



# Potential therapeutic effects of antagonizing adenosine A<sub>2A</sub> receptor, curcumin and niacin in rotenone-induced Parkinson's disease mice model

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## Abstract

Parkinson's disease (PD) is the second common age-related neurodegenerative disease. It is characterized by control loss of voluntary movements control, resting tremor, postural instability, bradykinesia, and rigidity. The aim of the present work is to evaluate curcumin, niacin, dopaminergic and non-dopaminergic drugs in mice model of Parkinson's disease through behavioral, biochemical, genetic and histopathological observations. Mice treated with rotenone rerecorded significant increase in adenosine A<sub>2A</sub> receptor (A<sub>2A</sub>R) gene expression,  $\alpha$  synuclein, acetylcholinesterase (AChE), malondialdehyde (MDA), angiotensin-II (Ang-II), c-reactive protein (CRP), interleukin-6 (IL-6), caspase-3 (Cas-3) and DNA fragmentation levels as compared with the control group. While, significant decrease in dopamine (DA), norepinephrine (NE), serotonin (5-HT), superoxide dismutase (SOD), reduced glutathione (GSH), ATP, succinate and lactate dehydrogenases (SDH & LDH) levels were detected. Treatment with curcumin, niacin, adenosine A<sub>2A</sub>R antagonist; ZM241385 and their combination enhanced the animals' behavior and restored all the selected parameters with variable degrees of improvement. The brain histopathological features of hippocampal and substantia nigra regions confirmed our results. In conclusion, the combination of curcumin, niacin and ZM241385 recorded the most potent treatment effect in Parkinsonism mice followed by ZM241385, as a single treatment. ZM241385 succeeded to antagonize adenosine A<sub>2A</sub> receptor by diminishing its gene expression and ameliorating all biochemical parameters under investigation. The newly investigated agent; ZM241385 has almost the same pattern of improvement as the classical drug; Sinemet®. This could shed the light to the need of detailed studies on ZM241385 for its possible role as a promising treatment against PD. Additionally, food supplements such as curcumin and niacin were effective in Parkinson's disease eradication.

**Keywords** Curcumin · Niacin · Rotenone · Parkinson's disease · ZM241385

## Introduction

Parkinson's disease (PD) is the second neurodegenerative disorder that affects elderly population [1]. The most predominant feature of the disease is motor impairments [2, 3]. The clinical feature of PD includes dopaminergic neuronal loss in substantia nigra region and depleted level of dopamine

in striatum [4]. The pathological evidence of PD is characterized by the presence of Lewy bodies, which consists of insoluble and fibrous aggregates of  $\alpha$ -synuclein with some filaments [5]. PD is also associated with oxidative stress, brain mitochondrial dysfunction, inflammation, and apoptosis [5].

The human brain needs about 20% of total body energy and 25% of glucose utilization. This high-energy consumption is due to the high number of neurons which need to maintain a delicate interplay between energy metabolism, neurotransmission, and plasticity. Disturbances to the energetic balance, to mitochondria quality control or to glial neuron metabolic interaction may lead to brain circuit malfunction or even severe disorders of the CNS [6].

Levodopa (L-dopa) is a precursor of dopamine that reduces some of PD motor symptoms, since it compensates for dopamine-producing cell loss by enhancing dopamine synthesis in the surviving neurons. Levodopa is used to treat motor

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symptoms but, has severe side-effects with its long-term use [7]. Besides it does not stop the neurodegenerative process, the gastrointestinal dysfunctions interferes with its absorption and attenuates its effectiveness [8].

Since most patients are treated with L-dopa, there is a need for complementary therapies that rescue them from such undesirable motor and non-motor symptoms. This encourages researchers to search for potential herbal products with anti-oxidant activity for PD treatment.

Curcumin is a polyphenol compound having antioxidant, anti-inflammatory, anticarcinogenic effect [9–11] and has an effective role against cognitive implications [12]. Moreover, curcumin can cross the blood–brain barrier (BBB), which is essential to exert its therapeutic effect for PD [13].

Niacin (nicotinic acid) is obtained in the diet from a variety of whole and processed foods, in fortified packaged foods and in some vegetable and animal sources [14]. Niacin can play a significant role in enhancing the anti-inflammatory immune responses in humans and animal models [15]. Niacin plays a potential role in endothelial function by improving endothelium-dependent vasodilatation in coronary heart disease patients [16] and in diabetic encephalopathy [17]. Niacin supplementation may influence the course of PD [18] and it may have neuroprotective role in mice-stroke model [19] by promoting vascular remodeling and functional outcome [20].

Adenosine  $A_{2A}$  receptor ( $A_{2A}R$ ) antagonist (4-(2-[7-amino-2-(2-furyl)[1,2,4]-triazolo[2,3-a][1,3,5]triazin-5-yl amino] ethyl) phenol); ZM241385 is one of the non-dopaminergic drugs which enhances motor activity [21]. Therefore, the aim of the present work is to evaluate the natural compounds; curcumin and niacin against rotenone-induced Parkinson's disease in mice with special emphasis on the role of the dopaminergic; Sinemet® and the non-dopaminergic agent; adenosine  $A_{2A}R$  antagonist (ZM241385). The evaluation has been performed through estimation of certain biomarkers as  $\alpha$ -synuclein level and adenosine  $A_{2A}R$  gene expression. The oxidative stress indices; MDA, SOD and GSH were estimated. The neurotransmitter levels; DA, 5-HT, NE and AchE enzyme were evaluated. The inflammatory mediators; IL-6, Ang-II and CRP levels were determined. The work was extended to estimate the apoptotic markers; Cas-3 and DNA fragmentation pattern. Additionally, the energetic indices; ATP, SDH and LDH were carried out. The brain histopathological analysis of hippocampal and substantia nigra regions were also done.

## Materials and methods

### Drugs and chemicals

Rotenone, niacin, curcumin, and ZM 241385 were purchased from Sigma Chemical Company, St. Louis, MO, USA. Sinemet® marketed by Merck and Co. Inc., Whitehouse

station (NJ, USA) (10 mg carbidopa/100 mg levodopa). All chemicals utilized in the existing study were of analytical grade.

### Animal and ethics

Adult male Swiss albino mice weighing 22–27 g were used in the current study. Mice were obtained from the animal house, National Research Centre, Dokki, Giza, Egypt. Mice were adapted for 2 weeks in 12 h light/dark cycle in a well-ventilated cage with free access of water and diet. Animal care during the experiments were carried out in keeping with agreement of the Medical Ethical Committee, National Research Centre, Egypt (Approval no. 16089) and Ethics Committee of Faculty of Pharmacy, Cairo University, Cairo, Egypt (BC 1839). Basic housing requirements and regular inspection of facilities that mandate the control of pain and suffering during the experiment were conducted. Euthanasia was done rapidly and painlessly to be sure that the animals do not suffer at any stage of the experiment. Getting rid of the animals after termination was done rapidly by the aid of the Safety and Health Committee, National Research Centre, Dokki, Giza, Egypt.

### Induction of Parkinsonism and treatments

Rotenone and all treatments were dissolved in a mixture of dimethyl sulfoxide (DMSO) and sun flower oil (1: 9, v/v). Treatments with curcumin, niacin, ZM241385, their combination and Sinemet® were run simultaneously with rotenone injection and continued daily for 12 days.

To induce experimental Parkinsonism, mice were subcutaneously injected with rotenone at a dose of 1.5 mg/kg/48 h, for total of 6 injections [22]. Curcumin was orally administered at a dose of 80 mg/kg/day [23], niacin was orally administered at a dose of 40 mg/kg/day [24], ZM 241385 was intraperitoneal injected at a dose of 3.3 mg/kg/day [21] and Sinemet® was orally administered at a dose of 10 mg levodopa/kg/day [25]. The dose of Sinemet® was adjusted to contain 10 mg levodopa. The co-administration of carbidopa with levodopa allows the use of smaller doses of levodopa and reduced the side effects resulting from the peripheral actions of dopamine by decarboxylation of levodopa in the peripheral tissues [7, 8].

### Experimental design

After 2 weeks of acclimatization period, the animals were randomly divided into 7 groups ( $n=6$ ). Group 1 served as control and received subcutaneously vehicle of 20  $\mu$ l DMSO/ml sun flower oil (1:9 v/v). Group 2 was subcutaneously injected with rotenone. Groups 3–7 were mice injected with rotenone and administered simultaneously with oral

curcumin, niacin, intraperitoneal ZM 241385, their combination and oral Sinemet®, daily for 12 days, respectively. Forty-eight hours after the last administration, animals were anesthetized by diethyl ether inhalation and sacrificed by decapitation.

### Behavioral study

Daily observation of animals was done for follow-up the development of PD symptoms as bradykinesia, and rigidity. Mice were further quantified by wire hanging test, where mice were suspended by its forelimbs on a metal rod (40 cm length and 0.50 cm in diameter) located approximately 50 cm above the surface. The time the animal remains on the rod (maximum 60 s) was recorded [26].

### Blood and tissue samples

Blood samples were drawn from the retro-orbital plexus into dry test tubes and centrifuged at 300×g for 15 min to separate serum, which was kept at –80 °C for the assays of IL-6, Ang-II and CRP levels.

The whole brain was detached immediately after decapitation, washed with ice-cold isotonic saline, the surface wash solution was removed from the brain with an absorbent paper, weighed, homogenized in 50 mM phosphate buffer (pH 7.4) and centrifuge at 300×g for 10 min at 4 °C. The supernatants were separated and stored at –80 °C for the assays of adenosine A<sub>2A</sub>R gene expression,  $\alpha$ -synuclein, AchE, Cas-3, NE, DA, 5-HT, GSH, MDA, SOD, LDH, SDH, ATP and DNA fragmentation levels.

### Evaluation of Parkinson's disease markers

For adenosine A<sub>2A</sub> receptor gene expression, brain total RNA was isolated and purified by TRIzol® reagent extraction method (cat#15596-026, Invitrogen, Germany). According to manufacturer's instructions, the purity of total RNA was assessed by the 260/280 nm ratio (between 1.8 and 2.1). The integrity was assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis. Aliquots were used for reverse transcription (RT). The complete Poly(A)+ RNA isolated from brain tissues was reverse transcribed into cDNA using RevertAid™ first-strand cDNA synthesis kit (MBI Fermentas, Germany). DNA amplification was done through quantitative real-time polymerase chain reaction (qRT-PCR). PCR (Veriti™ 96-Well Thermal Cycler, Applied Biosystems™, CA, USA) reactions were done using SYBR® Premix Ex Taq™ (TaKaRa, Biotech. Co. Ltd., Germany). The quantitative values of RT-PCR (qRT-PCR) of adenosine A<sub>2A</sub>R (Adora2a-F: 5'-cat catcgtggggctctttg-3', Adora2a-R:

5'-gaactcccg gat cctgta gg-3', NCBI Reference Sequence: NM-009630.3) gene were normalized on the bases of  $\beta$ -actin ( $\beta$ -actin-F: 5'-GTG GGC CGC TCT AGG CAC CAA-3',  $\beta$ -actin-R: 5'-CTC TTT GAT GTC ACG CAC GAT TT-3' expression [27]. At the end of each qRT-PCR, a melting curve analysis was performed at 95.0 °C to check the quality of primers [28].

Brain  $\alpha$ -synuclein was determined using ELISA kits (Cloud-Clone Company, TX, USA) according to the method of Cerri et al. [29].

### Estimation of AchE and neurotransmitter levels

AchE (Cusabio, TX, USA) was estimated according to the method of Wen et al. [30]. Neurotransmitters as NE, DA and 5-HT levels were determined by the method of Zagrodzka et al. [31], where their concentrations were determined using high-performance liquid chromatography with electrochemical detection (HPLC-ED). The flow rate of the column was adjusted to 1.4 ml/min and the concentrations of neurotransmitters in each sample were calculated from the integrated chromatographic peak area and expressed as ng/mg tissue. Absolute quantitation was performed by additional injection of standards containing defined concentration of specific amines. A standard curve was generated for each assay.

### Estimation of oxidative stress markers

Brain GSH level was estimated by the method of Moron et al. [32]. MDA was performed according to the method of Wills [33]. SOD was estimated according to the method of Kono [34].

### Estimation of brain energetic parameters

Brain ATP level was determined using ATP colorimetric assay kit supplied by Bio Vision Incorporated Co., Milpitas, USA. The assay depend on utilize the phosphorylation of glycerol to generate; a product that is easily quantified by colorimetric technique at 570 nm. SDH activity was estimated in brain tissue by the method of Rice and Shelton [35]. LDH activity was estimated in brain tissue by the method of Babson and Babson [36].

### Exploration of the inflammatory indices

Serum IL-6, Ang-II and CRP levels were measured by ELISA technique. IL-6 (Cloud-Clone Company, TX, USA) was estimated according to the method of Sun et al. [37], Ang-II level (Kamiya Biomedical Company, WA, USA) according to the method of Sun et al. [38] and CRP level

(Life Diagnostics, Inc., PA, USA) according to the method of Schreiber et al. [39].

### Estimation of brain apoptotic indices

Cas-3 was determined using an ELISA kit (Cloud-Clone Company, TX, USA) according to the method of Pradeep et al. [40].

DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA according to Lu et al. [41]. A 100-bp DNA ladder (Invitrogen, USA) was included as a molecular size marker and DNA fragments were visualized and photographed by exposing the gels to ultraviolet transilluminator. Six DNA fragmentation patterns displayed seven lanes of one mice/group were analyzed and one pattern was selected for illustration.

### Histopathological assay

Specimen of the brain substantia nigra and hippocampus regions were fixed in 10% neutral buffered formalin, embedded in paraffin wax, cut at 5  $\mu$ m thickness and stained with hematoxylin and eosin (H&E) after processing [42]. Six mice brains sections were analyzed in each group and one figure/group was selected for illustration.

### Statistical analysis and calculations

Data are expressed as mean  $\pm$  standard deviation (SD) of six mice in each group. Statistical differences among groups were assessed by one-way analysis of variance (ANOVA) test accompanied by Tukey–Kramer's test for intergroup comparisons with least significance level at  $P < 0.05$ . Significance levels at  $P < 0.0001$ ,  $< 0.001$ ,  $< 0.01$  and  $< 0.05$  had been given respective symbols.

$$\% \text{ Change} = ((\text{mean of control group} - \text{mean of treated group}) / \text{mean of control group}) \times 100$$

$$\% \text{ of improvement} = ((\text{mean of rotenone group} - \text{mean of treated group}) / \text{mean of control group}) \times 100.$$

## Results

### Wire hanging test

The wire hanging test revealed significant decrease ( $P < 0.0001$ ) in time spent by rats for being hanging with a wire in rotenone-treated group by 79.30%, as compared by control group. Treatment of rotenone-induced rats with curcumin ( $P < 0.05$ ), niacin ( $P < 0.0001$ ), ZM ( $P < 0.0001$ ), their combination ( $P < 0.0001$ ) and Sinemet® ( $P < 0.0001$ ) showed significant increase in time as compared with the

**Table 1** Wire hanging test after 2 weeks of treatment

| Groups                           | Hanging time (s)                 |
|----------------------------------|----------------------------------|
| Control                          | 23.00 <sup>a*</sup> $\pm$ 2.94   |
| Rotenone <sup>a</sup>            | 4.75 <sup>b*</sup> $\pm$ 1.70    |
| Rotenone + Curcumin              | 9.00 <sup>a##b*</sup> $\pm$ 2.16 |
| Rotenone + Niacin                | 11.25 <sup>a*b#</sup> $\pm$ 2.21 |
| Rotenone + ZM                    | 17.50 <sup>a*</sup> $\pm$ 2.08   |
| Rotenone + (Cur + Nia + ZM)      | 18.50 <sup>a*</sup> $\pm$ 1.73   |
| Rotenone + Sinemet® <sup>b</sup> | 16.50 <sup>a*</sup> $\pm$ 2.38   |

Data are expressed as mean  $\pm$  SD of six mice in each group

Superscript letters are significant difference between groups

Symbols \*, \*\*, #, ## are levels of significance at  $P < 0.0001$ , 0.001, 0.01, 0.05, respectively

Statistical analysis is carried out using one way ANOVA followed by Tukey–Kramer for multiple comparison test at  $P \leq 0.05$

rotenone group (Table 1). Therefore, the behavioral observation of rats through the wire hanging test showed improvement by 18.40, 28.20, 55.40, 59.70 and 51.08%, respectively.

### Parkinson's disease markers

Regarding to the adenosine A<sub>2A</sub>R gene expression level, it showed significant increase ( $P < 0.0001$ ) in rotenone group by 281.08% compared to the control group. Significant decrease in adenosine A<sub>2A</sub>R gene expression level was recorded after treatment with curcumin, niacin, ZM, their combination and Sinemet® (each  $P < 0.0001$ ) as compared with the rotenone-induced group (Table 2). Hence, enhancement in adenosine A<sub>2A</sub>R gene expression level was reached to 118.90, 135.10, 208.10, 224.00 and 170.20%, respectively.

$\alpha$ -synuclein level showed significant increase ( $P < 0.0001$ ) in rotenone-induced mice by 267.70% as compared with control group. Mice treated with curcumin, niacin, ZM,

their combination and Sinemet® (each  $P < 0.0001$ ) showed significant decrease in  $\alpha$ -synuclein as compared with rotenone group (Table 2). Improvement levels were observed after different treatment reached to 136.80, 149.70, 220.70, 241.70 and 185.60%, respectively.

### Neurotransmitter Index

DA, 5-HT and NE levels in rotenone-induced mice showed significant decrease ( $P < 0.0001$ ) by 56.50, 35.73 and 37.47%, respectively as compared with the control



**Table 2** Adenosine A<sub>2A</sub> receptor gene expression and  $\alpha$ -synuclein levels in brain of mice in different studied groups

| Groups                           | Relative gene expression of adenosine A <sub>2A</sub> R | $\alpha$ -synuclein (ng/ml)   |
|----------------------------------|---|-------------------------------|
| Control                          | 0.37 <sup>a*</sup> ± 0.06                               | 11.87 <sup>a*b*</sup> ± 1.65  |
| Rotenone <sup>a</sup>            | 1.41 <sup>b*</sup> ± 0.10                               | 43.57 <sup>b*</sup> ± 1.42    |
| Rotenone + Curcumin              | 0.97 <sup>a*</sup> ± 0.07                               | 27.33 <sup>a*b##</sup> ± 0.96 |
| Rotenone + Niacin                | 0.91 <sup>a*</sup> ± 0.03                               | 25.80 <sup>a*</sup> ± 1.31    |
| Rotenone + ZM                    | 0.64 <sup>a*</sup> ± 0.07                               | 17.37 <sup>a*</sup> ± 0.74    |
| Rotenone + (Cur + Nia + ZM)      | 0.58 <sup>a*</sup> ± 0.06                               | 14.87 <sup>a*b#</sup> ± 1.01  |
| Rotenone + Sinemet® <sup>b</sup> | 0.78 <sup>a*</sup> ± 0.08                               | 21.53 <sup>a*</sup> ± 0.86    |

Data are expressed as mean ± SD of six mice in each group

Superscript letters are significant difference between groups

Symbols \*, \*\*, #, ## are levels of significance at  $P < 0.0001$ , 0.001, 0.01, 0.05, respectively

Statistical analysis is carried out using one way ANOVA followed by Tukey–Kramer for multiple comparison test at  $P \leq 0.05$

The relative quantification of adenosine A<sub>2A</sub>R gene to the reference ( $\beta$ -actin) was determined by using the  $2^{-\Delta\Delta C_T}$  method

mice. Significant increase in the DA and serotonin levels were noted in curcumin ( $P < 0.01$ ), niacin, ZM, their combination and Sinemet® (each  $P < 0.0001$ ) as compared with the rotenone group. In addition, the NE level was increased significantly after treatments with curcumin, niacin (each  $P < 0.05$ ), ZM, their combination and Sinemet® (each  $P < 0.0001$ ) as compared to rotenone group. In contrast, AchE enzyme level was significantly increased ( $P < 0.0001$ ) by 153.49% in rotenone-induced mice as compared with the normal mice. Treatments with curcumin, niacin, ZM, their combination and Sinemet® (each  $P < 0.0001$ ) significantly decreased the AchE level as compared with rotenone-induced mice group (Tables 3, 4).

According to these results, the DA levels ameliorated after treatment by 11.48, 15.91, 14.83, 22.25 and 27.53%, respectively, while 5-HT level was improved by 7.06, 13.08, 12.86, 20.23 and 21.85%. Additionally, NE ameliorated by 6.07, 5.94, 11.14, 14.49 and 21.34% and AchE improved by 84.90, 90.12, 117.38, 133.95 and 118.11%, respectively.

### Oxidative stress markers

Concerning MDA level, the results revealed significant increase ( $P < 0.0001$ ) in rotenone induced mice by 90.95% as compared to control group. Treatment of rotenone-induced mice with curcumin ( $P < 0.01$ ), niacin, ZM, their combination and Sinemet® ( $P < 0.0001$ ) showed significant decrease as compared with the rotenone-induced mice (Table 5). Therefore, treatments showed improvement in MDA level by 52.85, 85.87, 81.74, 58.09 and 78.25%, respectively.

**Table 3** Levels of dopamine (DA) and noradrenaline (NA) in brain of mice in different studied groups

| Groups                           | DA (ng/gm)                   | NA (ng/gm)                      |
|----------------------------------|------------------------------|---------------------------------|
| Control                          | 61.06 <sup>a*b*</sup> ± 4.27 | 532.67 <sup>a*b*</sup> ± 9.58   |
| Rotenone <sup>a</sup>            | 26.54 <sup>b*</sup> ± 4.44   | 333.04 <sup>b*</sup> ± 17.72    |
| Rotenone + Curcumin              | 33.55 <sup>a#b*</sup> ± 1.48 | 365.41 <sup>a##b*</sup> ± 17.56 |
| Rotenone + Niacin                | 33.26 <sup>a*b#</sup> ± 1.35 | 364.71 <sup>a##b*</sup> ± 13.79 |
| Rotenone + ZM                    | 35.6 <sup>a*b**</sup> ± 2.24 | 392.43 <sup>a*b*</sup> ± 20.52  |
| Rotenone + (Cur + Nia + ZM)      | 40.13 <sup>a*</sup> ± 1.82   | 410.25 <sup>a*b#</sup> ± 14.23  |
| Rotenone + Sinemet® <sup>b</sup> | 43.35 <sup>a*</sup> ± 3.42   | 446.71 <sup>a*</sup> ± 23.78    |

Data are expressed as mean ± SD of six mice in each group

Superscript letters are significant difference between groups

Symbols \*, \*\*, #, ## are levels of significance at  $P < 0.0001$ , 0.001, 0.01, 0.05, respectively

Statistical analysis is carried out using one way ANOVA followed by Tukey–Kramer for multiple comparison test at  $P \leq 0.05$

In GSH level, a significant decrease ( $P < 0.0001$ ) was observed in rotenone-induced mice by 65.18% comparing to the control group. Treatment of rotenone induced mice with curcumin ( $P < 0.05$ ), niacin, ZM (each  $P < 0.01$ ), their combination ( $P < 0.0001$ ) and Sinemet® ( $P < 0.01$ ) showed significant increase as compared with the rotenone-induced mice (Table 5). Thus, treatments showed improvement in GSH level by 21.86, 26.13, 24.28, 31.29 and 25.02%, respectively.

Regarding to SOD enzyme activity, a significant decrease ( $P < 0.0001$ ) in rotenone induced mice by 75.93% was recorded as compared to the control group. Treatment of rotenone-induced mice with curcumin, niacin, ZM, their combination and Sinemet® (each  $P < 0.0001$ ) showed a significant increase compared with rotenone group

**Table 4** Levels of serotonin (5-HT) and acetylcholinesterase (AChE) in brain of mice in different studied groups

| Groups                           | 5-HT (ng/gm)                    | AChE (mU/mg)                  |
|----------------------------------|---------------------------------|-------------------------------|
| Control                          | 476.59 <sup>a*b**</sup> ± 11.04 | 9.72 <sup>a*b**</sup> ± 1.18  |
| Rotenone <sup>a</sup>            | 306.62 <sup>b*</sup> ± 12.50    | 24.64 <sup>b*</sup> ± 2.21    |
| Rotenone + Curcumin              | 340.30 <sup>a#b*</sup> ± 8.05   | 16.38 <sup>a*b**</sup> ± 1.14 |
| Rotenone + Niacin                | 368.99 <sup>a*b*</sup> ± 13.18  | 15.88 <sup>a*b#</sup> ± 1.29  |
| Rotenone + ZM                    | 367.51 <sup>a*b*</sup> ± 18.53  | 13.23 <sup>a*</sup> ± 0.92    |
| Rotenone + (Cur + Nia + ZM)      | 403.07 <sup>a*</sup> ± 17.59    | 11.62 <sup>a*</sup> ± 0.72    |
| Rotenone + Sinemet® <sup>b</sup> | 410.36 <sup>a*</sup> ± 13.77    | 13.16 <sup>a*</sup> ± 0.58    |

Data are expressed as mean ± SD of six mice in each group

Superscript letters are significant difference between groups

Symbols \*, \*\*, #, ## are levels of significance at  $P < 0.0001$ , 0.001, 0.01, 0.05, respectively

Statistical analysis is carried out using one way ANOVA followed by Tukey–Kramer for multiple comparison test at  $P \leq 0.05$

**Table 5** Glutathione (GSH), malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity in brain of mice in different studied groups

| Groups                          | MDA ( $\mu\text{g}/\text{mg}$ protein) | GSH ( $\mu\text{g}/\text{mg}$ protein) | SOD ( $\mu\text{g}/\text{mg}$ protein) |
|---------------------------------|--|--|--|
| Control                         | 6.30 <sup>a*</sup> $\pm$ 1.78          | 45.92 <sup>a*b*</sup> $\pm$ 7.45       | 239.75 <sup>a*b*</sup> $\pm$ 11.71     |
| Rotenone <sup>a</sup>           | 12.03 <sup>b*</sup> $\pm$ 3.31         | 20.13 <sup>b#</sup> $\pm$ 2.92         | 57.70 <sup>b*</sup> $\pm$ 18.07        |
| Rotenone + Curcumin             | 8.70 <sup>a##</sup> $\pm$ 1.07         | 30.17 <sup>a##</sup> $\pm$ 4.53        | 124.77 <sup>a*</sup> $\pm$ 16.85       |
| Rotenone + Niacin               | 6.62 <sup>a*</sup> $\pm$ 1.22          | 32.13 <sup>a#</sup> $\pm$ 6.13         | 156.51 <sup>a*</sup> $\pm$ 22.66       |
| Rotenone + ZM                   | 6.88 <sup>a*</sup> $\pm$ 1.31          | 31.28 <sup>a#</sup> $\pm$ 3.07         | 189.18 <sup>a*b#</sup> $\pm$ 19.65     |
| Rotenone + (Cur + Nia + ZM)     | 8.37 <sup>a*</sup> $\pm$ 1.44          | 34.50 <sup>a*</sup> $\pm$ 2.30         | 157.40 <sup>a*</sup> $\pm$ 25.32       |
| Rotenone + Sinemet <sup>b</sup> | 7.10 <sup>a*</sup> $\pm$ 0.49          | 31.62 <sup>a#</sup> $\pm$ 6.06         | 144.20 <sup>a*</sup> $\pm$ 13.33       |

Data are expressed as mean  $\pm$  SD of six mice in each group

Superscript letters are significant difference between groups

Symbols \*, \*\*, #, ## are levels of significance at  $P < 0.0001$ , 0.001, 0.01, 0.05, respectively

Statistical analysis is carried out using one way ANOVA followed by Tukey–Kramer for multiple comparison test at  $P \leq 0.05$

(Table 5). We noticed that treatment showed improvement in SOD activity by 27.97, 41.21, 54.84, 41.60 and 36.07%, respectively.

### Brain energetic indices

Regarding to ATP level, the rotenone group showed significant decrease ( $P < 0.0001$ ) by 49.23% as compared with the normal control mice. Treatment with curcumin, niacin, Sinemet®, ZM, their combination and Sinemet® (each  $P < 0.0001$ ) were significantly increased the ATP level as compared with the rotenone-induced group (Table 6). We noticed improvement in ATP levels after treatment with curcumin, niacin, ZM, their combination and Sinemet® reached to 15.14, 21.90, 15.18, 24.95 and 31.70%, respectively.

SDH enzyme showed significant decrease ( $P < 0.0001$ ) by 34.50% in rotenone-induced group as compared with the healthy mice. Curcumin, ZM and their combination showed no significant change in the enzyme level comparing to rotenone group, but showed significant increase with niacin and Sinemet® treatments (each  $P < 0.01$ ) as compared to

rotenone-induced group (Table 6). Therefore, these treatments recorded amelioration levels by 9.14, 21.10, 12.06, 17.01 and 22.75%, respectively.

In case of LDH enzyme activity, a significant decrease ( $P < 0.0001$ ) by 44.80% was recorded compared with the control mice. Significant increase in SDH levels were noticed after treatment with niacin ( $P < 0.01$ ), ZM ( $P < 0.05$ ), their combination and Sinemet® (each  $P < 0.05$ ) as compared with the rotenone group, but no significant change after treatment with curcumin was seen (Table 6). The enzyme recorded improvement values by 10.25, 20.51, 27.00, 21.83 and 21.90%, respectively.

### Inflammatory mediator index

Regarding to Ang-II, CRP and IL-6 levels, rotenone group showed significant increase (each  $P < 0.0001$ ) by 20.80, 113.97 and 334.41% as compared with the control mice. In Ang-II and IL-6 levels, treatments with curcumin, niacin, ZM, their combination and Sinemet® (each  $P < 0.0001$ ) were significantly decreased comparing to the rotenone-induced

**Table 6** Succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activities and ATP level in brain of mice in different studied groups

| Groups                          | SDH ( $\mu\text{g}/\text{mg}$ protein) | LDH ( $\mu\text{g}/\text{mg}$ protein) | ATP (Pmol/mg)                    |
|---------------------------------|--|--|----------------------------------|
| Control                         | 18.81 <sup>a*</sup> $\pm$ 1.88         | 15.11 <sup>a*b**</sup> $\pm$ 1.35      | 58.35 <sup>a*b*</sup> $\pm$ 5.09 |
| Rotenone <sup>a</sup>           | 12.32 <sup>b#</sup> $\pm$ 1.90         | 8.34 <sup>b**</sup> $\pm$ 1.34         | 29.62 <sup>b*</sup> $\pm$ 2.56   |
| Rotenone + Curcumin             | 14.04 $\pm$ 1.46                       | 9.89 $\pm$ 1.10                        | 38.40 <sup>a*b*</sup> $\pm$ 1.55 |
| Rotenone + Niacin               | 16.29 <sup>a#</sup> $\pm$ 1.55         | 11.44 <sup>a#</sup> $\pm$ 1.43         | 42.44 <sup>a*b#</sup> $\pm$ 2.30 |
| Rotenone + ZM                   | 14.59 $\pm$ 1.67                       | 12.42 <sup>a*</sup> $\pm$ 1.22         | 38.48 <sup>a*b*</sup> $\pm$ 1.53 |
| Rotenone + (Cur + Nia + ZM)     | 14.52 $\pm$ 1.51                       | 11.64 <sup>a**</sup> $\pm$ 0.82        | 44.18 <sup>a*</sup> $\pm$ 1.75   |
| Rotenone + Sinemet <sup>b</sup> | 16.60 <sup>a#</sup> $\pm$ 2.28         | 11.65 <sup>a**</sup> $\pm$ 1.47        | 48.17 <sup>a*</sup> $\pm$ 1.92   |

Data are expressed as mean  $\pm$  SD of six mice in each group

Superscript letters are significant difference between groups

Symbols \*, \*\*, #, ## are levels of significance at  $P < 0.0001$ , 0.001, 0.01, 0.05, respectively

Statistical analysis is carried out using one way ANOVA followed by Tukey–Kramer for multiple comparison test at  $P \leq 0.05$

group. CRP level showed significant decrease with curcumin ( $P < 0.001$ ), niacin, ZM, their combination and Sinemet® (each  $P < 0.0001$ ) treatments as compared with the rotenone group (Table 7). Therefore, treatment with curcumin, niacin, ZM, their combination and Sinemet® recorded improvement by 10.19, 6.93, 10.70, 10.70 and 11.70%, respectively, for Ang-II, while this improvement reached to 40.70, 47.77, 53.91, 64.05 and 74.19%, respectively, for CRP level and reached to 123.40, 137.60, 191.70, 248.70 and 147.70%, respectively, for IL-6 level.

### Apoptotic indices

Cas-3 and DNA fragmentation levels showed significant increase ( $P < 0.0001$ ) in rotenone-induced mice by 237.30 and 260.08%, respectively, as compared with the healthy mice. Curcumin, niacin, ZM, their combination and Sinemet® (each  $P < 0.0001$ ) significantly decreased the level of Cas-3 and DNA fragmentation level as compared to

the rotenone group. Hence, the treatments under investigation recorded amelioration in Cas-3 level by 115.90, 124.80, 160.90, 189.40 and 143.70%, respectively, while it amounted 82.40, 98.60, 209.40, 232.40 and 178.37%, respectively, for DNA fragmentation level (Table 8 and Fig. 1).

### Histopathological analysis

The histopathological pattern of control mice showed the cerebellum with normal histological features, a well-defined molecular, granular and Purkinje layers with normal structure of neuronal cells in the frontal cortex. In rotenone-treated mice, abnormal cerebral cortex as well as mild vacuolization and oedema of neuronal cells were seen. The brain of rotenone-induced mice treated with curcumin, niacin, Sinemet®, ZM and their combination showed almost normal histological structure represented by well-defined molecular, granular and Purkinje layers, and normal neurons with visible axons. Some

**Table 7** Angiotensin-II, c-reactive protein (CRP) and interleukin-6 (IL-6) levels in serum of mice in different studied groups

| Groups                           | Ang-II (Pg/ml)                     | CRP (Pg/ml)                       | IL-6 ( $\mu\text{g}/\text{mg}$ protein) |
|----------------------------------|------------------------------------|-----------------------------------|---|
| Control                          | 383.73 <sup>a*b*</sup> $\pm$ 14.22 | 6.51 <sup>a*b**</sup> $\pm$ 0.86  | 17.33 <sup>a*b*</sup> $\pm$ 1.53        |
| Rotenone <sup>a</sup>            | 463.58 <sup>b*</sup> $\pm$ 6.76    | 13.93 <sup>b*</sup> $\pm$ 0.90    | 75.23 <sup>b*</sup> $\pm$ 2.28          |
| Rotenone + Curcumin              | 424.46 <sup>a*</sup> $\pm$ 10.60   | 11.28 <sup>a**b#</sup> $\pm$ 0.67 | 53.83 <sup>a*</sup> $\pm$ 2.57          |
| Rotenone + Niacin                | 436.97 <sup>a*b#</sup> $\pm$ 4.52  | 10.82 <sup>a*</sup> $\pm$ 0.67    | 51.37 <sup>a*</sup> $\pm$ 2.28          |
| Rotenone + ZM                    | 422.46 <sup>a*</sup> $\pm$ 6.10    | 10.42 <sup>a*</sup> $\pm$ 0.94    | 42.13 <sup>a*</sup> $\pm$ 2.47          |
| Rotenone + (Cur + Nia + ZM)      | 424.67 <sup>a*</sup> $\pm$ 4.40    | 9.76 <sup>a*</sup> $\pm$ 1.64     | 32.13 <sup>a*b#</sup> $\pm$ 2.40        |
| Rotenone + Sinemet® <sup>b</sup> | 418.43 <sup>a*</sup> $\pm$ 9.78    | 9.10 <sup>a*</sup> $\pm$ 0.85     | 49.63 <sup>a*</sup> $\pm$ 1.86          |

Data are expressed as mean  $\pm$  SD of six mice in each group

Superscript letters are significant difference between groups

Symbols \*, \*\*, #, ## are levels of significance at  $P < 0.0001$ , 0.001, 0.01, 0.05, respectively

Statistical analysis is carried out using one way ANOVA followed by Tukey–Kramer for multiple comparison test at  $P \leq 0.05$

**Table 8** Caspase-3 activity and DNA fragmentation pattern in brain of mice in different studied groups

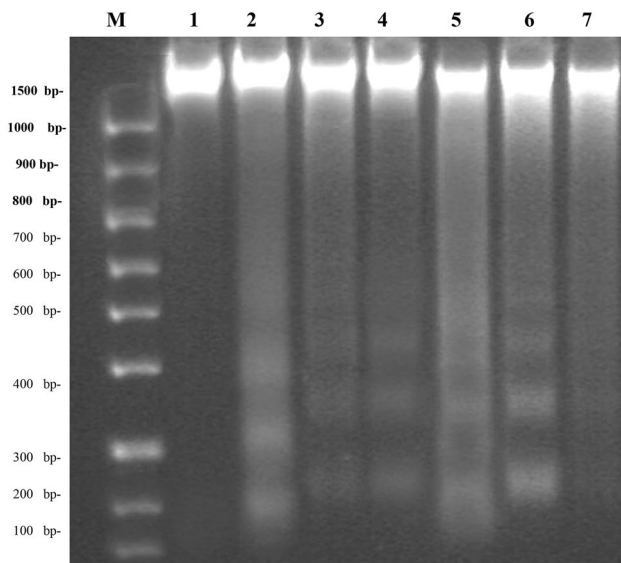
| Groups                           | Caspase3 (pmol/min/ml)           | DNA fragmentation ( $\mu\text{g}/\text{mg}$ protein) |
|----------------------------------|----------------------------------|--|
| Control                          | 19.20 <sup>a*b*</sup> $\pm$ 0.87 | 7.4 <sup>a*b*</sup> $\pm$ 0.30                       |
| Rotenone <sup>a</sup>            | 64.77 <sup>b*</sup> $\pm$ 1.45   | 26.7 <sup>b*</sup> $\pm$ 0.60                        |
| Rotenone + Curcumin              | 42.50 <sup>a*</sup> $\pm$ 0.78   | 20.6 <sup>a*b*</sup> $\pm$ 0.40                      |
| Rotenone + Niacin                | 40.80 <sup>a*</sup> $\pm$ 0.92   | 19.4 <sup>a*b*</sup> $\pm$ 0.30                      |
| Rotenone + ZM                    | 33.87 <sup>a*</sup> $\pm$ 1.25   | 11.2 <sup>a*b##</sup> $\pm$ 0.50                     |
| Rotenone + (Cur + Nia + ZM)      | 28.4 <sup>a*</sup> $\pm$ 0.82    | 9.5 <sup>a*b*</sup> $\pm$ 0.50                       |
| Rotenone + Sinemet® <sup>b</sup> | 37.17 <sup>a*</sup> $\pm$ 1.04   | 13.5 <sup>a*</sup> $\pm$ 0.40                        |

Data are expressed as mean  $\pm$  SD of six mice in each group

Superscript letters are significant difference between groups

Symbols \*, \*\*, #, ## are levels of significance at  $P < 0.0001$ , 0.001, 0.01, 0.05, respectively

Statistical analysis is carried out using one way ANOVA followed by Tukey–Kramer for multiple comparison test at  $P \leq 0.05$



**Fig. 1** Brain DNA fragmentation pattern analyzed by DNA gel electrophoresis laddering assay. M: DNA ladder. Lane 1: control mice. Lane 2: rotenone-induced mice. Lane 3: mice exposed to rotenone and treated with Sinemet®. Lane 4: mice exposed to rotenone and treated with ZM. Lane 5: mice exposed to rotenone and treated with curcumin. Lane 6: mice exposed to rotenone and treated with niacin. Lane 7: mice exposed to rotenone and treated with combination of curcumin, niacin and ZM

neurodegenerative changes as vacuolation and apoptotic nuclei were also seen (Figs. 2, 3).

## Discussion

There are great evidences suggest that  $\alpha$ -synuclein is responsible for initiating the pathological features in PD [5]. Within Lewy bodies (LB),  $\alpha$ -synuclein is deposited in fibrillary structures [43], where the  $\alpha$ -synuclein lesions are propagated and spread between the interconnected brain regions [44, 45]. Additionally, release of LB derived  $\alpha$ -synuclein into the cerebrospinal fluid and blood may represent additional evidence for spreading of pathological  $\alpha$ -synuclein in different organs.

These observations may have important implications for the development of different therapies for PD targeting expression levels, pathological conversion or transmission of  $\alpha$ -synuclein [46]. This was confirmed by our results through improvement in  $\alpha$ -synuclein level and histological diminution in Lewy bodies after treatment with curcumin, niacin, ZM, their combination and Sinemet®.

Although the efficiency of levodopa and dopamine agonists treatment is recommended, their long-term uses induce motor complications [7]. One of the most effective strategies for treatment is to use drugs that can modulate non-dopaminergic neurotransmitter systems in the basal

ganglia, such as adenosine [47]. In this regard, adenosine  $A_{2A}R$  was considered as a potential therapeutic target due to its close interaction within the dopaminergic system [48].

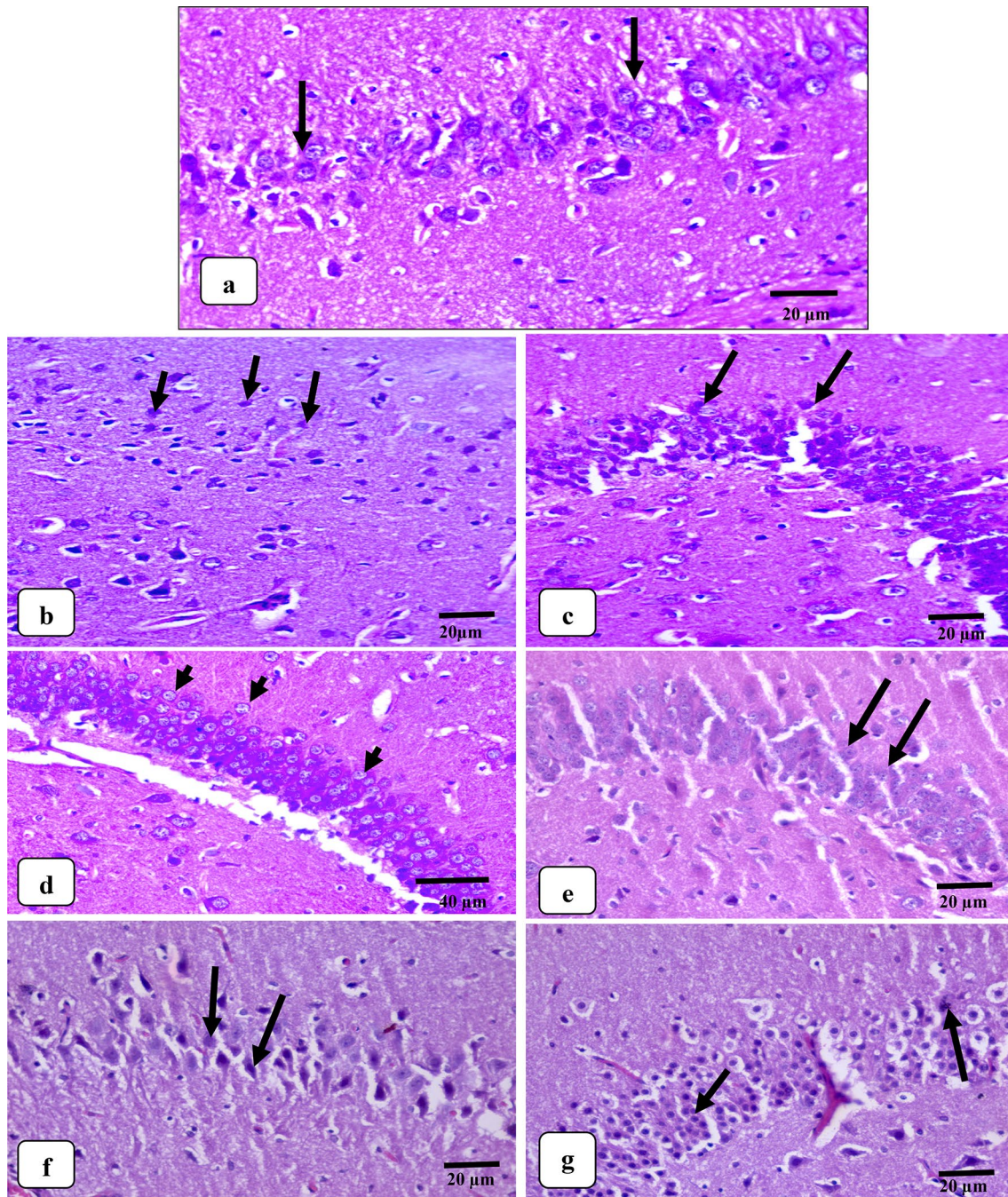
Treatment with adenosine  $A_{2A}R$  antagonists seems to be a promising candidate for non-dopaminergic therapy due to the high expression of adenosine  $A_{2A}R$  within the basal ganglia [49]. In parallel with our results, Calon et al. [50] and Kachroo et al. [51] mentioned high presence of adenosine  $A_{2A}R$ s in the brain of Parkinson's disease mice model. The last authors added that adenosine  $A_{2A}R$  gene disruption may also protect the Parkinsonism mice. In addition, reversal of  $\alpha$ -synuclein toxicity by adenosine  $A_{2A}R$  depletion raising the possibility that adenosine  $A_{2A}R$  antagonists produce their neuroprotective effects in PD models by preventing synuclein-induced toxicity [51]. Dungo and Deeks [52] found that Istradefylline (ZM 241385); a selective adenosine  $A_{2A}R$  antagonist efficiently crosses BBB, binds to adenosine  $A_{2A}R$  with high affinity, and potentiates L-dopa activity. Interestingly, it was found that the amyloid beta ( $A\beta$ ) is mainly detected in PD and Istradefylline acted as a modulator of  $A\beta$  generation through targeting adenosine  $A_{2A}R$  [53, 54].

One of the major features of PD is the depletion of neurotransmitters. In agreement with our results, Shen [55] and Perez-Lloret and Barrantes [56] suggested that the neurodegeneration of dopaminergic, noradrenergic, serotonergic, and cholinergic neurons in PD affected the release of DA, NE, 5-HT and AchE, respectively. In case of AchE, Perez-Lloret and Barrantes [56] added that there is a link between cholinergic alterations and PD motor symptoms that related to alteration of cholinergic tone in the striatum and/or to degeneration of cholinergic nuclei. Therefore, therapeutic strategies directed toward restoring normal neurotransmitters levels and inhibiting AchE enzyme may be effective in controlling PD symptoms [56]. Curcumin treatment may also modulate the expression of 5-HT; the rate-limiting enzyme for DA biosynthesis that serves to modulate locomotor activity [57]. This gives an additional supports through the observed restored levels of DA, NE, 5-HT and AchE after treatment with curcumin, niacin, ZM and their combination by variable degrees.

Many studies showed that mitochondrial dysfunction, oxidative stress, ATP depletion, caspase releasing and electron transport chain are the major features that occur during neuronal death in PD [57–59]. This in line with our results by depletion of GSH, MDA, SOD, ATP and increment of Cas-3 levels. Treatment with niacin in combination with levodopa can decrease the levels of free radicals associated with PD [60].

Mitochondrial SDH enzyme plays a potential role in the oxidative phosphorylation system, where it connects Krebs cycle to the electron transport chain. Thus, any inhibition of SDH can trigger mitochondrial dysfunction





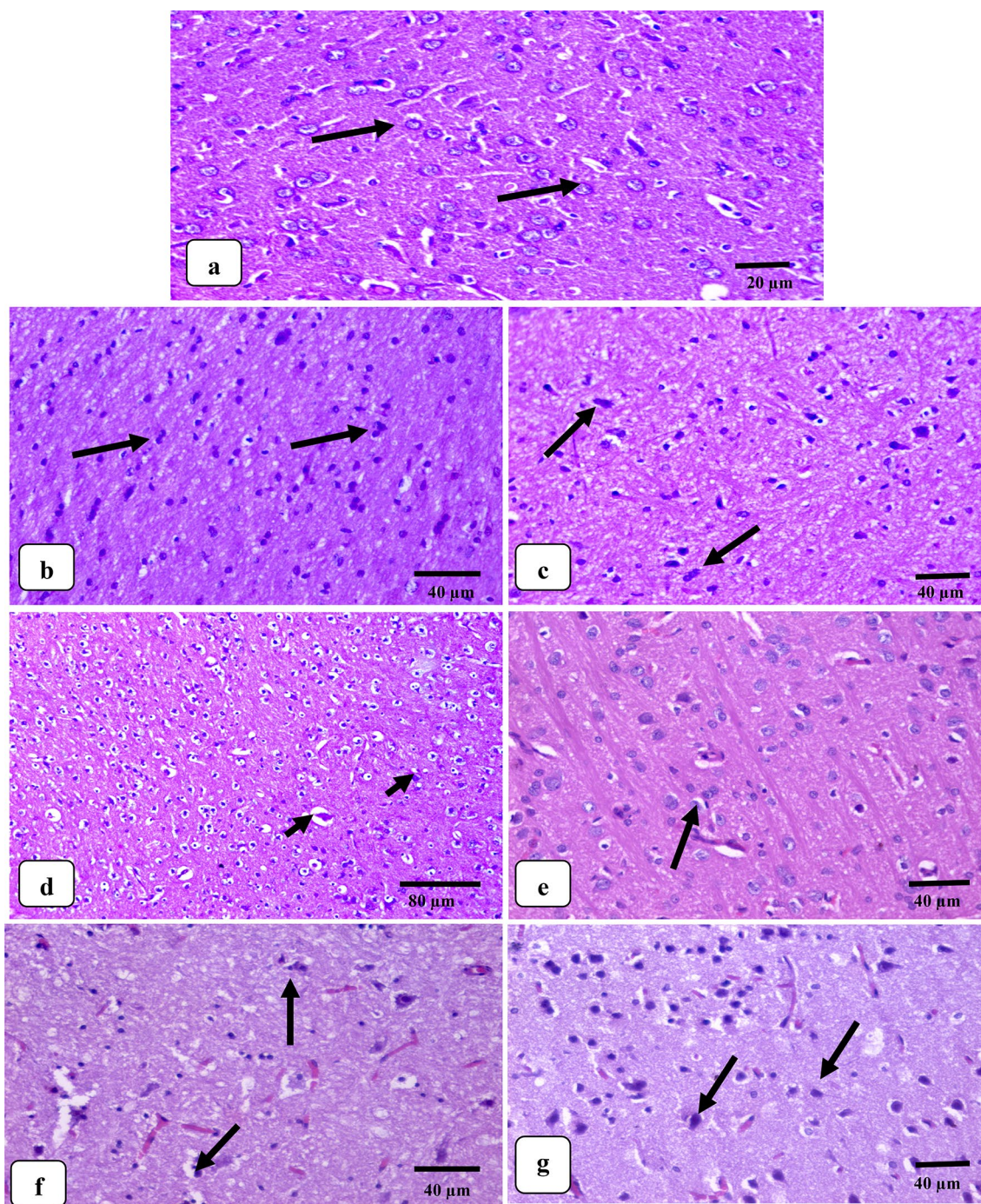
**Fig. 2** Photomicrograph (H&E) of the hippocampus region of control mice (**a**, $\times 400$ ), rotenone group (**b**, $\times 400$ ), rotenone+curcumin (**c**, $\times 400$ ), rotenone+niacin (**d**, $\times 200$ ), rotenone+ZM (**e**, $\times 400$ ), rotenone+curcumin+niacin+ZM (**f**, $\times 400$ ) and rotenone+Sinemet® (**g**, $\times 400$ ). Control mice showing normal structure of hippocampal region being formed of granular cells with large

rounded vesicular nuclei (arrows). Rotenone group shows increase in degenerated granular cells and shrunken deep cells (arrows). Treatment groups show nearly normal structure of this region being formed of granular cells with large rounded vesicular nuclei and mild number of degenerated granular cells (arrows). Scale bars represent the actual length of the cell before magnification

and ATP degeneration [61]. Ludtmann et al. [62] added that  $\alpha$ -synuclein accumulation destroys complex I-respiration system, enhances ATP synthase oxidation and the mitochondrial lipid peroxidation processes which lead to increase the permeability of the transition pore opening,

trigger mitochondrial swelling and eventually cell death. These observations are well-explained the diminished production of ATP and SDH levels. Bordone et al. [6] added that most ATP is consumed in the synaptic terminals by pumping ions to maintain resting membrane





**Fig. 3** Photomicrograph (H&E) of the substantia nigra region of control mice (**a**,  $\times 400$ ), rotenone group (**b**,  $\times 200$ ), rotenone + curcumin (**c**,  $\times 200$ ), rotenone + niacin (**d**,  $\times 100$ ), rotenone + ZM (**e**,  $\times 200$ ), rotenone + curcumin + niacin + ZM (**f**,  $\times 200$ ) and rotenone + Sinemet® (**g**,  $\times 200$ ). Control mice shows normal neuronal

cells (arrows) in the substantia nigra region. In rotenone group, brain shows a large number of degenerated cells (arrows). In treatment groups, normal neuronal cells and a few number of degenerated cells (arrows) are seen

potential, vesicle filling, vesicle transport, vesicle recycling, and enzymatic processing of synaptic transmitters within synapses. Additionally, the presynaptic ATP levels are consumed by  $\text{Na}^+/\text{K}^+$  pump,  $\text{Ca}^{2+}$  -ATPases in the

plasma membrane and endoplasmic reticulum, vacuolar  $\text{H}^+$  -ATPase, motor proteins and protein disassembly machineries. At postsynaptic regions, ATP is consumed for counterbalancing the ion fluxes through postsynaptic

receptors and on rebounding  $\text{Ca}^{2+}$  to intracellular stores and on mitochondrial trafficking. [6].

Ross et al. [63] showed an increase in brain lactate levels through a shift in the transcriptional activities of lactate dehydrogenase that promote pyruvate to lactate conversion. Over consumption of LDH enzyme to trigger this conversion will explain its reduction in rotenone induction rats in this study. In rats with intracerebral hemorrhage, Zhou et al. [64] mentioned that brain lactate accumulation promotes angiogenesis and neurogenesis; the vital brain repair process that may promote recovery via activation of NF- $\kappa$ B-signaling pathway. Accordingly, treating with phenolic antioxidants as curcumin could modulate oxidative stress and delay the progression of PD [65]. This gives an additional support of the improvement in GSH, MDA and SOD levels after treatment with curcumin.

Regarding to the animal behavior through the wire hanging test, we recorded enhancement in time spent for animals to be hanged by the wire after different treatments. Additionally, Muthian et al. [57] found that curcumin enhanced the animal behaviors in a dose-dependent manner exceeding the control group, which suggests that curcumin by itself will enhance performance.

The renin–angiotensin system (RAS) in the brain has a regulatory role of the brain's response to stress [66], while the peripheral RAS regulates blood pressure and volume [67]. Therefore, the circulating Ang-II binds to its type 1 receptor on vascular smooth muscle cells to induce vasoconstriction. In the present study and in accordance with Zawada et al. [59], it was found that dopamine neuron loss is accompanied by increased expression of Ang-II level in PD rodent model. Benigni et al. [68] postulated the pro-inflammatory and pro-fibrotic roles of Ang-II that contribute to progressive deterioration of organs function in PD.

Regarding to CRP, it has been identified as an inflammatory-related cytokine and correlates with vascular death [69]. In accordance with the present study, CRP is associated with the pathogenesis of neurological disorders and deemed to be a risk factor for Parkinson's disease [70].

In the present study, DNA damage was significantly increased in rotenone group. This is in agreement with Martin et al. [58] who stated that DNA damage is a form of cell stress and injury that has been implicated in the pathogenesis of many neurologic disorders. In addition, Khadrawy et al. [71] noticed certain pathological features in the midbrain of Parkinsonism rat like apoptosis, chromatin condensation and DNA fragmentation. In parallel with our results, Hegde et al. [72] stated that curcumin can change over inhibition of DNA in animal models and in neuroblastoma cells. Furthermore, Wang et al. [73] postulated the effect of curcumin in down regulation of DNA damage. Picada et al. [74] added that the derivatives oxidation of dopamine and L-dopa used in the therapy of PD might display genotoxic activities. Therefore,

this finding should be taken into consideration when the oxidized metabolites of dopamine agonists is used for PD treatment.

With respect to IL-6, it has been pointed to be an anti-inflammatory cytokine [75]. This cytokine increases leukocyte adhesion and migration, disrupt the BBB and induce glial cell activation. This could subsequently lead to the overproduction of reactive oxygen species, apoptosis and cytolysis [76]. Glial activation plays a potential role in neurodegenerative diseases even in the presence of other cellular insults such as hypoxia which is directly linked to ROS production, induced apoptosis, neuroinflammation and numbers of signaling cascades. Moreover, inflammation can also trigger hypoxia by damaging mitochondria and endothelial cells to impair blood flow regulation [77]. In agreement with our study, Hofmann et al. [78] found an increment in serum IL-6 level in PD patients, however, there was a positive correlation between IL-6 levels and the severity of PD indicating that patients with more severe disease have higher levels of IL-6. It was reported that levodopa in physiological concentrations, presented an immunomodulatory effect and caused a stimulation of IL-6 production [79]. We and Feingold et al. [15] found that niacin can play a significant role in triggering and boosting anti-inflammatory responses in PD. The role of niacin in attenuating PD symptoms is mainly due to the synthesis of nicotinamide adenine dinucleotide (NAD) and release nicotinamide by poly ADP-ribosylation that involved in many cellular processes; including cell signaling, DNA repair, gene regulation and apoptosis [60].

## Conclusion

Curcumin as a natural polyphenolic compound and niacin as a vitamin may be considered as promising food supplements for Parkinson's disease treatment. Treatment with Sinemet® (levodopa) and the newly adenosine  $\text{A}_{2\text{A}}$ R antagonist (ZM) had almost the same improvement levels, while the combination of ZM with niacin and curcumin recorded the most potent improvement level. As many side effects were recorded by the long term treatment of levodopa, thereafter further detailed studies on ZM are needed to consider it as a promising anti-Parkinsonism agent and to confirm the uses of curcumin and niacin as dietary supplements against PD.

**Author contributions** TKM, NAHS and MAH: Participated in study design, revised the statistical analysis and revised the manuscript. SAA: Analyzed the histopathological pictures, drafted and revised this section. WKBH: Conducted the genetic and DNA analysis, drafted and revised this section. YRA: Conducted the biochemical parameters, analyzed the data and drafted the article. All authors revised the final form of the paper and agreed for publication.



## Compliance with ethical standards

**Conflict of interest** The authors declared no conflict of interest.

**Ethical approval** Animals care during the experiments were carried out in keeping with agreement of the Medical Ethical Committee, National Research Centre, Egypt (Approval No. 16089) and Ethics Committee of Faculty of Pharmacy, Cairo University, Cairo, Egypt (BC 1839). Basic housing requirements and regular inspection of facilities that mandate the control of pain and suffering during the experiment were conducted. Euthanasia was done rapidly and painlessly to be sure that the animals do not suffer at any stage of the experiment. Getting-rid of the animals after termination was done rapidly by the aid of the Safety and Health Committee at National Research Centre, Dokki, Giza, Egypt.

## References

- Rajabally YA, Martey J (2011) Neuropathy in Parkinson disease: prevalence and determinants. *Neurology* 77:1947–1950
- Taylor TN, Greene JG, Miller GW (2010) Behavioral phenotyping of mouse models of Parkinson's disease. *Behav Brain Res* 211:1–10
- Betarbet R, Sherer TB, Greenamyre JT (2002) Animal models of Parkinson's disease. *Bio Essays* 24:308–318
- Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JTM (2009) A highly reproducible rotenone model of Parkinson's disease. *Neurobiol Dis* 34:279–290
- Dexter DT, Jenner P (2013) Parkinson disease: from pathology to molecular disease mechanisms. *Free Radic Biol Med* 62:132–144
- Bordone MP, Salman MM, Titus HE, Amini E, Andersen JV, Chakraborti B et al (2019) The energetic brain—a review from students to students. *J Neurochem* 151:139–165
- Crispo JAG, Fortin Y, Thibault DP, Emons M, Bjerre LM, Kohen DE, Perez-Lloret S, Mattison D, Willis AW, Krewski D (2015) Trends in inpatient antiparkinson drug use in the USA, 2001–2012. *Eur J Clin Pharmacol* 71:1011–1019
- Perez-Pardo P, Broersen LM, Kliet T, van Wijk N, Attali A, Garsen J, Kraneveld AD (2018) Additive effects of levodopa and a neurorestorative diet in a mouse model of Parkinson's disease. *Front Aging Neurosci* 10:237
- Jacob A, Wu R, Zhou M, Wang P (2007) Mechanism of the anti-inflammatory effect of curcumin: PPAR-gamma activation. *PPAR Res* 2007:89369
- Zhao LN, Chiu SW, Benoit J, Chew LY, Mu Y (2012) The effect of curcumin on the stability of  $\alpha\beta$  dimers. *J Phys Chem B* 116:7428–7435
- Trujillo J, Chirino YI, Molina-Jijón E, Andérica-Romero AC, Tapia E, Pedraza-Chaverrí J (2013) Reno-protective effect of the antioxidant curcumin: recent findings. *Redox Biol* 1:448–456
- Reeta KH, Mehla J, Gupta YK (2010) Curcumin ameliorates cognitive dysfunction and oxidative damage in phenobarbitone and carbamazepine administered rats. *Eur J Pharmacol* 644:106–112
- Tsai YM, Chien CF, Lin LC, Tsai TH (2011) Curcumin and its nano-formulation: the kinetics of tissue distribution and blood-brain barrier penetration. *Int J Pharm* 416:331–338
- Garg A, Sharma A, Krishnamoorthy P, Garg J, Virmani D, Sharma T, Stefanini G, Kostis JB, Mukherjee D, Sikorskaya E (2017) Role of niacin in current clinical practice: a systematic review. *Am J Med* 130:173–187
- Feingold KR, Moser A, Shigenaga JK, Grunfeld C (2014) Inflammation stimulates niacin receptor (GPR109A/HCA2) expression in adipose tissue and macrophages. *J Lipid Res* 55:2501–2508
- Chapman MJ (2004) Raising high-density lipoprotein cholesterol with reduction of cardiovascular risk: the role of nicotinic acid—a position paper developed by the European Consensus Panel on HDL-C. *Curr Med Res Opin* 20:1253–1268
- Motawi TK, Darwish HA, Hamed MA, El-Rigal NS, Aboul Naser AF (2017) A therapeutic insight of niacin and coenzyme Q10 against diabetic encephalopathy in rats. *Mol Neurobiol* 54:1601–1611
- Wakade C, Chong R (2014) A novel treatment target for Parkinson's disease. *J Neurol Sci* 347:34–38
- Rahman M, Muhammad S, Khan MA, Chen H, Ridder DA, Muller-Fielitz H, Pokorna B, Vollbrandt T, Stolting I, Nadrowitz R, Okun JG, Offermanns S, Schwaninger M (2014) The beta-hydroxybutyrate receptor HCA2 activates a neuroprotective subset of macrophages. *Nat Commun* 5:3944
- Ye X, Chopp M, Cui X, Zacharek A, Cui Y, Yan T, Shehadah A, Roberts C, Liu X, Lu M, Chen J (2011) Niaspan enhances vascular remodeling after stroke in type 1 diabetic rats. *Exp Neurol* 232:299–308
- Fathalla AM, Soliman AM, Ali MH, Moustafa AA (2016) Adenosine  $A_{2A}$  receptor blockade prevents rotenone-induced motor impairment in a rat model of Parkinsonism. *Front Behav Neurosci* 2016(10):1–5
- El Shebiney SA, El-Denshary ES, Abdel-Salam OME, Salem NA, El-Khyat ZA, El Shaffie N, Abdallah DM (2014) Cannabis resin extract in Parkinson's disease: behavioral, neurochemical, and histological evaluation. *Cell Biol Res Ther* 3:1
- Rajeswari A, Sabesan M (2008) Inhibition of monoamine oxidase-B by the polyphenolic compound, curcumin and its metabolite tetrahydrocurcumin, in a model of Parkinson's disease induced by MPTP neurodegeneration in mice. *Inflammopharmacology* 16:96–99
- Yan T, Chopp M, Ye X, Liu Z, Zacharek A, Cui Y, Roberts C, Buller B, Chen J (2012) Niaspan increases axonal remodeling after stroke in type 1 diabetic rats. *Neurobiol Dis* 46:157–164
- Alam M, Schmidt WJ (2004) L-DOPA reverses the hypokinetic behaviour and rigidity in rotenone-treated rats. *Behav Brain Res* 153:439–446
- Sanberg P, Martinez R, Shytle R, Cahill D (1996) The catalepsy test: is a standardized method possible? In: Sanberg PR, Ossenkopp KP, Kavaliers M (eds) *Motor activity and movement disorders*. Humana Press, New York
- Khalil WKB, Booles HF (2011) Protective role of selenium against over-expression of cancer-related apoptotic genes induced by o-Cresol in rats. *Arh Hig Rad Toksikol* 62:121–129
- Linjawi SAA, Khalil WKB, Salem LM (2014) Detoxified *Jatropha curcas* kernel meal impact against benzene-induced genetic toxicity in male rats. *Int J Pharm* 4:57–66
- Cerri S, Ghezzi C, Sampieri M, Siani F, Avenali M, Dornini G, Zangaglia R, Minafra B, Blandini F (2018) The exosomal/total  $\alpha$  synuclein ratio in plasma is associated with glucocerebrosidase activity and correlates with measures of disease severity in PD patients. *Front Cell Neurosci* 12:125
- Wen G, Hui W, Dan C, Xiao-Qiong W, Jian-Bin T, Chang-Qi L (2009) The effects of exercise-induced fatigue on acetylcholinesterase expression and activity at rat neuromuscular junctions. *Acta Histochem Cytochem* 42:137–142
- Zagrodzka J, Romaniuk A, Wiczorek M, Boguszewski P (2000) Bicuculline administration into ventromedial hypothalamus: effects on fear and regional brain monoamines and GABA concentrations in rats. *Acta Neurobiol Exp* 60:333–343

32. Moron MS, Depierre JW, Mannervik B (1979) Level of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. *Biochem Biophys Acta* 582:67–78
33. Wills ED (1966) Mechanism of lipid peroxide formation in animal tissue. *Biochem J* 99:667–676
34. Kono Y (1978) Generation of superoxide radical during auto-oxidation of hydroxylamine and an assay of superoxide dismutase. *Arch Biochem Biophys* 186:189–195
35. Rice ME, Shelton E (1957) Comparison of the reduction of two tetrazolium salts with succinoxidase activity of tissue homogenates. *J Nat Cancer Inst* 18:117–125
36. Babson AL, Babson SR (1973) Kinetic colorimetric measurement of serum lactate dehydrogenase activity. *Clin Chem* 19:766–769
37. Sun H, Li P, Chen W, Xiong X, Han Y (2012) Angiotensin II and angiotensin-(1-7) in paraventricular nucleus modulate cardiac sympathetic afferent reflex in renovascular hypertensive rats. *PLoS ONE* 7:1–11
38. Sun X, Wang D, Yu H, Hu L (2010) Serial cytokine levels during wound healing in rabbit maxillary sinus mucosa. *Acta Otorrhinolaringol* 130:607–613
39. Schreiber G, Tsykin A, Aldred AR, Thomas T, Fung WP, Dickson PW, Cole T, Birch H, De Jong FA, Milland J (1989) The acute phase response in the rodent. *Ann N Y Acad Sci* 557:61–85
40. Pradeep AR, Suke DK, Prasad MV, Singh SP, Martande SS, Nagpal K, Naik SB, Guruprasad CN, Raju AP, Singh P, Siddaya M (2016) Expression of key executioner of apoptosis caspase-3 in periodontal health and disease. *J Invest Clin Dent* 7:174–197
41. Lu T, Xu Y, Mericle MT, Mellgren RL (2002) Participation of the conventional calpains in apoptosis. *Biochem Biophys Acta* 1590:16–26
42. Bancroft J, Stevens A (1996) Theory and practice of histological techniques, 4th edn. Churchill Livingstone, London, pp 40–138
43. Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, Shen J, Takio K, Iwatsubo T (2002) Alpha-synuclein is phosphorylated in synucleinopathy lesions. *Nat Cell Biol* 4:160–164
44. Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, Hardy J, Leverenz JB, Del Tredici K, Wszolek ZK, Litvan I (2009) Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. *Lancet Neurol* 8:1150–1157
45. Ulusoy A, Rusconi R, Perez-Revelta BI, Musgrove RE, Helwig M, Winzen-Reichert B, Di Monte DA (2013) Caudo-rostral brain spreading of alpha-synuclein through vagal connections. *EMBO Mol Med* 5:1051–1059
46. El-Agnaf OM, Salem SA, Paleologou KE, Curran MD, Gibson MJ, Court JA, Schlossmacher MG, Allsop D (2006) Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. *FASEB J* 20:419–425
47. Grondin R, Bedard PJ, HadjTahar A, Gregoire L, Mori A, Kase H (1999) Antiparkinsonian effect of a new selective adenosine A<sub>2A</sub> receptor antagonist in MPTP-treated monkeys. *Neurology* 52:1673–1677
48. Ferre S, Popoli P, Gimenez-Llort L, Rimondini R, Muller CE, Stromberg I, Ögren SO, Fuxe K (2001) Adenosine/dopamine interaction: implications for the treatment of Parkinson's disease. *Parkinsonism Relat Disord* 7:235–241
49. Ascherio A, Zhang SM, Hernan MA, Kawachi I, Colditz GA, Speizer FE, Willett WC (2001) Prospective study of coffee consumption and risk of Parkinson's disease in men and women. *Ann Neurol* 50:56–63
50. Calon F, Dridi M, Hornykiewicz O, BeÂdard PJ, Rajput AH, Di Paolo T (2004) Increased adenosine A<sub>2A</sub> receptors in the brain of Parkinson's disease patients with dyskinesias. *Brain* 127:1075–1084
51. Kachroo A, Schwarzschild MA (2012) Adenosine A<sub>2A</sub> receptor gene disruption protects in an  $\alpha$ -synuclein model of Parkinson's disease. *Ann Neurol* 71:278–282
52. Dungo R, Deeks ED (2013) Istradefylline: first global approval. *Drugs* 73:875–882
53. Lu J, Cui J, Li X, Wang X, Zhou Y, Yang W, Chen M, Zhao J, Gang Pe (2016) An anti-Parkinson's disease drug via targeting adenosine A<sub>2A</sub> receptor enhances amyloid- $\beta$  generation and  $\gamma$ -secretase activity. *PLoS ONE* 11:e0166415
54. Compta Y, Parkkinen L, Kempster P, Selikhova M, Lashley T, Holton JL, Lees AJ, Revesz T (2014) The significance of  $\alpha$ -synuclein, amyloid- $\beta$  and tau pathologies in Parkinson's disease progression and related dementia. *Neurodegener Dis* 13:154–156
55. Shen J (2010) Impaired neurotransmitter release in Alzheimer's and Parkinson's diseases. *Neurodegener Dis* 7:80–83
56. Perez-Lloret S, Barrantes FJ (2016) Deficits in cholinergic neurotransmission and their clinical correlates in Parkinson's disease. *NPJ Parkinson's Dis* 2:16001
57. Muthian G, Mackey V, Prasad K, Charlton C (2018) Curcumin and an antioxidant formulation protect C57BL/6 J mice from MPTP-induced Parkinson's disease like changes: potential neuroprotection for neurodegeneration. *J Parkinsonism Restless Legs Synd* 8:49–59
58. Martin LJ (2008) DNA damage and repair: relevance to mechanisms of neurodegeneration. *Neuropathol Exp Neurol* 67:377–387
59. Zawada WM, Mrak RE, Biedermann JA, Palmer QD, Gentleman SM, Aboud O, Griffin WST (2015) Loss of angiotensin II receptor expression in dopamine neurons in Parkinson's disease correlates with pathological progression and is accompanied by increases in Nox4- and 8-OH guanosine-related nucleic acid oxidation and caspase-3 activation. *Acta Neuropathol Commun* 3:9
60. Saeed A, Shakir L, Khan MA, Ali A, Yousaf M, Zaidi AA (2017) Haloperidol induced Parkinson's disease mice model and motor-function modulation with Pyridine-3-carboxylic acid. *Biomed Res Ther* 4:1305–1317
61. Farshbaf MJ (2017) Succinate dehydrogenase in Parkinson's disease. *Front Biol* 12:175–182
62. Ludtmann MHR, Angelova PR, Horrocks MH, Choi ML, Rodrigues M, Baev AY, Berezhnov AV, Yao Z, Little D, Banushi B, Al-Menhali AS, Ranasinghe RT, Whiten DR, Yapom R, Dolt KS, Devine MJ, Gissen P, Kunath T, Jaganjac M, Pavlov EV, Klenerman D, Abramov AY, Gandhi S (2018)  $\alpha$ -synuclein oligomers interact with ATP synthase and open the permeability transition pore in Parkinson's disease. *Nat Commun* 12:2293
63. Ross JM, Öberg J, Brené S, Coppotelli G, Terzioglu M, Pernold K, Goinyng M, Sitnikov R, Kehr J, Trifunovic A, Larsson N, Hoffer BJ, Olson L (2010) High brain lactate is a hallmark of aging and caused by a shift in the lactate dehydrogenase A/B ratio. *PNAS* 107:20087–20092
64. Zhou J, Liu T, Guo H, Cui H, Li P, Feng D, Hu E, Huang Q, Yang A, Zhou J, Luo J, Tang T, Wang Y (2018) Lactate potentiates angiogenesis and neurogenesis in experimental intracerebral hemorrhage. *Exp Mol Med* 50:78
65. Jha N, Jurma O, Lalli G, Liu Y, Pettus EH, Greenamyre JT, Liu RM, Forman HJ, Andersen JK (2000) Glutathione depletion in PC12 results in selective inhibition of mitochondrial complex I activity: implications for Parkinson's disease. *J Biol Chem* 275:260996
66. Jiang T, Gao L, Lu J, Zhang YD (2013) ACE2-Ang-(1–7)-mas axis in brain: a potential target for prevention and treatment of ischemic stroke. *Curr Neuropharmacol* 11:209–217
67. Garrido-Gil P, Valenzuela R, Villar-Cheda B, Lanciego JL, Labandeira-Garcia JL (2013) Expression of angiotensinogen and receptors for angiotensin and prorenin in the monkey and human substantia nigra: an intracellular renin-angiotensin system in the nigra. *Brain Struct Funct* 218:373–388



68. Benigni A, Cassis P, Remuzzi G (2010) Angiotensin II revisited: new roles in inflammation, immunology and aging. *EMBO Mol Med* 2:247–257
69. Sawada H, Oeda T, Umemura A, Tomita S, Kohsaka M, Park K, Yamamoto K, Sugiyama H (2015) Baseline C-reactive protein levels and life prognosis in Parkinson disease. *PLoS ONE* 10:e0134118
70. Qiu X, Xiao Y, Wu J, Gan L, Huang Y, Wang J (2019) C-reactive protein and risk of Parkinson's disease: A systematic review and meta-analysis. *Neurology, Front*. <https://doi.org/10.3389/fneur.2019.00384>
71. Khadrawy YA, Salem AM, El-Shamy KA, Ahmed EK, Fadl NN, Hosny EN (2017) Neuroprotective and therapeutic effect of caffeine on the rat model of Parkinson's disease induced by rotenone. *J Diet Suppl* 14:553–572
72. Hegde ML, Hegde PM, Holthauzen LM, Hazra TK, Rao KS, Mitra S (2010) Specific inhibition of NEIL-initiated repair of oxidized base damage in human genome by copper and iron: potential etiological linkage to neurodegenerative diseases. *J Biol Chem* 285:28812–28825
73. Wang XS, Zhang ZR, Zhang MM, Sun MX, Wang WW, Xie CL (2017) Neuroprotective properties of curcumin in toxin-based animal models of Parkinson's disease: a systematic experiment literatures review. *BMC Comp Alt Med* 17:412
74. Picada JN, Flores DG, Zettler CG, Marroni NP, Roesler R, Henriques JA (2003) DNA damage in brain cells of mice treated with an oxidized form of apomorphine. *Mol Brain Res* 114:80–85
75. Sawada M, Imamura K, Nagatsu T (2006) Role of cytokines in inflammatory process in Parkinson's disease. *J Neural Transm Suppl* 70:373–381
76. Hirsch EC, Hunot S, Hartmann A (2005) Neuroinflammatory processes in Parkinson's disease. *Parkinsonism Relat Disord* 11:S9–S15
77. Yang R, Dunn JF (2019) Multiple sclerosis disease progression: contributions from a hypoxia-inflammation cycle. *Mult Scler* 25:1715–1718
78. Hofmann KW, Schuh AFS, Saute J, Townsend R, Fricke D, Leke R, Souza DO, Portela LV, Chaves MLF, Rieder CRM (2009) Interleukin-6 serum levels in patients with Parkinson's disease. *Neurochem Res* 34:1401–1404
79. Bessler H, Djaldetti R, Salman H, Bergman M, Djaldetti M (1999) IL-1b, IL-2, IL-6 and TNF-a production by peripheral blood mononuclear cells from patients with Parkinson's disease. *Biomed Pharmacother* 53:141–145

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