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ORIGINAL ARTICLE

# Neuromodulatory Potential of Aqueous Extracts of Cumin, Cinnamon: Evidence from Rotenone Model in Drosophila: Implications to Parkinson's Disease

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## ABSTRACT

**AIM:** Spice extracts and their bioactive molecules have been well recognized for their innumerable beneficial effects against various chronic diseases. However, experimental data regarding their potential to abrogate oxidative stress and neurotoxicity in animal models of Parkinson's disease (PD) are rather limited. In the current study, we aimed to assess the neuromodulatory potential of aqueous extracts of spices viz., cumin and cinnamon and their bioactives (Cuminaldehyde (CU) and Cinnamaldehyde (CN) using a rotenone (ROT) model of neurotoxicity in Drosophila.

**MATERIALS AND METHODS:** Adult male flies (Oregon K) were fed medium enriched (0.1-0.2%) with aqueous extract of Cumin or Cinnamon and their bio-actives (CU/ CN) with without ROT (500 μM) for 7 days. The propensity of extracts or bio-actives to protect flies against ROT-induced lethality, locomotor phenotype, oxidative stress and neurotoxicity was determined. While both the extracts significantly protected the flies against ROT-induced mortality, the survivors exhibited improved locomotor phenotype. Further both CU and CN-enrichment markedly reduced the ROT -induced lethality, diminished locomotor deficits and significantly abrogated the degree of oxidative impairments. Both bio-actives also augmented the antioxidant enzyme activities and restored ROT-induced mitochondrial dysfunctions. Interestingly, ROT -induced elevation of the activity of acetylcholinesterase and depletion of dopamine levels were also restored. Further, flies provided prophylactic treatment with bio-actives exhibited significant resistance to an acute exposure to Paraquat (PQ). In a parallel study, both bio-actives were found to significantly delay the onset of locomotor deficits among ROT-stressed flies besides extending their survival.

**CONCLUSION:** We hypothesise that the efficacy aqueous extract and their bioactives to attenuate ROT-mediated neurotoxicity may be largely related to the combined antioxidant activity of bio-actives resulting in improved locomotor performance, abrogation of oxidative stress and mitochondrial dysfunction. Based on these results, we propose that cumin and cinnamon extracts may be exploited as therapeutics against PD and other neurodegenerative diseases.

**Key words:** Cuminaldehyde; Cinnamaldehyde; *Drosophila*; Rotenone; Oxidative stress; Neurotoxicity; Parkinsonism



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## INTRODUCTION

Spices are primarily used as flavoring agents in food products but also, they are also employed in the preservation of food and afford nutritional and health benefits. Different components in spices viz., fiber, carbohydrate, fat, sugar, protein, gum, ash, volatile (essential oils), and other non-volatile components impart the particular flavor, color, nutritional, health, or preservative effects[1]. Phytomedicines are known for safety, efficacy, and lesser side effects[2]. Several studies have shown that habitual ingestion of phytochemicals benefit health by improving mental and physical performance, increasing neuronal cell survival and up regulate the antioxidant defences[3,4]. Natural phytochemicals are becoming popular both in developed and developing countries. However, since these traditional herbal medicines are commonly prepared from crude materials, current research aims to re-examine their specific effects, reproducibility, and mechanism of action and the identification of active ingredients[5].

Cumin (Cuminum cyminum) a flowering plant (family Apiaceae) is a native from the east Mediterranean to East India and is well known for its antioxidant properties. The most important chemical components of cumin seeds are α-pinene, Myrcene, limonene, 1-8- cineole, cymene, pinene, D-terpinene, cuminaldehyde, cuminyl alcohol, etc. Cuminaldehyde (CU) is the major component in cumin. Studies on Cumin essential oil and non-volatile extracts have shown robust antioxidant properties (*in vitro*) and inhibitory properties against free radicals[6]. Cinnamon (Cinnamomum verum) is obtained from the inner bark of trees of tropical medicine belongs to the Lauraceae family. Being one of the most important spices used in culinary practices all over the world, Cinnamon comprises an array of resinous compounds, including cinnamaldehyde, cinnamate, cinnamic acid, and numerous essential oils. Cinnamaldehyde (CN), the major component in cinnamon is well known for its antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, lipid-lowering, and cardiovascular-disease-lowering properties[7].

The predominant physiological symptoms of degenerative diseases include elevated oxidative/nitrosative stress, mitochondrial dysfunction, protein misfolding/aggregation, synapse loss, and decreased neuronal survival[8]. Parkinson's disease (PD), one of the common neurodegenerative disorders which affect the aging human is a synucleinopathy, with the accumulation of misfolded α-synuclein that forms intracellular inclusions in neurons, Lewy bodies and Lewy neurites. Loss of dopaminergic (DAergic) neurons in the substantia nigra of the midbrain is the distinctive neuropathological trait in PD while the prominent clinical features include motor symptoms (bradykinesia, tremor, stiffness and postural unsteadiness) and non-motor related symptoms (olfactory deficits, autonomic dysfunction, depression, cognitive deficits and sleep disorders)[9].

Drosophila melanogaster has been extensively employed as an experimental model to obtain basic insights into the genetics and pathophysiology of several human neurodegenerative diseases[10]. It is a unique minuscule system with rapid generation time. Pioneering studies have demonstrated that, chronic rotenone exposure causes selective loss of dopaminergic neurons and severe locomotor dysfunctions in fly models[10-12]. It has been extensively employed to screen a broad range of phytochemicals/bio-actives for their potential to prevent or ameliorate biochemical/phenotypical aberrations induced in chemical models and or/transgenic models of Alzheimer's and PD[13-14]. Accordingly, we have successfully employed Drosophila melanogaster model to assess the neuroprotective potential of various plant extracts[15-17] and spice bio-actives[18] against neurotoxin exposure.

Owing to their well-known antioxidant and anti-inflammatory properties, we hypothesized that extracts of cumin and cinnamon and their bioactives are likely to alleviate rotenone (ROT) -mediated oxidative stress and neurotoxic implications in the fly model. First, we assessed if the aqueous extracts of cumin and cinnamon possess the propensity to modulate the endogenous levels of oxidative markers in flies. Further, in a co-treatment regime, we determined the efficacy of cuminaldehyde and cinnamaldehyde to ameliorate locomotor deficits, oxidative impairments, cholinergic and mitochondrial enzyme dysfunctions and decreased dopamine levels induced by ROT. Further, their ability to offer resistance to an acute paraquat (PQ) exposure was also studied in an oxidative stress bioassay.

## MATERIALS AND METHODS

### Chemicals

Cuminaldehyde (CU), Cinnamaldehyde (CN), Rotenone (ROT), Paraquat (PQ), and Dopamine (DA) were purchased from M/s Sigma Chemical Co., (St. Louis, USA). All other chemicals were purchased from Sisco Research Laboratory Chemicals (Mumbai, India).

### Preparation of aqueous extracts of cumin and cinnamon

Cumin seeds and cinnamon bark purchased from local market were dried, powdered. 10% spice powder in double distilled water kept under continuous stirring for 24 h at room temperature. The extract was centrifuged and evaporated to dryness and the dry residue was stored at 4°C until use.

### Quantification of major bioactives in cumin/cinnamon extracts

The bioactives in the extracts were quantified by HPLC using an isocratic mobile phase of acetonitrile: water (76:24%) at a flow rate of 1.0 mL/min.

### Drosophila culture

D. melanogaster, wild (Oregon K), adult flies maintained and cultured in our research institute were employed for the study and they were maintained as described previously from our laboratory[15]. Age-synchronized adult male flies (9-10 d old, 50 per replicate, 3 replicates per group) were used for each study.

### Experimental design

**Effect of aqueous extracts of Cumin/ Cinnamon on endogenous levels of oxidative markers:** Adult male flies were maintained for 7 days on medium enriched either with cumin or cinnamon extract (0.05-0.1%). Terminally, the extent of oxidative stress (enzymatic and non-enzymatic markers) was determined.

**Cumin/cinnamon extracts rescue rotenone (ROT)-induced locomotor phenotype:** Employing a co-exposure paradigm, 8-10d old flies were exposed to medium containing ROT (500 µM) and cumin/cinnamon extract (0.01-0.1%) for 7 days. The extent of survival of the flies and the locomotor phenotype were monitored in all the experimental groups.

**Efficacy of cuminaldehyde (CU) and cinnamaldehyde (CN) to ameliorate ROT-induced locomotor phenotype, oxidative impairments and neurotoxicity:** We assessed the potency of cuminaldehyde and cinnamaldehyde to ameliorate ROT-induced neurotoxicity. The concentration of ROT (500 µM, 7d) used was selected based on our earlier studies[14,15]. At the end of each day, flies were examined for mortality and locomotor deficits in all the experimental groups. Two concentrations (10 and 25 µM) of CU/CN were as employed for all the biochemical investigations. Terminally, various biochemical determinations were made in both head and body regions.

**Oxidative stress bioassay: Prophylactic efficacy of CU/CN against Paraquat (PQ) exposure***:* In this study, flies maintained on

CU and CN (10-25 µM) -enriched medium for 10 d were exposed to PQ (20mM, in 5% sucrose solution) for 5 d and survival and locomotor deficits were monitored as described previously[15].

**Efficacy of CU / CN enriched diet to enhance Longevity of flies:** Two groups of flies were maintained - medium enriched with CU and CN per se (10-25 µM) and co-exposure group (ROT + CU/CN). Mortality (daily) and climbing ability (at intervals of 5 d) was assessed.

**Assessment of locomotor performance by negative geotaxis assay**

Locomotor performance of flies was assessed employing a negative geotaxis assay as described previously[11]. Data was expressed as percent flies escaped a minimum distance of 10 cm in 20 s.

**Sample preparation and biochemical assays**

Flies were anesthetized mildly using diethyl ether as described earlier[15] and oxidative stress markers, activities of mitochondrial enzymes and MTT reduction were assayed. Both head and body regions were used for the assays.

Reactive oxygen species (ROS) generated was assayed as described earlier with minor modifications[19]. The levels of HP were determined using FOX reagent as described previously[20]. The levels of nitric oxide (NO) were assayed by employing Griess reagent[21]. The levels of reduced glutathione were measured by following o-pthalaldehyde fluorescence[22] while the total thiols levels were determined following a previously described method with minor modifications[22]. Superoxide dismutase (SOD) activity was assayed by monitoring the inhibition of quercetin auto-oxidation[23]. Catalase activity was determined by monitoring the breakdown of $H_2O_2$[24]. Thioredoxin reductase (TR) activity in the sample was measured by monitoring the reduction of DTNB[25]. Glutathione-S-transferase (GST) activity was quantified by monitoring the conjugation of glutathione to CDNB[26].

AChE activity was estimated according to a standard method[27] by employing acetylthiocholine iodide as the substrate. Dopamine (DA) content was analyzed by HPLC[28]. The sample was eluted using the mobile phase 0.2% aqueous trifluoroacetic acid and methanol (70:30, v/v) at the flow rate of 1 mL/min.

NADH–cytochrome C reductase activity was measured following the reduction of cytochrome C[29]. The activity of succinate-cytochrome-C reductase (complex II-III) was measured by following the reduction of cytochrome-C by the sample[29]. The MTT reduction 5-dimethylthiazol-2-ylwas measured as described earlier[30]. Protein content in the samples was measured using Folin–Ciocalteau's phenol reagent[31].

**Statistical analysis**

Data were expressed as mean ± standard error (SE) for each experimental group. The various statistical analyses were performed using Graph Pad prism version-5.0.

## RESULTS

**Constituents of cumin and cinnamon extracts**

The major constituents, cuminaldehyde (λmax- 350) from cumin and cinnamaldehyde ($\lambda_{max}$- 280) from cinnamon were analysed by HPLC. The calculated amount of cuminaldehyde in the cumin aqueous extract and cinnamaldehyde in cinnamon aqueous extract was 4.15 and 8.94 mg/mL respectively.

**Cumin/Cinnamon extracts modulate endogenous levels of oxidative markers**

While the lowest concentration (0.01%) of spice extracts had no effect, a significant decrease in the oxidative stress markers was evidenced at higher concentrations (0.05 and 0.1%). The endogenous ROS levels were significantly diminished (Cumin: 20-30%; Cinnamon: 25-36%) (Table1). Cumin treatment markedly diminished the levels of HP (head - 48%; body -32%). A similar result was also obtained with cinnamon extract. While the basal NO levels were decreased, the levels of GSH and total thiols were markedly enhanced by both the extracts (Table 2).

**Protective effect of Cumin/Cinnamon aqueous extracts and their bioactives**

ROT (500 µM)-induced significant lethality from 3d which progressed with the duration of exposure and the cumulative mortality was nearly 50%. Marked protection against ROT-induced mortality was evident with both the extracts (Figure 1A). While the protection at the lowest concentration (0.01%) was only marginal, the protection was robust (80-85%) with higher concentrations. ROT alone caused a severe (65%) impairment in locomotor activity (on day 7). In contrast, marked improvement (55- 68%) in locomotor performance was observed in the co-exposure paradigm (Figure 1B). Similarly, spice bioactives CU and CN proved effective in combating ROT toxicity. ROT caused significant (55%) lethality on day 7 and survivors exhibited marked motor deficits (68%). However, in the co-exposure paradigm, both CU and CN provided a concentration related protection which was evident from the reduced incidence of lethality (CU 29-57%; CN 40-52%)(Fig 1C). Furthermore, with CU and CN enrichment, flies showed marked improvement in locomotor performance at both the concentrations (Figure 1D).

**Modulatory effect on oxidative damage, enzymic antioxidants and NO levels**

In general, cytosolic ROS and HP levels were markedly enhanced with ROT exposure. In contrast, the levels were significantly diminished with CU and CN enrichment (Figure 2 A, B). Both the bioactives caused significant restoration of GSH levels and the activity levels of GST. Interestingly, ROT induced elevated levels of NO were also restored with CU and CN enrichment (Table 3). The activity levels of various enzymic antioxidants (viz., catalase, SOD, TRR) were significantly diminished with ROT exposure suggesting a state of oxidative stress *in vivo*. In general, co-exposure with bioactives caused varying degree of restoration in the activity levels of enzymes (Table 4).

**AChE activity and dopamine (DA) content**

AChE activity was significantly elevated in ROT flies, while the levels were restored to normal levels by CU supplementation. CN was effective at lower concentration (10 µM) (Figure 3A). ROT exposure significantly depleted the DA levels (head -52%; body -51%). Both the bioactives significantly restored the DA levels (CU: 29-36%; CN: 37-43%) (Figure 3B).

**Effect on mitochondrial enzymes**

ROT induced a significant reduction in the activity levels of complexes I–III (37%). Co-exposure of flies to CU and CN showed marked elevation in the activity levels of complex I-III in head (CU: 27%; CN: 17%) and body (CU: 16%; CN: 28%) (Table 5). Likewise, ROT induced a significant reduction in the activity levels of complex II-III (head: 34%; body: 48%) which was attenuated by both bioactives. Further, ROT also caused a marked diminution in MTT reduction in mitochondria (head -37%; body -31%), while both bioactives caused significant restoration. CN seemed to be efficient at the lowest concentration (10 µM) (Table 5).

**Table 1** Status of endogenous levels of oxidative stress markers in adult *Drosophila melanogaster* fed with aqueous extracts of cumin and cinnamon enriched diet for 7 days.

| Biochemical parameters | Region | Control | Cumin | | Cinnamon | |
|---|---|---|---|---|---|---|
| | | | 0.05% | 0.10% | 0.05% | 0.10% |
| ROS[a] | Head | 49.18±2.51 | 36.46±1.62* | 33.30±0.49* | 37.95±1.44* | 38.36±0.97* |
| | Body | 5.13±0.22 | 3.38±0.08* | 6.72±0.23* | 4.10±0.04* | 3.47±0.14* |
| HP[b] | Head | 0.25±0.02 | 0.16±0.012* | 0.14±0.005* | 0.16±0.012* | 0.15±0.014* |
| | Body | 0.33±0.014 | 0.23±0.003* | 0.21±0.006* | 0.22±0.012* | 0.24±0.008* |
| NO[c] | Head | 2.52±0.07 | 2.36±0.026 | 3.09±0.04* | 1.94±0.10* | 2.12±0.09* |
| | Body | 0.45±0.025 | 0.26±0.011* | 0.32±0.012* | 0.26±0.011* | 0.32±0.012* |

Values are mean ± SE ($n$ = 50 flies/replicate; three replications/group). Data analyzed by one-way ANOVA followed by Dunnett's test ($p < 0.05$); *Significantly different compared to control; Fly Medium enriched with spice extracts at 0.05 and 0.1% levels; ROS-Reactive oxygen species, HP-Hydroperoxides, NO-Nitric oxide. a: pmolDCF/min/mg protein; b: µmol hydroperoxides/min/mg protein; c: nmolnitricoxide/min/mg protein.

**Table 2** Status of reduced glutathione (GSH) and total thiols (TSH) in adult *Drosophila melanogaster* fed with aqueous extracts of cumin -and cinnamon -enriched diet for 7 days.

| | | GSH[a] | | TSH[b] | |
|---|---|---|---|---|---|
| | | Head | Body | Head | Body |
| Control | 0 | 59.56 ± 0.77 | 229.42 ± 4.73 | 25.62 ± 0.55 | 24.82 ± 1.16 |
| Cumin | 0.05% | 78.28 ± 1.5* | 284.97 ± 7.65* | 32.13 ± 0.72* | 35.58 ± 1.12* |
| | 0.10% | 72.75 ± 1.38* | 267.26 ± 7.81* | 38.39 ± 0.7* | 31.56 ± 1.15* |
| Cinnamon | 0.05% | 73.59 ± 1.14* | 275.34 ± 5.85* | 36.78 ± 0.73* | 38.01 ± 1.08* |
| | 0.10% | 80.67 ± 0.85* | 238.78 ± 3.74* | 39.22 ± 0.59* | 36.70 ± 1.76* |

Values are mean ± SE ($n$ = 50 flies/replicate; three replications/group). Data analyzed by one-way ANOVA followed by Dunnett's test ($p < 0.05$); * Significantly different compared to control; Fly Medium enriched with spice extracts at 0.05 and 0.1% levels. a: µmol GSH/mg protein; b: nmol thiols/mg protein.

**Table 3** Effect of Cuminaldehyde , Cinnamaldehyde - enriched diet on Rotenone (500µM) induced alterations on reduced glutathione (GSH), Nitric oxide ( NO) levels and acactivity level s of glutathione transferase in adult *Drosophila melanogaster*.

| Treatment group | Region | GSH[a] | GST[b] | NO[c] |
|---|---|---|---|---|
| CTR | Head | 48.82 ± 2.12 | 149.79 ± 3.79 | 0.21 ± 0.01 |
| | Body | 167.71 ± 4.0 | 163.35 ± 4.03 | 2.10 ± 0.02 |
| ROT | Head | 29.01 ± 0.84* | 105.60 ± 2.63* | 0.38 ± 0.01* |
| | Body | 144.69 ± 2.88* | 116.1 ± 3.29* | 3.58 ± 0.05* |
| ROT+CU1 | Head | 32.81 ± 1.50* | 119.5 ± 0.56*# | 0.26 ± 0.01# |
| | Body | 132.95 ± 3.29*# | 128.70 ± 3.14*# | 3.24 ± 0.03*# |
| ROT+CU2 | Head | 40.28 ± 0.69*# | 125.59 ± 2.71*# | 0.23 ± 0.01# |
| | Body | 157.8 ± 1.37# | 153.28 ± 2.58# | 3.03 ± 0.04*# |
| ROT+CN1 | Head | 35.18 ± 2.18* | 124.47 ± 2.57*# | 0.26 ± 0.01# |
| | Body | 144.11 ± 3.27*# | 146.62 ± 2.09*# | 2.87 ± 0.06*# |
| ROT+CN2 | Head | 43.08 ± 2.15# | 116.52 ± 2.51* | 0.32 ± 0.01# |
| | Body | 133.11 ± 3.20*# | 131.49 ± 4.08*# | 3.04 ± 0.01*# |

Values are mean ± SE ($n$ = 50 flies/replicate; three replications/group). Data analyzed by one-way ANOVA followed by Tukey's test ($p < 0.05$); * significantly different compared to control; # significantly different compared to Rotenone. CU: Cuminaldehyde, CN: Cinnamaldehyde, (CU1: 10 µM, CU2: 25 µM, CN1: 10 µM, CN: 25 µM); a: µmol GSH/mg protein, b: nmol NADPH oxidised/min/mg protein; c:m nmol nitric oxide/min/mg protein.

**Table 4** Effect of Cuminaldehyde , Cinnamaldehyde enriched diet against Rotenone (500 µM) induced alterations on antioxidant enzyme activity levels.

| Treatment group | Region | SOD[a] | CAT[b] | TR[c] |
|---|---|---|---|---|
| CTR | Head | - | 88.11 ± 1.33 | 9.99 ± 0.85 |
| | Body | 114.42 ± 3.74 | 172.23 ± 1.84 | 18.54 ± 0.47 |
| ROT | Head | - | 51.78 ± 1.21* | 5.83 ± 0.98* |
| | Body | 61.65 ± 4.11* | 225.30 ± 5.83* | 10.59 ± 0.49* |
| ROT+CU1 | Head | - | 67.49 ± 0.77*# | 7.12 ± 0.07* |
| | Body | 79.31 ± 1.86*# | 192.44 ± 3.77*# | 13.05 ± 0.09*# |
| ROT+CU2 | Head | - | 77.25 ± 1.39*# | 8.39 ± 0.29# |
| | Body | 93.06 ± 4.74*# | 176.80 ± 1.93*# | 15.31 ± 0.22*# |
| ROT+CN1 | Head | - | 72.67 ± 0.98*# | 7.31 ± 0.08* |
| | Body | 93.39 ± 2.96*# | 182.79 ± 3.44*# | 14.98 ± 0.44*# |
| ROT+CN2 | Head | - | 65.01 ± 0.89*# | 8.66 ± 0.17# |
| | Body | 89.33 ± 4.16*# | 171.93 ± 5.19*# | 15.39 ± 0.12*# |

Values are mean ± SE ($n$ = 50 flies/replicate; three replications/group). Data analyzed by one-way ANOVA followed by Tukey's test ($p < 0.05$); * Significantly different compared to control; # significantly different compared to Rotenone. CU: Cuminaldehyde, CN: Cinnamaldehyde, (CU1: 10 µM, CU2: 25 µM, CN1: 10 µM, CN2: 25 µM). SOD: Superoxide dismutase, CAT: Catalase, TR: Thioredoxin reductase. a: units/mg protein; b: nmol HP decomposed/mg protein; c: nmol adduct formed/min/mg protein.

**Prophylaxis with bioactives alleviates PQ induced phenotype and lethality**

Adult flies given prophylactic treatment (10 d) with CU and CN exhibited significant resistance against PQ- induced lethality. While PQ caused 60% mortality (at the end of 5 d), flies pre-treated with CU and CN, showed a low incidence of mortality and the effect was concentration dependent. The degree of protection was higher with a lower concentration of CN (10 µM-33%) compared with that of CU (25 µM -23%) (Figure 4A). PQ exposure also induced marked impairment of locomotor activity (65%). Among untreated controls, more than 90% flies were able to reach the top of the vial in 20 sec, while PQ exposed flies exhibited a significant decrease in climbing ability (only 35% climbed). In contrast, prophylaxis with both bioactives markedly improved the locomotor performance of flies exposed to PQ (CU-30%; CN-42%) (Figure 4B).

**Life span and locomotor performance of flies exposed to ROT.**

ROT (50 µM) exposure caused 100% mortality by day 55, while flies maintained on spice active -enriched diet survived longer, and their maximum life was significantly extended (CU: 15-18% (8-10 d); CN: 11-15% (6-8d) (Figure 5 A, B). Significant motor deficits were evident in flies exposed to ROT which developed over the experimental period and on day 45, they exhibited marked (36%) motor deficits compared to control flies. There was a marked decrease (36-48%) in locomotor deficits among flies provided CU and CN enriched diet (Data not shown).

## DISCUSSION

The primary objective of the present study was to evaluate the neuroprotective effects of commonly used spices, cumin and cinnamon and their major bioactive molecules viz., cuminaldehyde (CU) and cinnamaldehyde(CN) employing a rotenone model of neurotoxicity in Drosophila. Both these spices are extensively used in culinary practices in the Indian subcontinent and elsewhere[32] and are known to possess multiple pharmacological properties. Cumin is a potent antioxidant capable of scavenging a variety of free radical such as hydroxyl, peroxy and DPPH radicals in vitro and thus inhibits radical mediated lipid peroxidation *in vivo*[33]. Several studies have also reported the antioxidant, antimicrobial[34,35] and antidiabetic potential of CN. Further, a recent study demonstrated the inhibitory effect of CN on α-synuclein fibrillation and cytotoxicity in PC12 cells suggesting its therapeutic potential in neurodegenerative conditions[36]. Likewise, CN is also known to possess antimicrobial[37], antioxidant[38], and antidiabetic[39] properties. Despite these reports, the neuroprotective efficacy of the cuminaldehyde and cinnamaldehyde has not been examined in animal models. Hence, we sought to address their potential to alleviate neurotoxicity in a PD model of Drosophila.

In recent times, the potential of natural compounds to attenuate the endogenous redox status *in vivo* has been considered as an effective approach to achieve neuroprotection[40,41]. Since the antioxidant effects of both cumin and cinnamon are well known, we initially

**Table 5** Protective effect of Cuminaldehyde, Cinnamaldehyde enricheddiet on the on activities of NADH-cyt C reductase (A), Succinate-cyt C reductase (B) and MTT reduction (C) in mitochondria of flies exposed to ROT (500 µM).

| Treatment group | Region | Complex I-III [a] | Complex II-III[b] | MTT reduction[c] |
|---|---|---|---|---|
| CTR | Head | 8.67 ± 0.35 | 12.85 ± 0.12 | 9.87 ± 0.19 |
| | Body | 8.58 ± 0.19 | 10.6 ± 0.31 | 9.93 ± 0.16 |
| ROT | Head | 5.49 ± 0.13* | 8.68 ± 0.12* | 6.24 ± 0.04* |
| | Body | 5.42 ± 0.30* | 5.56 ± 0.48* | 6.83 ± 0.34* |
| ROT+CU1 | Head | 6.18 ± 0.11* | 10.46 ± 0.30*# | 7.26 ± 0.14*# |
| | Body | 6.48 ± 0.20*# | 7.04 ± 0.16*# | 8.12 ± 0.06*# |
| ROT+CU2 | Head | 7.57 ± 0.09*# | 11.61 ± 0.15*# | 8.34 ± 0.06*# |
| | Body | 6.34 ± 0.05*# | 8.45 ± 0.24*# | 9.06 ± 0.04*# |
| ROT+CN1 | Head | 8.0 ± 0.04* | 11.29 ± 0.09*# | 7.31 ± 0.06*# |
| | Body | 7.56 ± 0.15*# | 8.52 ± 0.26*# | 8.3 ± 0.02*# |
| ROT+CN2 | Head | 6.65 ± 0.16*# | 10.42 ± 0.11*# | 6.65 ± 0.19* |
| | Body | 6.5 ± 0.08*# | 7.48 ± 0.15*# | 7.77 ± 0.07*# |

Values are mean ± SE ($n$ = 50, 3 replicates); Data analyzed by One-way ANOVA ($p \leq 0.05$) followed by Tukey's Multiple Comparison Test. *- Significantly different compared to control; # Significantly different compared to rotenone. CU: Cuminaldehyde, CN: Cinnamaldehyde; (CU1: 10 µM, CU 2: 25 µM, CN1: 10 µM, CN2: 25 µM); a: nmol cytochrome-C reduced/min/mg protein; b: nmol cytochrome-C reduced/min/mg protein; c: - OD/mg protein.

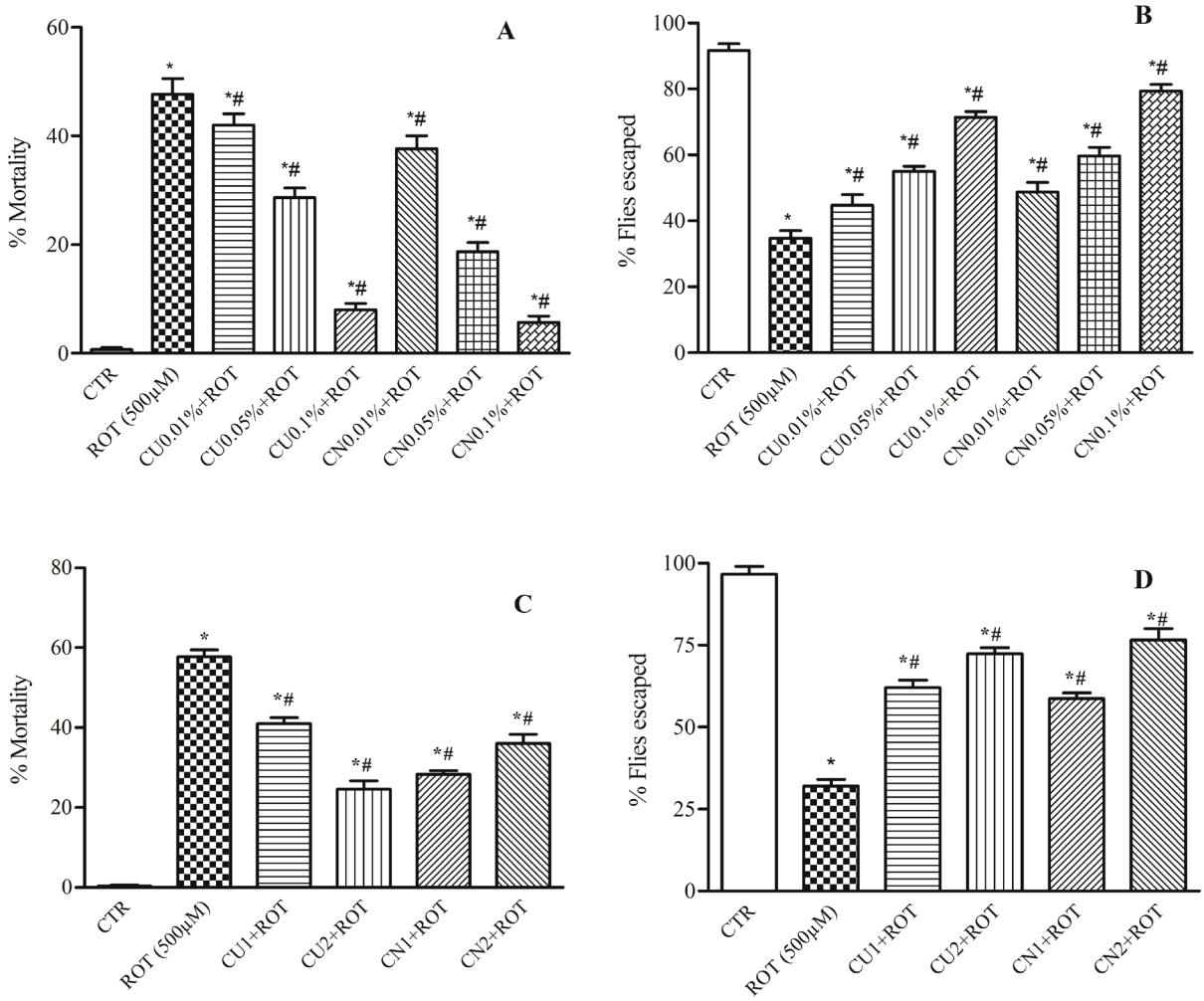


**Figure 1** Effect of cumin and cinnamon aqueous extracts and their actives cuminaldehyde (CU) and cinnamaldehyde (CN) against Rotenone (500µM) induced mortality (A), (C) and locomotor deficits (B), (D) in adult male *Drosophila melanogaster*; (CU1 and CN1-10µM, CU2 and CN2-25 µM). Values are mean ± SD ($n$=25, 3 replicates). Data analyzed by One-way ANOVA ($p \leq 0.0001$) followed by Tukey's Multiple Comparison Test. *significantly different compared to control; # Significantly different compared to ROT.

examined the modulatory potential of extracts on endogenous redox markers in head and body regions of Drosophila employing a dietary approach. Enrichment with extracts brought about significant reduction in the levels of various oxidative in head and body of flies. In addition, it also increased the activity levels of enzymic antioxidant defenses. The diminished levels of oxidative markers along with enhanced antioxidant enzyme activities strongly suggest the antioxidative property of the extracts and biactives *in vivo*. This evidence further substantiates the previous findings on the antioxidative and anti-inflammatory potential of cumin and cinnamon extracts in other experimental models[38,39].

Pioneering studies have demonstrated the behavioural effects of a sub-lethal chronic exposure to ROT and the development of Parkinson-like symptoms and neurodegeneration in Drosophila model[42,43]. Owing to this, ROT model is extensively employed to understand the neuroprotective potential of several molecules, phytomedicines, and plant extracts. The neurotoxic effects of ROT are multifactorial. In animal models, besides inhibition of complex-I and ROS generation, ROT is demonstrated to be involved in the activation of microglia, ATP-depletion, oxidative damage of biomolecules, induction of apoptosis and acceleration of α-synuclein aggregation and fibrillation. Hence, a combination of all these factors may underlie the selective degeneration of dopaminergic neurons caused by ROT[44,45]. The mechanism by which ROT induces PD like symptoms following chronic exposure is not clearly known although the participation of oxidative stress is generally well accepted both in fly[9,12] and rodent models[46]. Hence, we chose to examine the potential of spice bio-actives to alleviate ROT-induced oxidative impairments/mitochondrial dysfunctions in this model.

In the present study, exposure of flies to ROT (500 µM) resulted in marked oxidative stress which corroborates with our previous findings[14-17,47]. While flies exposed to ROT alone exhibited marked locomotor deficits, diet-enrichment with spice extracts/ bio-actives (CU and CN) resulted in significant improvement in the locomotor performance. This is suggestive of their potential to ameliorate the oxidative stress-mediated effects through their antioxidant action. These results also corroborate with the free radical scavenging ability of cumin and cinnamon[48,49]. Further, the decreased incidence of lethality (increased survival rate) evidenced among flies maintained on CU and CN enriched diet in the co-exposure regime clearly suggests their ability to promote survival pathways which are atleast in part may be mediated through antioxidant action. In the present study, ROT exposure resulted in a significant depletion of reduced GSH levels with an associated decrease in the activity of several antioxidant enzymes clearly indicating that the flies were subjected to oxidative stress. Both CU and CN enrichment resulted in enhanced GSH levels in flies with concomitant elevation in the activities of antioxidant enzymes. The potential of CN appeared to be higher compared to CU, a finding consistent with a recent report which showed that pre-treatment with CN extract could ameliorate toxic oxidative effects of bisphenol-induced pathological changes in several organs such as kidney, brain and testis[50]. Since GSH and antioxidant enzymes provide the cell with numerous defences against various toxic products[51,52] we speculate that both CU and

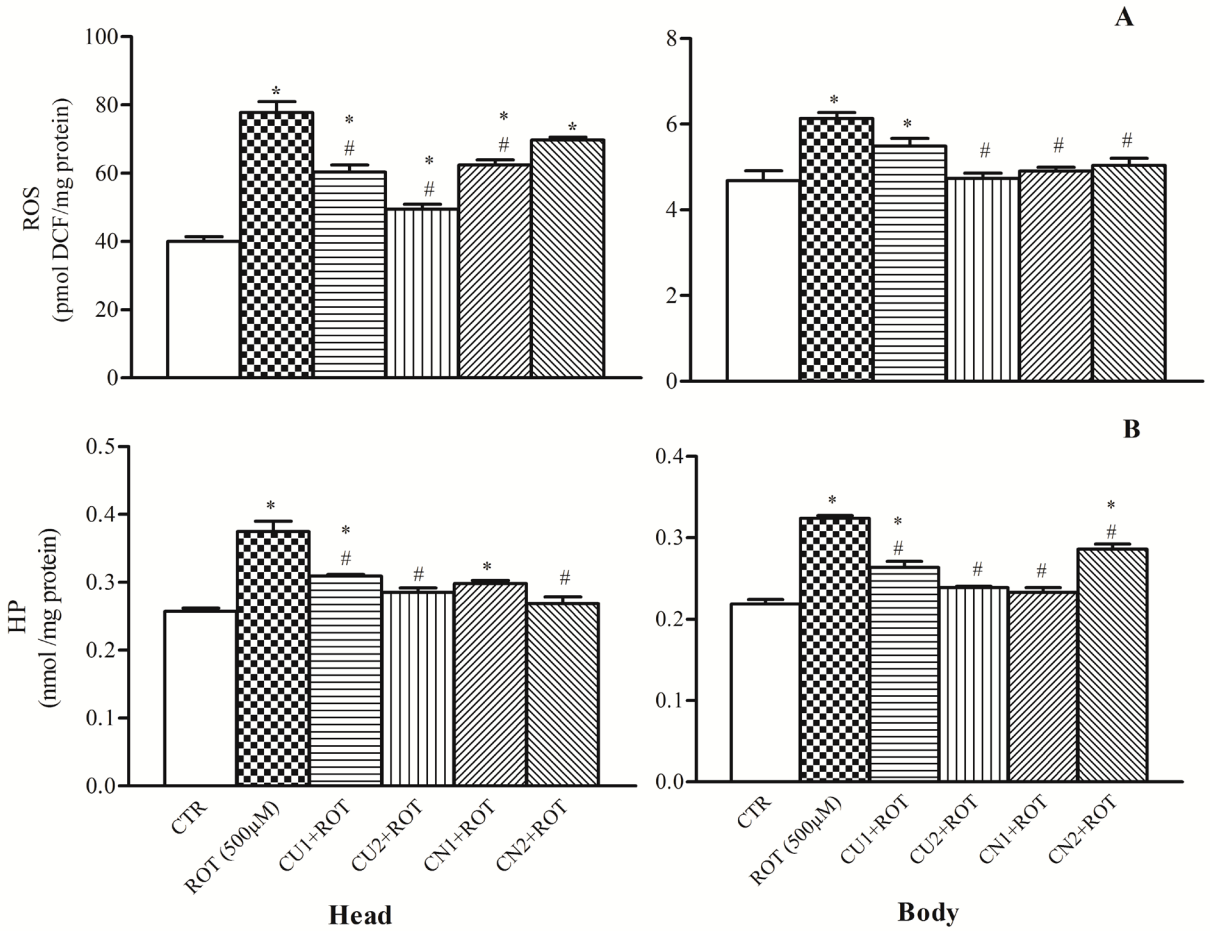


**Figure 2** Effect of CU/CN enriched diet on Rotenone (500 µM) induced alterations in ROS (A) and HP levels (B) in adult male Drosophila melanogaster; (CU1 and CN1-10µM, CU2 and CN2-25 µM). Values are mean ± SE ($n$ = 50, 3 replicates). Data analyzed by One-way ANOVA ($P \leq 0.05$) followed by Tukey's Multiple Comparison Test. *significantly different compared to control; # Significantly different compared to rotenone.

CN possess the potential to up-regulate the pathways regulating the GSH/TSH[53]. Although we have not assessed the contribution of the anti-inflammatory effect of these spice actives in the present study, marked decrease in nitric oxide (NO) levels with spice active enrichment under ROT co-exposure does not preclude such a possibility. However, further studies are required in this direction.

Evidence from various animal models of PD clearly suggests that mitochondrial dysfunction/s occur early in PD pathogenesis and appears to be a general feature in both idiopathic and genetic forms of PD. The general mitochondrial abnormalities encountered are mitochondrial electron transport chain impairment[54]. In the present study, as anticipated ROT induced diminution in vital mitochondrial metabolic enzymes activities in flies which could also trigger the decline in ATP levels[55], which may partially account for the locomotor deficits among flies exposed to ROT. Further, under ROT exposure, the ability of spice bio-actives (CU and CN) to significantly restore the activity of mitochondrial enzymes and MTT reduction property clearly suggests their potential to abrogate mitochondrial dysfunction.

ROT exposure has been reported to cause significant depletion of DA levels and elevated oxidative stress specifically in the dopaminergic neurons of experimental animals and flies[56,57]. In the present model, we found significant depletion of DA levels with ROT exposure which were to a large extent restored with spice active enrichment. Further, the decrease in the locomotor ability of flies exposed to ROT explains the functional deficit and interestingly, the phenotype was considerably returned to near normal levels with spice active enrichment. It was proposed that hypofunction of cholinergic neurons results during aging and neurodegeneration[58]. AChE deficiency is reported to decrease apoptosis and protect dopaminergic neurons in the neurotoxin model of PD[59]. ROT exposure significantly resulted in elevated levels of AChE activity in flies which were restored to normalcy by spice active enrichment, suggesting their efficacy, at least in part, to attenuate cholinergic function. We speculate that the ability of the bio-actives to attenuate cholinergic activity may be largely responsible for the improved locomotor performance of flies.

Paraquat (PQ), a prototypic toxin is known to exert detrimental effects through oxidative stress and by induction of cell death in a various cell types and tissues[60]. Studies on PQ neurotoxicity have offered valuable insights into the mechanisms of neuronal cell death induced by environmental toxicants[61]. In the prophylactic study with bioactives, our principal findings viz., low incidence of mortality and the associated improved locomotor behavior among PQ exposed flies suggests their potential to decrease oxidative stress and increase survival pathways. This corroborates with our previous findings wherein flies developed significant resistance to the neurotoxic effects of PQ following their treatment with several plant extracts[15,62], Curcumin[63], and other molecules such as creatine[64] and Crocin[47]. We speculate it may be predominantly related to the induction of antioxidant mechanisms in the fly model rendering the fly nervous system less susceptible to PQ toxicity.

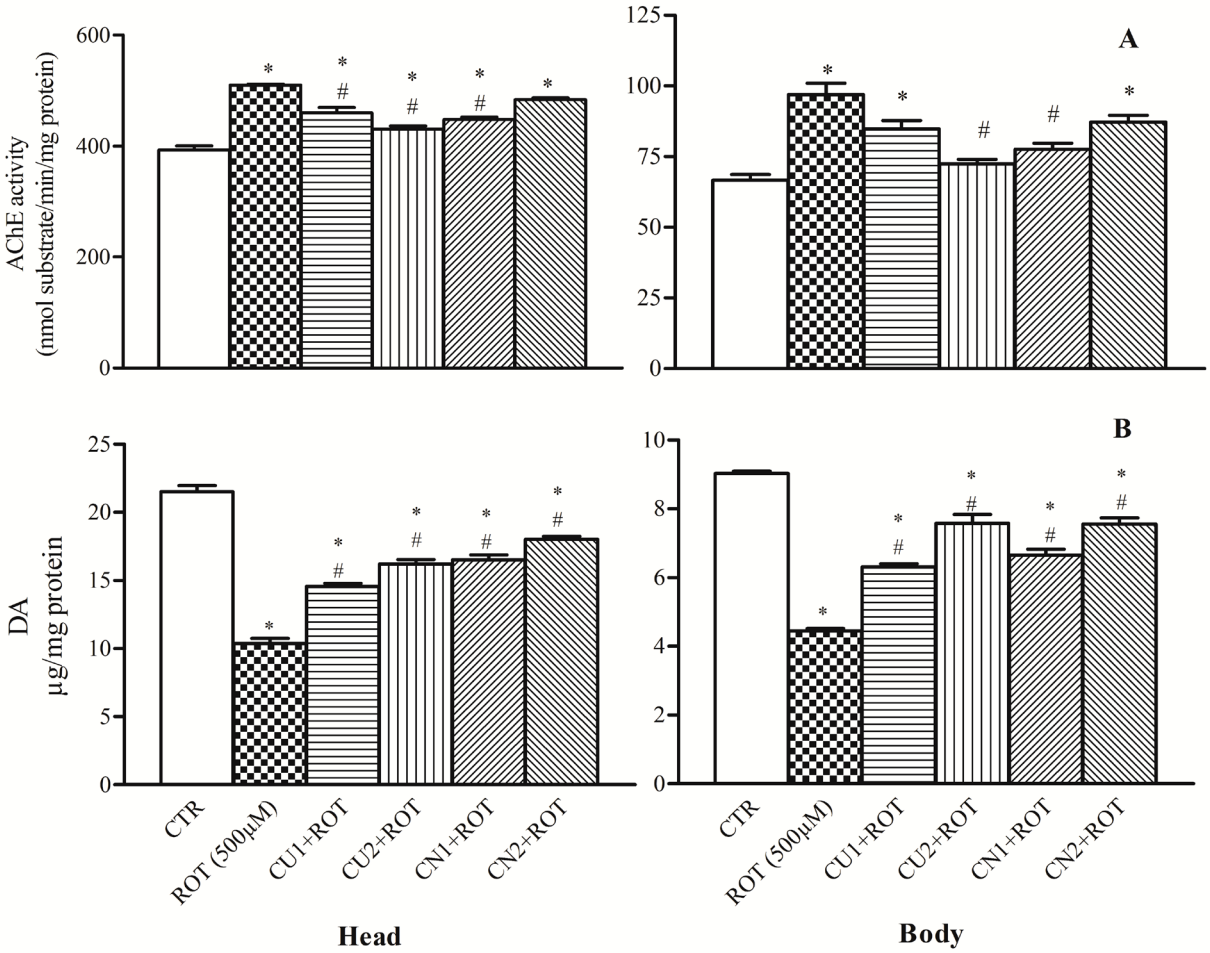


**Figure 3** Protective effect of CU/CN enriched diet on Rotenone (500 µM) induced alterations in AChE (A) DA (B) in adult male Drosophila melanogaster; (CU1 and CN1-10 µM, CU2 and CN2-25 µM). Values are mean ± SE ($n$ = 50, 3 replicates); Data analyzed by One-way ANOVA ($p \leq 0.05$) followed by Tukey's Multiple Comparison Test. *significantly different compared to control; # Significantly different compared to rotenone.

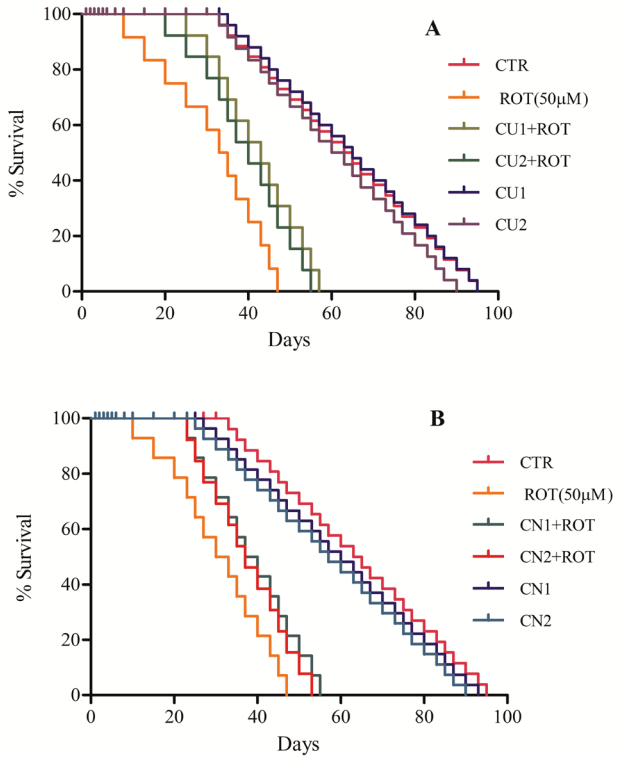


**Figure 4** Prophylactic efficacy of CU/CN on paraquat (PQ-30 mM) induced lethality (A) and locomotor phenotype (B) in adult male Drosophila melanogaster; (CU1 and CN1-10µM, CU2 and CN2-25 µM). Values are mean ± SE ($n$ = 25, 3 replicates). Data analyzed by One-way ANOVA ($p \leq 0.05$) followed by Tukey's Multiple Comparison Test. *significantly different compared to control; # significantly different compared to PQ.

Aging is characterized by a progressive loss of physiological integrity, resulting in impaired function and increased susceptibility to death. Hence, a major challenge has been to identify pharmaceutical targets to improve human health during aging with minimal side effects[65,66]. Environmental chemical exposure and diet are known to impact the process of aging significantly[67]. It is well known that with age, there is a progressive decline in the functional capacity and age-related locomotor dysfunction which is a direct reflection of functional deficits in the nervous system. Hence, mechanisms that interrupt age-related locomotor impairments could eventually lead to treatment of age-related defects especially processes that govern functioning of the nervous system or musculature[68]. It is opined that such treatment protocols would offer considerable gains in quality of life in the aged populations. Our data obtained from the longevity study, suggests that both CU and CN enrichment led to a marked extension of lifespan and improvement in the locomotor performance among ROT-exposed flies. This observation merits further study.

## CONCLUSIONS

In conclusion we propose that commonly used spices, cumin and cinnamon and their bioactives (cuminaldehyde and cinnamaldehyde) possess a potential to attenuate rotenone-induced oxidative stress owing to their antioxidative nature and their capacity to modulate the activities of antioxidant defenses. The neuroprotective properties of cuminaldehyde and cinnamaldehyde were discernible by their ability to abrogate rotenone-induced oxidative stress, mitochondrial dysfunction, restoration of dopamine levels, and extension of life span under chronic ROT exposure. Hence, the use of these spices is likely to provide a therapeutic benefit under oxidative stress mediated neurodegenerative conditions such as PD.

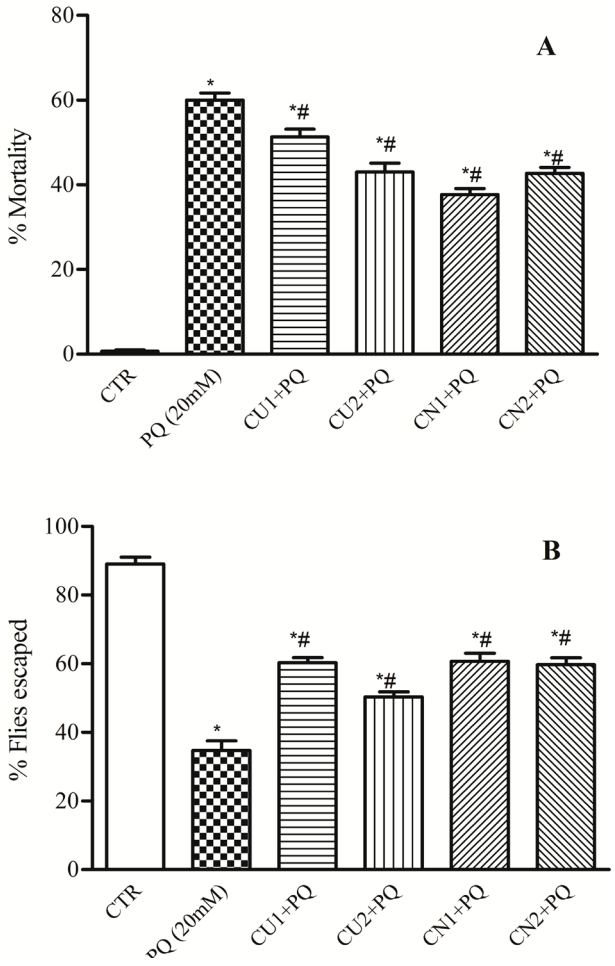


**Figure 5** Effect of CU (A) and CN (B) on the survival of flies under stressed condition (with ROT 50 µM); Values are percent (%) survival ($n$ = 25, 4 replicates). Data analyzed by Kaplan-Meier ($p \leq 0.0001$) followed by Log-rank (Mantel-Cox) Test; (CU1 and CN1-10 µM, CU2 and CN2-25 µM).

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## REFERENCES

1 Nilius B, Appendino G. Spices: the savory and beneficial science of pungency. *Rev Physiol Biochem Pharmacol*. 2013; **164**: 1-76. [PMID: 23605170; DOI: 10.1007/112_2013_11].

2 Phanikumar G and Khanum F. Neuroprotective potential of phytochemicals. *Pharmacognosy Rev*. 2012; **8(12)**: 81-90 [PMID: 23055633; DOI: 10.4103/0973-7847.99898].

3 Kannappan R, Gupta SC, Kim JH, Reuter S, Aggarwal BB. Neuroprotection by Spice-Derived Nutraceuticals: You Are What You Eat! *Mol Neurobiol* 2011; **44**: 142-159. [PMID: 21360003; DOI: 10.1007/s12035-011-8168-2].

4 More SV, Kumar H, Kang SM, Song S-Y, Lee K, Choi DK. Advances in Neuroprotective Ingredients of Medicinal Herbs by Using Cellular and Animal Models of Parkinson's Disease. *Evid Based Complement Alternat Med* 2013: e957875. [PMID: 24173012; DOI: 10.1155/2013/957875].

5 Venkatesan R, Ji E, Kim SY. Phytochemicals That Regulate Neurodegenerative Disease by Targeting Neurotrophins: A Comprehensive Review. *BioMed Res Int* 2015: e814068. [PMID: 21360003; DOI: 10.1007/s12035-011-8168-2].

6 Rebey IB, Bourgou S, Debez IBS, Karoui IJ, Sellami IH, Msaada K, Limam F, Marzouk B. Effects of Extraction Solvents and Provenances on Phenolic Contents and Antioxidant Activities of Cumin (Cuminum cyminum L.) Seeds. *Food Bioprocess Technol* 2011; **5**: 2827-2836. [http: //link.springer.com/article/10.1007/s11947-011-0625-4; DOI: 10.1007/s11947-011-0625-4].

7 Rao PV, Gan SH. Cinnamon: A Multifaceted Medicinal Plant. Evid Based Complement Alternat Med: 2014; e642942 [PMID: 24817901; DOI: 10.1155/2014/642942].

8 Winner B, Kohl Z, Gage FH. Neurodegenerative disease and adult neurogenesis. *Eur J Neurosci* 2011; **33**: 1139-1151. [http: //onlinelibrary.wiley.com/doi/10.1111/j.1460-9568.2011.07613.x/abstract; DOI: 10.1111/j.1460-9568.2011.07613.x].

9 Hwang O. Role of Oxidative Stress in Parkinson's Disease. *Exp Neurobiol* 2013; **22**: 11-19. [PMID: 23605170; DOI: 10.1007/112_2013_11].

10 Feany MB, Bender WW. A Drosophila model of Parkinson's disease. *Nature* 2000; **404**: 394-398. [http: //www.nature.com/nature/journal/v404/n6776/abs/404394a0.html; DOI: 10.1038/35006074].

11 Coulom H, Birman S. Chronic exposure to rotenone models sporadic Parkinson's disease in Drosophila melanogaster. *J Neurosci* 2004; **24**: 10993-8. [PMID: 23605170; DOI: 10.1007/112_2013_11].

12 Hirth F. Drosophila melanogaster in the study of human neurodegeneration. *CNS Neurol Disord Drug Targets* 2010; **9**: 504-523. [PMID: 20522007].

13 Saini N, Oelhafen S, Hua H, Georgiev O, Schaffner W, Büeler H. Extended lifespan of Drosophila parkin mutants through sequestration of redox-active metals and enhancement of anti-oxidative pathways. *Neurobiol Dis* 2010; **40**: 82-92. [http: //www.sciencedirect.com/science/article/pii/S0969996110001622; DOI: 10.1016/j.nbd.2010.05.011].

14 Sudati JH, Vieira FA, Pavin SS, Dias GRM, Seeger RL, Golombieski R, Athayde ML, Soares FA, Rocha JBT, Barbosa NV. Valeriana officinalis attenuates the rotenone-induced toxicity in Drosophila melanogaster. *Neuro Toxicology* 2013; **37**: 118-126. [PMID: 23639798; DOI: 10.1016/j.neuro.2013.04.006].

15 Hosamani R, Muralidhara. Neuroprotective efficacy of Bacopa monnieri against rotenone induced oxidative stress and neurotoxicity in Drosophila melanogaster. *Neuro Toxicology* 2009; **30**: 977-985. [PMID: 19744517; DOI: 10.1016/j.neuro.2009.08.012].

16 Girish C, Muralidhara. Propensity of Selaginella delicatula aqueous extract to offset rotenone-induced oxidative dysfunctions and neurotoxicity in Drosophila melanogaster: Implications for Parkinson's disease. *Neuro Toxicology* 2012; **33**: 444-456. [PMID: 22521218; DOI: 10.1016/j.neuro.2012.04.002].

17 Manjunath MJ, Muralidhara. Standardized extract of Withania somnifera (Ashwagandha) markedly offsets rotenone-induced locomotor deficits, oxidative impairments and neurotoxicity in Drosophila melanogaster. *J Food Sci Technol* 2015; **52**: 1971-1981. [PMID: 19744517; DOI: 10.1007/s13197-013-1219-0].

18 Prasad SN, Muralidhara. Neuroprotective Efficacy of Eugenol and Isoeugenol in Acrylamide-Induced Neuropathy in rats: Behavioral and Biochemical evidence. *Neurochem Res* 2012; **38**: 330-345. [PMID: 24231732; DOI: 10.1007/s11064-012-0924-9].

19 Chandrashekar KN, Muralidhara. Oxidative alterations induced by d-aspartic acid in prepubertal rat testis in vitro: A mechanistic study. *Theriogenology* 2008; **70**: 97-104. [PMID: 18439665; DOI: 10.1016/j.theriogenology.2008.02.004].

20 Wolff SP. Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. In: Enzymology 1994; **233**: 182-189. [http://www.sciencedirect.com/science/article/pii/S0076687994330212].

21 Choi JS, Chung HY, Kang SS. The structure-activity relationship of flavonoids as scavengers of peroxynitrite. *Phytotherapy Res.* **16**: 232-235. [PMID: 12164267; DOI: 10.1002/ptr.828]

22 Mokrasch LC, Teschke EJ. Glutathione content of cultured cells and rodent brain regions: A specific fluorometric assay. *Anal Biochem* 1984; **140**: 506-509. [PMID: 6486436].

23 Kostyuk VA, Potapovich AI. Superoxide--driven oxidation of quercetin and a simple sensitive assay for determination of superoxide dismutase. *Biochem Int* 1989; **19**: 1117-1124. [PMID: 2561443].

24 Aebi H. Catalase *in vitro*. *Methods Enzymol* 1984; **105**: 121–126. [PMID: 6727660; http: //ci.nii.ac.jp/naid/10018913812/].

25 Luthman M, Holmgren A. Rat liver thioredoxin and thioredoxin reductase: purification and characterization. *Biochemistry (Mosc)* 1982; **21**: 6628-6633. [http://dx.doi.org/10.1021/bi00269a003; PMID: 7159551].

26 Guthenberg C, Ålin P, Mannervik B Glutathione transferase from rat testis. In: Alton Meister editor. *Methods Enzymol* 1985; 507-510. [http://www.sciencedirect.com/science/article/pii/S0076687985130673].

27 Ellman GL, Courtney KD, Andres Jr. V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; **7**: 88-95. [http://www.sciencedirect.com/science/article/pii/S0076687985130673; DOI: 10.1016/0003-9861(59)90090-6].

28 Dalpiaz A, Filosa R, de Caprariis P, Conte G, Bortolotti F, Biondi C, Scatturin A, Prasad PD, Pavan B. Molecular mechanism involved in the transport of a prodrug dopamine glycosyl conjugate. *Int J Pharm* 2007; **336**: 133-139. [PMID: 17184941; DOI: 10.1016/j.ijpharm.2006.11.051].

29 Navarro A, Gomez C, Lo'pez-Cepero JM, Boveris A. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress and mitochondrial electron transfer. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 2004; **286**: 505-511. [PMID: 14615275; DOI: 10.1152/ajpregu.00208.2003].

30 Berridge MV, Tan AS. Characterization of the Cellular Reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular Localization, Substrate Dependence, and Involvement of Mitochondrial Electron Transport in MTT Reduction. *Arch Biochem Biophys* 1993; **303**: 474-482. [PMID: 8390225; DOI: 10.1006/abbi.1993.1311].

31 Lowry O, Rosebrough N, Farr A, Randall R. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-275. [http://www.garfield.library.upenn.edu/classics1977/A1977DM02300001.pdf]

32 Raghavan, S (2007) Handbook of spices, seasonings, and flavorings (2nd ed.). Boca Raton: CRC, Taylor & Francis Group [Google-Books-ID: bePKBQAAQBAJ].

33 Thippeswamy NB, Naidu KA. Antioxidant potency of cumin varieties—cumin, black cumin and bitter cumin—on antioxidant systems. *Eur Food Res Technol* 2005; **220**: 472-476. [http://link.springer.com/article/10.1007/s00217-004-1087-y; DOI: 10.1007/s00217-004-1087-y].

34 Raeisi S, Quek SY, Ojagh SM, Alishahi AR. Effects of Cumin (Cuminum Cyminum L.) Seed and Wild Mint (Mentha Longifolia L.) Leaf Extracts on the Shelf Life and Quality of Rainbow Trout (Oncorhynchus Mykiss) Fillets Stored at 4o±1. *J Food Saf* 2015; [http://onlinelibrary.wiley.com/doi/10.1111/jfs.12240/abstract; DOI: 10.1111/jfs.12240].

35 Lee HS. Cuminaldehyde: Aldose Reductase and α-Glucosidase Inhibitor Derived from Cuminum cyminum L. Seeds. *J Agric Food Chem* 2005; **53**: 2446-2450. [PMID: 15796577; DOI: 10.1021/jf048451g].

36 Morshedi D, Aliakbari F, Tayaranian-Marvian A, Fassihi A, Pan-Montojo F, Pérez-Sánchez H. Cuminaldehyde as the Major Component of Cuminum cyminum, a Natural Aldehyde with Inhibitory Effect on Alpha-Synuclein Fibrillation and Cytotoxicity.

*J Food Sci* 2015; **80**: H2336-H2345. [PMID: 26351865; DOI: 10.1111/1750-3841.13016].

37 Rajkovic K, Pekmezovic M, Barac A, Nikodinovic-Runic J, Arsić Arsenijević V. Inhibitory effect of thyme and cinnamon essential oils on Aspergillus flavus: Optimization and activity prediction model development. *Ind Crops Prod* 2015; **65**: 7-13. [http: //www.sciencedirect.com/science/article/pii/S0926669014007468; DOI: 10.1016/j.indcrop.2014.11.039].

38 Pandey M, Chandra DR. Evaluation of Ethanol and Aqueous extracts of Cinnamomum verum Leaf Galls for Potential Antioxidant and Analgesic activity. *Indian J Pharm Sci* 2015; **77**: 243-247. [PMID: 26009661; http: //www.ncbi.nlm.nih.gov/pmc/articles/PMC4442477/].

39 Shen Y, Honma N, Kobayashi K, Jia LN, Hosono T, Shindo K, Ariga T, Seki T. Cinnamon Extract Enhances Glucose Uptake in 3T3-L1 Adipocytes and C2C12 Myocytes by Inducing LKB1-AMP-Activated Protein Kinase Signaling. *PLoS ONE* 2014; **9**: e87894. [PMID: 24551069; DOI: 10.1371/journal.pone.0087894].

40 Rio MJD, Martinez CG, Pardo CV. The effects of polyphenols on survival and locomotor activity in Drosophila melanogaster exposed to Iron and Paraquat. *Neurochem Res* 2009; **35**: 227-38. [http://link.springer.com/article/10.1007/s11064-009-0046-1; DOI: 10.1007/s11064-009-0046-1].

41 Dumont MF, Beal M. Neuroprotective strategies involving ROS in Alzheimer disease. *Free Radic Biol Med* 2011; **51**: 1014-26. [PMID: 21130159; DOI: 10.1016/j.freeradbiomed.2010.11.026].

42 Whitworth AJ, Wes PD, Pallanck LJ. Drosophila models pioneer a new approach to drug discovery for Parkinson's disease. *Drug Discov Today* 2006; **11**: 119-126. [PMID: 16533709; DOI: 10.1016/S1359-6446(05)03693-7].

43 Navarro JA, Heßner S, Yenisetti SC, Bayersdorfer F, Zhang L, Voigt A, Schneuwly S, Botella JA. Analysis of dopaminergic neuronal dysfunction in genetic and toxin-induced models of Parkinson's disease in Drosophila. *J Neurochem* 2014; **131**: 369-382 [PMID: 14615275; DOI: 10.1152/ajpregu.00208.2003].

44 Greenamyre JT, Cannon JR, Drolet R, Mastroberardino PG. Lessons from the rotenone model of Parkinson's disease. *Trends Pharmacol Sci* 2010; **31**: 141-142. [PMID: 20096940; DOI: 10.1016/j.tips.2009.12.006].

45 Spivey A. Rotenone and Paraquat Linked to Parkinson's Disease: Human Exposure Study Supports Years of Animal Studies. *Environ Health Perspect* 2011; **119**: A259. [PMID: 21628118; DOI: 10.1289/ehp.119-a259a].

46 Sanders LH, Timothy Greenamyre J.Oxidative damage to macromolecules in human Parkinson disease and the rotenone model. *Free Radic Biol Med* 2013; 62: 111-120. [PMID: 23328732; DOI: 10.1016/j.freeradbiomed.2013.01.003].

47 Rao SV, Muralidhara, Yenisetti SC, Rajini PS. Evidence of neuroprotective effects of saffron and crocin in a Drosophila model of parkinsonism. *Neuro Toxicology* 2016; **52**: 230-242. [PMID: 26705857; DOI: 10.1016/j.neuro.2015.12.010].

48 El-Ghorab AH, Nauman M, Anjum FM, Hussain S, Nadeem M. A Comparative Study on Chemical Composition and Antioxidant Activity of Ginger (Zingiber officinale) and Cumin (Cuminum cyminum). *J Agric Food Chem* 2010; **58**: 8231-8237. [PMID: 20590154; DOI: 10.1021/jf101202x].

49 Durak A, Gawlik-Dziki U, Pecio Ł. Coffee with cinnamon- Impact of phytochemicals interactions on antioxidant and anti-inflammatory in vitro activity. *Food Chem* 2014; **162**: 81-88. [PMID: 24874360; DOI: 10.1016/j.foodchem.2014.03.132].

50 Morgan AM, El-Ballal SS, El-Bialy BE, EL-Borai NB. Studies on the potential protective effect of cinnamon against bisphenol A- and octylphenol-induced oxidative stress in male albino rats. *Toxicol Rep* 2014; 1: 92-101. [PMID: 20590480; DOI: 10.1089/acm.2009.0342]

51 Schmidt MM, Dringen R (2012) Glutathione Synthesis and Metabolism. In: Choi I-Y, Gruetter R, (Eds). Neural Metabolism In Vivo Springer US. p. 1029-1050. [http://link.springer.com/chapter/10.1007/978-1-4614-1788-0_36; DOI: 10.1007/978-1-4614-1788-0_36].

52 Missirlis F, Phillips JP, Jäckle H. Cooperative action of antioxidant defense systems in Drosophila. *Curr Biol* 2001; **11**: 1272–1277. [PMID: 11525742; DOI: 10.1016/S0960-9822(01)00393-1].

53 Calabrese V, Cornelius C, Trovato-Salinaro A, Cambria MT, Locascio MS, Rienzo LD, Condorelli DF, Mancuso C, De Lorenzo A, Calabrese EJ.The Hormetic Role of Dietary Antioxidants in Free Radical-Related Diseases. *Curr Pharm Des* 2010; **16**: 877-883. [PMID: 20388101; DOI: 10.2174/138161210790883615].

54 Subramaniam SR, Chesselet MF. Mitochondrial dysfunction and oxidative stress in Parkinson's disease. *Prog Neurobiol* 2013; **106**: 17-32. [PMID: 23643800; DOI: 10.1016/j.pneurobio.2013.04.004].

55 Giordano S, Lee J, Darley-Usmar VM, Zhang J. Distinct Effects of Rotenone, 1-methyl-4-phenylpyridinium and 6-hydroxydopamine on Cellular Bioenergetics and Cell Death. *PLoS ONE* 2012; **7**: e44610. [PMID: 22970265; DOI: 10.1371/journal.pone.0044610].

56 Bayersdorfer F, Voigt A, Schneuwly S, Botella JA. Dopamine-dependent neurodegeneration in Drosophila models of familial and sporadic Parkinson's disease. *Neurobiol Dis* **40**: 113-119. [PMID: 20211259; DOI: 10.1016/j.nbd.2010.02.012].

57 Cannon JR, Greenamyre JT (2014) Rotenone as Preclinical Model Compound in Parkinson Disease. In: Kostrzewa RM, editor. Handbook of Neurotoxicity Springer New York. p. 995-1012. [http://link.springer.com/referenceworkentry/10.1007/978-1-4614-5836-4_8; DOI: 10.1007/978-1-4614-5836-4_8].

58 Schliebs R, Arendt T. The cholinergic system in aging and neuronal degeneration. *Behav Brain Res*. 2011; **221(2)**: 555-63 [PMID: 21145918; DOI: 10.1016/j.bbr.2010.11.058].

59 Zhang X, Lu L, Liu S, Ye W, Wu J, Zhang X. Acetylcholinesterase deficiency decreases apoptosis in dopaminergic neurons in the neurotoxin model of Parkinson's disease. *Int J Biochem Cell Biol* 2013; **45**: 265-272. [PMID: 23201480; DOI: 10.1016/j.biocel.2012.11.015].

60 Bove J, Prou D, Perier C, Przedborski S. Toxin-Induced Models of Parkinson's Disease. *NeuroRx* 2005; **2**: 484-494. [PMID: 16389312; DOI: 10.1602/neurorx.2.3.484].

61 González-Polo RA, Pedro JMB-S, Gómez-Sánchez R, Pizarro-Estrella E, Niso-Santano M, Fuentes JM. 2014. Links Between Paraquat and Parkinson's Disease. In: Kostrzewa RM, editor. Handbook of Neurotoxicity Springer New York. p. 819-842. [http://link.springer.com/referenceworkentry/10.1007/978-1-4614-5836-4_4; DOI: 10.1007/978-1-4614-5836-4_4; ].

62 Hosamani R, Muralidhara. Prophylactic treatment with Bacopa monneri leaf powder mitigates Paraquat induced oxidative perturbations and lethality in Drosophila melanogaster. *Ind J Biochem Biophy.* 2010; **4**: 75-82. [PMID: 20521619; http: //www.pubfacts.com/detail/20521619/Prophylactic-treatment-with-Bacopa-monnieri-leaf-powder-mitigates-paraquat-induced-oxidative-perturb].

63 Phom L, Achumi B, Alone DP, Muralidhara, Yenisetti SC. Curcumin's Neuroprotective Efficacy in Drosophila Model of Idiopathic Parkinson's Disease Is Phase Specific: Implication of its Therapeutic Effectiveness. *Rejuvenation Res* 2014; **17**: 481-489. [PMID: 25238331; DOI: 10.1089/rej.2014.1591].

64 Hosamani R, Ramesh SR, Muralidhara. Attenuation of Rotenone-Induced Mitochondrial Oxidative Damage and Neurotoxicity in Drosophila melanogaster Supplemented with Creatine. *Neurochem Res* 2010; **35**: 1402-1412. [PMID: 20514516; DOI: 10.1007/s11064-010-0198-z]

65 López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The Hallmarks of Aging. *Cell* 2013; **153**: 1194-1217. [PMID: 23746838; DOI: 10.1016/j.cell.2013.05.039].

66 Tatar M. The plate half-full: Status of research on the mechanisms of dietary restriction in Drosophila melanogaster. *Exp*

*Gerontol* 2011; **46**: 363-368. [PMID: 21187139; DOI: 10.1016/j.exger.2010.12.002].

67 Le Couteur DG, Sinclair DA. A blueprint for developing therapeutic approaches that increase healthspan and delay death. *J Gerontol A Biol Sci Med Sci*. 2010; **65**: 693-694. [PMID: 20375077; DOI: 10.1093/gerona/glq048].

68 Jones MA, Grotewiel M. Drosophila as a model of for age-related impairment in locomotor and other behaviours. *Exp Gerontol* 2011; **46**: 320-325. [PMID: 20800672; DOI: 10.1016/j.exger.2010.08.012].

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