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Effect of L-dopa or bromocryptine alone or in combination with cannabis on oxidative stress, inflammation and neurodegeneration in experimental Parkinson's disease

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ABSTRACT

This study aimed to investigate whether cannabis would modulate the effects of the antiparkinsonian drugs L-dopa or bromocryptine on striatal oxidative stress, neuroinflammation, and neuronal integrity in an experimental Parkinson's disease evoked by injection of the inflammogen lipopolysaccharide (LPS) into the rat striatum. Rats received intrastriatal injections of LPS or vehicle. They were treated with L-dopa (25 mg/kg), bromocryptine (0.6 mg/kg) or cannabis (20 mg/kg) combined with either L-dopa or bromocryptine once a day for 15 days. In striatal homogenates, the following parameters were measured: total antioxidant capacity (TAC), paraoxonase-1 activity (PON-1), reduced glutathione (GSH), glutathione peroxidase (GPx), nitric oxide, malondialdehyde (lipid peroxidation), and tumour necrosis factor-α (TNF-α). Brain histology and caspase-3 immunohistochemistry were done to assess neuronal damage. Results indicated that injection of LPS causes a significant increase in malondialdehyde, and nitric oxide along with decreased GSH, TAC and PON-1 activity in the striatum. There was also a substantial decrease in striatal GPx and increased TNF-α. LPS resulted in marked neuronal apoptosis. We found that treatment with either L-dopa or bromocryptine was associated with a significant reduction in malondialdehyde together with increased GSH levels. Nitric oxide showed a significant reduction by either drug and there was a modest though a significant reduction in striatal TNF- α. Additionally, PON-1 activity increased by L-dopa or bromocryptine. Neither bromocryptine nor L-dopa demonstrated a discernible protection against histologic neuronal injury. Combining L-dopa or bromocryptine with cannabis has an extra impact on oxidative stress and neurodegeneration. Nonetheless, apoptotic neuronal cells, pyknotic nuclei, and

neuropil vacuolation remained. These results do not point to a cannabis-related benefit for Parkinson's disease patients.

Keywords: Cannabis; L-dopa; bromocryptine; lipopolysaccharide; neuroinflammation; oxidative stress; neurodegeneration

1. INTRODUCTION

Parkinson's disease is a chronic hypokinetic motor disorder of the basal ganglia. It is due to a preferential and continued death of dopaminergic neurons in the substantia nigra and consequent depletion of striatal dopamine (Hughes et al., 1992; Graybiel, 2000; Abdel-Salam et al., 2023a; Abdel-Salam et al., 2023b). Cardinal symptoms of Parkinson's disease patients include difficulty initiating voluntary movements, muscle rigidity, and hand tremors (Beitz, 2014). In 95 percent of cases the disorder is essentially sporadic and has no known origin, a condition known as idiopathic Parkinson's disease (Pankratz and Foroud, 2007; Wirdefeldt et al., 2011). Nevertheless, the most widely accepted hypothesis to explain Parkinson's disease is that it results from exposure to environmental pollutants such pesticides or organic solvents and a genetic susceptibility (Firestone et al., 2005; Ritz et al., 2016). Furthermore, oxidative stress and neuroinflammation are strongly suggested to be the primary processes causing dopamine cell death in Parkinson's disease based on a wealth of evidence (Drechsel and Patel, 2008; Miller et al., 2009).

The gold standard of Parkinson's disease therapy is the dopamine precursor levodopa (L-3,4-dihydroxyphenylalanine), with the aim to correct the biochemical deficit by providing dopamine to the striatum. Dopamine receptor agonists e.g., bromocryptine are also used either as an adjunctive treatment with L-dopa or as first line drugs in mild cases and to delay the emergence of motor complications of L-dopa (Rascol et al., 2011; Tambasco et al., 2018). There are currently only symptomatic treatments for Parkinson's disease; there is no known cure. Other medications include catechol-O-methyl transferase inhibitors and monoamine oxidase type B inhibitors, which alleviate motor symptoms (Rascol et al., 2011; Abdel-Salam, 2015a). Cannabis preparations from the plant *Cannabis Sativa* have long been known for their psychotropic effects, such as euphoria, relaxation, and sensory perceptual alterations when smoked (Huestis, 2002).

The active constituent is delta-9-tetrahydrocannabinol (Δ9-THC), which produces its effects by acting on two types of G-protein-coupled receptors. Cannabinoid receptor 1 is primarily expressed in the central nervous system, while cannabinoid receptor 2 is primarily found on peripheral immune cells. The use of cannabis-based medications for the treatment of various neurological conditions, such as multiple sclerosis Zajicek and Apostu, (2011) and epilepsy Porter and Jacobson, (2013), has gained popularity throughout the past ten years. Cannabis is also used by individuals with Parkinson's disease, and some data indicate that it helps with motor impairment, lowers pain complaints, and improves sleep (Erga et al., 2022). In light of the above, the present study was designed to investigate the effect of the combined administration of cannabis and L-dopa or bromocryptine on brain oxidative stress neuroinflammation, and striatal pathology in experimental Parkinson's disease caused by intrastriatal LPS.

2. MATERIALS AND METHODS

Animals

The study used adult male Sprague-Dawley rats weighing 160–170 g. The National Research Center's animal house colony provided the rats that were purchased. The animals were kept in standard housing conditions, with a temperature between 24 and 27 °C, 60% humidity, and 12-hour cycles of light and dark. Water and standard lab pellets were provided to the rats without restriction.

Surgical procedures

Rats were given an intraperitoneal (i.p.) dosage of sodium pentobarbital at a rate of 40 mg/kg to induce anesthesia. Following hair removal from the front-occipital region, 2% iodine solution was used to induce antisepsis. Using an orthodontic roof motor and number 2 drills, a 0.5 cm hole was created to the right of the bregma until the dura mater was visible. With the help of a Hamilton syringe fitted with a 30-gauge needle, lipopolysaccharide endotoxin (LPS) was injected into the right striatum at the dose of 5μg/rat (3 μL/rat). As a negative control, a single group of rats underwent the identical surgical procedure but were given a saline injection. Following the injection, bone wax was used to carefully seal the burr hole and antibiotic powder (neosporin) was applied to the wound.

Drugs and chemicals

The Ministry of Justice (Egypt) offered the *Cannabis sativa* plant. An Escherichia coli endotoxin (Serotype 055: B50) that had been purified using lyophilization, purchased from Sigma, St Louis, MO, USA, was utilized. It was diluted in sterile physiological saline, aliquoted, and stored at -20 °C. For every experiment, the same stock solutions were employed. The last of the chemicals and reagents were bought from Sigma in St. Louis, USA.

Preparation of cannabis extract

After drying, the flowering tops and leaves of Cannabis sativa were extracted. Reported extraction process was used with certain adjustments. Briefly, a mortar and pestle were used to grind 10 g of the dried plant material. The plant material was decarboxylated by heating it to 100 °C for two hours in an oven, while it was enclosed in a glass test tube, and covered with aluminum foil. Analytical grade 10 ml of chloroform was used for the extraction, and it was allowed to react overnight. Three extractions of the cannabis material were made. After that, fractions were mixed, filtered through a filter paper, and gathered. A little stream of nitrogen was applied to evaporate the filtrate. The residue was kept in a jar coated in aluminum at four °C and shielded from light. In order to conduct the studies, the residue was again suspended in two milliliters of 96% ethanol, then saline was added to bring the volume up to 100 milliliters. Using chromatography–mass spectrometry, the extract's Δ9-THC concentration was quantified and found to be approximately 10%.

Experimental groups

Different groups of rats (n = 6/group) were used in the study. Rats were randomly assigned to treatment groups as follows:

Group 1 received the vehicle (intrastriatal saline) (negative control).

Group 2 received intrastriatal LPS (5 μg/rat) (positive control).

Group 3 received intrastriatal LPS + L-dopa 25 mg/kg s.c.

Group 4 received intrastriatal LPS + L-dopa 25 mg/kg s.c. + cannabis 20 mg/kg.

Group 5 received intrastriatal LPS + bromocryptine 0.6 mg/kg s.c.

Group 6 received intrastriatal LPS + bromocryptine 0.6 mg/kg s.c. + cannabis 20 mg/kg.

Drug treatments (cannabis, L-dopa, and bromocryptine) were administered daily for 15 days starting on the day of surgical treatment and LPS injection.

Determination of lipid peroxidation

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in brain supernatants using the method of (Ruiz-Larrea et al., 1994).

Determination of nitric oxide

Nitric oxide was measured spectrophotometrically in supernatants as nitrite using Griess reagent in accordance with (Moshage et al., 1995).

Determination of reduced glutathione

The sulfhydryl group content in supernatants was determined by the 2.2-dithiobisnitrobenzoic acid assay as described by (Ellman, 1959).

Determination of total antioxidant capacity

A commercially available kit test (Biodiagnostics, Egypt) was used to determine total antioxidant capacity. The response of antioxidants in the sample with a predetermined volume of hydrogen peroxide supplied exogenously is how this assay calculates antioxidant capacity. A portion of the peroxide is removed by the antioxidants. An enzymatic reaction involving the conversion of 3,5-dichloro-2-hydroxybenzenesulfate to a colorful product allows for the colorimetric determination of residual peroxide (Koracevic et al., 2001).

Determination of glutathione peroxidase activity

Using a glutathione peroxidase kit from Biodiagnostics (Egypt), the GPx activity in the supernatants of homogenized striata samples was measured spectrophotometrically at 340 nm through the analysis of NADPH oxidation in accordance with the Paglia and Valentine, (1967) technique. The quantity of protein that oxidized 1 mM NADPH per minute is known as one unit of GPx activity. The unit of measurement for GPx activity is mU/mL

Determination of tumour necrosis factor-alpha

TNF-α was determined in supernatants by enzyme-linked immunosorbent assay using TNF- α kit (Biosource International, USA) according to the instructions provided by the manufacture.

Determination of paraoxonase-1 activity

Using phenyl acetate as a substrate, the arylesterase activity of paraoxonase was quantified spectrophotometrically in supernatants. In this assay, phenyl acetate is cleaved by arylesterase/paraoxonase, leading to the production of phenol. The rise in absorbance at 270 nm at 25°C is used to calculate the rate at which phenol is formed. The working reagent was 20 mM Tris/HCl buffer (pH 8.0) with four mM phenyl acetate serving as the substrate and one mM calcium chloride. After adding samples that have been diluted 1:3 in buffer, a 20-second lag period is used to record the change in absorbance. Using a UV-Vis Recording Spectrophotometer (Shimadzu Corporation), absorbance at 270 nm was measured every 15 s for 120 s. One microgram of phenol produced every minute is equivalent to one unit of arylesterase activity. Based on the phenol extinction coefficient of 1310 M/cm at 270 nm, pH 8.0, and 25°C, the activity is represented in kU/L. To account for the spontaneous hydrolysis of phenyl acetate, blank samples that include water are utilized (Haagen and Brock, 1992).

Brain histopathology

Formalin 10% phosphate-buffered was used to preserve brain samples. Slices of brain tissue (3-5 mm) were fixed overnight, then cleaned in xylene, dehydrated in increasing alcohol grades, and embedded in paraffin wax (58–60°C). Using a microtome, blocks with a thickness of 5 μm were created and divided. The tissue sections were stained with hematoxylin and eosin (Hx & E) and observed under a light microscope (Olympus, Japan).

Immunohistochemistry for caspase-3

Streptoavidin-biotin was used for the immunohistochemical staining of anti-caspase-3 antibodies. Sections that were four micrometers thick were deparaffinized, and they were then incubated for 30 minutes at room temperature with fresh 0.3% hydrogen peroxide in methanol. Following that, a 1:100 dilution of the primer antibody, anti-caspase-3, was incubated with the specimens. The samples underwent H & E counterstaining. For each primary antibody, normal mouse serum was used to create the negative controls.

Statistics

The data is given as mean ± SE. One-way analysis of variance was used to evaluate the data, and Duncan's multiple range test was then used to compare the group means post hoc. Effects were considered significant when they had a probability of $p < 0.05$.

3. RESULTS

Biochemical results

Lipid peroxidation

In the striatum of LPS-injected rats, MDA level showed a significant increase by 192.9% compared to vehicle/saline control levels (53.6 ± 1.8 *vs.* 18.3 ± 0.5 nmol/g.tissue). The level of MDA significantly decreased by treatment with L-dopa or combined with cannabis (20 mg/kg). The combination treatment, however, was not superior to treatment with L-dopa alone. Thus, MDA decreased by 15.7 and 21.68% by L-dopa or cannabis + L-dopa, respectively (45.2 ± 1.28 and 42.0 ± 1.62.*vs.* 53.6 ± 1.8 nmol/g.tissue). After treatment with bromocryptine or cannabis + bromocryptine, MDA decreased in the striatum by 22.4 and 27.4%, respectively (41.6 ± 1.81 and 38.93 ± 1.41 *vs.* 53.6 ± 1.8 nmol/g.tissue) (Figure 1).

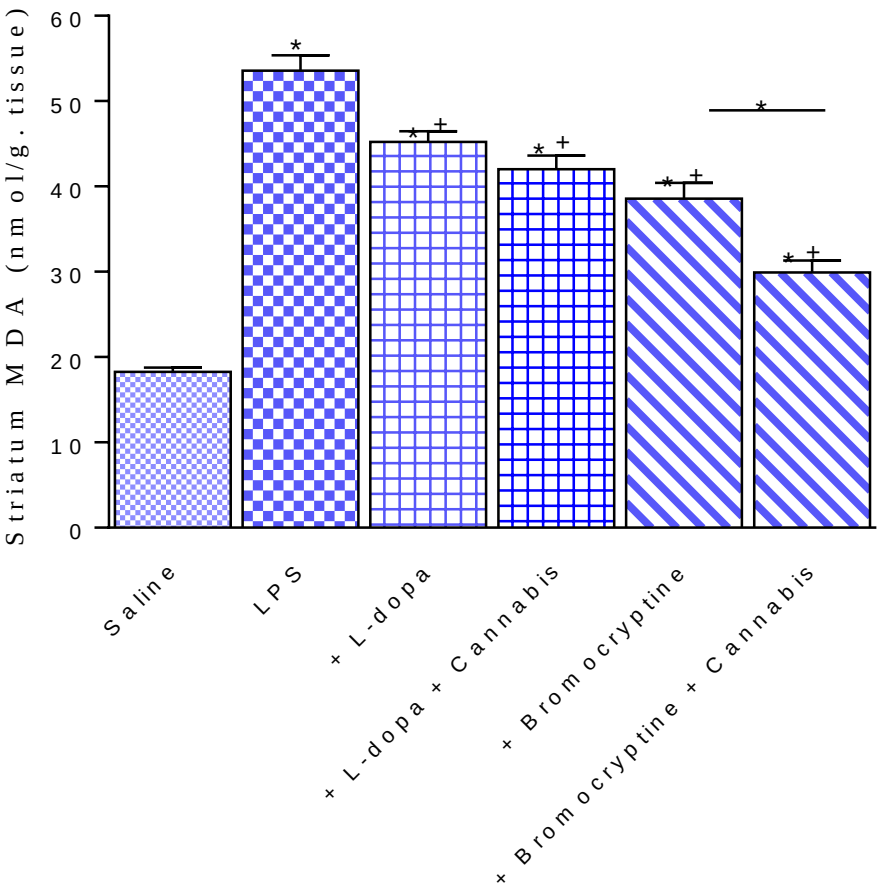

Figure 1 Malondialdehyde (MDA) levels in the striatal tissue after intrastriatal lipopolysaccharide injection and the effect of L-dopa or bromocryptine alone or in combination with cannabis. Significant differences across groups and from the saline control group are indicated by asterisks, as seen in the graph. The plus sign denotes a significant difference from the LPS control group.

Nitric oxide

In LPS-injected rats, nitric oxide content significantly increased by 303% compared to vehicle/saline control levels (98.0 ± 3.2 *vs.* 24.3 ± 1.0 μmol/g.tissue). A significant and marked decrease in nitric oxide was observed by 31.6 and 71% after treatment with L-dopa alone or in combination with cannabis with respect to LPS control levels (67.0 ± 3.5 and 28.4 ± 1.38 *vs.* 98.0 ± 3.2 μmol/g.tissue). A significant reduction in nitric oxide in different brain areas by 24% occurred after treatment with bromocryptine compared to LPS control levels (74.5 ± 4.1 *vs.* 98.0 ± 3.2 μmol/g.tissue). Cannabis given in combination with bromocryptine resulted in a significant decrease in nitric oxide by 30.5% compared to treatment with only bromocryptine (51.8 ± 2.9 *vs.* 74.5 ± 4.1 μmol/g.tissue) (Figure 2).

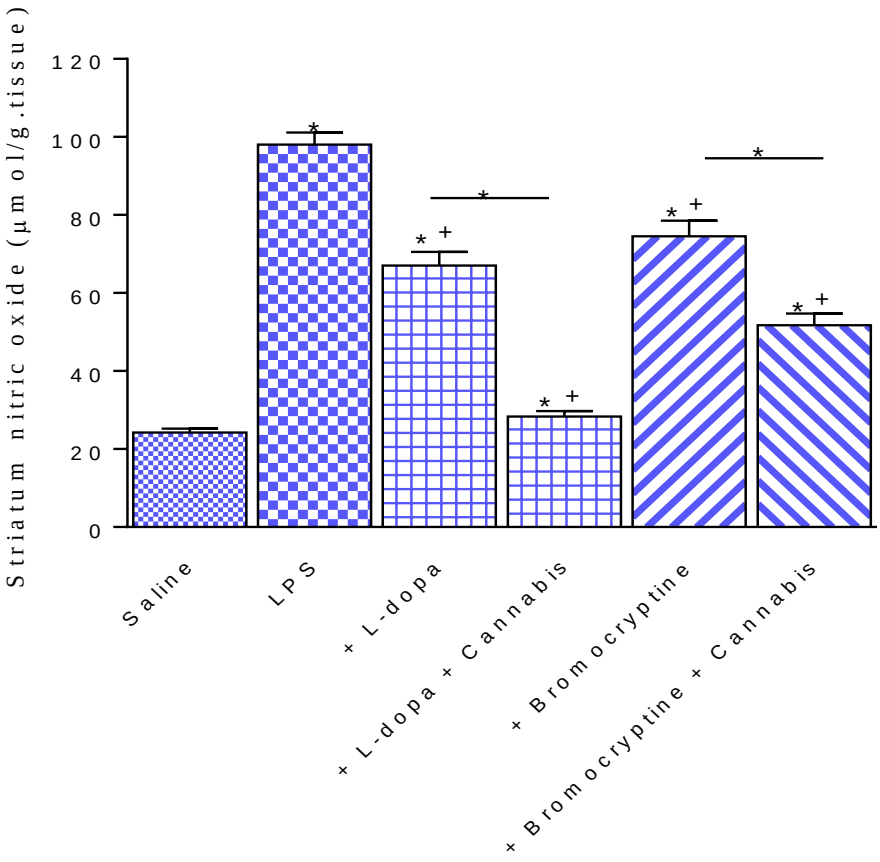

Figure 2 The increased striatum nitric oxide content after intrastriatal LPS and the effect of different treatments: L-dopa, bromocryptine, cannabis /L-dopa or cannabis/bromocryptine. Significant differences across groups and from the saline control group are indicated by asterisks, as seen in the graph. The plus sign denotes a significant difference from the LPS control group.

Reduced glutathione

In LPS-injected rats, GSH significantly decreased by 45% compared to the vehicle/saline control level (2.0 ± 0.03 *vs.* 3.63 ± 0.1 μmol/g.tissue). A significant and marked increase in GSH was observed after treatment with L-dopa alone or in combination with cannabis compared to LPS control level by 25 and 71%, respectively (2.5 ± 0.06 and 3.42 ± 0.11 *vs.* 2.0 ± 0.03 μmol/g.tissue). Following bromocryptine treatment, a significant increase in GSH concentrations was observed by 40% (2.8 ± 0.05 *vs.* 2.0 ± 0.03 μmol/g.tissue). Rats treated with bromocryptine and cannabis exhibited higher GSH level by 17.9% compared with bromocryptine only-treated rats (3.3 ± 0.07 *vs.* 2.8 ± 0.05 μmol/g.tissue) (Figure 3).

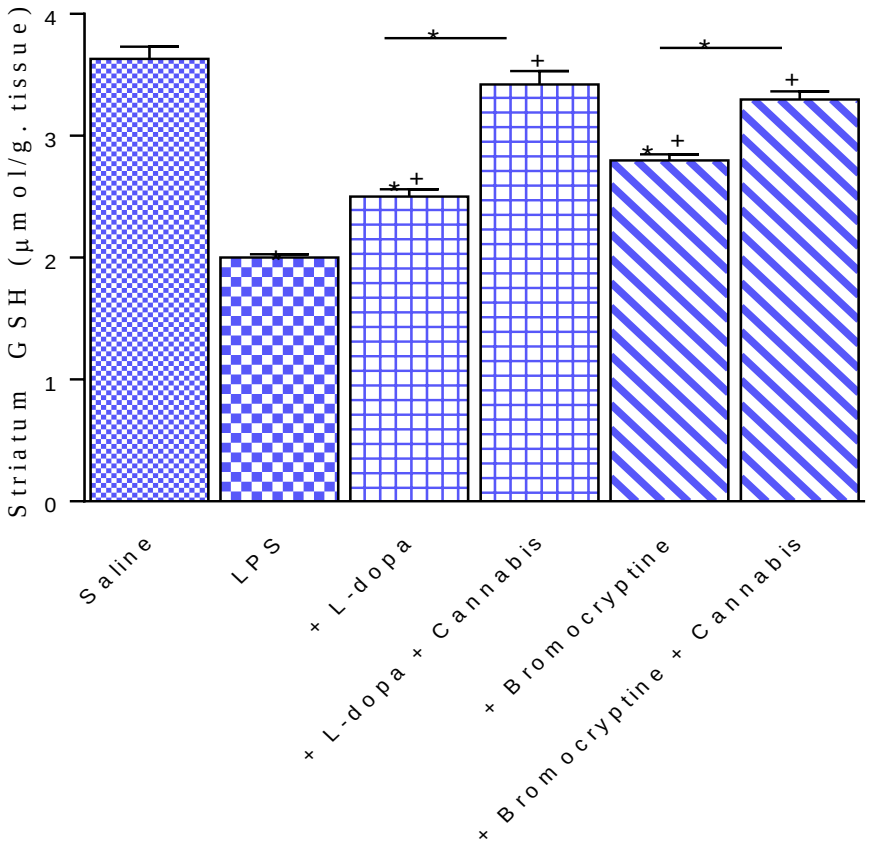

Figure 3 Reduced glutathione (GSH) in the striatum after intrastriatal LPS and the effect of L-dopa, bromocryptine alone, or combined with cannabis. Significant differences across groups and from the saline control group are indicated by asterisks, as seen in the graph. The plus sign denotes a significant difference from the LPS control group.

Total antioxidant capacity

There was a significant decrease in TAC in LPS-treated rats by 42.4% compared to the vehicle/saline control value (1.32 ± 0.09 *vs.* 2.29 ± 0.08 μmol/g.tissue). In LPS + L-dopa-treated rats, TAC did not significantly change (1.40 ± 0.02 *vs.* 1.32 ±.09 μmol/g.tissue). Yet, the co-administration of L-dopa + cannabis resulted in a significant increase in TAC by 22.9% compared with L-dopa only-treated rats (1.72 ± 0.05 *vs.* 1.40 ± 0.02 μmol/g.tissue). In LPS + bromocryptine-treated rats, there was a significant increase in TAC in the striatum by 18.2% compared to LPS control group (1.56 ± 0.01 *vs.* 1.32 ± 0.09 μmol/g.tissue). Rats treated with bromocryptine + cannabis showed an increase in TAC by 24.4% compared with the bromocryptine only-treated group (1.94 ± 0.01 *vs.* 1.56 ± 0.01 μmol/g.tissue) (Figure 4).

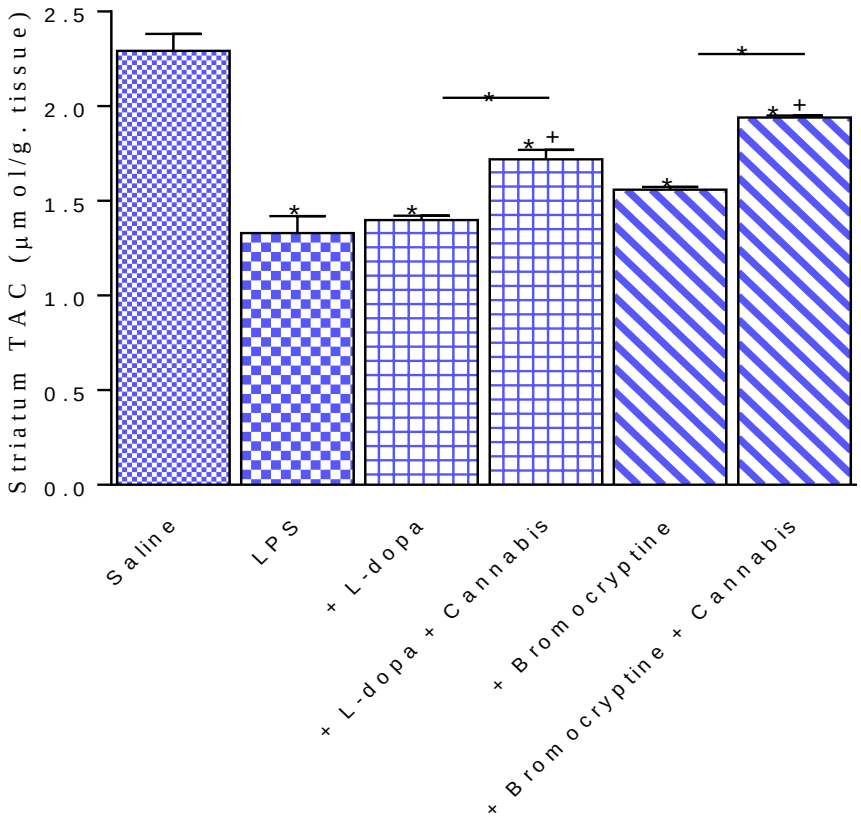

Figure 4 Total antioxidant capacity (TAC) in the striatum of rats after intrastriatal lipopolysaccharide injection, and the effect of only cannabis, L-dopa, bromocryptine alone, or combined with cannabis. Significant differences across groups and from the saline control group are indicated by asterisks, as seen in the graph. The plus sign denotes a significant difference from the LPS control group.

Paraoxonase 1

Following LPS injection, PON-1 activity showed a significant decrease by 80.5% compared to the vehicle/saline control level (1.95 ± 0.4 *vs.* 10.0 ± 0.76 kU/l). A marked increase in PON-1 activity was observed after treatment with L-dopa alone, or in combination with cannabis with respect to LPS controls levels. Thus, in rats treated with L-dopa alone or cannabis + L-dopa, PON1 activity increased by 134.9 and 203%, respectively (4.58 ± 0.26 and 5.91 ± 0.41 *vs.* 1.95 ± 0.4 kU/l). In rats treated with bromocryptine, PON-1 activity decreased in the striatum by 92.8%, compared to LPS control (3.76 ± 0.13 *vs* 1.95 ± 0.4 kU/l). A significant increase in PON-1 activity by 44% was seen after treatment with bromocryptine + cannabis compared with only bromocryptine (5.43 ± 0.18 *vs.* 3.76 ± 0.13 kU/l) (Figure 5).

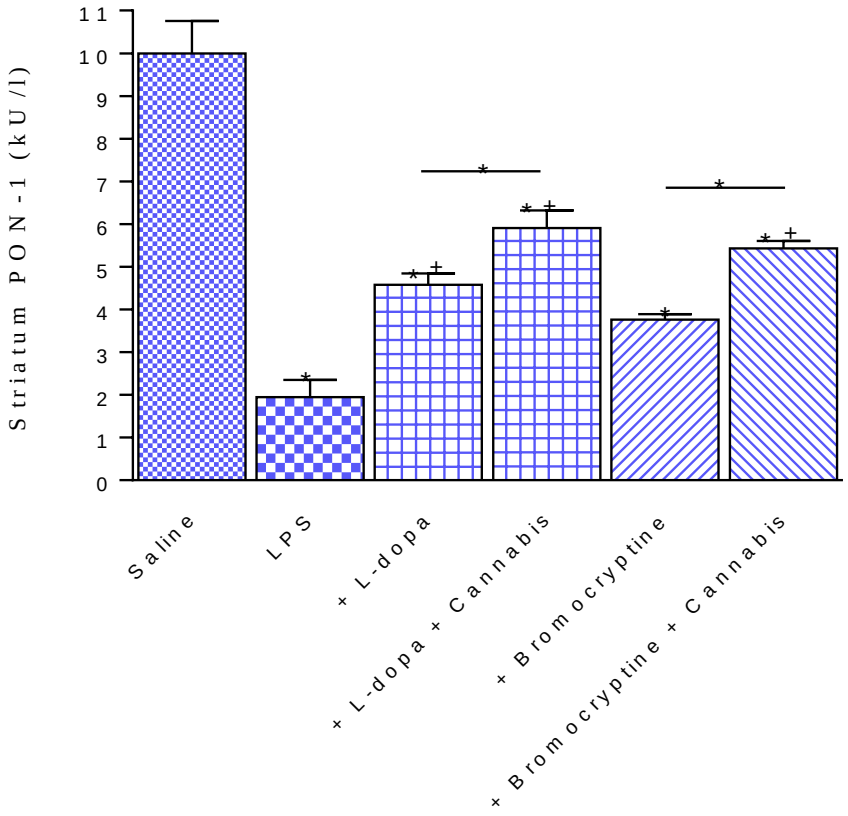

Figure 5 Paraoxonase-1 (PON-1) activity in the striatum of rats after intrastriatal LPS and the effect of L-dopa, bromocryptine alone, or combined with cannabis. Significant differences across groups and from the saline control group are indicated by asterisks, as seen in the graph. The plus sign denotes a significant difference from the LPS control group.

Glutathione peroxidase

In the striatum of LPS-injected rats, GPx activity decreased significantly by 38.1% compared to the vehicle/saline control group (289.0 ± 5.9 *vs.* 179.0 ± 4.9 mU/ml). GPx activity was unaltered by L-dopa, but significantly increased by 23.6% after bromocryptine treatment. Rats treated with cannabis combined with either L-dopa or bromocryptine exhibited a 22.6% and 15.7% decrease in GPx activity compared with those treated with only L-dopa or bromocryptine, respectively. Values were 178.4 ± 3.8 and 218.7 ± 7.0 for L-dopa or L-dopa/cannabis and 221.2 ± 9.3 and 256.0 ± 11.0 for bromocryptine and bromocryptine/cannabis *vs.* LPS control value of 179.0 4.9 mU/ml) (Figure 6).

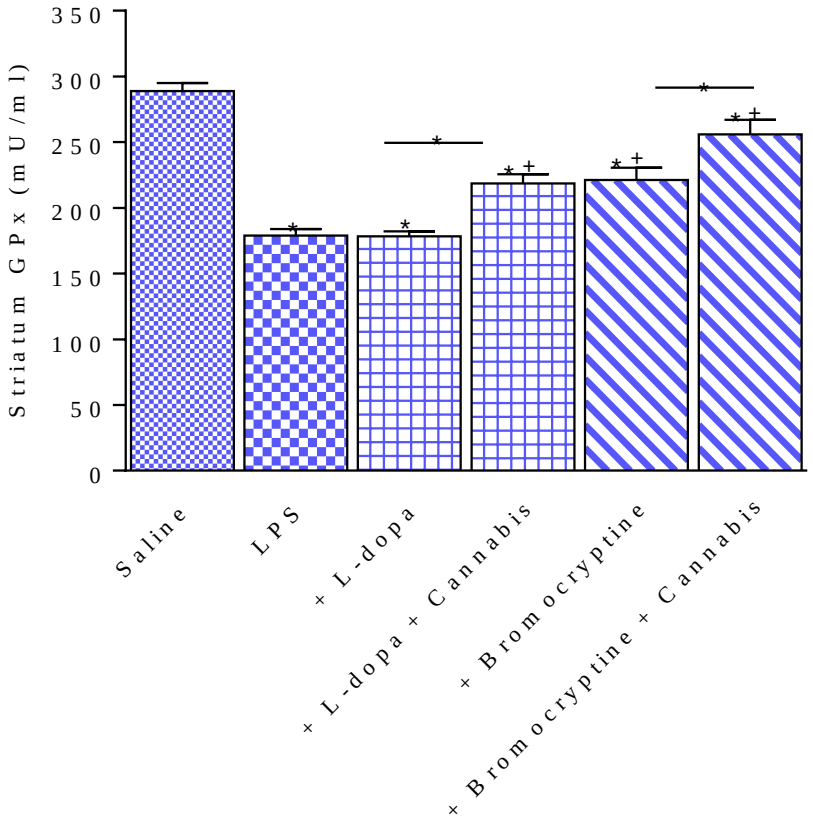

Figure 6 Glutathione peroxidase (GPx) activity in the striatum of rats after intrastriatal lipopolysaccharide LPSm and the effect of different treatments. Significant differences across groups and from the saline control group are indicated by asterisks, as seen in the graph. The plus sign denotes a significant difference from the LPS control group.

Tumour necrosis factor-α

The injection of LPS into the striatum led to a robust increase in striatal TNF-α by 874% (293.3 ± 8.4 *vs.* saline control value of 30.1 ± 0.9 pg/ml). TNF-α showed a 36.9 and 81.9% decrease after L-dopa and L-dopa + cannabis, respectively (185 ± 6.9 and 53.3 ± 1.2 *vs.* 293.3 ±8.4 pg/ml). In addition, a 31.8 and 86.4% decrease in TNF-α occurred in rats treated with bromocryptine or cannabis + bromocryptine, respectively (200 ± 9.2 and 40 ± 1.3 *vs*, 293.3 ±8.4 pg/ml) with respect to LPS control value (Figure 7).

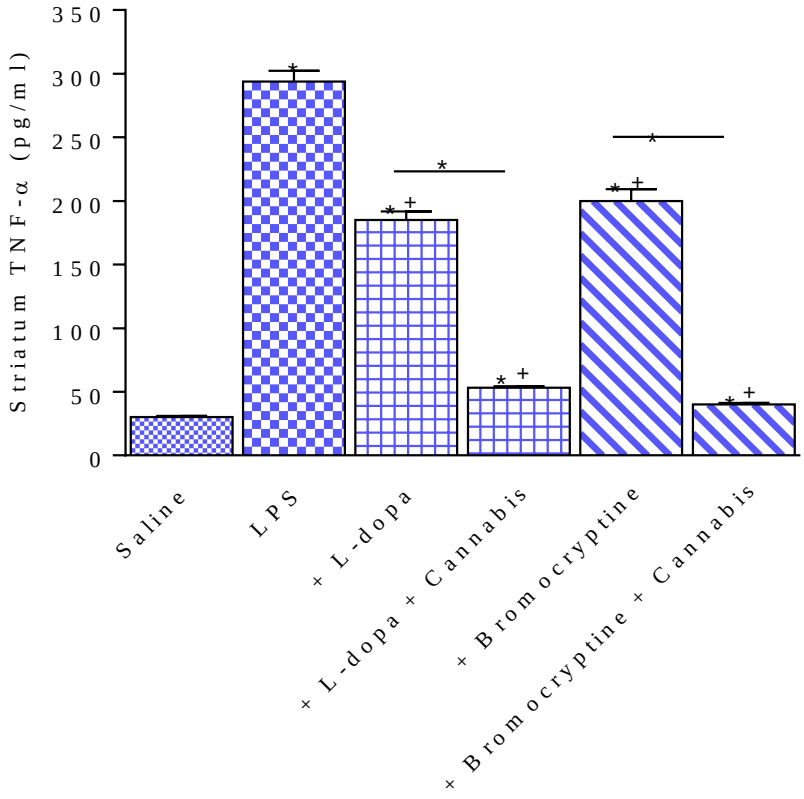

Figure 7 Tumour necrosis factor-α (TNF-α) in the striatum of rats after intrastriatal lipopolysaccharide injectionm and the effect of different treatments. Significant differences across groups and from the saline control group are indicated by asterisks, as seen in the graph. The plus sign denotes a significant difference from the LPS control group.

Histopathological results

In rats that received LPS/L-dopa, or LPS/L-dopa/cannabis, there were darkly stained, pyknotic, apoptotic cells, and a slight vacuolation of neuropil, with dilated blood vessels (Figure 8A & B). Rats treated with LPS/bromocryptine showed pyknotic, apoptotic cells, slight vacuolation of neuropil, and dilated blood vessels (Figure 8C & D). Rats treated with LPS/bromocryptine/cannabis showed less severe changes.

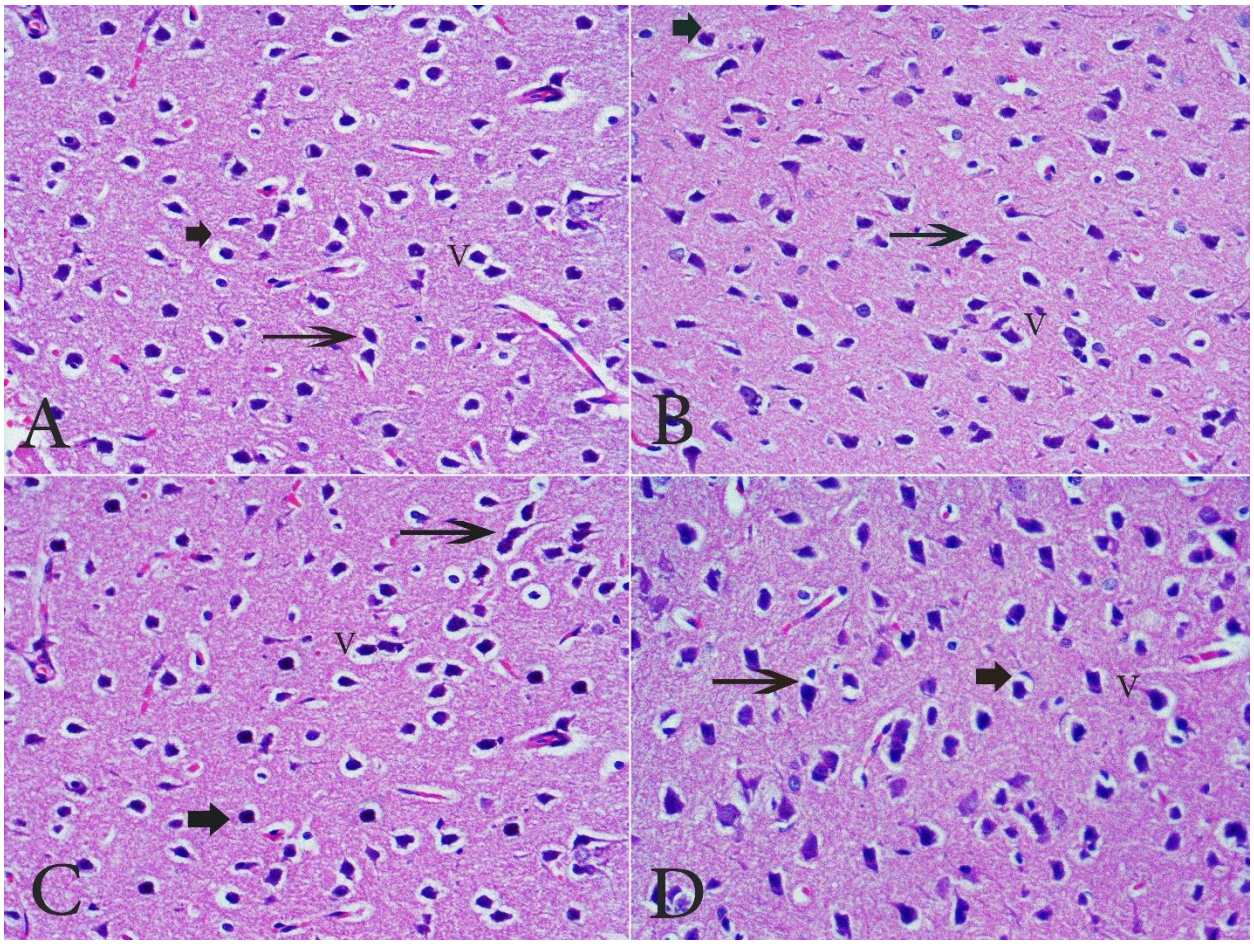

Figure 8 Hx & E stained sections of the rat striatum: (A) LPS and L-dopa showed neuronal damage with shrunken cells, pyknotic nuclei (arrow), apoptotic neurons (arrowhead), and vacuolation of neuropil (V); (B) LPS and L-dopa and cannabis showed few to mild pyknotic nuclei (arrow), apoptotic neurons (arrowhead), and slight vacuolation of neuropil (V); (C**)** LPS and bromocryptine showed marked cell damage, shrinkage of neurons, pyknotic nuclei (arrow), apoptotic cells (arrowhead), and vacuolation of neuropil (V); (D**)** LPS and bromocryptine and cannabis showed mild cell damage, pyknotic nuclei (arrow), apoptotic cells (arrowhead), and a slight vacuolation of neuropil (V) (H & E, X 400).

Caspase-3 results

The results of immunohistochemical reaction in the group that received LPS/L-dopa LPS/bromocryptin showed positive reactivity of caspase-3 in the striatum (Figure 9A & B). On the other hand, examination of sections in the striatum showed mild to moderate reactivity of caspase-3 in groups treated with cannabis and LPS/L-dopa or LPS/bromocryptine (Figure 9C & D).

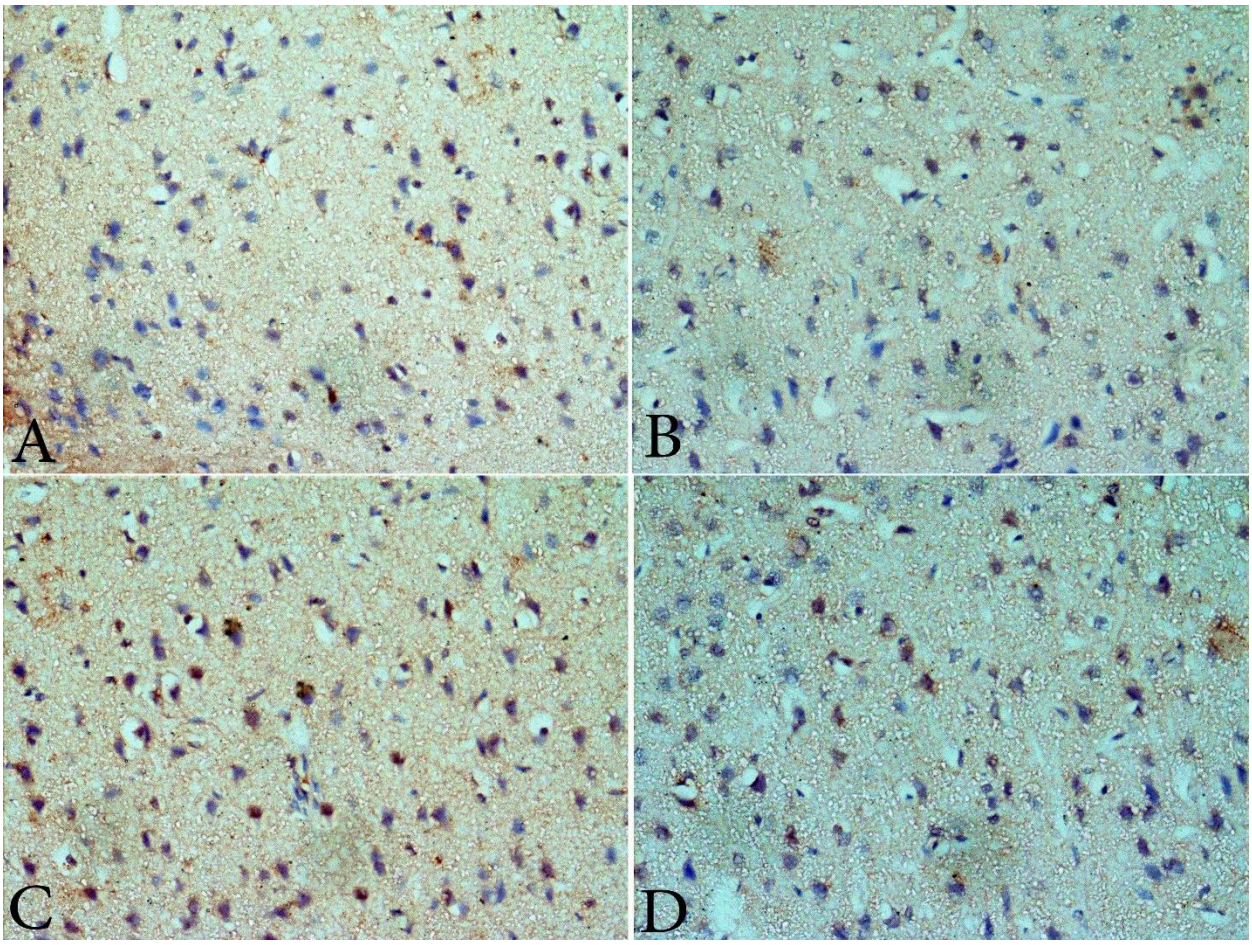

Figure 9 Immunohistochemical staining for capase-3: (A) LPS and L-dopa showed positive reactivity of caspase-3, indicated by the presence of the brown color; (B) LPS and L-dopa and cannabis showed mild to moderate reactivity of caspase-3; (C) LPS and bromocryptine showed positive reactivity of caspase-3; (D**)** LPS and bromocryptine and cannabis showed mild to moderate reactivity of caspase-3 (H & E, X 400).

4. DISCUSSION

The intrastriatal injection of LPS in rodents is considered a reliable model of Parkinson's disease in which microglia activation, and inflammatory response occur (Choi et al., 2009; Deng et al., 2021). We have also reported increased oxidative stress, inducible nitric oxide synthase, caspase-3 immunoreactivities, and decreased tyrosine-hydroxylase-immunoreactivity in the striatum, and neuronal degeneration in the striatum and substantia nigra after the systemic administration of LPS in mice (Abdel-Salam et al., 2014). The aim of this study was to (i) investigate the effect of the antiparkinsonian drugs L- dopa and bromocryptine on oxidative stress, neuroinflammation and neurodegeneration in the model of Parkinson's disease induced by intrastriatal injection of LPS in the rat; (ii) examine the possible modulation by cannabis of the effects of L-dopa and bromocryptine.

We found that injection of LPS into the rat striatum caused a significant oxidative stress indicated by the increase in brain level of the lipid peroxidation product malondialdehyde, and the decrease in the antioxidant and free radical scavenger reduced glutathione,

and striatal glutathione peroxidase. Levels of nitric oxide were also significantly increased in the striatum. High levels of nitric oxide are harmful to neurons *via* mechanisms that involve the formation of the oxidant radical peroxynitrite (Brown and Borutaitem, 2001; Pacher et al., 2007). These findings indicate increased oxidative/nitrosative stress by LPS injection, and the consequent depletion of the cellular antioxidants. We also observed a significant decrease in total antioxidant capacity and inhibition of paraoxonase-1 activity (PON-1) in the striatum. Paraoxonase-1 enzyme is essential in the detoxification of organophosphorus insecticides and lipid hydroperoxides.

By preventing the release of pro-inflammatory cytokines such as tumour necrosis factor-α, interleukin-6, and reactive oxygen species from macrophages, it demonstrated anti-inflammatory and antioxidant properties (Rozenberg et al., 2003; Aharoni et al., 2013). Therefore, the cell's resistance to oxidative stress is probably going to be compromised by the decrease in enzyme's activity. Furthermore, research indicates that a decrease in the enzyme's catalytic efficiency raises the vulnerability to the neurotoxic effects of organophosphate insecticides (Lee et al., 2013). These actions are associated with a higher risk of Parkinson's disease development (Manthripragada et al., 2010; Narayan et al., 2013). Therefore, a decrease in PON-1 activity may raise the risk of Parkinson's disease among those who have been exposed to pesticides, such as farmers. Our results also showed that LPS caused marked increase in the proinflammatory cytokine tumour necrosis factor-α (TNF-α) in the striatum, indicative of an inflammatory response following the injection of LPS.

The histological study indicated the presence of marked neurodegeneration in the striatum. These findings indicated the presence of significant oxidative stress, and inflammatory process in brain of LPS-injected rat that is associated with brain neurodegeneration. L-dopa is the gold-standard drug in the treatment of Parkinson's disease. It is administered to correct the biochemical deficit in midbrain by providing dopamine when combinated with the peripheral decarboxylase inhibitor carbidopa (Rascol et al., 2011). There has been contradictory data as regards the effect of L-dopa on oxidative stress and dopaminergic cell integrity *in vitro*. L-dopa or dopamine was shown to be toxic to cultured dopaminergic cells. Reactive oxygen species, dopamine quinones, and semiquinones are produced when dopamine is oxidized by enzymes or by auto-oxidation. This process results in the apoptosis or death of dopamine neurons and may be mediated by mechanisms such as mitochondrial failure and reaction with glutathione released from astrocytes.

This neurotoxicity of dopamine is increased by glutathione depletion and prevented by antioxidants (Berman and Hastings, 1999; Hirrlinger et al., 2002; Mytilineou et al., 2003). Other studies showed that dopamine (also apomorphine and apocodeine) conferred protection against glutamate neurotoxicity in rat cortical neurons and mouse hippocampal nerve cells *via* dopamine D4 receptor-mediated mechanism. L-dopa increased antioxidant activity, which could be involved at least in part in its neuroprotective effect (Ishige et al., 2001). Additionally, L-dopa or L-dopa/carbidopa was shown to have a protective effect *in vitro* against DNA damage caused by reactive oxygen species in human neuroblastoma cells (Colamartino et al., 2012). Nigrostriatal neurons were not damaged *in vivo* by giving L-dopa at high doses (200 mg/mouse) to mice for a period of 18 months Hefti et al., (1981) or by giving L-dopa to rat pups either alone or in combination with a glutathione production inhibitor (Mytilineou et al., 2003).

Our present findings suggest an antioxidant effect for L-dopa. We found that treatment with L-dopa was associated with a significant decrease in MDA and nitric oxide levels as well as increased levels of GSH in the striatum. Meanwhile, PON-1 and GPx activities were increased by L-dopa treatment. Moreover, a moderate and significant decrease in striatal TNF-α was observed. Cannabis given to LPS/L-dopa treated rats resulted in better decrease in lipid peroxidation and increased antioxidant parameters and TNF- α compared to only LPS/L-dopa. These effects of cannabis are in agreement with earlier observationsm which indicated an antioxidative action Abdel-Salam et al., (2014), and a decrease in the elevated TNF-α in the rat striatum after treatment with intrastriatal rotenone Abdel-Salam et al., (2015b) or in rat cerebral cortex after injection of ketamine (Abdel-Salam et al., 2021).

The administration of L-dopa alone or combined with cannabis, however did not result in significant histologic protection in LPS-treated rats as there was still substantial neuronal damage. Parkinson's disease patients are treated with dopamine agonists, such as bromocriptine. In the early stages of the disease, they are given as monotherapy or in conjunction with L-dopa when the disease is further advanced (Blandini and Armentero, 2014). Because dopamine agonists don't undergo oxidative metabolism, these medications don't have the same potential to produce cytotoxic free radicals as dopamine. Furthermore, their suppression of endogenous dopamine release may provide protection against the potentially harmful effects of excessive dopamine on neurons (Brooks, 2000).

In this study, treatment with bromocryptine decreased oxidative stress in the striatum. GPx, however, was unchanged but a significant decrease in TNF-α was observed, though to much less degree than that caused by the combined administration of bromocryptine and cannabis. On the other hand, administration of cannabis to LPS/bromocryptine treated rats resulted in a further

decrease in oxidative stress and striatal levels of TNF-α. Despite the beneficial effect of bromocryptine or cannabis/bromocryptine on the antioxidants and TNF-α, these treatments did not substantially protect against the striatal neurodegeneration caused by intrastriatal LPS.

5. CONCLUSIONS

In this study, the intrastriatal injection of LPS in rats produced an increased oxidative stress, and neuroinflammation, associated with marked neurodegeneration in the striatum. The administration of L-dopa or bromocryptine was associated with lower levels of oxidative stress and improved antioxidants. There was an additional effect on oxidative stress and neurodegeneration from combining either L-dopa or bromocryptine with cannabis. This, however, was not reflected in significant neuroprotection. These results do not suggest a benefit for using cannabis in Parkinson's disease.

Statement of Ethical approval

Animal procedures followed the guidelines of the institute's ethics committee for using animals in experimental studies (Project number 10001004) and the Guide for the Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Informed consent

Not applicable.

Author contribution

Abdel-Salam OME and Sleem AA designed the study and conducted the research and analysis. Omara EA performed the histopathology and its interpretation. Youness ER performed biochemical studies. Abdel-Salam OME performed the interpretation of data, wrote and prepared the manuscript. Abdel-Salam OME, Sleem AA, Youness ER and Omara EA approved the final version of the manuscript.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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