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Simultaneous administration of coffee and rasagiline/L-dopa protects against paraquat-induced neurochemical and motor behavior impairments in vivo

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Abstract

Background: Caffeine is a natural alkaloid present in a variety of highly consumed popular drinks such as coffee, tea and soft drinks as well as chocolate. Its consumption elicits beneficiary psychostimulant that has been linked to a reduced risk of developing Parkinson's disease (PD). The aim of the present study is to investigate the possible synergistic neuroprotective effects of co-administration of caffeine (CAF) or coffee (COF) with rasagiline (R) or L-dopa against paraquat (PQ)-induced neurochemical and motor behavior impairments in mice.

Results: In behavioral tests, R + COF increased the locomotor activity in rotarod test compared to L-dopa + COF. L-Dopa combinations decreased the immobility time in FST compared to rasagiline combinations; L-dopa + CAF provided a similar increase in locomotor activity compared to R + CAF. Combination of CAF or COF with L-dopa or rasagiline resulted in a substantial improvement in brain neurotransmitter and antioxidant levels as they significantly increased dopamine and super oxide dismutase but significantly decreased nitric oxide levels as compared to L-dopa or rasagiline, respectively. Furthermore, they also exerted a protective effect against the neurodegenerative histo-pathological changes induced by PQ.

Conclusions: Our findings demonstrated co-administration of COF or CAF, adenosine 2A receptor antagonists, along with L-dopa or rasagiline possesses a new therapeutic strategy for the management of PD neurochemical disturbances and motor behavior impairments through preservation of the brain dopamine and serotonin content, antioxidants level and histological features.

Keywords: Parkinson's disease, Paraquat, Coffee, Rasagiline, Caffeine, Neurochemical disturbances, Motor impairments, Behavior tests

Background

Parkinson's disease (PD) is one of the most common neurodegenerative diseases, for which currently there is no cure. It affects more than 1% in elderly population (Ikawa et al. 2020). PD develops as a result of progressive loss of dopaminergic neurons in the substantia nigra (SN), leading to striatal dopamine (DA) depletion. This neurological condition caused by the degeneration of dopaminergic nigrostriatal system is largely responsible for the classical clinical motor symptoms including bradykinesia, muscle rigidity, tremor at rest and postural instability (Pires et al. 2017). In addition, non-dopaminergic degeneration also occurs in other brain areas which appears to be responsible for a suite of other non-motor symptoms such as anosmia, depressed mood, psychosis, emotional

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Aboutabl *et al. Bulletin of the National Research Centre*   (2021) 45:219

Page 2 of 20

and cognitive deficits, sensory disturbances and pain, sleep disorder, autonomic, gastrointestinal and sensory dysfunction that precede the classical motor symptoms in PD and also recognized as relevant symptoms in PD patients (Hirao et al. 2015; Olanow et al. 2009).

Despite several familial forms of PD associated with specific genetic mutations, the majority of patients suffer from idiopathic PD (De Deurwaerdère et al. 2017). The etiology of the disease is not completely understood. The underlying molecular pathogenesis involves several pathways and mechanisms such as oxidative stress, α-synuclein proteostasis, calcium homeostasis, neuroinflammation, mitochondrial function and axonal transport (Poewe et al. 2017).

Paraquat (PQ) (1,10-dimethyl-4-4-bipyridinium) is a herbicide used to induce several neurotoxic effects similar to PD symptoms in experimental animals (Chanyachukul et al. 2004; Liou et al. 1996). Systemic administration of PQ into mice induces a dose-dependent decrease in dopaminergic nigral and striatal neurons, which was accompanied by reduction in ambulatory movements. Oxidative stress is the mechanisms by which PQ-induced nigrostriatal dopaminergic neurodegeneration leads to the PD (Singhal et al. 2011). Several studies in rodents have indicated that paraquat causes neurotoxicity via the formation of reactive oxygen species (ROS) and production of apoptosis-related molecules. PQ initiates intracellular formation of ROS through three different pathways that involves formation of the reduced form of PQ by NADPH-cytochrome P450 reductase followed by redox cycle with the involvement of super oxide dismutase (SOD) and glutathione, inhibition of mitochondrial electron transport chain and interaction with another enzymes as NADPH oxidase, nitric oxide synthase, xanthine oxidase and thioredoxin reductase (Gonzalez-Polo et al. 2004).

L-3,4-Dihydroxyphenylalanine (levodopa or L-dopa), the endogenous and metabolic precursor of DA, is given exogenously to patients to raise levels of DA in DA-denervated tissues. It has been used for decades for the symptomatic relief of PD (Cenci 2014; Oertel 2017). Over the years, L-dopa, either administrated alone or co-administrated with inhibitors of peripheral amino acid decarboxylase in order to protect it from its peripheral degradation, remains the gold-standard medication for PD (Goshima et al. 2019). L-Dopa is effective in most PD patients; however, chronic treatment results in motor and behavioral side effects that include dyskinesias or involuntary movements and impulse control disorders (Bastide et al. 2015; Voon et al. 2017). There is controversy over whether the side effects of L-dopa administration could be attributed to the production of free radicals (Ahmadinejad et al. 2017).

Rasagiline [R(+)-*N*-propargyl-1-aminoindane] is a potent selective irreversible monoamine oxidase B (MAO-B) inhibitor. Rasagiline possesses neuroprotective properties through prolongation of dopamine duration of action in the brain, thus improving the motor symptoms some non-motor symptoms in both early and advanced PD patients (Im et al. 2019; Stocchi et al. 2015).

Phase III clinical studies have declared the significant efficacy of the use of rasagiline for PD treatment, either as an adjunct therapy to L-dopa (Rabey et al. 2000) or alone in early PD conditions (Group 2002). The neuroprotective effect of rasagiline has been markedly confirmed in different in vivo models. In fact, rasagiline increased the survival of dopaminergic neurons in the substantia nigra of 6-hydroxydopamine (6-OHDA)-induced rats (Blandini et al. 2004) and upregulated the tyrosine kinase receptor (Trk)–phosphatidylinositol 3 (PI3) kinase–Akt pathway leading to increased dopaminergic cell survival in the midbrain region of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mice (Sagi et al. 2007). Several epidemiological studies have anticipated the association between coffee and caffeine intake and a reduced risk of subsequent development of PD (Cho et al. 2018; Hong et al. 2020; Moccia et al. 2016). Caffeine is a natural xanthine alkaloid that is widely consumed in coffee, tea, chocolate and soft drinks (Nuhu 2014). It elicits psychostimulatory effect as it can readily cross the blood brain barrier, due to its hydrophobic nature causing alertness and reducing fatigue, leading to more effective functioning in psychomotor tasks demanding fast reactions upon average levels of consumption (Kolahdouzan and Hamadeh 2017; Ullrich et al. 2015). Caffeine is a non-selective A_1/A_{2A} adenosine receptor antagonist (Rascol et al. 2015). The A_{2A} receptors, specifically located in the basal ganglia (BG) circuit, are of particular interest in PD as they interact functionally and are co-expressed with dopaminergic D2 receptors on the striatal GABAergic neurons of the "indirect" BG pathway directing from the striatum to the globus pallidus (Schiffmann et al. 2007). Preclinical studies showed that the blockade of the A_{2A} receptor subtype contributes to most neuroprotective effect (Fathalla et al. 2017). Antagonists of the adenosine A_{2A} (but not A_1) receptor mimicked the effects of caffeine in acute MPTP and 6-OHDA toxin models of PD (Bove et al. 2005; Pierri et al. 2005). In addition, genetic disruption of the A_{2A} receptor attenuated acute neurotoxicity in a mouse model of PD. Prospective epidemiologic studies demonstrated the inverse relationship between caffeine intake and PD incidence (Ross et al. 2000). Consumption of caffeine greater than 12 oz/day was linked with reduction in dyskinesia compared with less than 4 oz/day intake (Wills et al. 2013).

Aboutabl *et al. Bulletin of the National Research Centre*   (2021) 45:219

Page 3 of 20

Thus, identification of any promising therapeutic intervention for PD management is of a huge importance. Therefore, the aim of the present study is to investigate the possible synergistic neuroprotective effects of co-administration of caffeine or coffee with L-dopa or rasagiline on PQ-induced neurochemical and behavior impairments in mice.

Methods

Animals

Adult old male albino mice, aged 8–9 weeks and weighing 30–35 g, were used in the present study (McCormack and Di Monte 2003). Mice were purchased from the Animal House Colony of the National Research Centre, Dokki, Giza, Egypt. Throughout the period of investigation, the animals were housed in wire mesh plastic cages in groups of five and maintained under suitable laboratory conditions of room temperature (23 ± 2 °C), relative humidity ($55 \pm 5\%$) and 12-h light /12-h dark cycle. Animals were provided with standard pellet diet (protein: 23%, fat: 3.25%, carbohydrates and fiber: $\sim 68\%$) and allowed free access to tap water. Mice were periodically checked to verify their pathogen free condition All animal procedures were performed according to the Ethics Committee of the National Research Center [ethics approval no. 08020] and in accordance with the recommendations for the proper care and use of laboratory animals "Canadian Council on Animal Care Guidelines, 2011."

Drugs and kits

Levodopa (L-dopa) and caffeine were a gift from El Nasr Pharmaceutical Company ($>98\%$). Rasagiline mesylate was purchased from Clearsynth Labs Ltd., Mumbai, India (98%). Caffeinated coffee (Nescafe) was purchased from market. PQ was purchased from Sigma−Aldrich (St. Louis, MO, USA). Malondialdehyde and superoxide dismutase kits were purchased from Biodiagnostic, Giza, Egypt. Nitric oxide (NO^{2-}/NO^{3-}) assay kit was purchased from Assay Designs, Inc., Ann Arbor, MI, USA. Dopamine and serotonin ELISA kits were purchased from MyBioSource, Inc., San Diego, CA, USA.

Experimental design

Mice ($n = 8$) were treated with single or combination of drugs: levodopa (L-dopa), rasagiline (R), caffeine (CAF) and coffee (COF)] as assigned in the groups below. Thirty minutes later, a single dose of PQ was injected in mice for the induction of motor behavior and neurochemical disturbances (Chanyachukul et al. 2004; McCormack and Di Monte 2003). The course of treatment was carried out according to Chanyachukul et al. (2004) with modifications. After 90 min from PQ treatment, behavioral tests were carried out followed by scarification of the mice by cervical dislocation. The brains were excised immediately from all animals in different groups and washed with physiological saline. One set was stored at -80 °C for estimation of different parameters. Other brain sets were stored in 10% neutral formalin for histopathological examination.

Animal groups

Animals were randomly allocated into two main groups: Group 1: animals receiving the vehicle and serve as normal control group and Group 2: mice were injected with PQ (30 mg/kg, *i.p.*) (Chanyachukul et al. 2004; McCormack and Di Monte 2003). This group was further subdivided into: (1) untreated mice, (2) mice treated with L-dopa [100 mg/kg, i.p.] (McCormack and Di Monte 2003), (3) mice treated with rasagiline (R) [10 mg/kg, i.p.] (Finberg and Youdim 2002), (4) mice treated with caffeine (CAF) [10 mg/kg, i.p.] (Brothers et al. 2010), (5) mice treated with coffee (at a dose which corresponds to regular human intake of three cups of coffee $\approx$ 10 mg/kg, i.p. of caffeine) (Costa et al. 2008), (6) mice treated simultaneously with L-dopa and CAF, (7) mice treated simultaneously with L-dopa and COF, (8) mice treated simultaneously with R and CAF and (9) mice treated simultaneously with R and COF. All treatments were carried out at 30 min prior to PQ administration.

Behavioral tests

All experiments were carried out in similar environmental conditions in the morning between 9 am and 3 pm to diminish circadian effects.

Rotarod test

The rotarod test has been used to assess motor coordination and balance alterations after PQ injection. Mice were trained to balance on a rotating black grip striated rod (diameter: 3 cm) separated in five lanes 57 mm wide, 20 cm high from five tilting planks (UGO BASILE, Varese, Italy). Ninety minutes after PQ administration, the treated animals were placed on the rod that rotated at 40 rpm for 60 s (Rozas et al. 1997). Neurotoxicity was determined by the inability of the animal to remain on the rod.

Forced swim test

The "behavioral despair" test was performed where mice were individually plunged into a transparent plexiglass cylinder (13 cm diameter X 24 cm high) containing water (22 °C $\pm$ 2 °C) to a depth of 40 cm (UGO Basile S.R.L., Italy. The duration of immobility for the last 4 min of the 6-min test was measured (Castagne et al. 2010).

Aboutabl *et al. Bulletin of the National Research Centre*   (2021) 45:219

Page 4 of 20

Biochemical tests
Preparation of mouse brain tissue homogenate

Tissue homogenates were prepared according to the kit instructions. Brain tissues were rinsed in ice-cold phosphate-buffered saline (PBS) (0.02 mol/L, pH 7.0–7.2) to remove excess blood thoroughly and weighed before homogenization. The brain tissues were homogenized (10 mg tissue to 100 µl PBS) with a glass homogenizer (Pyrex® glass pestle tissue grinder, Corning Life Sciences, USA) on ice. The resulting brain homogenates were centrifuged (Z323K, Hermle Labortechnik, Germany) for 15 min at 5000 rpm. The supernatants were stored at -80 °C freezer (Nuaire Ultra-Low Freezer, Japan).

Measurement of brain dopamine (DA) level

Competitive enzyme immunoassay mouse DA ELISA kit was used. This technique utilizes a monoclonal anti-DA antibody and DA-HRP conjugate. The brain homogenate sample and buffer are incubated together with DA-HRP conjugate in pre-coated plate and then incubated with a substrate for HRP enzyme. The product of the enzyme–substrate reaction is measured spectrophotometrically at 450 nm using a microplate reader (TECAE, A5082, Austria). The intensity of the color is inversely proportional to the DA concentration since DA from brain samples and DA-HRP conjugate compete for the anti-DA antibody binding site. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The DA concentration in each sample is interpolated from this standard curve.

Measurement of brain serotonin level

The mouse brain serotonin content was estimated by adopting the quantitative sandwich ELISA kit. The optical density (O.D.) was measured at 450 nm using the microplate reader.

Measurement of brain total nitric oxide (NO^{2-}/NO^{3-})

Total nitric oxide was measure in the brain homogenates of both treated and normal mice using nitric oxide (NO^{2-}/NO^{3-}) assay kit. The measurement of total nitric oxide involves the enzymatic conversion of nitrate to nitrite, by the enzyme nitrate reductase, followed by the colorimetric detection of nitrite as a colored azo dye product of the Griess reaction that absorbs visible light at 540 nm using the microplate reader. For most accurate determination of total nitric oxide production, both nitrate and nitrite concentrations were quantized in the brain samples.

Measurement of brain superoxide dismutase (SOD) level

SOD was determined by colorimetric method using commercially available SOD kit. SOD was assayed in terms of its ability to inhibit the radical-mediated chain-propagating autoxidation of epinephrine. The enzyme assay based on adrenochrome absorption at 480 nm has been improved by measuring the absorption change at 320 nm using a Cary series UV–Vis spectrophotometer, Agilent Technologies (Sun and Zigman 1978).

Measurement of brain malondialdehyde (MDA) level

MDA was determined by colorimetric method using commercially available MDA kit (Yagi 1998). MDA reacts with thiobarbituric acid in acidic medium at 95 °C for 30 min to form thiobarbituric acid reactive product. The absorbance of the resultant pink product was measured at 534 nm using the UV–Vis spectrophotometer.

Histopathological examination of brain
Hematoxylin and eosin staining

At the end of the experimental period, a replicate set of animal groups were killed by cervical dislocation and whole brains were dissected immediately. Tissues were fixed in 10% buffered formalin, processed through ascending grades of alcohol, cleared in xylene and prepared into paraffin blocks. Serial sections 5 microns thick were prepared from each block and stained with hematoxylin and eosin for routine histopathological study (Drury et al. 1976).

Immunohistochemical analysis

Immunohistochemical (IHC) study was done on additional sections of mice brain tissues to detect glial fibrillary acidic protein (GFAP) for demonstration of astrogliosis. Deparaffinization was performed. Antigen retrieval was done by boiling tissue sections in 10 mM citrate buffer, pH 6.0 for 20 min, followed by cooling at room temperature for 20 min. Primary antibody rabbit polyclonal anti-GFAP antibody (ab7260, Abcam, USA) was applied 5–10 µg/mL for 30 min at room temperature. UltraVision plus detection system anti-polyvalent, horseradish peroxidase/3, 3'-diaminobenzidine (HRP/DAB) was applied to detect GFAP+ve astrocytes (Li et al. 2020). Counterstaining was performed by applying Mayer's hematoxylin for 30 s. Sections of the human brain were run with each batch of IHC stains to act as positive control. Sections omitting the primary antibody and using diluent instead were used as negative control. One negative control and one positive control were used in each run. Positive immunoreactivity was considered when brownish cytoplasmic coloration was seen.

Microscopic examination and photomicrography

Hematoxylin- and eosin-stained tissue sections were examined using an Olympus CX41 research microscope at the Pathology Department, National Research Centre. Slide

Aboutabl *et al. Bulletin of the National Research Centre*   (2021) 45:219

Page 5 of 20

tissue microphotography was done using a CCD digital camera Olympus DP-12 attached to the Olympus CX41 research microscope. Digital photomicrographic sections were taken at various magnifications.

Morphometric image analysis

The morphometric image analysis was performed at the Pathology Department, National Research Centre, using the Leica Qwin 500 Image analyzer (LEICA Imaging Systems Ltd, Cambridge, UK), which consisted of a Leica DM-LB microscope with a JVC color video camera attached to a computer system.

The examined slides were placed on the stage of the microscope. The light source was set to the required level. Successful adjustment of illumination was checked for on the monitor. The morphometric image analysis was carried out on hematoxylin- and eosin-stained slides to measure parameters of neuronal cells and pigmented neurons and also carried out on GFAP-immunostained slides. Area measurements of neurons, pigmented neurons and GFAP positively stained cells were taken at magnification 200×. The selected nuclei were surrounded by a line to be covered automatically by a green mask, which is called a binary image. The parameters of this binary image appeared automatically in the form of a table in micrometers, and finally, the mean and standard variation of all parameters examined were determined. The area percentage of the binary image is calculated automatically by the software. The mean percentage of all fields examined is determined.

Optical density measurements of pigmented neurons and GFAP+ve astrocytes were performed with a computer-assisted image analysis system. The mean optical density of each region was bilaterally measured on selected brain regions, using consecutive sections in each subject. The degree of reaction was chosen by the color-detect menu. The image was transformed to gray image (a grid of pixels each representing the intensity or the brightness at that point by a range of numbers, from 0 (black) to 255(white) (Kunchok et al. 2019).

Statistical analysis

Data are presented as mean ± S.E.M. Differences between groups were statistically analyzed using a one-way analysis of variance followed by a Student–Newman–Keuls post hoc comparison. Data were analyzed using the Sigma Stat software version 11. A result was considered statistically significant where $p < 0.05$.

Results

Behavioral studies

Rotarod test

In the current study, mice were treated with a single injection of PQ preceded by an injection with either saline (positive control), ʟ-dopa, R or their combination with COF or CAF. PQ administration was associated with significant decrease ($P < 0.001$) in locomotor activity (13.80 ± 1.66 s) which is fourfold lower than that of normal group. All treatments demonstrated threefold to fourfold significant increase ($P < 0.001$) in locomotor activity compared to the PQ group. Interestingly, treatment with ʟ-dopa + CAF, R, R + COF or R + CAF normalized the locomotor activity. Moreover, treatment with R alone demonstrated significant improvement ($P = 0.001$) in motor coordination by 36.36% when compared to treatment with ʟ-dopa alone. Similarly, R + COF combination improved significantly ($P = 0.039$) the motor coordination by 16.55% when compared to ʟ-dopa + COF (Fig. 1A).

Forced swim test

The PQ-induced mice showed sharp significant ($P < 0.001$) increase in the immobility time by 165% compared to normal value. Treatment with ʟ-dopa, or ʟ-dopa with either CAF or COF significantly decreased ($P < 0.001$) the immobility by 58, 93 and 59%, respectively, compared to PQ group (Figure 1B). In addition, treatment with R, R + CAF or R + COF significantly decreased ($P < 0.001$) the immobility by 59, 66 and 65%, respectively, compared to PQ group. Meanwhile, treatments with ʟ-dopa (63.33 ± 2.91 s) or ʟ-dopa + COF (62.83 ± 1.56 s) as well as R (63 ± 1.73 s) or R + CAF (51.33 ± 2.40 s) or R + COF (53 ± 3.22 s) were able to return the immobility time to the normal level. Interestingly, combined treatment with ʟ-dopa + CAF (10.5 ± 0.5 s) significantly decreased ($p = 0.001$) the immobility time by 82% compared to the normal value (Fig. 1B).
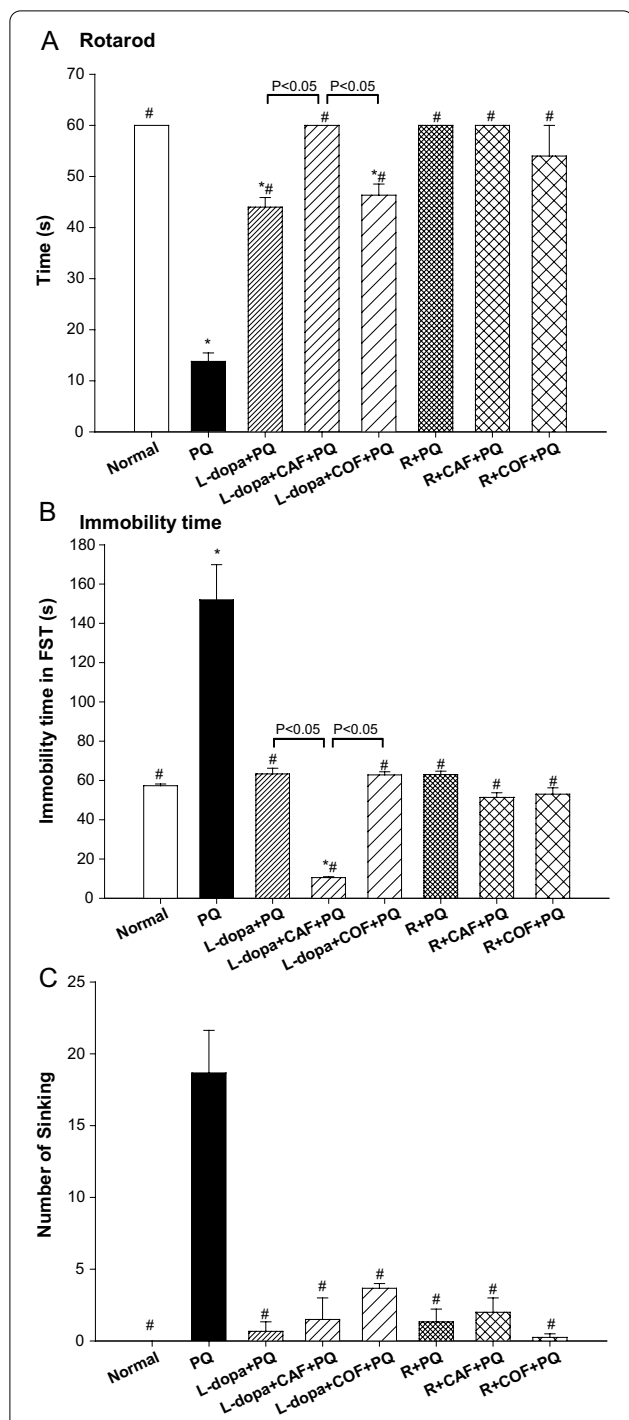
Number of sinking

In forced swim test, PQ administration exhibited a significant increase ($P < 0.001$) in the number of sinking (18.67 ± 2.96) done by mice compared to normal group. Mice treated with ʟ-dopa, ʟ-dopa + CAF or ʟ-dopa + COF as well as R, or R + CAF demonstrated significant reduction ($P < 0.001$) in the number of sink attempts by 96, 92, 80, 93, 89 and 99%, respectively, compared to the PQ group (Fig. 1C).

Biochemical tests

Brain neurotransmitters

Dopamine level Dopamine level was significantly decreased in PQ-induced mice brain tissue homogenates (4.58 ± 0.26 ng/g tissue) compared to normal (31.78 ± 0.88 ng/g tissue, $P < 0.001$). However, treatment with ʟ-dopa, ʟ-dopa + CAF or ʟ-dopa + COF significantly increased ($P < 0.001$) the brain dopamine level by 2.6-, 5.5- and 3.2-fold, respectively, compared to PQ. In addition,

Aboutabl *et al. Bulletin of the National Research Centre*   (2021) 45:219

Page 6 of 20

Fig. 1 Effect of each L-dopa and rasagiline (R) alone or in combination with caffeine (CAF) or coffee (COF) on behavioral activity in paraquat (PQ)-treated mice. **A** Rotarod test, **B** Immobility time in forced swim test, **C** Number of sinking in forced swim test. Data are presented as mean ± S.E.M. Differences between groups were statistically analyzed using a one-way analysis of variance followed by a Student–Newman–Keuls post hoc comparison. *Significant from normal group *P* < 0.05. #Significant from PQ group *P* < 0.05. Lines represent significance *P* < 0.05
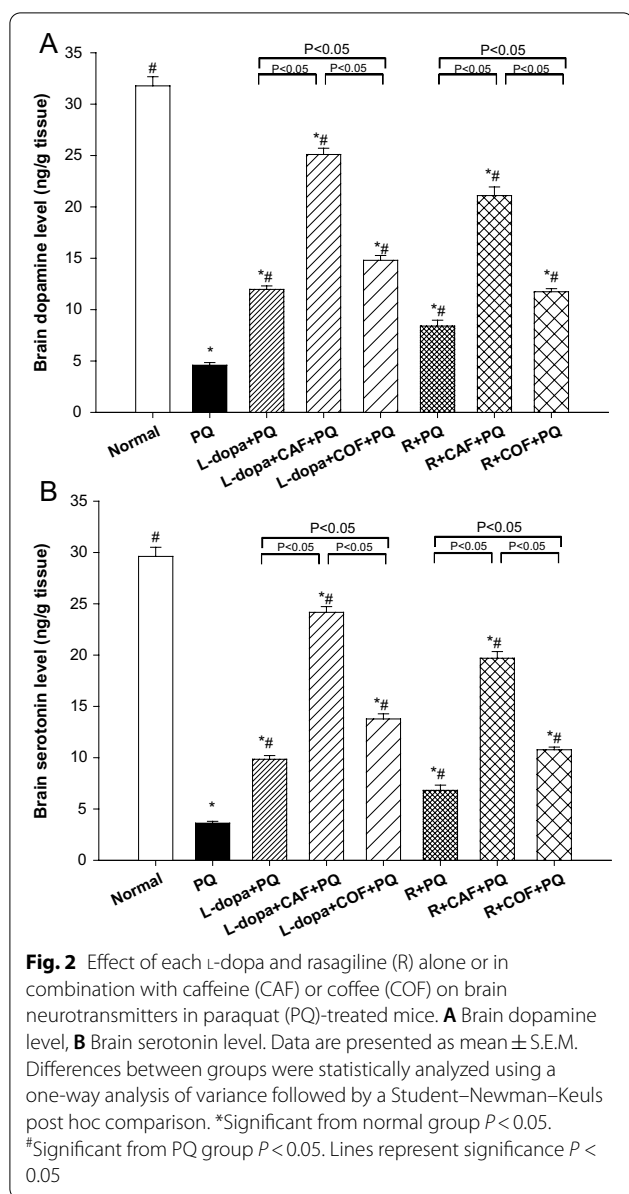
treatment with R, R + CAF, or R + COF demonstrated significant increase (P < 0.001) in brain dopamine level by 1.8-, 4.6- and 2.6-fold, respectively, compared to PQ.

Moreover, treatment with CAF demonstrated a pronounced significant effect (P < 0.001) in elevating the brain dopamine level when combined with L-dopa or R compared to L-dopa and L + COF or R and R + COF, respectively. Interestingly, treatment with L-dopa, L-dopa + CAF or L-dopa + COF significantly increased the brain dopamine level compared to treatment with R (P < 0.001), R + CAF (P < 0.001), or R + COF (P = 0.002), respectively. All the tested treatments failed to significantly (P < 0.001) restore the brain dopamine level to the normal level (Fig. 2A).

Serotonin level   Treatment with PQ induced a significant (P < 0.001) decrease in serotonin level (3.62 ± 0.18 ng/g tissue) in mouse brain homogenate compared to the normal group (29.62 ± 0.88 ng/g tissue). Treatment with L-dopa, L-dopa + CAF, L-dopa + COF, R, R + CAF or R + COF exhibited significant (P < 0.001) increase in brain serotonin content by 2.7-, 6.7-, 3.8-, 1.9-, 5.4- and 3.0-fold, respectively, compared to PQ-treated animals. All the test groups did not restore the serotonin level to normal values. Moreover, treatment with L-dopa + CAF (24.17 ± 0.56 ng/g tissue) significantly increased (P < 0.001) the brain serotonin level compared to L-dopa (9.85 ± 0.35 ng/g tissue) or L-dopa + COF (13.78 ± 0.5 ng/g tissue). In a similar manner, treatment with R + CAF significantly increased (P < 0.001) the brain serotonin level compared to R or R + COF. In addition, treatment with L-dopa, L-dopa + CAF and L-dopa + COF demonstrated significantly higher serotonin level compared to the corresponding groups treated with R (Fig. 2B).

Brain antioxidant level
Nitric oxide brain level   Administration of PQ induced a significant increase (P < 0.001) in the nitric oxide (NO) brain level compared to normal and all treated groups (Figure 3A). Treatment with L-dopa (13.87 ± 0.46 μmole/g tissue), L-dopa + CAF (6.62 ± 0.29 μmole/g tissue) or L-dopa + COF (10.5 ± 0.3 μmole/g tissue) significantly reduced (P < 0.001) the PQ (30.4 ± 1.13 μmole/g tissue) mediated significant increase in NO brain level by 54, 78 and 65%, respectively. However, this reduction did not reach the normal nitric oxide brain level. Interestingly, treatment with L-dopa + CAF demonstrated significant reduction (P < 0.001) in brain nitric oxide level than the treatment with either L-dopa or L-dopa + COF. Treatment with R, R + CAF or R + COF significantly decreased (P < 0.001) NO brain level by 28, 71 and 58%, respectively, compared to PQ group. In a similar manner, R + CAF

Aboutabl *et al. Bulletin of the National Research Centre* (2021) 45:219

Page 7 of 20

Fig. 2 Effect of each L-dopa and rasagiline (R) alone or in combination with caffeine (CAF) or coffee (COF) on brain neurotransmitters in paraquat (PQ)-treated mice. **A** Brain dopamine level, **B** Brain serotonin level. Data are presented as mean ± S.E.M. Differences between groups were statistically analyzed using a one-way analysis of variance followed by a Student–Newman–Keuls post hoc comparison. *Significant from normal group *P* < 0.05. #Significant from PQ group *P* < 0.05. Lines represent significance *P* < 0.05

(8.74 ± 0.25 μmole/g tissue) significantly decreased (P < 0.001) NO brain level compared to R (21.98 ± 1.05 μmole/g tissue) or R + COF (12.82 ± 0.4 μmole/g tissue). Moreover, treatment with L-dopa, L-dopa + CAF and L-dopa + COF demonstrated significant reduction in NO brain level compared to R (P < 0.001), R + CAF (P = 0.023) or R + COF (P = 0.018), respectively (Fig. 3A).

Superoxide dismutase brain level PQ administration induced a significant decrease by 69% in mouse SOD brain level compared to the normal group. Treatment with L-dopa, L-dopa + CAF, L-dopa + COF, R, R + CAF or R + COF exhibited significant increase (P < 0.001) in

brain SOD content by 2.2-, 2.8-, 2.6-, 2.9-, 3.1- and 3.0-fold, respectively, compared to PQ-treated mice. All treatments did not restore the SOD level to the normal level. Moreover, treatment with L-dopa + CAF (761.44 ± 8.43 U/g tissue) significantly increased (P < 0.001) brain SOD level compared to L-dopa (618.86 ± 6.61 U/g tissue) and L-dopa + COF (716.58 ± 5.02 U/g tissue) by 18.7 and 5.9%, respectively. Similarly, treatment with R + CAF (858.34 ± 8.19 U/g tissue) significantly increased the brain SOD level by 8.2% (P < 0.001) and 4.1% (P = 0.005) compared to R (788.15 ± 4.39 U/g tissue) and R + COF (823.38 ± 5.75 U/g tissue), respectively. In addition, treatment with R, R + CAF or R + COF demonstrated significantly elevated (P < 0.001) SOD level compared to the corresponding groups: L-dopa, L-dopa + CAF, L-dopa + COF, respectively (Fig. 3B).

Malondialdehyde brain level Mice administered PQ induced a significant (P < 0.001) increase in MDA brain level (10.88 ± 1.13 nmole/g tissue) by 186% compared to the normal level (3.8 ± 0.13 nmole/g tissue). Treatment with L-dopa, L-dopa + CAF, L-dopa + COF, R, R + CAF or R + COF, normalized the MDA brain level as they significantly decreased (P < 0.001) its level by 60, 67, 60, 67, 78 and 74%, respectively, compared to PQ treatment (Fig. 3C).

Histopathological examination
Haematoxylin and eosin stained tissue sections
Sections of normal mice brain showed pyramidal neuronal cells with abundant pigment within cytoplasm together with other non-pigmented neuronal cells with average overall cellularity. Organized cellular Purkinje cell layer with abundant cells was seen within white matter. Cerebellar tissue showed organized cellular granular cell layer in between molecular layer and white matter (Fig. 4A–D).

Administration of PQ caused neurodegenerative changes in the brain tissue, consisting mainly of severe neuronal loss and marked depletion of pigmented neuronal cells as compared to normal (Table 1). The few surviving neuronal cells showed depletion of melanin content within cytoplasm as well as irregularity in size and shape, with irregular contours and deeply basophilic, hyperchromatic nuclei. Several pyknotic nuclei were noticed. Astrogliosis and microgliosis were seen as well. Vacuolar degeneration was seen, being prominent within the background material. Disorganization of Purkinje cell layer was observed (Fig. 5A–D). All treatments showed improvement as compared to PQ; cellularity being restored, neuronal cells increased, pigmented pyramidal neuronal cells increased as well. In addition,
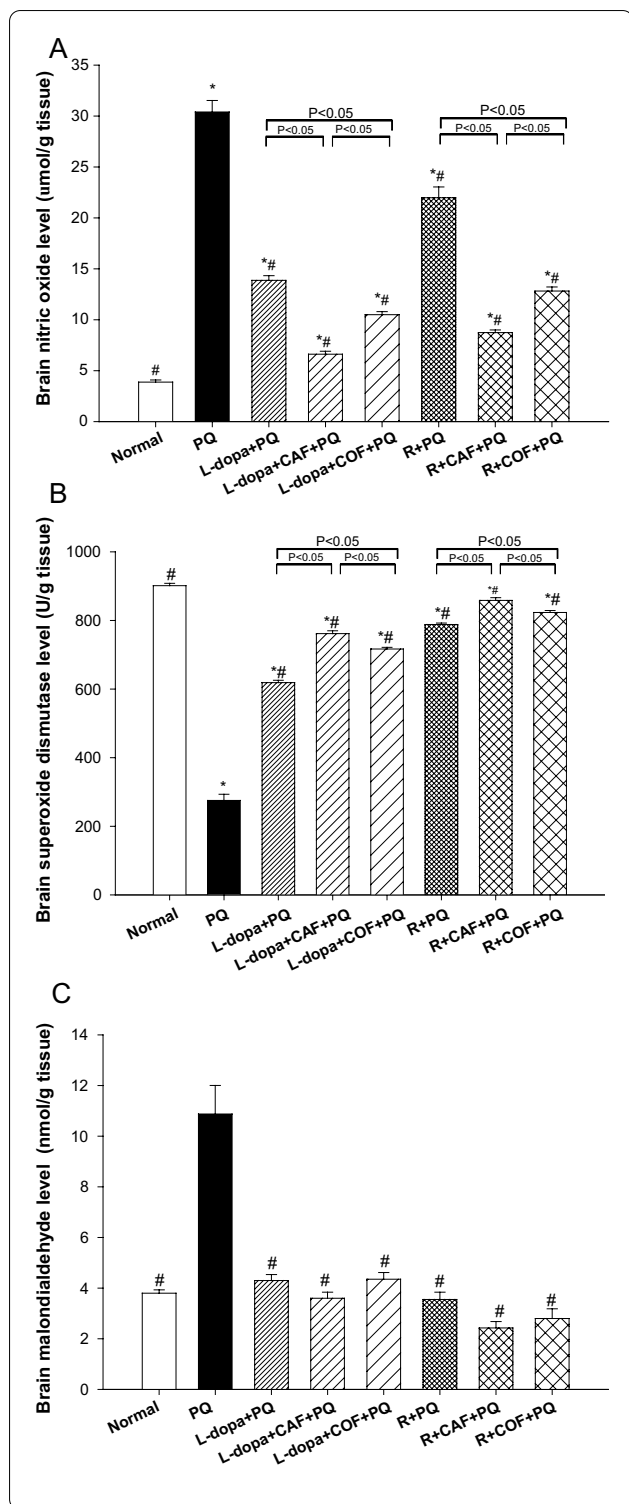
Aboutabl *et al. Bulletin of the National Research Centre*   (2021) 45:219

Page 8 of 20

A

B

C

Neuronal cells became evenly distributed throughout the brain tissue. Pyknosis within nuclei diminished. Irregularity in shape and size of neuronal cells diminished (Figs. 6, 7, 8, 9 and 10; Table 1). The most prominent improvement was in the following descending order: mice administrated PQ and treated with L-dopa + CAF (Fig. 7A–D) > L-dopa + COF (Fig. 8A–D) > L-dopa (Fig. 6A, B) > R + CAF (Fig. 9A–D) > R + COF (Fig. 10A–D) > R (Fig. 6C, D).

### Morphometric image analysis on hematoxylin and eosin stained tissue sections

Results of the morphometric image analysis on hematoxylin- and eosin-stained tissue sections are shown in Table 1. A significant decrease (P < 0.001) in the mean area percentage of neuronal cells and the pigmented neuronal cells in paraquat-treated mice was observed by 76.47% and 83.87%, respectively, as compared to normal mice. Treatment with L-dopa + CAF, L-dopa + COF, L-dopa and R + CAF in PQ-induced brain neurodegenerative mice showed an increase in the mean area percentage of neuronal cells by 266.67%, 75%, 116.67% and 8.33%, respectively, compared to paraquat; this increase is significant (P < 0.001) in the treatment with L-dopa + CAF. In addition, treatment with L-dopa + CAF, L-dopa + COF, L-dopa, R + CAF, R + COF and R demonstrated an increase in the mean area percentage of pigmented neurons by 440%, 380%, 820%, 220%, 160% and 140%, respectively, compared to PQ; this increase is significant in the treatment with L-dopa + CAF (P = 0.002), L-dopa + COF (P = 0.008) and L-dopa (P < 0.001).

Immunohistochemical analysis

GFAP (immunohistochemistry and morphometric study) The mean area percentage of GFAP+ve small normal neuronal cells in normal mice brain was 3.44 ± 0.48% (Table 2; Fig. 11A). Astrogliosis was demonstrated in brain of PQ-induced neurodegenerative mice group with significant increase (P < 0.001) in GFAP+ve large neuronal cells showing numerous branching processes (Table 2; Fig. 11B) reaching 123.26% compared to normal mice. GFAP+ve astrocytes showed signifi-

melanin pigment within the neuronal cells also increased. Astrogliosis as well as microgliosis diminished. Vacuolar degeneration within background material also diminished. Purkinje cell layer organization was restored.
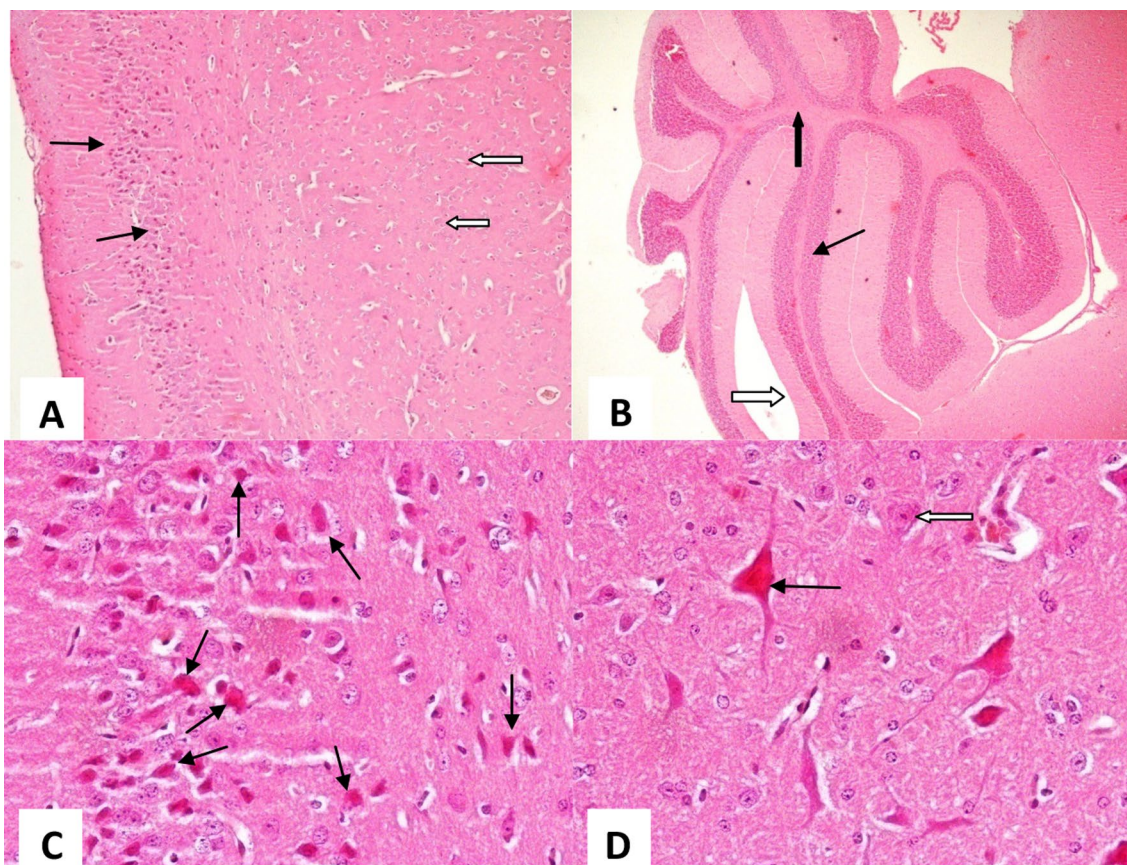
Fig. 4 **A** Brain tissue of normal mice showing cellular Purkinje cell layer (black arrows) and abundant cells within white matter (white arrows) (H&E X100). **B** Cerebellar tissue of normal mice showing granular cell layer (thin black arrow) in between molecular layer (white arrow) and white matter (thick black arrow) (H&E X100). **C** Brain tissue of normal mice showing numerous pigmented pyramidal neuronal cells (black arrows) (H&E X400). **D** Brain tissue of normal mice showing pyramidal neuronal cells with abundant pigment within cytoplasm (black arrow), together with other non-pigmented neuronal cells (white arrow) (H&E X400)

Table 1 Effect of each of ʟ-dopa and rasagiline (R) alone or in combination with caffeine (CAF) or coffee (COF) on mean area percentage of neuronal cells and pigmented neurons as well as mean optical density of pigmented neurons in brain tissue sections in paraquat (PQ)-treated mice

Groups	Mean area percentage		Mean optical density Pigmented neurons
	Neuronal cells	**Pigmented neurons**	
Control	0.51 ± 0.06	0.31 ± 0.04	99.41 ± 4.80
Paraquat	$0.12 \pm 0.02^*$	$0.05 \pm 0.01^*$	$24.57 \pm 1.21^*$
ʟ-Dopa	$0.26 \pm 0.03^*$	$0.46 \pm 0.07^{*\#}$	$127.83 \pm 0.91^{*\#}$
ʟ-Dopa + CAF	$0.44 \pm 0.10^{\#\$}$	$0.27 \pm 0.04^{\#\$}$	$112.21 \pm 5.29^{*\#\$}$
ʟ-Dopa + COF	$0.21 \pm 0.01^{*€}$	$0.24 \pm 0.02^{\#\$}$	$116.87 \pm 3.25^{*\#}$
R	$0.09 \pm 0.03^*$	$0.12 \pm 0.02^*$	$166.05 \pm 7.42^{*\#}$
R + CAF	$0.13 \pm 0.02^*$	$0.16 \pm 0.02^*$	$135.83 \pm 3.51^{*\#\&}$
R + COF	$0.12 \pm 0.02^*$	$0.13 \pm 0.02^*$	$142.35 \pm 3.62^{*\#\&}$

Values are presented as mean ± S.E.M. *Significant from normal group $P < 0.05$. #Significant from paraquat group $P < 0.05$. $Significant from ʟ-dopa group $P < 0.05$. €Significant from ʟ-dopa + CAF group $P < 0.05$. &Significant from R group $P < 0.05$
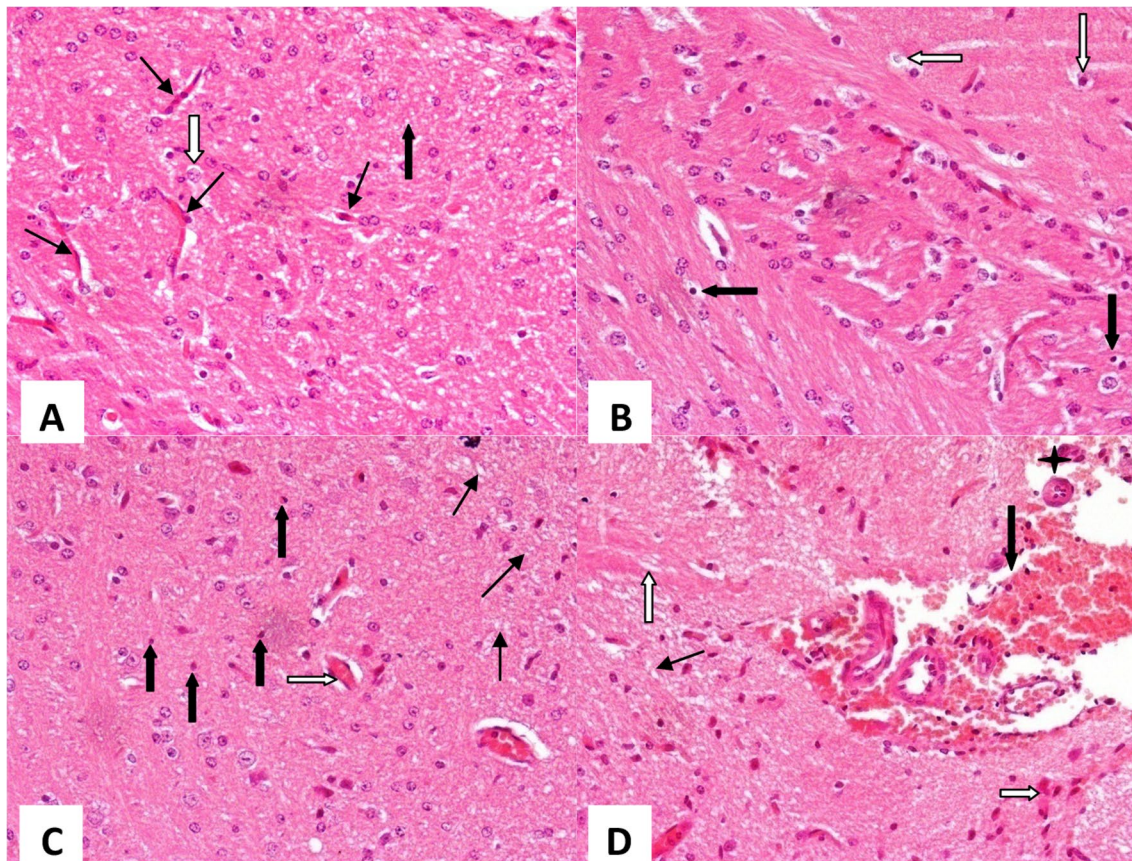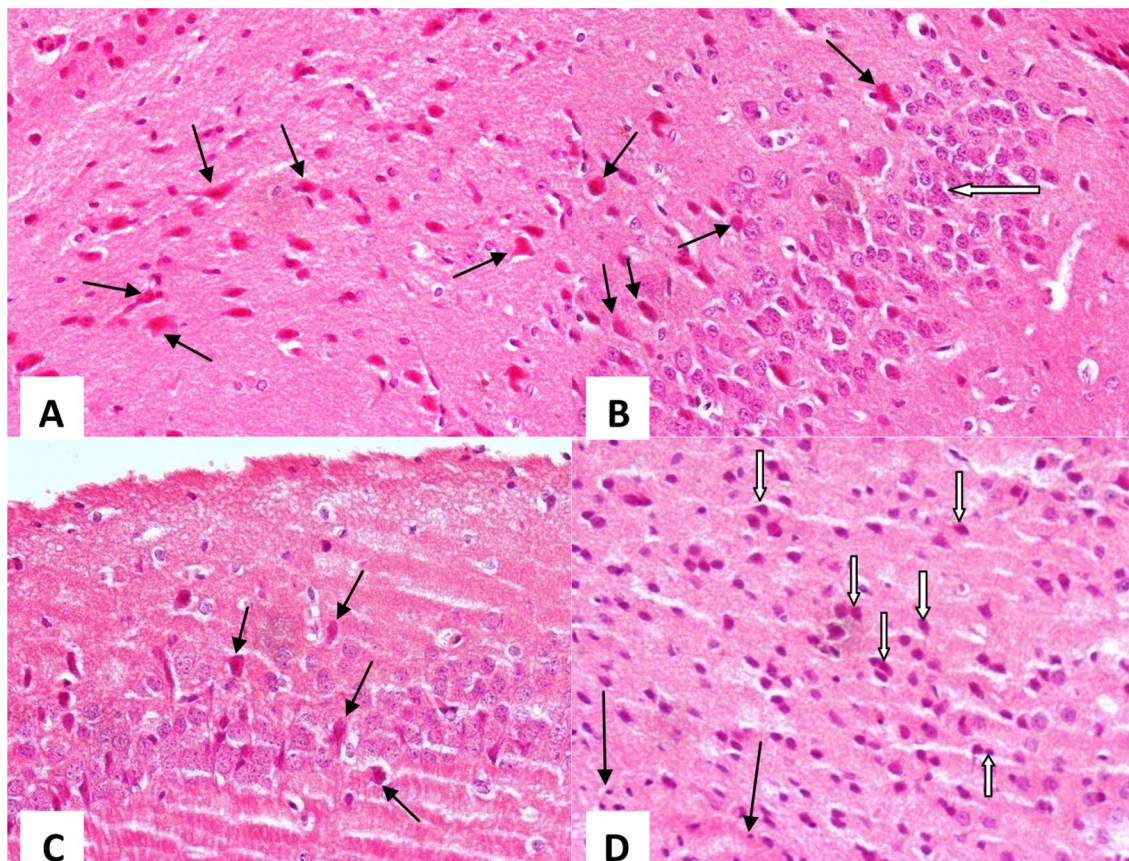
Fig. 5 **A** Brain tissue of mice treated with PQ showing scattered oligodendroglial cells (white arrow) amidst vacuolated degenerated background (thick black arrow). Scattered blood vessels were seen (thin black arrows) with absence of pigmented neuronal cells. **B** Brain tissue of mice treated with PQ showing scattered oligodendroglial cells (white arrows) with absence of pigmented neuronal cells. Pyknotic nuclei were seen (thick black arrows). **C** Brain tissue of mice treated with PQ showing hypocellularity and absence of pigmented neuronal cells. Several pyknotic nuclei were seen (thick black arrows). Vacuolated, degenerated background was noticed (thin black arrows) with ordinary blood vessels (white arrow). **D** Brain tissue of mice treated with PQ showing degenerated, vacuolated background with congestion (thick black arrow), thick walled blood vessels (star) and attempts at gliosis (white arrow) (H&E X400)

cant decrease in ʟ-dopa + CAF (Fig. 11C), ʟ-dopa + COF (Fig. 11D), ʟ-dopa (Fig. 11E), R + CAF (Fig. 11F), R + COF (Fig. 11G) and R (Fig. 11H) by 55.86% (P = 0.002), 57.68% (P = 0.002), 58.72% (P = 0.003), 60.81% (P = 0.003), 69.40% (P < 0.001) and 73.83% (P < 0.001), respectively, compared to PQ group. Treatment with ʟ-dopa + CAF, ʟ-dopa + COF, ʟ-dopa, R + CAF, R + COF and R demonstrated significant reduction in the mean optical density of GFAP+ve astrocytes in brain tissue sections by 25.96% (P < 0.001), 19.87% (P < 0.001), 12.97% (P < 0.001), 8.42% (P < 0.001) and 4.23% (P = 0.015), respectively, compared to PQ (Table 2).

Discussion

The core therapeutic strategies in the developments PD treatments have mainly focused on: (1) improvement of DA replacement therapies for better management or prevention of the onset of motor complications and (2) discovery of compounds that could modify the course of neurodegeneration. Remarkably, the manipulation of adenosine neurotransmission is considered one of the most valuable therapeutic approaches aimed at addressing these objectives (Simola et al. 2014). Therefore, the current study examined the possible preventive effect of co-administration of caffeine or coffee with ʟ-dopa or rasagiline in paraquat-induced motor behavior alterations and neurochemical disturbances. The present findings demonstrated that combined administration of coffee and caffeine with ʟ-dopa and rasagiline produced

Aboutabl *et al. Bulletin of the National Research Centre*   (2021) 45:219

Page 11 of 20

Fig. 6 A Brain tissue of mice treated with (L-dopa + PQ) showing restoration of cellularity with numerous neuronal pyramidal cells showing brownish pigment within cytoplasm (black arrows). **B** Brain tissue of mice treated with (L-dopa + PQ) showing restored cellular Purkinje cell layer (white arrow) with numerous pigmented neuronal cells (black arrows) (H&E X 400). **C** Brain tissue of mice treated with (R + PQ) showing cellular, restored Purkinje cell layer with several prominent pigmented neuronal cells (black arrows). **D** Brain tissue of mice treated with (R + PQ) showing dense cellularity, scattered cells appeared with irregular contours and deeply basophilic, hyperchromatic nuclei (white arrows). Still, some pyknotic nuclei were seen (thin black arrows) (H&E X400)

synergistic effects that targeted the brain dopaminergic system resulting in re-balancing of behavior response, neurotransmitters and antioxidant parameters in mouse brain as well as a protective effect from histopathological alterations and astrogliosis, causing their amelioration, as demonstrated by histopathological, GFAP immunohistochemical and image analysis morphometric studies.

In the present work, mice were exposed to PQ which provides a valuable model for understanding the mechanisms of toxicant-induced dopaminergic cell injury and neurodegeneration mainly through increased oxidative stress, synuclein aggregation, mitochondrial dysfunction, α-excitotoxicity and autophagy (Colle and Farina 2021; McCormack and Di Monte 2003). Previous studies reported that the peak concentration of PQ in plasma and brain (Naylor et al. 1995; Widdowson et al. 1996) as well as reduction in the exploratory behavior (motor behavioral activities) of animals was achieved 30 min post-PQ

injection (Chanyachukul et al. 2004). Our results demonstrated that PQ significantly resulted in motor impairment, increased immobility time and number of sinking in the forced swim test. In agreement with our results, single subcutaneous systemic injection of PQ (5, 10 and 20 mg/kg) induced significant reductions in locomotive, stereotypic and rotational behavioral activities as compared to control, 30 min after PQ administration. Moreover, this reduction was reversed on day 3 post-single PQ injection and approached control level over 30-day observation period (Chanyachukul et al. 2004).

Impairment in motor activity is usually accompanied by neurochemical alterations, where a good correlation between the reduction in striatal dopamine levels and rotational behavior was shown 30 min after PQ injection (Chanyachukul et al. 2004). The mechanism of PQ-induced histopathological changes in mice brain involves: oxidative stress and metabolic processes

Aboutabl *et al. Bulletin of the National Research Centre*   (2021) 45:219

Page 12 of 20
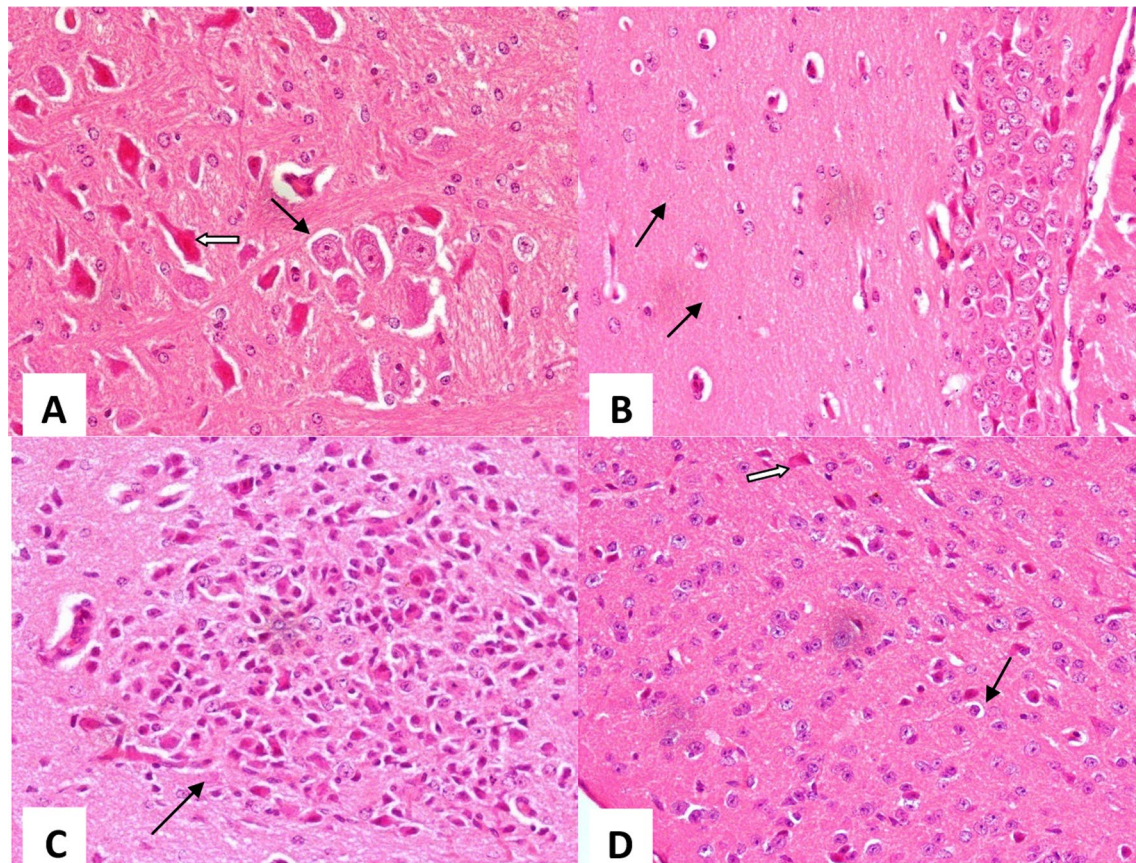
Fig. 7 A Brain tissue of mice treated with (L-dopa + CAF + PQ) showing restored cellularity with several neuronal cells, non-pigmented with large rounded nuclei and prominent nucleoli (black arrow). Adjacent pyramidal pigmented neuronal cells were seen as well (white arrow). **B** Brain tissue of mice treated with (L-dopa + CAF + PQ) showing organized, cellular Purkinje cell layer with adjacent tissues showing marked improvement with no vacuolar degeneration (black arrows). **C** Brain tissue of mice treated with (L-dopa + CAF + PQ) showing prominent restored cellularity (black arrow). **D** Brain tissue of mice treated with (L-dopa + CAF + PQ) showing cellular tissue, with several oligodendroglial (black arrow) and pigmented neuronal cells (white arrow) together with markedly improved background with minimal residual vacuolar degeneration (H&E X400)

of PQ-inducing excitotoxicity. Also, autophagy, development of α-synuclein aggregates, dopamine catabolism variation and tyrosine hydroxylase inactivation are considered as reasons for dopaminergic cells loss (Zhang et al. 2016). In the current study, PQ significantly decreased brain neurotransmitters level: dopamine and serotonin which was confirmed by the histopathological results which revealed histopathological changes including neuronal loss and depletion of pigmented neuronal cells in addition to astrogliosis, as compared to normal, demonstrated by morphometric and immunohistochemical results. These results are in agreement with previous finding that reported that treatment of mice with PQ resulted in loss of dopaminergic neurons in the SNc which is the primary neurodegenerative feature of PD (McCormack et al. 2002). Moreover, a significant decrease in dopamine, dopamine metabolites and norepinephrine content in some brain areas was determined

30 min after a single systemic injection of PQ (20 mg/kg, *s.c.*). Treatment with PQ 10–20 mg/kg reduced striatal dopamine content to 74–82%, suggesting that PQ may preferentially target dopaminergic neurons rather than other neurons in the same deeper area (Chanyachukul et al. 2004).

PQ is a potent free radical generator actively accumulated in various tissues as lung and brain (Awadalla 2012). Our results showed that PQ significantly altered the brain antioxidant levels of NO, SOD and DA (oxidative stress biomarkers). The significant increase in brain NO level in PQ-treated mice could lead to the formation of peroxynitrite which is a potent oxidant that can severely damage several biological targets (Ugusman et al. 2019). Over production of NO leads to neurotoxicity by several mechanisms that involve excitotoxicity, DNA damage or post-translational modification of proteins (Zhang et al. 2006). Increased oxidative stress and
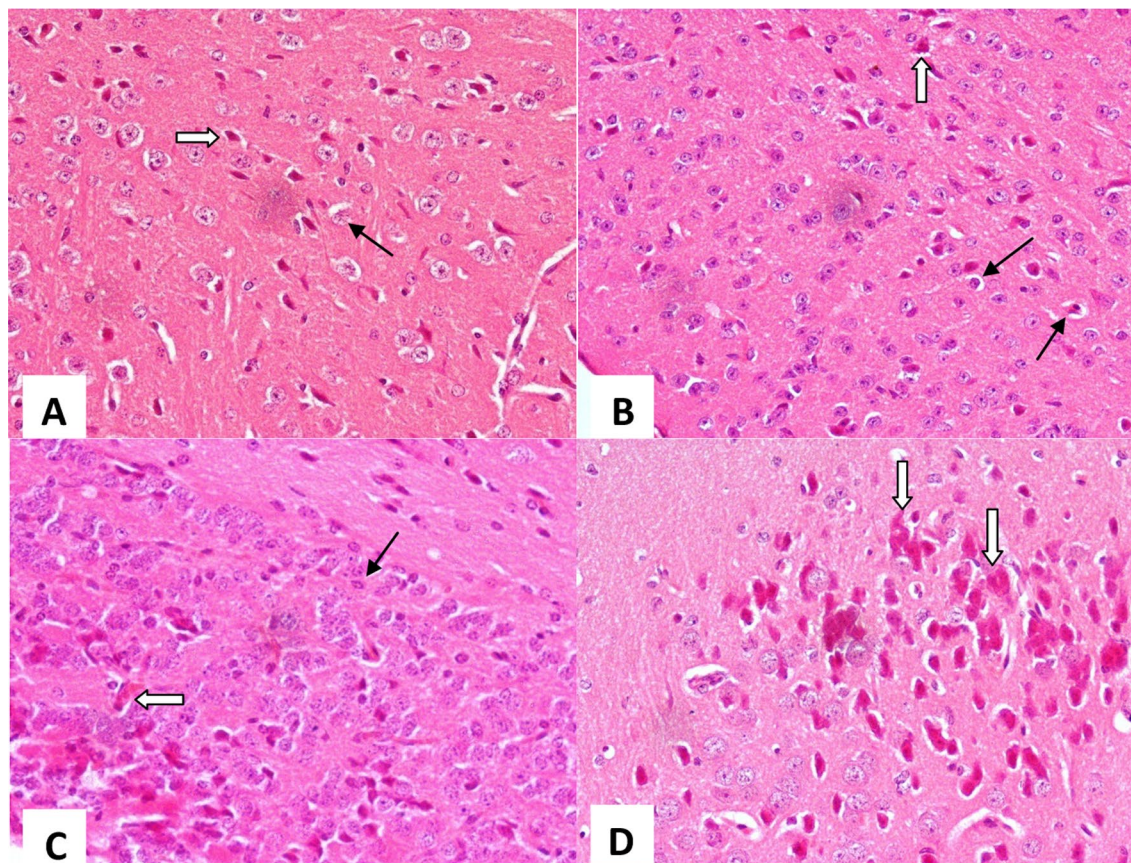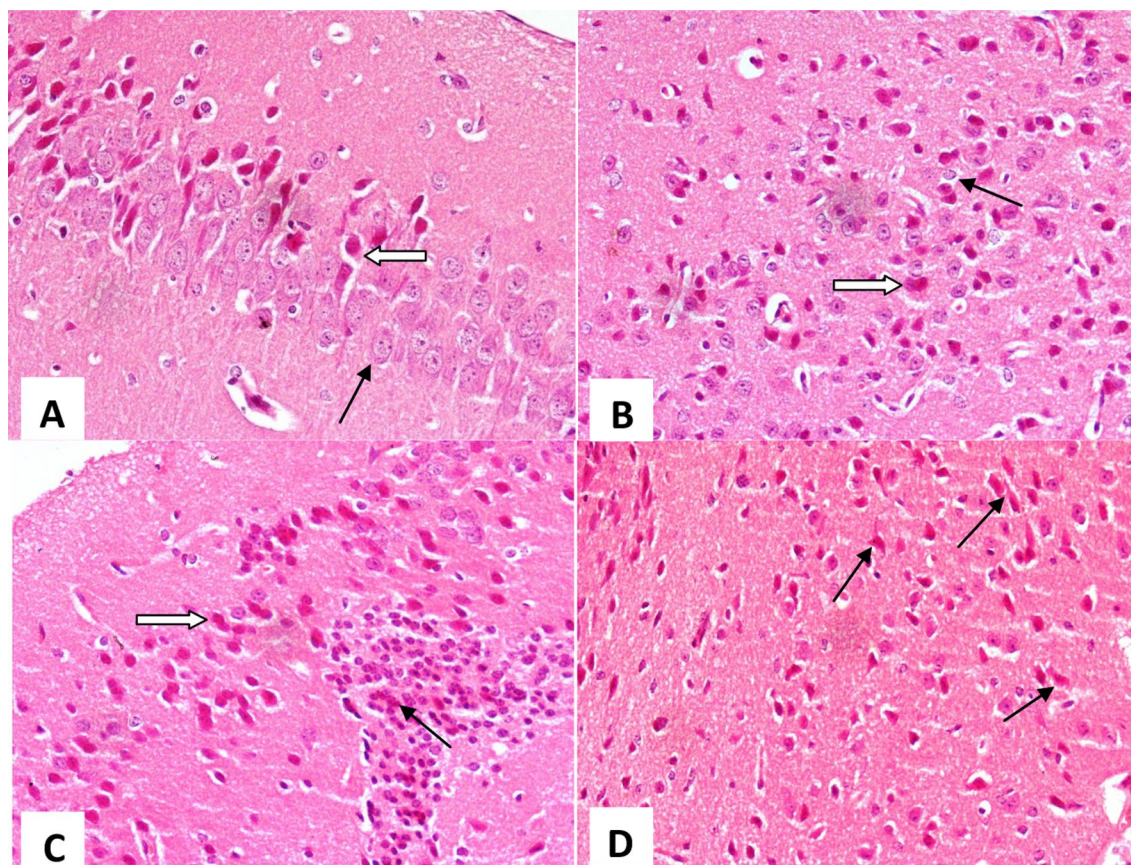
Fig. 8 **A** Brain tissue of mice treated with (ʟ-dopa + COF + PQ) showing numerous pigmented pyramidal neuronal cells (white arrow) among several other oligodendroglial cells (black arrow). **B** Brain tissue of mice treated with (L + COF + PQ) showing restored prominent cellularity with numerous oligodendroglial cells (black arrows) with scattered pigmented pyramidal neuronal cells (white arrow). **C** Brain tissue of mice treated with (ʟ-dopa + COF + PQ) showing cellular, organized Purkinje cell layer (black arrow) with scattered pigmented neuronal cells (white arrow). **D** Brain tissue of mice treated with (ʟ-dopa + COF + PQ) showing restored marked pigmentation within numerous neuronal pyramidal pigmented cells (white arrows) (H&E X400)

nitric oxide synthase (NOS)-mediated nitric oxide production were found to play a role in microglial activation. Several signaling pathways such as tyrosine kinase pathway, p38 mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-kB) were reported to be involved in iNOS-mediated nitrite oxide production and neuronal injury (Gupta et al. 2010).

In addition, our study demonstrated that PQ significantly reduced the brain antioxidant level of SOD which is reported to play an important role in the protection against oxidative toxicity as it catalyzes the dismutations of superoxide anion free radicals to less toxic forms that involves hydrogen peroxide and molecular oxygen (Ighodaro and Akinloye 2018). During PQ exposure, large amounts of superoxide ion are generated due to redox cycling of the herbicide. NO reacts faster with superoxide ion $O_2^{\bullet-}$ than any other known biological molecule and is produced in high enough concentrations to outcompete endogenous levels of SOD. Once ONOO⁻ is formed, it can chemically change nucleic acids, amino acids and thiol-containing proteins and peptides (Moran et al. 2010). In agreement with our results, Zeinvand-Losertani et al. (2018) reported that acute administration of PQ significantly decreased SOD activity, which led to the depletion of antioxidant stores in liver tissue and consequently oxidative damage, in addition to decrease in total thiol groups content in liver homogenate and increased hepatic lipid peroxidation (Zeinvand-Lorestani et al. 2018). Moreover, SOD protected against the development of acute PQ toxicity when injected 60 min post-PQ administration (Wasserman and Block 1978). Knowing that PQ belongs to a broad class of redox cycling compounds known to produce ROS, mitochondria are strongly implicated as a major source of paraquat-induced ROS generation in rat brain (Castello
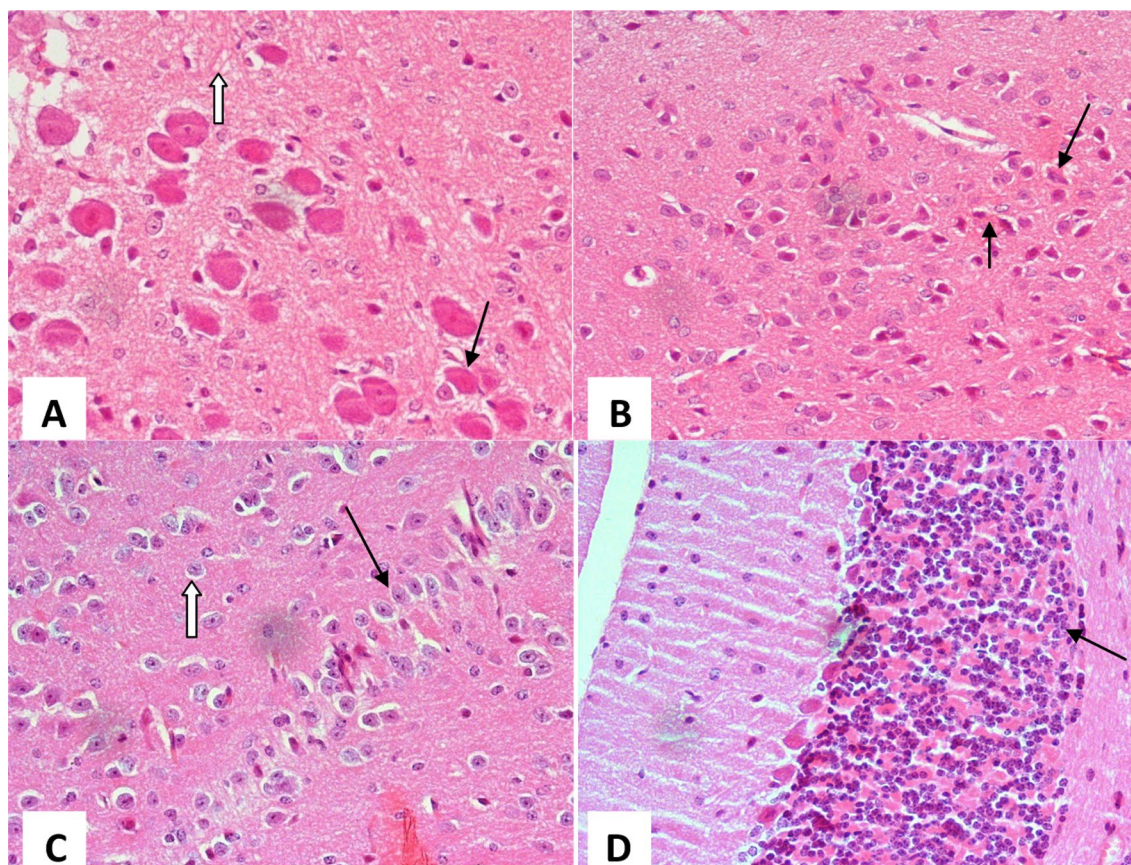
Fig. 9 A Brain tissue of mice treated with (R + CAF + PQ) showing preserved, organized Purkinje cell layer (black arrow) with numerous pigmented pyramidal neuronal cells (white arrow). **B** Brain tissue of mice treated with (R + CAF + PQ) showing restored marked cellularity with numerous pigmented neuronal cells (white arrow) intermixed with several oligodendroglial cells (black arrow). **C** Cerebellar tissue of mice treated with (R + CAF + PQ) showing organized, cellular, granular cell layer (black arrow) with adjacent numerous pigmented pyramidal neuronal cells (white arrow). **D** Brain tissue of mice treated with (R + CAF + PQ) showing prominent restored cellularity and marked pigmentation (black arrows) (H&E X400)

et al. 2007). Superoxides generated by mitochondrial dysfunction can amplify NLRP3 inflammasome signaling pathways, which augments the dopaminergic neurodegenerative process (Ikawa et al. 2020; Sarkar et al. 2017). In addition, induction of oxidative stress affects the astrocyte in terms of morphologic and phenotypic changes that can lead to astrogliosis mostly identified by increased production of GFAP (Li and Stary 2016).

Restoration of neurochemical parameters was noticed and documented histologically when using treatment with each of L-dopa, R, CAF and COF in the brain of mice exhibiting PQ histopathological changes. The present study showed that treatment with L-dopa significantly improved the behavioral response, brain DA and ST, the brain antioxidant parameters and improved the histopathological features in PQ-induced neurochemical disturbances and motor behavior impairment in mice. This could be attributed to the capability of L-dopa in

preventing PQ neurotoxicity possibly by acting as competitive uptake inhibitors and thus limiting its access into the CNS (Shimizu et al. 2001). Pretreatment with L-dopa, which is transported across the blood brain barrier through the same neutral amino acid carrier (system L carrier) as PQ, demonstrated a neuroprotective effect when administered 30 min prior to PQ via reducing the ROS, hydroxyl radical HO· content in the striatal tissue and reduced the accumulation of PQ in midbrain sections of mice as indicated by the lack of paraquat immunoreactivity in this part of the brain thus modifying the effects of toxicants targeting the nigrostriatal system (Ghosh et al. 2018; McCormack and Di Monte 2003; Rai and Singh 2020). On the other hand, in vitro studies of L-dopa using physiologically relevant concentrations showed certain antioxidant properties combined with potentially cytotoxic pro-oxidant characteristics. Also, L-dopa can undergo auto-oxidation, producing

Aboutabl *et al. Bulletin of the National Research Centre* (2021) 45:219

Page 15 of 20

Fig. 10 **A** Brain tissue of mice treated with (R + COF + PQ) showing prominent cellularity with numerous pyramidal pigmented neuronal cells (black arrow); however, still, residual vacuolar degeneration was observed in the background (white arrow). **B** Brain tissue of mice treated with (R + COF + PQ) showing restored cellularity; however, pigmented pyramidal neuronal cells still showed some changes with irregular contour (black arrows). **C** Brain tissue of mice treated with (R + COF + PQ) showing organized, yet hypocellular Purkinje cell layer (black arrow) with adjacent numerous oligodendroglial cells (white arrow). **D** Brain cerebellar tissue of mice treated with (R + COF + PQ) showing preserved, organized, cellular, granular layer (black arrow) (H&E X400)
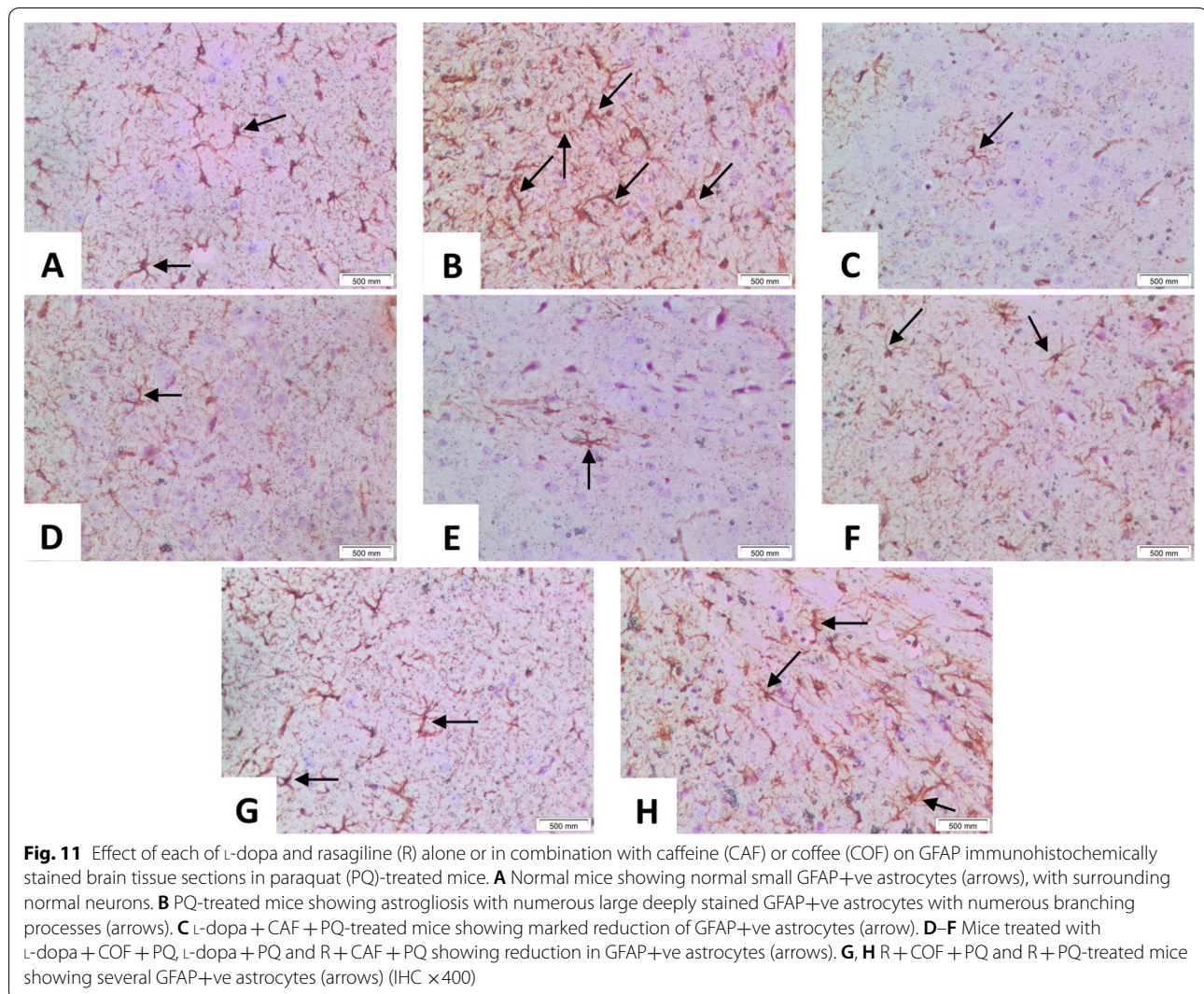
Table 2 Effect of each of ʟ-dopa and rasagiline (R) alone or in combination with caffeine (CAF) or coffee (COF) on mean area percentage and mean optical density of GFAP+ve astrocytes in brain tissue sections in paraquat (PQ)-treated mice

Groups	Mean area percentage	Mean optical density
Control	3.44 ± 0.48	69.94 ± 1.26
Paraquat	$7.68 \pm 1.52^{*}$	$96.07 \pm 0.26^{*}$
ʟ-dopa	$3.17 \pm 0.23^{\#}$	$83.61 \pm 0.81^{*\#}$
ʟ-dopa + CAF	$3.39 \pm 0.34^{\#}$	$71.13 \pm 1.67^{\#\$}$
ʟ-dopa + COF	$3.25 \pm 0.91^{\#}$	$76.98 \pm 1.30^{*\#\$\epsilon}$
R	$2.01 \pm 0.59^{\#}$	$95.28 \pm 0.51^{*}$
R + CAF	$3.01 \pm 0.31^{\#}$	$87.98 \pm 0.58^{*\#\&}$
R + COF	$2.35 \pm 1.00^{\#}$	$92.01 \pm 0.43^{*\#\&\pi}$

Values are presented as mean ± S.E.M. *Significant from normal group $P < 0.05$. #Significant from paraquat group $P < 0.05$. $Significant from ʟ-dopa group $P < 0.05$. €Significant from ʟ-dopa + CAF group $P < 0.05$. &Significant from R group $P < 0.05$. πSignificant from R + CAF group $P < 0.05$

the neurotoxin, 6-hydroxydopamine (6-OHDA), dopamine oxidation product, and toxic quinones and several ROS, such as hydroxyl radicals (Borah and Mohanakumar 2010). Our histopathological results revealed the improvement caused by treatment with ʟ-dopa in the form of restoration of cellularity with numerous neuronal pyramidal cells showing brownish pigment within cytoplasm as well as restored cellular Purkinje cell layer. Shin el al., 2009 agreed with our results declaring that ʟ-dopa showed a neuroprotective effect elaborated immunohistochemically in MPTP-treated mice by the marked elevation in the number of tyrosine hydroxylase immunoreactive and Nissl-stained cells (Shin et al. 2009). In addition, treatment with ʟ-dopa in rotenone-induced striatal histopathological alterations showed that some neurons regain their normal size (Badawi et al. 2020).

In the present study, treatment with rasagiline normalized the locomotor activity and immobility time and

Fig. 11 Effect of each of L-dopa and rasagiline (R) alone or in combination with caffeine (CAF) or coffee (COF) on GFAP immunohistochemically stained brain tissue sections in paraquat (PQ)-treated mice. **A** Normal mice showing normal small GFAP+ve astrocytes (arrows), with surrounding normal neurons. **B** PQ-treated mice showing astrogliosis with numerous large deeply stained GFAP+ve astrocytes with numerous branching processes (arrows). **C** L-dopa+CAF+PQ-treated mice showing marked reduction of GFAP+ve astrocytes (arrow). **D**–**F** Mice treated with L-dopa+COF+PQ, L-dopa+PQ and R+CAF+PQ showing reduction in GFAP+ve astrocytes (arrows). **G**, **H** R+COF+PQ and R+PQ-treated mice showing several GFAP+ve astrocytes (arrows) (IHC ×400)

significantly lowered number of sink attempts compared to PQ. In addition, rasagiline significantly increased brain DA, ST and SOD, but decreased NO and MDA levels compared to PQ. In agreement with the present results, rasagiline prevented neurodegradation in the brain striatum in mice (Baranyi et al. 2016). This was also documented by the histopathological results of brain tissue of mice receiving PQ and treated with rasagiline which showed cellular restored Purkinje cell layer with several prominent pigmented neuronal cells and dense cellularity, which was also confirmed by morphometric study. In agreement with our results, treatment with rasagiline in rotenone-induced histopathological alteration mouse model showed improvement in the form of moderate increase in neuronal size and number (Badawi et al. 2020).

The neuroprotective mechanism of rasagiline against apoptosis involves reduction in caspase-3 activation, activation of protein kinase c (PKC) α and ε, regulation of the Bcl-2 family proteins and mitochondrial membrane stabilization (Chau et al. 2010). Rasagiline raised the survival of dopaminergic neurons in the substantia nigra and abolished the motor stereotypes behaviors associated with nigrostriatal lesion induced by 6-OHDA (Blandini et al. 2004). Rasagiline inhibited *N*-methyl(R)salsolinol (endogenous dopaminergic neurotoxin), 6-OHDA and peroxynitrite toxin-induced mitochondrial impairment-related apoptosis in human neuroblastoma SH-SY5Y cells (Maruyama et al. 2000). It increased dopaminergic cell survival in the midbrain by up-regulation of the tyrosine kinase receptor (Trk)−phosphatidylinositol 3 (PI3) kinase−Akt pathway when administered chronically to post-MPTP-lesioned mice (Sagi et al. 2007). In addition, treatment with rasagiline was reported to increase ST level and decrease its turnover (Weinreb et al. 2015).

Aboutabl *et al. Bulletin of the National Research Centre*   (2021) 45:219

Page 17 of 20

Rasagiline treatment induced the antioxidant enzymes SOD and catalase in substantia nigra and striatum (Carrillo et al. 2000) and attenuated microglia activation related oxidative stress in vitro (Trudler et al. 2014). In nigral toxicity model induced by paraquat in neuroblastoma cell line, and cells over-expressing wild-type and A53T mutation of alpha-synuclein, rasagiline increased cellular glutathione levels and reduced superoxide generation, suggesting that its protective actions incorporate an antioxidant effect (Chau et al. 2010).

In the current study, the combined treatment of COF or CAF with ʟ-dopa or rasagiline was targeted to investigate the best potential approach in the management PQ-induced neurochemical disturbances and motor behavior impairments. In behavioral tests, rasagiline combination with COF increased the locomotor activity in rotarod test compared to ʟ-dopa+COF. In addition, ʟ-dopa combinations decreased the immobility time in the forced swim test compared to rasagiline combinations, while simultaneous co-administration of ʟ-dopa and caffeine provided similar increase in locomotor activity compared to co-administration of rasagiline and CAF. The administration of rasagiline together with COF or CAF possessed some beneficial effects where it significantly increased the DA and SOD but decreased MDA brain level compared to the respective combination with ʟ-dopa. On the other hand, ʟ-dopa combination with coffee or caffeine significantly increased serotonin and decreased the NO brain levels compared to the respective rasagiline combination.

The neuroprotective effects of caffeine were demonstrated in several in vivo experimental PD models through a variety of mechanisms such as antagonism of A_{2A} receptor and activation of the PI3K/Akt pathways signaling pathway (Ikram et al. 2020; Nakaso et al. 2008). Blocking of A_{2A} receptor by caffeine leads to downregulation of the presynaptic release of excitatory neurotransmitter glutamate through lowering Ca^{2+} influx, thus avoiding excessive calcium releasing-related neurotoxicity (Hong et al. 2020). In addition, inhibition of microglia activation through blocking of A2A receptor by caffeine leads to inhibition of the release of inflammatory cytokines and hence protection from neuroinflammation as well as regulating reactive oxygen species and other factors responsible for neuronal loss (Ikram et al. 2019; Liu et al. 2016).

Consumption of coffee has been associated with reduced risk of developing PD (Xu et al. 2005). In a large prospective study, the role of caffeine in caffeinated coffee was found to be linked with lower PD risk, but not in decaffeinated coffee (Ascherio et al. 2001). An inverse relationship between caffeine and PD risk is possibly due to the direct neuroprotective effect of caffeine. Caffeine could significantly restore the levels of DA and its metabolites in the striatum (Xu et al. 2010) and counteracted the neuroinflammation induced by acute exposure to the dopaminergic neuron toxin MPTP at a dose in mice corresponding to human consumption of a single cup of coffee (Carta et al. 2009).

In a similar manner, caffeine reduced nigrostriatal neuron injury triggered by direct intracerebral infusion of 6-OHDA in rats that was accompanied with restoration of the levels of DA and its metabolites in the striatum (Aguiar et al. 2006). Moreover, in chronic paraquat as well as "dual pesticide" model of PD in mice (PQ and maneb), caffeine attenuated the degradation of DA neurons through significantly reducing the tyrosine hydroxylase immunoreactivity neuron loss (Kachroo et al. 2010). In addition, treatment with caffeine in rotenone PD rat model improved the histopathological alterations in the neurons of the substantia nigra parts compacta (Soliman et al. 2016). Such pharmacological findings came in agreement with the histopathological and GFAP results of the current study, where combined treatment with coffee or caffeine demonstrated prominent improvement in neurodeteriorations caused by PQ compared to treatment with ʟ-dopa or rasagiline alone (Khan et al. 2019; Machado et al. 2020).

Conclusions

The approach of co-administration of coffee or caffeine as A2A receptor antagonists with ʟ-dopa or rasagiline can synergistically affect the histopathological and biochemical changes as well as neurotransmitters in paraquat-induced mice. Therefore, it possesses a new therapeutic strategy for PD management through using caffeine in its pure form or as a diet component. Future studies are needed on the inclusion of caffeine or coffee in diet strategies of patients undergoing PD medications, as well as normal population for future prevention of neurodegenerative diseases.

Abbreviations
PD: Parkinson's disease; PQ: Paraquat; ʟ-Dopa: Levodopa; R: Rasagiline; CAF: Caffeine; COF: Coffee; DA: Dopamine; NO: Nitric oxide; SOD: Super oxide dismutase; MDA: Malondialdehyde; SN: Substantia nigra; ROS: Reactive oxygen species; 6-OHDA: 6-Hydroxydopamine; Trk: Tyrosine kinase receptor; MPTP: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; BG: Basal ganglia; GFAP: Glial fibrillary acidic protein.

Authors' contributions
MA contributed to the design of the experiments, performed the pharmacology part and interpreted the data of the pharmacology section. MA wrote and revised the paper. YM designed the experiments, interpreted the data of the pharmacology section and revised the paper. AS contributed to the design of the experiments and performed the pharmacology part. AG designed and

Aboutabl *et al. Bulletin of the National Research Centre*   (2021) 45:219

Page 18 of 20

performed the histopathological experiments, interpreted the data and wrote and revised the histopathology section. All authors read and approved the final manuscript.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
All animal procedures were performed according to the Ethics Committee of the National Research Center [ethics approval no. 08020] and in accordance with the recommendations for the proper care and use of laboratory animals "Canadian Council on Animal Care Guidelines, 2011."

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Page 19 of 20

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Page 20 of 20

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