothesized that CBD would have a protective effect on the organs by alleviating oxidative stress induced by haloperidol irrespective of the duration of administration.

**Materials and methods**

***Animals***

Male adult Wistar rats used in this study were obtained from the Nigerian Institute of Medical Research (NIMR) colony, Yaba, Lagos, Nigeria. The animals 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 acclimatized 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 is a component of a larger study approved by the Institutional Review Board (IRB) of NIMR, 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, Praha, Czech Republic) was supplied in fine granule form with the amount administered weekly calculated and dissolved in 70% ethanol, as recommended by the manufacturer and diluted with distilled water. Cannabidiol was administered orally. Haloperidol tablets (Janssen Pharmaceuticals, Beerse, Belgium) were dissolved in 0.5% acetic acid and distilled water and administered orally. Rapid-acting parenteral haloperidol 5 mg/ml and slow-releasing parenteral haloperidol 50 mg/ml (Janssen Pharmaceuticals, Beerse, Belgium) were also used.

***Experimental design***

There were six experimental groups (n=53): sub-chronic haloperidol administration (SC-HAL)(n=9); subchronic haloperidol before CBD administration (SC-HAL-CBD)(n=10); cannabidiol only (CBD)(n=9); chronic haloperidol administration (CH-HAL)(n=8); chronic haloperidol before CBD administration (CH-HAL-CBD)(n=7); and a control group (n=10). Effective doses of CBD in rats’ range between 2.5-10 mg/kg (Guimaraes et al., 1990).

The administration of pharmacological agents was as follows: SC-HAL (haloperidol at 5 mg/kg IP), SC-HAL-CBD (haloperidol 5 mg/kg IP before administration of CBD at 5 mg/kg p.o.), CBD (CBD at 5 mg/kg p.o.), control (2ml distilled water p.o.), CH-HAL (Haloperidol decanoate at 50 mg/kg IM), CH-HAL-CBD (haloperidol decanoate at 50 mg/kg IM before administration of CBD at 5 mg/kg p.o.) (Table 1).

For the SC-HAL, CBD, and control groups, the agents were administered once daily for 21 days (Sasaki et al. 1995; Naidu & Kulkarni 2001a, 2001b,2001c; Bishnoi & Boparai, 2012). A 5 mg/kg dose of haloperidol was administered IP (Bishnoi and Boparai, 2012) to the SC-HAL group.

For SC-HAL-CBD, haloperidol was administered for 21 days followed by CBD which was commenced 24 h to 48 h after discontinuation of haloperidol and s administered for a further 21 days.

For the C-HAL group, slow-releasing IM haloperidol decanoate 50 mg/kg was administered monthly (Andreassen et al., 2001) on three consecutive occasions. For the CH-HAL-CBD, slow-releasing IM haloperidol decanoate 50 mg/kg monthly was also administered for three consecutive months, but administration of CBD 5mg/kg for 21 days was commenced 24 h to 48 h after the last dose of IM haloperidol.

SC-HAL and SC-HAL-CBD were classified as IP haloperidol groups and received IP haloperidol either alone or in combination with CBD, while CH-HAL and CH-HAL-CBD were classified as IM haloperidol groups and received IM haloperidol either alone or in combination with CBD.

Blood was collected from the lateral saphenous vein of each animal on the last day of each experiment after all the parameters of interest had been assessed. Animals were later sacrificed on the same day by first anesthetizing with phenobarbitone before cervical dislocation and dissection by opening the abdomen. The liver and kidneys of rats were isolated and dissected on ice. 10% w/v of the organs sample (0.03 M sodium phosphate buffer, pH 7.4) was homogenized. The homogenates generated from processed tissues were then used for oxidative stress, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and nitric oxide (NO) assays.

***Antioxidant indices***

The following antioxidant indices were determined spectrometrically:

*Malondialdehyde (MDA; an index of lipid peroxidation)*

MDA was determined using the method of Buege and Aust (1978): 1. Rats were sacrificed, and the liver and kidneys were removed carefully, immediately weighed, and homogenized with ice 1.15% KCl to make 10% homogenate; 2. 1 ml of tissue homogenate was combined with 2 ml 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 revolutions per min 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 malondialdehyde concentration of the sample was calculated using an extinction coefficient of 1.56 × 105 M-1cm-1.

MDA concentration = Absorbance at 532nm/1.56 × 105

*Glutathione (GSH)*

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 nm, Ʃ =1.34 × 104 M-1cm-1.

*Catalase activity (CA)*

CA was determined according to the method of Sinha (1972). It was assayed colourimetrically at 620 nm and expressed as (µmol of H2O2 consumed/min/mg protein at 25ºC. The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate, and 0.4 ml of 2 Mole (M) H2O2. 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 a 1:3 ratio). Ʃ=40 M-1cm-1.

*Superoxide dismutase activity (SOD)*

SOD was determined as described by Sun and Zigma (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 N HCl was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of the substrate (epinephrine), and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min. Ʃ=4020 M-1cm-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 our 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 naphthyl ethylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min. The absorbance (Abs) was then measured at 540 nm. The amount of nitric oxide 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 hydroxytoluene (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.

**Statistical analysis**

Data were analyzed using the IBM SPSS Statistics for Windows, Version 28.0 (Armonk, NY: IBM Corp). Data generated from oxidative stress enzyme assays were treated as continuous variables and were observed to be normally distributed and therefore described using means and standard deviations as measures of central tendency and dispersion. A comparison of the equality of means between groups was done using a one-way ANOVA test. When the F-statistic was significant (<0.05), depending on the violation of the homogeneity of variance, Tukey’s HSD test or the Games Howell post hoc test was used to identify the differences between groups.

**Liver oxidative stress indices**

The six oxidative stress parameters in the liver were compared among the groups. There were significant changes in these indices between the SC-HAL, SC-HAL-CBD, CH-HAL, CH-HAL-CBD, oral CBD, and control groups: CAT (p=0.000), SOD (p=0.000), GSH (p=0.000),(Scavenging activity in DPPH assay) (p=0.000), NO (p=0.000), and MDA (p=0.000). The SC-HAL group showed higher activity of SOD and CAT but a lower activity of GSH compared to all other groups (see Table 1).

**Table 1: Liver Oxidative Stress Parameters**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Liver antioxidant** | **SC-HAL**  Mean ± SD | **SC-HAL-CBD**  Mean ± SD | **CBD**  Mean ± SD | **Control**  Mean ± SD | **CH-HAL**  Mean ± SD | **CH-HAL-CBD**  Mean ± SD | **F-**  **statistics** | ***p-*value** |
| CAT  µmol/ml/min/mg pro | 46.8 ± 29.2 | 6.1 ± 2.8 | 8.2 ± 2.8 | 27.1 ± 2.5 | 11.5 ± 4.3 | 5.9 ± 3.2 | 16.044 | <0.001 |
| GSH µmol/ml | 14.3 ± 4.3 | 27.6 ± 9.2 | 17.6 ± 3.4 | 40.7 ± 10.5 | 10.9 ± 3.9 | 26.9 ± 9.2 | 19.421 | <0.001 |
| SOD  µmol/ml/min/mg pro) | 8.1 ± 4.0 | 0.8 ± 0.2 | 2.5 ± 0.5 | 4.9 ± 0.7 | 2.0 ± 0.6 | 0.8 ± 0.2 | 25.302 | <0.001 |
| MDA µmol/ml) | 3.5 ± 2.0 | 1.7 ± 1.1 | 2.3 ± 1.6 | 2.1 ± 0.4 | 3.6 ± 1.1 | 5.9 ± 3.2 | 6.561 | <0.001 |
| NO µmol/dl) | 35.0 ± 10.6 | 8.2 ± 2.3 | 6.2 ± 2.3 | 12.6 ± 3.8 | 14.5 ± 3.5 | 8.2 ± 2.8 | 35.963 | <0.001 |
| DPPH IC50/  µg/ml) | 75.8 ± 6.3 | 54.0 ± 11.5 | 60.1 ± 9.1 | 57.8 ± 15.0 | 46.3 ± 23.6 | 71.8 ± 7.7 | 5.348 | <0.001 |

SC-HAL: subchronic haloperidol administration; SC-HAL-CBD: subchronic haloperidol before CBD administration; CBD: cannabidiol; CH-HAL: chronic haloperidol administration; CH-HAL-CBD: chronic haloperidol before CBD administration; Control: 2ml distilled water.

*Liver CAT*

Post-hoc analysis revealed a significant difference between SC-HAL and SC-HAL-CBD groups (p=0.000), SC-HAL and CBD groups (p=0.000), SC-HAL and control groups (p=0.011), SC-HAL and CH-HAL groups (p=0.000), SC-HAL and CH-HAL-CBD groups (p=0.000), SC-HAL-CBD and control groups ( p=0.003), CBD and control groups (p=0.013), and control and CH-HAL-CBD groups ( p=0.008).

*Liver GSH*

Post-hoc analysis revealed a significant difference between SC-HAL and SC-HAL-CBD groups (p=0.007), SC-HAL and control groups (p=0.000), SC-HAL and CH-HAL-CBD groups (0.026), SC-HAL-CBD and control groups ( p=0.004), SC-HAL-CBD and CH-HAL groups (p=0.000), CBD and control groups (p=0.000), control and CH-HAL groups (0.001), control and CH-HAL-CBD groups (0.006), and CH-HAL and CH-HAL-CBD groups (0.002).

*Liver SOD*

Post-hoc analysis revealed a significant difference between SC-HAL and SC-HAL-CBD groups (p=0.000), SC-HAL and CBD groups (p=0.000), SC-HAL and control groups (p=0.002), SC-HAL and CH-HAL groups (p=0.000) SC-HAL and CH-HAL-CBD groups (p=0.000), SC-HAL-CBD and control groups (p=0.000), CBD and control groups ( 0.029), control and CH-HAL groups (p=0.006), and control and CH-HAL-CBD groups ( 0.000).

*Liver NO*

Post-hoc analysis revealed a significant difference between SC-HAL and SC-HAL-CBD groups (p=0.000), SC-HAL and CBD groups (p=0.000), SC-HAL and control groups (p=0.000), SC-HAL and CH-HAL groups (p=0.000) SC-HAL and CH-HAL-CBD groups (p=0.000), and CBD and SC-HAL groups ( p=0.020).

*Liver MDA*

Post-hoc analysis revealed a significant difference between SC-HAL-CBD and CH-HAL-CBD groups (p=0.000), and CBD and CH-HAL-CBD groups (p=0.002)

*Liver DPPH*

Post-hoc analysis revealed a significant difference between SC-HAL and SC-HAL-CBD groups (p=0.017), SC-HAL and CH-HAL groups (p=0.000), and CH-HAL and CH-HAL-CBD groups ( p=0.008).

**Kidney oxidative stress indices**

In the kidney, the six oxidative stress indices were compared among the groups. There were significant changes in kidney oxidative stress indices between the SC-HAL, SC-HAL-CBD, CH-HAL, CH-HAL-CBD, oral CBD, and control groups: CAT (p=0.000), SOD (p=0.002), GSH (p=0.000),(Scavenging activity in DPPH assay) (p=0.000), NO (p=0.000), and MDA (p=0.000).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Kidney antioxidant** | **SC-HAL**  **Mean ± SD** | **SC-HAL-CBD**  **Mean ± SD** | **CBD**  **Mean ± SD** | **Control**  **Mean ± SD** | **CH-HAL**  **Mean ± SD** | **CH-HAL-CBD**  **Mean ± SD** | **F-Statistics** | ***p*-value** |
| CAT (µmol/ml/min/mg pro) | 41.6 ± 33.8 | 8.9 ± 3.9 | 21.5 ± 5.4 | 24.3 ± 6.2 | 11.9 ± 3.0 | 8.7 ± 4.7 | 6.558 | <0.001 |
| GSH (µmol/ml) | 14.8 ± 2.4 | 14.2 ± 3.2 | 15.0 ± 4.5 | 40.7 ± 15.2 | 16.4 ± 5.5 | 14.3 ± 3.1 | 19.123 | <0.001 |
| SOD (µmol/ml/min/mg pro) | 5.8 ± 3.9 | 2.2 ± 1.0 | 3.0 ± 1.2 | 4.0 ± 0.9 | 3.1 ± 1.1 | 2.4 ± 1.1 | 4.521 | 0.002 |
| MDA (µmol/ml) | 3.9 ± 2.1 | 0.5 ± 0.5 | 1.7 ± 1.0 | 1.9 ± 0.7 | 4.4 ± 0.6 | 0.5 ± 0.2 | 21.493 | <0.001 |
| NO (µmol/dl) | 30.5 ± 11.4 | 7.3 ± 1.9 | 5.0 ± 1.6 | 17.5 ± 7.1 | 16.1 ± 2.1 | 8.0 ± 1.6 | 23.697 | 0.001 |
| DPPH (IC50/µg/ml) | 70.6 ± 10.8 | 54.9 ± 13.2 | 60.1 ± 9.1 | 57.7 ± 17.1 | 34.6 ± 14.8 | 74.6 ± 6.2 | 9.766 | <0.001 |

**Table 2:** Kidney Oxidative Stress Parameters

SC-HAL: subchronic haloperidol administration; SC-HAL-CBD: subchronic haloperidol before CBD administration; CBD: cannabidiol; CH-HAL: chronic haloperidol administration; CH-HAL-CBD: chronic haloperidol before CBD administration; Control: 2ml distilled water.

*Kidney* CAT

Post-hoc analysis revealed a significant difference between SC-HAL and SC-HAL-CBD - (p=0.000), SC-HAL and CH-HAL (p=0.002), and SC-HAL and CH-HAL-CBD groups (p=0.000).

*Kidney GSH*

Post-hoc analysis revealed a significant difference between SC-HAL and control (p=0.001), SC-HAL-CBD and control (p= 0.001), CBD and control (p=0.001), control and CH-HAL (p=0.001) CH-HAL and CH-HAL-CBD groups (p=0.001)

*Kidney SOD*

Post-hoc analysis revealed a significant difference between SC-HAL and SC-HAL-CBD (p=0.002), SC-HAL and CBD (p=0.030), and SC-HAL and CH-HAL-CBD groups (p=0.010).

*Kidney NO*

Post-hoc analysis revealed a significant difference between SC-HAL and SC-HAL-CBD (p=0.000), SC-HAL and CBD (p=0.000), SC-HAL and control (p=0.000), SC-HAL and CH-HAL (p=0.000), SC-HAL and CH-HAL-CBD (p=0.000), SC-HAL-CBD and control (p=0.004), SC-HAL-CBD and CH-HAL-CBD ( p=0.030), CBD and control ( p=0.000), CBD and CH-HAL (p=0.004), CH-HAL and CH-HAL-CBD( p=0.021).

*Kidney MDA*

Post-hoc analysis revealed a significant difference between SC-HAL and SC-HAL-CBD (p=0.000), SC-HAL and CBD (p=0.000), SC-HAL and control (p=0.02) SC-HAL and CH-HAL-CBD (p=0.000), SC-HAL-CBD and CH-HAL (p=0.000), CBD and CH-HAL (p=0.000), Control and CH-HAL (p=0.000), and CH-HAL and CH-HAL-CBD (p=0.000) groups.

*Kidney DPPH*

Post-hoc analysis revealed a significant difference between SC-HAL and CH-HAL (p=0.000), SC-HAL-CBD and CH-HAL (p=0.018), SC-HAL-CBD and CH-HAL-CBD (p=0.031), CBD and CH-HAL (p=0.002), control and CH-HAL (p=0.005), and CH-HAL and CH-HAL-CBD groups (p=0.000).

**Discussion**

This study compared the effects of chronic exposure to haloperidol alone for three months with that of subchronic exposure to haloperidol alone for twenty-one days on oxidative stress parameters in the liver and kidney. The effects of CBD on oxidative stress parameters were also examined. The overarching findings were the differences in the effects of sub-chronic and chronic haloperidol administration on oxidative stress parameters expression in the two organs and the ability of CBD to reduce oxidative stress.

SOD is found in the mitochondria of the hepatocytes and protects the cells from oxidative damage by converting superoxide radicals to hydrogen peroxide (Weisiger et al., 1973; Elchuri et al.,2005; Holley et al.,2011; Bhattacharya et al.,2015). We found elevated SOD activity in the liver and kidney in the SC-HAL group compared to other groups. Our observation in the liver and kidney may represent a response to increase free radicals’ production in the SC-HAL group compared to others (Harris et al., 1992; Dakhale et al.,2004; Boskovic et al.,2011) and indicated a compensatory increase in SOD activity in response to increased oxidative stress in the SC-HAL group. We did not observe an increase in oxidative stress in other groups with adjunctive CBD and in the CBD alone interventions, which means CBD ameliorated oxidative stress in the groups with adjunctive CBD and in the CBD alone group (Costa et al.,2007; Fouad et al.,2011; Fouad et al.,2012; Atalay et al.,2020).

The CH-HAL group had less SOD activity in the liver when compared to the SC-HAL and the control group, which indicates that the activity of SOD in the liver is reduced after prolonged administration of haloperidol. There was no difference in the SOD activity of the CH-HAL group and the CH-HAL-CBD group which suggests that CBD had no effects on SOD activity after prolonged haloperidol administration. We did not come across a study on the influence of chronic administration of haloperidol on liver antioxidants but Boskovic et al (2011) in a study observed a decrease in antioxidant enzyme activity with increased age and duration of illness in patients with schizophrenia following treatment with antipsychotics.

In the CH-HAL and CH-HAL-CBD groups, SOD activity in the kidney also followed the same pattern as in the liver except for the difference with the control group was not significant.

In the SC-HAL group, CAT activity in the liver was higher when compared with other groups. This may also be a compensatory response to increase free radical production. The lower CAT activity in the SC-HAL-CBD and CBD alone group when compared with SC-HAL and the control groups, suggests that CBD may have a protective effect in this group by reducing the level of free radical production through another mechanism without necessarily affecting CAT or SOD concentrations. Mukhopadhyay et al. (2012) demonstrated that CBD exerts a protective effect against liver ischemic injury by attenuating major pro-inflammatory and stress signalling pathways.

In the liver, we also found that the CH-HAL group CAT activity was significantly lower than SC-HAL and the control group which indicates that chronic administration of haloperidol did not increase CAT activity unlike that observed with subchronic administration of haloperidol. There was, however, no significant difference between the CH-HAL and CH-HAL-CBD groups which suggested CBD did not influence CAT activity with prolonged administration of haloperidol unlike what we observed with subchronic haloperidol administration.

CAT activity in the kidney in the SC- HAL group also showed a similar pattern as that of the liver with increased activity SC-HAL group compared to the other groups. CAT activity in the CH-HAL group was also increased but this was not significant compared with the control group, but the SC-HAL-CBD group had a significantly lower CAT activity than other groups suggesting CBD may have a protective effect in this group by reducing the level of free radical production and the compensatory effect of increase CAT activity may not be needed here. CBD has also been shown to be effective against ischemia/reperfusion kidney injury in an animal model by decreasing lipid and protein oxidative damage (Soares et al., 2015).

*GSH*

We observed a lower level of GSH in the liver in the SC-HAL and CH-HAL groups when the two groups were compared with SC-HAL-CBD and CH-HAL-CBD groups, respectively. The two groups also had lower GSH levels than the control group. The low level of reduced GSH in the SC-HAL and the CH-HAL groups suggested increased activity of glutathione peroxidase (GPx), the enzyme responsible for the conversion of reduced GSH to the oxidized form ( GSSG), with the help of hydrogen peroxide, which is converted to water and oxygen in the process ( Burk & Hill 2010; Ursini & Maiorino 2013). An increase in the activity of GPx implies more antioxidant activity in SC-HAL and CH-HAL groups likely due to increased free radical productions in these groups. These observations indicate that CBD increased the level and action of GSH.

GSH level in the kidney was significantly higher in the control compared to other groups but we did not detect a significant difference in GSH level between SC-HAL and SC-HAL-CBD groups. There was also no difference in GSH between CH-HAL and CH-HAL-CBD groups. This suggests increased peroxidase activity and therefore increased antioxidant activity in the other groups relative to the control group but does not confirm the protective effect of CBD on haloperidol-induced oxidative stress. Earlier investigators have however reported on the oxidative protective effects of CBD in the kidney (Cassol-Jr et al.,2010; Fouad et al.,2012).

In this study, there was strong evidence that CBD reduced NO activity in the liver and the kidney with subchronic administration of haloperidol because SC-HAL had a significantly higher value of NO activity than SC-HAL-CBD, CBD, and control groups, while CBD alone had significantly lower NO activity than the SC-HAL group but not the SC-HAL-CBD group. Our observations agree with most studies on the effect of CBD on NO produced because of oxidative stress (Booz 2011, Soares et al., 2015; Chen 2016) and the mechanism of action of CBD involved suppression of inducible nitric oxide synthase (iNOS) that generates a large amount of NO (Chen et al.,2016). CBD, however, did not lower NO with prolonged administration of haloperidol because the CH-HAL-CBD group did not have a lower NO activity than the CH-HAL group. Some authors have reported an association between prolonged administration of haloperidol and low NO activity (Iwahashi et al., 1996; Harvey and Bester, 2000).

There was no evidence of haloperidol-induced lipid peroxidation in the liver in this study because we did not observe a higher MDA activity in both the SC-HAL or the CH-HAL groups) compared to the other groups. SC-HAL-CBD and CBD alone groups, however, had a lower MDA activity than CH-HAL-CBD groups, suggesting CBD may have reduced MDA activity in the SC-HAL-CBD groups. The low activity of MDA observed in the liver may be related to the liver’s high content of antioxidants which prevented lipid peroxidation (Cobley et al., 2018).

Interestingly there was an increase in MDA activity in the kidney in both SC-HAL and CH-HAL groups compared to the other groups suggesting a greater increase in free radical production and lipid peroxidation compared to other interventions. This is in the same direction as other studies (Consroe et al., 1991; Kudo & Ishizaki 1999; Patil et al.,2012; Kamyar et al.,2016). We also observed a decrease in MDA in SC-HAL-CBD and CH-HAL-CBD groups compared to SC-HAL and CH-HAL groups, indicating that CBD is effective as an antioxidant when given after haloperidol (Luvone et al., 2004; Mechoulam et al., 2007; Pisanti 2017).

The DPPH assay was designed to measure the free radical scavenging activity of compounds in organic solvents (Sanchez-Moreno et al.,1998; Kedare and Singh 2011). and has been used to assess the antioxidant capacity of hydrolysed porcine tissues (Damgaard et al., 2014). In our study, we used DPPH to assess the total antioxidant activity of each sample.

There was a noticeable increase in free radical scavenging activity in the SC-HAL group compared to the SC-HAL-CBD group. indicating that the liver had more antioxidant activity here as a compensatory mechanism for the increase in free radical production observed in this group. Rao and Balachandran (2002) proposed that disequilibrium between free-radical metabolism and the antioxidant system can produce excessive reactive oxygen species (ROS) and other enzymes such as SOD, glutathione peroxidase ( GSH-Px), and CAT. DPPH, a stable radical (Sagar and Singh, 2020) interacts with the antioxidant enzymes which were produced more in the SC-HAL groups in this study. This may explain why DPPH activity in the liver was increased mostly in the SC-HAL group. This study also found low DPPH scavenging activity in the CH-HAL group compared to the CH-HAL-CBD group in the liver. This suggests the inability of the liver to increase antioxidant production and activity after prolonged administration of haloperidol and the ability of CBD to reverse the detrimental effects of chronic haloperidol administration in the liver.

Reduced DPPH scavenging activity in the CH-HAL group compared to the other groups was also observed in the kidneys. This strengthened earlier observations in the liver that prolonged administration of haloperidol decreased antioxidants production and induced oxidative stress (Boskovic et al.,2013), the difference between the CH-HAL group, and CH-HAL-CBD group indicated CBD alleviate the oxidative stress induced by prolonged haloperidol administration in the kidneys. We also noted that there was no significant difference between the SC-HAL group compared to the SC-HAL-CBD group unlike what was obtained in the liver, this suggested that sub-chronic administration of haloperidol did not alter the oxidative stress indices in the kidney.

**Conclusion**

In summary, there were differences in the activity of antioxidant enzymes, between chronic and subchronic administration of haloperidol and the effects of CBD on the antioxidant enzymes were less pronounced with chronic administration of haloperidol suggesting prolonged administration of haloperidol induced oxidative stress in the two end organs and may impair their functioning. A clinical study on the effects of long-term use of haloperidol and other conventional antipsychotics on the liver and kidney is highly desirable in a country like Nigeria where conventional antipsychotics are still widely used, and some patients have been on these medications for decades.

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**Declaration of Generative AI (Artificial intelligence) and AI-assisted technologies in the writing process.**

During the preparation of this work, the authors did not use generative AI and AI-assisted technologies in the writing process.

**Animal Welfare Ethical Statement**

The animals were properly housed in clean polypropylene cages with well-ventilated and hygienic compartments, fed with standard rodent pellets (Ladokun Feed Plc., Ibadan, Nigeria) and water *ad libitum,* and kept in surroundings appropriate to their species and acclimatized 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 approved by the Institutional Review Board (IRB) of NIMR, Yaba, Lagos, Nigeria (IRB/16/329) and Stellenbosch University’s Health Research Ethics Committee: Animal Care and Use (SU-ACUD16-00137).

**Ethical Standards**

The authors assert that all procedures contributing to this work comply with the International Guiding Principles for Biomedical Research Involving Animals, as issued by the Council for the International Organizations of Medical Sciences.

REFERENCES

Andreassen OA, Meshul CK, Moore C, Jørgensen HA. Oral dyskinesias and morphological changes in rat striatum during long-term haloperidol administration. Vol. 157, Psychopharmacology. 2001. p. 11–9.

Andreazza AC, Barakauskas VE, Fazeli S, Feresten A, Shao L, Wei V, et al. Effects of haloperidol and clozapine administration on oxidative stress in rat brain, liver and serum. Neurosci Lett [Internet]. 2015;591:36–40. Available from: <http://dx.doi.org/10.1016/j.neulet.2015.02.028>

Atalay S, Jarocka-Karpowicz I, Skrzydlewska E, Skrzydlewskas E. Antioxidative and anti-inflammatory properties of cannabidiol. Antioxidants. 2020;9(1):1–20.

Bhattacharya A, Hegazy AN, Deigendesch N, Kosack L, Cupovic J, Kandasamy RK, et al. Superoxide Dismutase 1 Protects Hepatocytes from Type I Interferon-Driven Oxidative Damage. Immunity. 2015 Nov 17;43(5):974–86.

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

Booz GW. Free Radical Biology & Medicine Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. Free Radic Biol Med [Internet]. 2011;51(5):1054–61. Available from: <http://dx.doi.org/10.1016/j.freeradbiomed.2011.01.007>

Bošković M, Grabnar I, Terzič T, Kores Plesničar B, Vovk T. Oxidative stress in schizophrenia patients treated with long-acting haloperidol decanoate. Psychiatry Res. 2013;210(3):761–8.

Boskovic M, Vovk TT, Kores Plesničar B, Grabnar I, Vovk TT, Kores Plesnicar B, et al. Oxidative Stress in Schizophrenia. Curr Neuropharmacol. 2011;9(2):301–12.

Bowen WD, Moses EL, Tolentino PJ, Michael Walker J. Metabolites of haloperidol display preferential activity at σ receptors compared to dopamine D-2 receptors. Eur J Pharmacol. 1990 Feb 27;177(3):111–8.

Brown J, Winterstein A. Potential Adverse Drug Events and Drug–Drug Interactions with Medical and Consumer Cannabidiol (CBD) Use. J Clin Med. 2019;8(7):989.

Buege JA, Aust SD. Microsomal Lipid Peroxidation. Methods Enzymol. 1978;52:302-310.

Burk RF, Hill KE. Glutathione Peroxidases. In: Comprehensive Toxicology, Second Edition. [Volume 4](https://www.sciencedirect.com/science/referenceworks/9780080468846), 2010, Pages 229-242.

Cassol-Jr OJ, Comim CM, Silva BR, Hermani F V., Constantino LS, Felisberto F, et al. Treatment with cannabidiol reverses oxidative stress parameters, cognitive impairment and mortality in rats submitted to sepsis by cecal ligation and puncture. Brain Res. 2010 Aug 21;1348:128–38.

Chen J, Hou C, Chen X, Wang D, Yang P, He X, et al. Protective effect of cannabidiol on hydrogen peroxide-induced apoptosis, inflammation, and oxidative stress in nucleus pulposus cells. Mol Med Rep. 2016 Sep 1;14(3):2321–7.

Cho CH, Lee HJ. Oxidative stress and tardive dyskinesia: Pharmacogenetic evidence. Prog Neuro-Psychopharmacology Biol Psychiatry [Internet]. 2013;46:207–13. Available from: <http://dx.doi.org/10.1016/j.pnpbp.2012.10.018>

Cobley JN, Fiorello ML, Bailey DM. 13 Reasons Why the Brain Is Susceptible To Oxidative Stress. Redox Biol [Internet]. 2018;15(January):490–503. Available from: <https://doi.org/10.1016/j.redox.2018.01.008>

Consroe P, Laguna J, Allender J, Snider S, Stern L, Sandyk R, et al. Controlled clinical trial of cannabidiol in Huntington’s disease. Pharmacol Biochem Behav. 1991;40(3):701–8.

Costa B, Trovato AE, Comelli F, Giagnoni G, Colleoni M. The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. Eur J Pharmacol. 2007 Feb 5;556(1–3):75–83.

Council NR. Guide for the Care and Use of Laboratory Animals: Eighth Edition. Vol. 184, The Biochemical Journal. 2010.

Dakhale G, Khanzode S, Khanzode S, Saoji A, Khobragade L, Turankar A. Oxidative damage, and schizophrenia: The potential benefit by atypical antipsychotics. Neuropsychobiology. 2004;49(4):205–9.

Damgaard TD, Otte JAH, Meinert L, Jensen K, Lametsch R. Antioxidant capacity of hydrolyzed porcine tissues. Food Sci Nutr. 2014 May 1;2(3):282–8.

Devinsky O, Cilio MR, Cross H, Fernandez-Ruiz J, French J, Hill C, et al. Cannabidiol: Pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. Epilepsia. 2014;55(6):791–802.

El-Awdan SA, Abdel Jaleel GA, Saleh DO. Alleviation of haloperidol-induced oxidative stress in rats: Effects of sucrose vs grape seed extract. Bull Fac Pharmacy, Cairo Univ. 2015 Jun;53(1):29–35.

Eftekhari A, Ahmadian E, Azarmi Y, Parvizpur A, Hamishehkar H, Eghbal MA. In vitro/vivo studies towards mechanisms of risperidone-induced oxidative stress and the protective role of coenzyme Q10 and N-acetylcysteine. Toxicol Mech Methods. 2016 Sep 1;26(7):520–8.

Elchuri S, Oberley TD, Qi W, Eisenstein RS, Roberts LJ, Van Remmen H, et al. CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. Oncogene. 2005 Jan 13;24(3):367–80.

Forsman A, Larsson M, Lundborg H, Renstrom P. On the distribution and elimination of haloperidol in cholecystectomized patients. Eur J Drug Metab Pharmacokinet. 1981 Oct;6(4):249–53.

Fouad AA, Jresat I. Therapeutic potential of cannabidiol against ischemia/reperfusion liver injury in rats. Eur J Pharmacol. 2011 Nov 16;670(1):216–23.

Fouad AA, Al-Mulhim AS, Jresat I. Cannabidiol treatment ameliorates ischemia/reperfusion renal injury in rats. Life Sci. 2012 Sep 17;91(7–8):284–92.

Guimarães, F.S., Chiaretti, T.M., Graeff, F.G. *et al.* Antianxiety effect of cannabidiol in the elevated plus-maze. *Psychopharmacology* **100**, 558–559 (1990).

Halici Z, Dursun H, Keles ON, Odaci E, Suleyman H, Aydin N, et al. Effect of chronic treatment of haloperidol on the rat liver: A stereological and histopathological study. Naunyn Schmiedebergs Arch Pharmacol. 2009;379(3):253–61.

Harris ED. Regulation of antioxidant enzymes 1. FASEB J. 1992 Jun;6(9):2675–83.

Harvey BH, Bester AM. Withdrawal-associated changes in peripheral nitrogen oxides and striatal cyclic GMP after chronic haloperidol treatment. Behav Brain Res. 2000;111(1–2):203–11.

Holley AK, Bakthavatchalu V, Velez-Roman JM, St. Clair DK. Manganese superoxide dismutase: Guardian of the powerhouse. Vol. 12, International Journal of Molecular Sciences. 2011. p. 7114–62.

Iffland K, Grotenhermen F. An Update on Safety and Side Effects of Cannabidiol: A Review of Clinical Data and Relevant Animal Studies. Cannabis Cannabinoid Res. 2017;2(1):139–54.

Iwahashi K, Yoneyama H, Ohnishi T, Nakamura K, Miyatake R, Suwaki H, et al. Haloperidol inhibits neuronal nitric oxide synthase activity by preventing electron transfer. Neuropsychobiology. 1996;33(2):76–9.

Iuvone T, Esposito G, Esposito R, Santamaria R, Di Rosa M, Izzo AA. Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on β-amyloid-induced toxicity in PC12 cells. J Neurochem. 2004;89(1):134–41.

Kajero JA, Seedat S, Ohaeri J, Akindele A, Aina O. Investigation of the effects of cannabidiol on vacuous chewing movements, locomotion, oxidative stress, and blood glucose in rats treated with oral haloperidol. World J Biol Psychiatry. 2020;21(8).

Kamyar M, Razavi BM, Vahdati Hasani F, Mehri S, Foroutanfar A, Hosseinzadeh H. Crocin prevents haloperidol-induced orofacial dyskinesia: Possible an antioxidant mechanism. Iran J Basic Med Sci. 2016;19(10):1070–9.

Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 2011;48(4):412–22.

Kiriakakis V, Bhatia KP, Quinn NP, Marsden CD. The natural history of tardive dystonia. A long-term follow-up study of 107 cases. Brain. 1998;121(11):2053–66.

Kudo S, Ishizaki T. Pharmacokinetics of haloperidol. An update. Vol. 37, Clinical Pharmacokinetics. Adis International Ltd; 1999. p. 435–56.

Kulkarni S, Naidu P. Oxidative stress and tardive dyskinesia: role of natural antioxidants. Iran J Pharm Res [Internet]. 2010 Nov 20 [cited 2021 May 16];0(Supplement 1):11–11. Available from: <http://ijpr.sbmu.ac.ir/article_85.html>

Lerner PP, Miodownik C, Lerner V. Tardive dyskinesia (syndrome): Current concept and modern approaches to its management. Vol. 69, Psychiatry and Clinical Neurosciences. Blackwell Publishing; 2015. p. 321–34.

Mechoulam R, Peters M, Murillo-Rodriguez E, Hanuš LO. Cannabidiol - Recent advances. Vol. 4, Chemistry and Biodiversity. 2007. p. 1678–92.

Mukhopadhyay P, Rajesh M, Horváth B, Bátkai1 S, Park O, Tanashian G, Gao R Y, Patel V V, Wink D A, Liaudet L, Haskó G, Mechoulam R, and Pacher P. Cannabidiol protects against hepatic ischemia/reperfusion injury by attenuating inflammatory signalling and response, oxidative/nitrative stress, and cell death. Free Radic Biol Med 2011 May 15; 50(10) 1368–1381.

Naidu PS, Kulkarni SK. Effect of 5-HT1A and 5-HT2A/2C receptor modulation on neuroleptic-induced vacuous chewing movements. Eur J Pharmacol. 2001 Sep 28;428(1):81–6.

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

Naidu PS, Kulkarni SK. Excitatory mechanisms in neuroleptic-induced vacuous chewing movements (VCMs): Possible involvement of calcium and nitric oxide. Behav Pharmacol. 2001;12(3):2

Patil R, Hiray Y, Shinde S, Langade P. Reversal of haloperidol-induced orofacial dyskinesia by Murraya koenigii leaves in experimental animals. Pharm Biol. 2012;50(6):691–7.

Peres FF, Lima AC, Hallak JEC, Crippa JA, Silva RH, Abílio VC. Cannabidiol as a promising strategy to treat and prevent movement disorders? Vol. 9, Frontiers in Pharmacology. 2018.

Pisanti S, Malfitano AM, Ciaglia E, Lamberti A, Ranieri R, Cuomo G, et al. Cannabidiol: State of the art and new challenges for therapeutic applications. Pharmacol Ther. 2017;175:133–50.

Rao A V., Balachandran B. Role of oxidative stress and antioxidants in neurodegenerative diseases. Vol. 5, Nutritional Neuroscience. 2002. p. 291–309.

Roversi K, Benvegnú DM, Roversi K, Trevizol F, Vey LT, Elias F, et al. Haloperidol-loaded lipid-core polymeric nanocapsules reduce DNA damage in blood and oxidative stress in the liver and kidneys of rats. J Nanoparticle Res. 2015;17(4).

Sánchez-Moreno C, Larrauri JA, Saura-Calixto F. A procedure to measure the antiradical efficiency of polyphenols. J Sci Food Agric. 1998 Feb;76(2):270–6.

Samara E, Bialer M, Harvey DJ. Metabolism of cannabidiol by the rat. Eur J Drug Metab Pharmacokinet. 1991;16(4).

Sasaki H, Hashimoto K, Maeda Y, Inada T, Kitao Y, Fukui S, et al. Rolipram, a selective c-AMP phosphodiesterase inhibitor suppresses oro-facial dyskinetic movements in rats. Life Sci. 1995;56(25).

Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman’s reagent. Anal Biochem. 1968;25(C).

Sinha AK. Colourimetric assay of catalase. Anal Biochem. 1972;47(2):389–94.

Soares RZ, Vuolo F, Dall’Igna DM, Michels M, Crippa JA de S, Hallak JEC, et al. Evaluation of the role of the cannabidiol system in an animal model of ischemia/reperfusion kidney injury. Rev Bras Ter Intensiva. 2015;27(4).

Sonego AB, Prado DS, Vale GT, Sepulveda-Diaz JE, Cunha TM, Tirapelli CR, et al. Cannabidiol prevents haloperidol-induced vacuous chewing movements and inflammatory changes in mice via PPARγ receptors. Brain Behav Immun. 2018 Nov 1;74:241–51.

Sun M, Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Anal Biochem. 1978;90(1):81-9. doi: 10.1016/0003-2697(78)90010-6. PMID: 727489.

Umar MU. Phenomenology of tardive syndromes: Clinical presentation and manifestations. In: Tardive Dyskinesia: Current Approach. 2018.

Ursini F, Maiorino M. Glutathione Peroxidases. In: Encyclopedia of Biological Chemistry: Second Edition. Elsevier Inc.; 2013. p. 399–404.

Uyanik A, Unal D, Halici Z, Cetinkaya R, Altunkaynak BZ, Keles ON, et al. Does haloperidol have side effects on histological and stereological structure of the rat kidneys? Ren Fail. 2009;31(7):573–81.

Weisiger RA, Fridovich - I. Mitochondrial superoxide dismutase. Site of synthesis and intramitochondrial localization. J Biol Chem. 1973;248(13).

Yin J, Barr AM, Ramos-Miguel A, Procyshyn RM. Antipsychotic Induced Dopamine Supersensitivity Psychosis: A Comprehensive Review. 2016;1–10.

Zhang XY, Yao JK. Oxidative stress and therapeutic implications in psychiatric disorders. Prog Neuro-Psychopharmacology Biol Psychiatry [Internet]. 2013;46:197–9. Available from: <http://dx.doi.org/10.1016/j.pnpbp.2013.03.003>