Neurochem Res (2013) 38:2616–2624
DOI 10.1007/s11064-013-1179-9

ORIGINAL PAPER

Neuroprotective Effect of *Decalepis hamiltonii* in Paraquat-Induced Neurotoxicity in *Drosophila melanogaster*: Biochemical and Behavioral Evidences

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Received: 18 May 2013 / Revised: 8 October 2013 / Accepted: 10 October 2013 / Published online: 31 October 2013

Abstract In this paper, we have demonstrated for the first time, the antioxidant and neuroprotective effects of *Decalepis hamiltonii* (*Dh*) root extract against paraquat (PQ)-induced oxidative stress and neurotoxicity in *Drosophila melanogaster*. Exposure of adult *D. melanogaster* (Oregon K) to PQ induced oxidative stress as evidenced by glutathione depletion, lipid peroxidation and enhanced activities of antioxidant enzymes such as catalase, superoxide dismutase as well as elevated levels of acetylcholine esterase. Pretreatment of flies by feeding with *Dh* extract (0.1, 0.5 %) for 14 days boosted the activities of antioxidant enzymes and prevented the PQ-induced oxidative stress. Dietary feeding of *Dh* extract prior to PQ exposure showed a lower incidence of mortality and enhanced motor activities of flies in a negative geotaxis assay; both suggesting the neuroprotective potential of *Dh*. Based on the results, we contemplate that the roots of *Dh* might prevent and ameliorate the human diseases caused by oxidative stress. The neuroprotective action of *Dh* can be attributed to the antioxidant constituents while the precise mechanism of its action needs further investigations.

Keywords *Decalepis hamiltonii* · *Drosophila melanogaster* · Oxidative stress · Neuroprotection · Paraquat · Neurotoxicity

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Introduction

Reactive oxygen species (ROS) is an inevitable byproduct of aerobic respiration and it can be induced by exposure to certain toxicants. ROS causes oxidative damage to DNA, lipids, and also affect antioxidant enzyme defenses [1]. Accumulation of free radicals and consequent neurodegeneration in the brain plays a decisive role in the aging process that has been linked to a variety of neurodegenerative diseases (NDD) [2–4]. Paraquat (PQ) (1,1-dimethyl-4-4-bipyridynium dichloride) (PQ), is widely used as herbicide. In vivo studies employing *Drosophila melanogaster* [5], *Rattus norvegicus* [6] and *Mus musculus* [7] have revealed that PQ is neurotoxic which affects dopaminergic neurons. The toxicity of PQ is a consequence of generation of ROS mainly hydrogen peroxide and hydroxyl radical [8]. Overproduction of ROS is believed to be the cause of death in dopaminergic neurons which leads to the movement disorder similar to that of Parkinson's disease [9]. It has been shown that PQ causes damage to the number of dopaminergic neurons due to its ability to penetrate the blood–brain barrier (BBB) [10]. PQ has been well attributed to damage in mitochondria and mitochondrial complex I inhibition by producing superoxide anions [11]. *Drosophila* is widely used as a model to understand mechanisms involved in neurodegenerative disorders (NDD) [12] and environmental toxin-induced parkinsonism [13]. *Drosophila* is also used as a convenient model to screen therapeutic agents for their effects on the cellular, biochemical and genetic systems [14]. Moreover, a number of *Drosophila* transgenic lines that mimic human diseased conditions are employed to understand precisely, the effects at the gene level [15]. The use of *Drosophila* as a model to study the role of oxidative stress in neurodegeneration is advantageous over other models for several

reasons: First, the high degree of evolutionary conservation of the fly and human genome, and homologous signal transduction pathways makes *Drosophila* a suitable model. Second the fly central nervous system has much smaller number of glial and neuronal cells and it works in the same way as in mammalian systems. Third: owing to feasible methods currently are used for the genetic manipulation of the fly, *Drosophila* has been served as a powerful model to study human genetic.

Antioxidants, especially those derived from natural sources are useful in ameliorating the oxidative stress and therefore, could serve as preventive therapeutic agents for neurodegenerative diseases [16, 17]. The tuberous roots of *Dh* are consumed as pickles and as juice since they are believed to possess health promoting properties. It is also used in folk medicine and ayurvedic preparations as a general vitalizer [18]. The roots of *Dh* possess potent antioxidant properties and several novel antioxidant molecules have been isolated and characterized which could be associated with their alleged health benefits [19–21]. It was earlier reported that the aqueous extract of *Dh* has more antioxidant properties compared to the different solvent extracts [20, 22].

Studies on albino rats [23, 24], that revealed antioxidant and neuroprotective potential of root extract of *Dh* prompted us to undertake present investigations to unfold the neuroprotective activity of *Dh* against PQ toxicity in *D. melanogaster*.

In this study, we tried to answer questions related to the possible neuropharmacological properties of *Dh* extract against PQ-induced neurotoxicity. First, following 14 days of feeding flies with *Dh* extract, its ability to ameliorate the levels of oxidative markers was measured. Second, neuroprotective propensity of *Dh* extract against PQ-induced mortality and oxidative stress was investigated. Oxidative stress response was qualified by measuring the activities of superoxide dismutase (SOD) and catalase (CAT) enzymes and malondialdehyde. PQ-induced neurotoxicity was determined by geotaxis assay.

Experimental Procedure

Materials

Acetylthiocholine iodide, PQ, pyrogallol, thiobarbituric acid (TBA), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co., St Louis, USA. TRIzol reagent was procured from Invitrogen, Carlsbad, CA, USA. QPCR cDNA synthesis kit, Brilliant II SYBR Green qPCR Master mix, and Stratagene Mx3005P platform were purchased from Agilent Technologies Inc., Santa Clara, CA, USA. All other chemicals used were of the highest purity grade and were purchased from Sisco Research Laboratories, Mumbai, India.

Preparation of the Root Powder and Extraction

The roots of *Dh* that were collected from the forests of B.R hills, Karnataka, India were washed with water, followed by crushing with a roller in order to separate the inner woody core from the outer fleshy layer. The fleshy portion thus collected was cut into smaller pieces and dried at 40 °C, before they were ground to get fine powder. The aqueous extract of the root was prepared by homogenizing the 100 g of powder in 1 l warm water (50 °C) which was allowed to stand for 24 h at room temperature. The homogenate was then filtered with Whatman filter paper No. 1 and the filtrate was lyophilized and weighed (19 g).

Drosophila Culture and *Dh* Treatment

Two-day-old wild type males of *D. melanogaster* (Oregon K) collected from experimental laboratory populations that were raised on standard wheat cream agar medium with a light/dark cycle of 12:12 h maintained at 22 ± 1 °C and 60–70 % relative humidity were used for *Dh* treatment. In a preliminary study, to determine the optimum concentrations of *Dh*, 200 of these flies were fed on culture media containing 0.01, 0.1, 0.5, 0.75 and 1.0 % (w/v) of aqueous extract of *Dh* per replicate. They were allowed to feed on *Dh*-supplemented media for 14 days. This experiment was repeated four times. We selected only two concentrations of *Dh* (0.1, 0.5 %) based on preliminary studies, wherein *Dh* concentrations of 0.01, 0.1, 0.5, 0.75, 1.0 % were screened. Two concentrations (0.1, 0.5 %) of *Dh* were selected based on survival and negative geotaxis assays. These concentrations (0.1, 0.5 %) of *Dh* represent lower and higher concentrations to measure the neuroprotective effect on PQ-induced changes in negative geotaxis assay and oxidative stress response. There was distinct difference at the lower (0.1 %) and higher (0.5 %) concentrations of *Dh* on PQ-induced oxidative stress and higher concentrations did not show higher protection. Therefore, 0.5 % was chosen as the higher effective concentration.

Feeding Assay

In order to see if the presence of *Dh* in the media affects feeding of the flies, Food intake was visually confirmed by feeding flies on media containing the food dye FD&C Blue No. 1. [25]. For this experiment, we mixed FD&C Blue No. 1 dye (1 mg/ml) in the media containing *Dh* aqueous extract. The flies were allowed to feed on the media for 14 days. In order to prevent interference of the eye pigment with the absorbance spectrum of Blue No. 1 dye, heads

were separated from the bodies of flies, and homogenized in PBS buffer (pH 7.2) and centrifuged (12,000 rpm) for 15 min. The supernatant was used to measure the absorbance at 628 nm using spectrophotometer. There was no significant difference in the values between the flies fed on normal diet and *Dh*-diet implying *Dh* extract does not affect food uptake during or after treatment.

Paraquat Toxicity Assay

In a preliminary study, the male flies fed with *Dh* extract were exposed to PQ. The parallel replicates fed on standard medium (without *Dh*) served as control. These flies were starved in empty vials for 3 h at 25 °C and transferred to vials with a filter paper soaked with 50 µl of 5 % sucrose solution, air dried and then soaked with different concentrations of PQ (10, 15, 20, 25 and 30 mM) in for 24–96 h to determine mortality. The filter papers soaked with PQ and sucrose were replaced every 24 h. The LC50 was determined 24 h after PQ treatment. Three replicates of 200 adult males were used to determine the LC50. Only sub lethal concentration of PQ (15 mM) was used to investigate the neuroprotective action of *Dh* on *Drosophila*.

Survival Assay

To investigate the effect of *Dh* feeding on survival rate of flies after PQ treatment, only one concentration of PQ (20 mM) and two concentrations of *Dh* (0.1, 0.5 %) were used. For this assay, 100 adult male flies per replicate (three replicates) were exposed to *Dh* extract, or PQ alone, or a combination of *Dh* and PQ for 48 h. The survivorship of flies was documented and showed in percentage.

Negative Geotaxis Assay

The locomotory function of the adults was determined by negative geotaxis assay [26]. Briefly, 30 adult males were placed in a graduated empty plastic jar (25 cm length, 2 cm diameter). After a 10 min rest period, the flies were gently tapped to the bottom of the jar and were allowed to climb up to the top of the jar. The number of flies that climbed from the bottom to 20 cm mark in 1 min was counted. The assay was repeated four times and the data was expressed as an average of four trials per replicate. Similar steps were followed to score for controls also.

Biochemical Assays

Eighty fly heads were separated from the body with a shape blade and then homogenized in 1 ml sodium-phosphate buffer (0.1 M, pH 8.0) and centrifuged at 2,500*g* for 10 min at 4 °C and the supernatant filtered through nylon mesh was used for biochemical assays.

Catalase (CAT)

Catalase activity was determined by the method of Aebi [27]. The reaction was initiated by adding 50 µl of the sample to 1 ml reaction mixture containing 8.8 mM H_2O_2 (3 %) in 0.1 M phosphate buffer (pH 7.4) and the change in absorbance at 240 nm was monitored. The decrease in absorbance due to enzyme activity was expressed as µmol of H_2O_2 utilized/min/mg protein.

Superoxide Dismutase (SOD)

Superoxide dismutase activity was determined by monitoring inhibition of pyrogallol auto oxidation [28]. The reaction was started by adding 0.5 ml of 2 mM pyrogallol in a total volume of 3 ml reaction mixture containing 200 µl sample and 0.1 M Tris HCl buffer (pH 8.2) and the change in absorbance was monitored at 412 nm. The specific activity of the enzyme was expressed as units; wherein 1 unit was equal to 50 % inhibition of pyrogallol autoxidation.

Acetylcholine Esterase (AchE)

Acetylcholine esterase activity was measured by the method of [29]. The assay reaction mixture consisted of 5,5-dithiobis 2-nitrobenzoic acid (DTNB), 10 mM, 50 µl sample and 1 ml phosphate buffer (0.1 M, pH 8.0). The reaction was started by adding acetylthiocholine iodide and the change in absorbance was monitored at 412 nm for 3 min. The enzyme activity was expressed as nmoles of substrate hydrolyzed/min/mg protein.

Reduced Glutathione (GSH)

Reduced glutathione content was measured following the fluorimetric method using O-phthal-aldehyde (OPT) as per [30]. The volume of 50 µl of whole body homogenate (stable reduced gluthatione) was added to 0.1 M formic acid and spun at 5,200*g* for 10 min to precipitate protein. The supernatant was kept at room temperature for 30 min to react with OPT (1 mg/ml in methanol) and the fluorescence was measured at excitation of 345 nm and emission at 425 nm. Glutathione level was calculated from a standard curve and expressed as µg GSH/mg protein.

Lipid Peroxidation (LPO)

Lipid peroxidation was measured by using TBA [31]. Briefly, the reaction mixture containing 1.5 ml of 20 %

acetic acid (pH 3.5), 500 µl fly homogenate, 1.5 ml of TBA (0.8 % w/v), and sodium lauryl sulphate (SDS) (8 % w/v). The mixture was heated in boiling water bath for 50 min and adducts formed were extracted into 3 ml of 1-butanol. The absorbance was measured at 532 nm and quantified as malondealdehyde equivalents.

Gene Expression

To investigate the effects of *Dh* extract on expression levels of *sod1* and *cat* in flies, RT-PCR amplification was carried out on adult males after 14 days of feeding with *Dh* aqueous extract. Total RNA from *Drosophila* whole bodies was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized using RevTra Ace (TOYOBO, Osaka, Japan). cDNAs were then analyzed by quantitative real-time PCR using an Applied Biosystems model 7500 (Applied Biosystems Inc, CA, USA) and conventional PCR. Rpl32 transcripts were used for normalization. The primers for *D. melanogaster* genes *sod1* and *cat* were manually designed using Gene Runner version 3.05. Primer sequences used were as follows: for catalase 5′-TCA ACATCACCGACTCCAAG and 3′-CAGCGTTGCCCGTT GACTT and for sod 5′-GAACTACTTTGCTGAGGTGG and 3′-GGATCTGCAAGTAGTTCGGT and for rpl32 5′-A GGGTATCGACAACAGAGTG and 3′-GAACTTCTTGA ATCCGGTGG. The primers were validated using CONTROL sample and amplicon sizes were confirmed by Bioanalyzer. Using the affinity Script qPCR cDNA synthesis kit (Agilent-Lot# 6077352), 1 µg of DNase treated RNA was reverse transcribed to make 50 ng/µl of cDNA. Relative quantification by qPCR was done using Brilliant II SYBR Green qPCR Master mix (Lot#6109083). Each sample was run in duplicates for each gene.

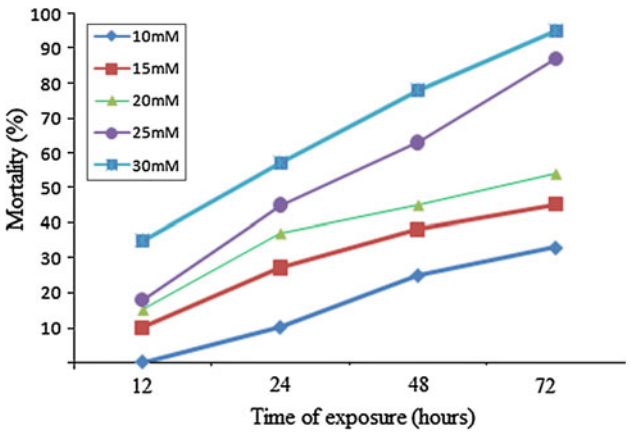

Fig. 1 Dose and time dependent mortality in *Drosophila melanogaster* exposed to PQ. Mortality pattern among adult flies exposed to five different concentration of parquet (10, 15, 20, 25 and 30 mM). PQ exposure induced a concentration-dependent lethality

Determination of Protein

Protein concentrations in the whole body homogenates were measured by using bovine serum albumin as standard [32].

Statistical Analysis

All data are expressed as mean ± standard error (SE) for each experimental group. Statistical significance was analyzed by one-way ANOVA followed by Duncan's test; *P* values less than 0.01 were taken as statistically significant.

Results

Mortality

Exposure of adult male flies to PQ showed a dose-dependent mortality over 3 days of experimental period. There was no mortality in adult males that were exposed to 10 mM of PQ after 12 h. Dose-dependent mortality was observed at higher concentrations (15, 20 and 30 mM) up to 12 h. However, at 24 h, survivorships at the higher concentrations were lower, indicating PQ was highly toxic at concentrations of more than 20 mM. PQ-exposed flies for 24 h showed remarkable distribution patterns compared to the other time points. Therefore, LC50 value of PQ toxicity to flies was determined after 24 h of exposure. The LC50 was found to be 25 mM (Fig. 1).

Modulatory Effect of *Dh* on PQ-Induced Mortality

In this study, only one concentration of PQ (20 mM) was used to determine the modulatory effect of *Dh* extract on PQ-induced mortality of flies. Exposure of adult flies to PQ induced significant mortality after 48 h. There was marked reduction in mortality in *Dh* pretreated flies that were exposed to PQ. A significant reduction in PQ induced lethality indicates protective effect of *Dh* against PQ (Fig. 2).

Negative Geotaxis

We measured the PQ-induced locomotor deficits of adult male flies by measuring negative geotaxis. More than 95 % of the control (*Dh* unfed) flies showed movement to the top of the vial within a minute, in contrast to the flies exposed to 15 mM PQ for 24 h, that showed marked decrease in the climbing ability. The PQ-treated flies tended to stay at the middle or bottom of the vial. While, climbing ability of *Dh* fed flies exposed to PQ was comparable to the control

group. *Dh* extract showed significant improvement of fly locomotion deficits caused by PQ indicating its neuroprotective effect (Fig. 3).

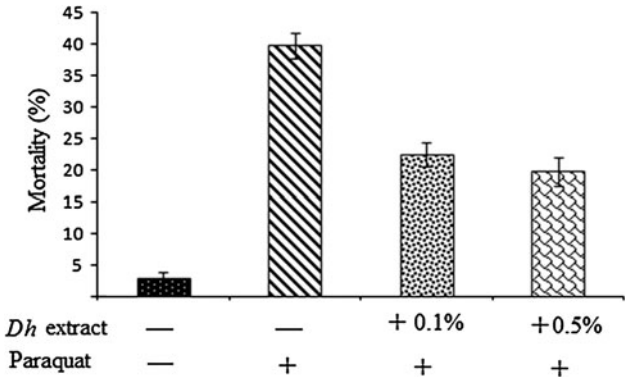

Fig. 2 Protective effect of *Dh* on PQ-induced mortality of flies. Pretreatment of flies with *Dh* extract showing significant increase of survivorship after 48 h of PQ (20 mM) exposure. Significance was determined by one-way ANOVA followed by Duncan's test ($n = 3$, $p < 0.01$). *Error bars* represent mean ± SE

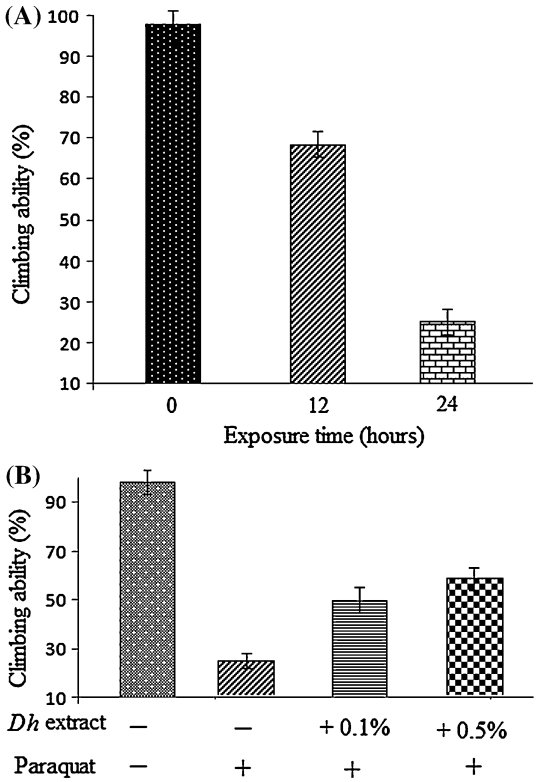

Fig. 3 PQ-induced changes in locomotary behavior of *Drosophila* and ameliorative effect of *Dh* root extract in negative geotaxis assay. **a** Time-course of the effect of PQ (15 Mm) on negative geotaxis (0 h = control). **b** modulation of PQ-induced locomotor deficits in flies pre-fed with *Dh* extract. Flies treated with *Dh* extract showed significant improvement in climbing activities compared to control group. Significance was analyzed by one-way ANOVA followed by Duncan's test ($n = 4$, $p < 0.01$). *Error bars* represent mean ± SE

Oxidative Markers and Antioxidant Enzymes

PQ treatment led to marked depletion of GSH in flies and *Dh* pretreatment prevented depletion of GSH (Fig. 4). Therefore, Adult male flies pre-fed with *Dh* extract were able to restore the depletion level of GSH. Flies exposed to PQ showed increased LPO when compared to the control group. Feeding of *Dh* extract to adult male flies significantly diminished the basal levels of LPO (Fig. 5). PQ treatment increased SOD activity which was restored to control level in *Dh* pre-treated flies (Fig. 6). Similarly, the PQ treatment also increased CAT activity in the flies while *Dh* pretreatment suppressed the induction of CAT activity and restored it closer to untreated control flies (Fig. 7). Interestingly, *Dh* treatment alone boosted antioxidant defense mechanism by increasing the activity of both SOD

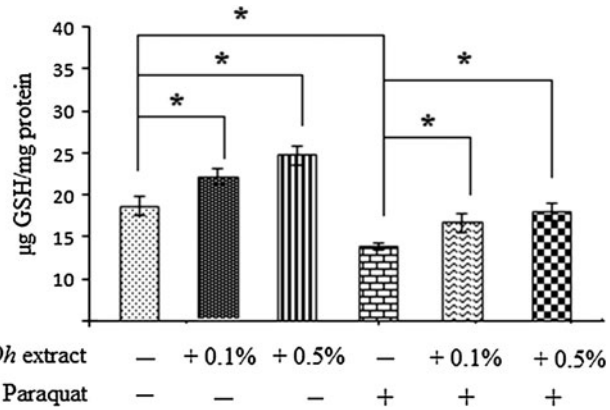

Fig. 4 Effect of *Dh* on PQ induced alterations in GSH. *Dh* reversed PQ-induced GSH depletion and by itself enhanced the GSH level. Data was analyzed by one-way ANOVA followed by Duncan's test ($n = 4$, $p < 0.01$). *Error bars* represent mean ± SE

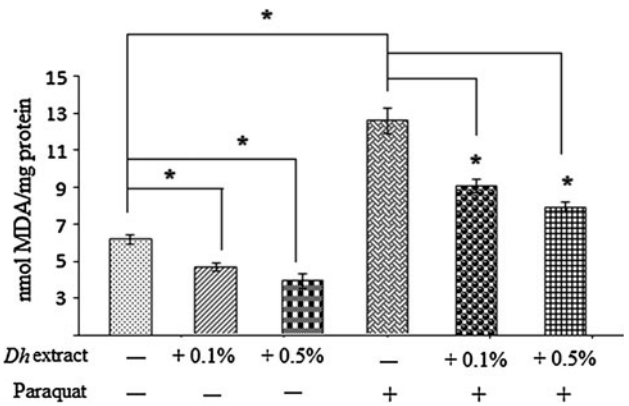

Fig. 5 Protective effect of *Dh* extract on LPO as a marker of oxidative stress in the whole body homogenate of adult *Drosophila* after exposure to 15 mM PQ. *Dh* supplementation significantly decreases the level of MAD in *Dh*-treated flies compared to control and PQ-exposed flies. Significance was analyzed by one-way ANOVA followed by Duncan's test ($n = 4$, $p < 0.01$). *Error bars* represent mean ± SE

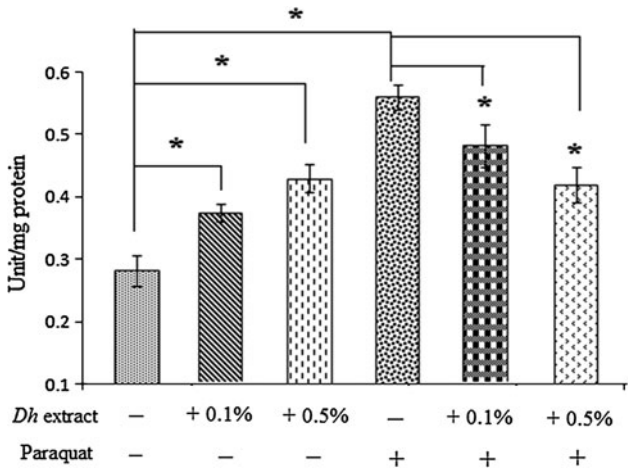

Fig. 6 Effect of *Dh* on SOD activity in flies exposed to PQ with or without *Dh* pretreatment. PQ increased the activity of SOD. *Dh*-treated flies showed significant decrease in the activity of SOD after PQ exposure. (One-way ANOVA, Duncan's test, $n = 4$, $p < 0.01$). *Error bars* represent mean ± SE

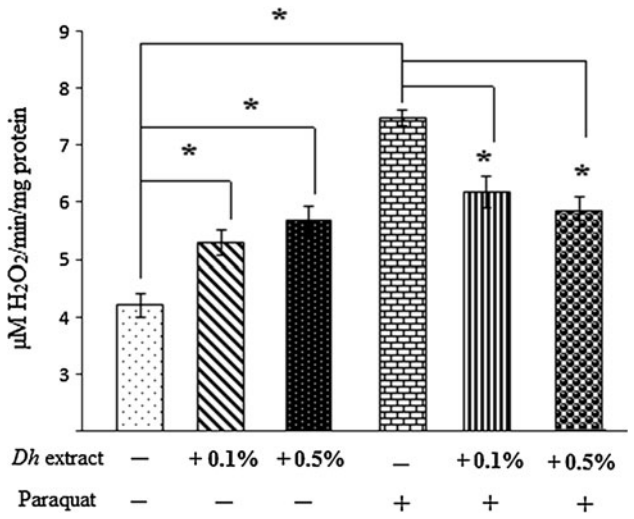

Fig. 7 Effect of *Dh* on CAT activity in flies exposed to PQ. PQ (15 Mm) increased the activity of CAT. *Dh* treatment returned the activity of CAT to a nearly normal state compared to control. Data was analyzed by one-way ANOVA followed by Duncan's test ($n = 4$, $p < 0.01$). *Error bars* represent mean ± SE

and CAT enzymes. In this study, the activity of CAT and SOD enzymes induced by PQ were significantly lower in the flies pre-fed with *Dh* extract compared to the flies only exposed to PQ. It indicates that *Dh* extract has strong effects to modulate anti-oxidative mechanisms by restoring CAT and SOD activities.

Acetylcholine Esterase Activity

PQ-exposed flies showed significant elevation in the AchE activity, compared to untreated flies. *Dh* treatment restored

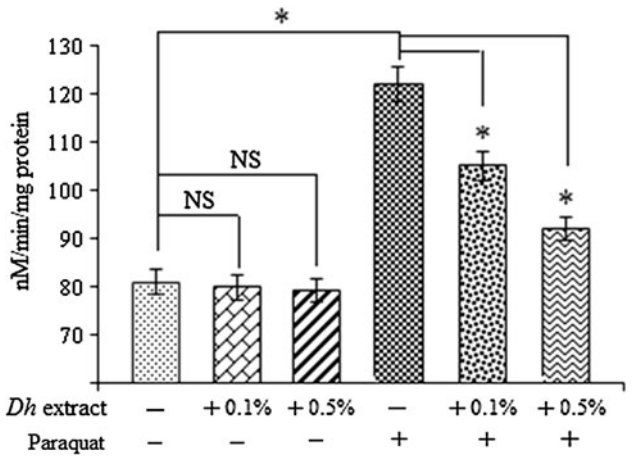

Fig. 8 PQ induced elevated level of AchE in flies after 24 h of exposure compared to control. But *Dh*-treated flies showed significant decrease in the activity of AchE after PQ exposure, *Dh* treatment alone did not exchange AchE activities. (One-way ANOVA, Duncan's test, $n = 4$, $p < 0.01$). *Error bars* represent mean ± SE

the activity of AchE to the levels found in control flies (Fig. 8).

Effects of *Dh* on Gene Expression Related to Oxidative Stress

The effect of *Dh* treatment on gene expression was studied by quantitative RT-PCR by measuring specific mRNAs in flies fed with aqueous extract. Expression of SOD and CAT did not change significantly in flies fed on *Dh* containing diet but *Dh* treatment resulted in only a marginal upregulation of both *sod1* and *cat* genes (Fig. 9).

Discussion

In aerobic organisms, around 2 % of oxygen metabolized forms superoxide anion radical (O_2^-) that ultimately yields toxic hydrogen peroxide and then hydroxyl radicals. Hence ROS is the inevitable by-product of normal cellular respiratory metabolism. The level of ROS production would be in equilibrium with the antioxidant capacity when present in transient amounts. However, at high and sustained levels they chemically modify unsaturated fatty acids of membranes, proteins and DNA. Oxidative stress induced neurodegeneration in the brain has been proposed as a causal factor in the progression of neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's disease (PD) [4]. Substantial evidence suggests that environmental risk factors including agricultural chemicals such as herbicides (PQ), pesticides (rotenone) and heavy metals are capable of reacting with DNA causing DNA damage which is implicated as a causative factor in PD [33]. PQ (1,1-dimethy

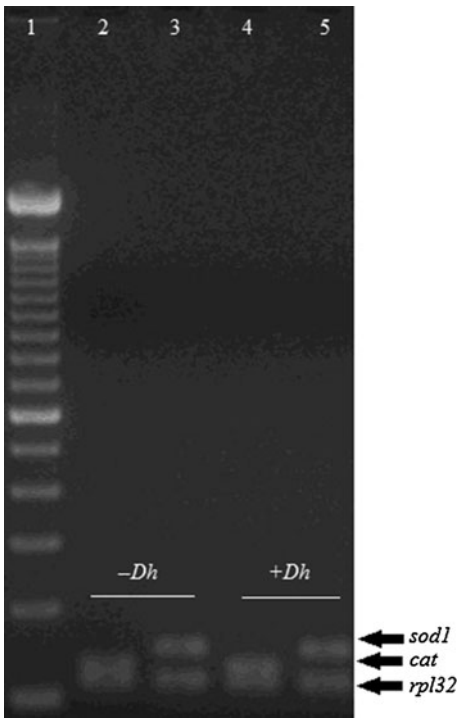

Fig. 9 Real Time-PCR analysis for the expression of *sod1* and *cat* in adult male flies treated with *Dh* extract. Results showed no significant difference in the expression level of *sod1* and *cat* between *Dh*-treated and control flies ($p > 0.05$ by *t* test). *rpl32* was used as housekeeping gene

l-4-4-bipyridynium dichloride) and neurotoxin such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) are highly toxic to humans and animals due to generation of superoxide anion that increases ROS levels in vivo [34, 35] and thereby, causing oxidative stress in the mitochondria of the cell leading to cell death and NDD [36]. PQ is considered as a risk factor for PD, because of its close structural similarity with MPP^+ (1-methyl-4-phenyl pyridium ion), the active form of PD-inducing agent (MPTP) [37]. It has shown that PQ penetrates the mitochondrial inner membrane and inhibits mitochondrial complex 1 by producing superoxide anions and other redox products [11]. Repeated exposure of *Drosophila* to sub lethal doses of PQ results in selective degeneration of dopaminergic neurons causing locomotor defects. *Drosophila* is being widely used as a model to understand the mechanisms involved in NDD, to model environmental toxin-induced parkinsonism and a convenient system to screen therapeutic agents for their neuroprotective effects, prior to their testing in mammalian model [38]. Our results show that the PQ-exposed flies exhibited locomotion deficits and stayed at the bottom in negative geotaxis assay, as they could not coordinate their legs while climbing. Interestingly, the flies that were fed with *Dh* extract prior to PQ exposure showed improved locomotory activity in negative geotaxis assay, compared to flies exposed to PQ, indicative the ameliorative effect of *Dh* on the PQ-induced locomotory deficit. Our results show that acute PQ exposure of adult males is highly toxic with no survivors. However, the lower incidence of mortality among the *Dh*-treated flies indicates the protective effect of the bioactive compounds in *Dh* against the neurotoxic effects of PQ. Our findings show that, the neuroprotective activity of *Dh* was comparable to that of curcumin, quercetin, zedoariaerhizoma and *Sanguisorba officinalis* in negative geotaxis assay [39]. PQ-exposed flies showed significant induction of oxidative stress as evidenced by the marked LPO, depletion of GSH, and altered antioxidant enzyme profile. *Dh* treatment resulted in suppression of PQ-induced oxidative stress as shown by oxidative markers. LPO causes membrane damage by reaction of oxygen with poly-unsaturated fatty acids (PUFAS). GSH detoxifies ROS by reacting directly with radicals. GSH depletion in flies pre-fed with *Dh* extract was significantly reduced; suggesting that the protective effect of *Dh* could be attributed to scavenging of free radicals as well as increased synthesis of GSH. The activity of both SOD and CAT enzymes were induced by PQ in the fly which was reversed in *Dh* fed flies. These results indicate that *Dh* extract modulates antioxidant pathways in *Drosophila* by restoring the redox status. Treatment of flies with *Dh* alone boosted the antioxidant capacity as evident by increased activity of SOD and CAT, which may reflect the adaptive response. Increased activity of CAT and SOD after *Dh* treatment is not reflected in an increased expression level of the genes as assessed by RT-PCR. These findings fall in line with earlier investigations on flies fed with green tea extract or blueberry or *Bacopa monnieri* that elevated both SOD and CAT activities in *Drosophila* [40–42] AchE is the most important biological component of the cholinergic function. AchE is involved in membrane integrity and changes in membrane permeability occurring for synaptic transmission and conduction [43]. Enhancement of AchE activity causes more acetylcholine degradation and consequent reduction in acetylcholine receptor stimulation which leads to a decrease in cholinergic neurotransmission and related functions such as cell proliferation and enhanced apoptosis [43–45]. Deficiency in acetylcholine and consequent cholinergic neurotransmissions is one of the cognitive symptoms of Alzheimer's disease (AD) [46]. It has been also shown that, acetylcholine is involved in reduction of LPO by scavenging superoxide anions [47]. Environmental toxins cause elevation in AchE activity and is associated with oxidative stress in neurological dysfunction, owing to decrease in acethylcholine levels in the brain [48]. Previous studies have demonstrated that rotenone and acrylamide also increased the activity of AchE in *Drosophila* [49, 50]. In the present study, PQ exposure also caused elevation of AchE activity, and it can be as an

evident for dopaminergic neurotransmission to be largely affected. The edible root of *Dh* is a good source of natural antioxidants [20, 21]. We have already shown that *Dh* protects against age-induced changes in the neuronal function such as cognitive deficit in fly brains [51]. The in vivo ameliorative potential of natural antioxidants from *Dh* roots have been reported in the laboratory rat [21, 52]. In this study, significant increase in the activity of AchE has been found in PQ-exposed flies, compared to control. Treatment of flies with *Dh* can reduce the activity of AchE and protect *Drosophila* against neurotoxic effect of PQ. Further, present finding of *Dh* treatment ameliorating PQ-induced oxidative stress in *Drosophila* is consistent with the reports in the rat [21]. In conclusion, Neuroprotective efficiency of *Dh* extract has been demonstrated by its ability to abrogate PQ-induced neurotoxicity and oxidative stress in *Drosophila* brain. As brain is deficient in antioxidant defenses and is rich in oxidisable substrates, it is susceptible to oxidative stress. Therefore, neuroprotective effect of *Dh* extract is evident by attenuation of PQ-induced oxidative stress, restoration of AchE activity and GSH in the fly brain and improvement of fly locomotion performance and survival assay after treatment with *Dh* extract. Understanding the biochemical processes involved in neurotoxicity in *Drosophila* that cause phenotype manifestations such as motor dysfunction, could be useful to identify new therapies. Hence, a therapy that augments antioxidative defense system may be useful. This study being the first report on the neuroprotective potential of *Dh* in the *Drosophila* model against PQ induced oxidative stress needs further investigations by employing specific genetic strains to unravel the mechanisms of its action at cellular and at organism levels.

Acknowledgments We thank the Chairman, Department of Studies in Zoology, University of Mysore, for the facilities.

Conflict of interest None.

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