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Protective potential of *Lannea microcarpa* fruit pulp extract on paraquat-induced oxidative stress and locomotor deficits in *Drosophila melanogaster*

H. O. B. Oloyede[1,3], A. O. Adeleye[1,2], F. A. Ariyo[1,3], M. O. Salawu[1,3], A. I. Ganiyu[1,3], and B. O. Yusuf[1,3]

[1]Department of Biochemistry, University of Ilorin, Ilorin, Nigeria
[2]Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria
[3]Department of Chemical Sciences, Summit University, Offa, Kwara State, Nigeria

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ABSTRACT: Exposure to environmental toxins like paraquat (PQ) produces a characteristic feature of Parkinson's disease (PD) due to its ability to induce dopaminergic cell death and oxidative stress. The aim of this study was to investigate the protective potential of *L. microcarpa* fruit pulp extract (LMFE) on paraquat-induced oxidative stress and locomotor deficits in *Drosophila melanogaster*. The flies were randomly distributed into six groups. Group A was maintained on standard diet for 7 days; group B, PQ (20 mM, 24 hrs); group C and D maintained on diet containing 0.25 and 0.5% LMFE respectively; group E and F were pretreated with 0.25 and 0.5% of LMFE respectively for 6 days followed by PQ exposure for 24 h. PQ exposure resulted in high mortality and impairment of locomotor coordination in flies as well as elevated activity of acetylcholine esterase. Also, there were significant alterations ($p<0.05$) in activity of antioxidant enzymes, glutathione, malondialdehyde, protein carbonyl and fragmented DNA concentrations. Pretreatment of the flies with LMFE significantly improved the survival and locomotor ability of the flies as well as attenuating the PQ-induced increase in activities of acetylcholine esterase, catalase, superoxide dismutase and glutathione transferase. Paraquat-induced decrease in concentration of glutathione was completely reversed by LMFE. Furthermore, LMFE completely reversed the PQ-induced increase in levels of malondialdehyde, protein carbonyl and fragmented DNA. This study concludes that LMFE differentially reversed PQ-induced oxidative stress by restoring alterations in oxidative markers, antioxidant enzymes, acetylcholine esterase and by modulating locomotor coordination of the flies.

Keywords: Paraquat; Oxidative Stress; Locomotor; *Drosophila melanogaster*

Introduction

Paraquat (PQ) (1,1-dimethyl-4-4-bipyridynium dichloride) is widely used as a herbicide and also considered a risk factor for neurodegenerative diseases due to its structural similarity to MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a classical parkinsonism inducing agent (Abdulwahid and Ahmad,

2010; Goldman *et al.*, 2012). A growing body of evidence suggests that exposure to neurotoxins such as rotenone and paraquat can lead to Parkinson disease (PD) like pathology and symptoms (Valko *et al.*, 2007; Cicchetti *et al.*, 2009; Goldman *et al.*, 2012).

Epidemiological as well as experimental studies provide evidence of the association of PQ- induced PD phenotype in experimental models including laboratory rats, mice and *Drosophila* (Liou *et al.*, 1997; Chaudhuri *et al.*, 2007; Zhang *et al.*, 2016; Wang *et al.*, 2018).

While the mechanism of PQ induced PD remains elusive, its toxic effect has been well attributed to altered redox equilibrium, free radical overproduction and mitochondria dysfunction (Peng *et al.*, 2004; Chaudhuri *et al.*, 2007; Shukla *et al.*, 2014; Michel *et al.*, 2016). Intracellularly, PQ is reduced to a free radical form via mono-cation radical (PQ^+) that is re-oxidized by oxygen to initiate an oxidative cascade that includes the generation of superoxide radicals (Thiruchelvam *et al.*, 2005; Cocheme and Murphy, 2008). In addition, high aerobic metabolic activity of nervous tissue coupled with the high lipid content exacerbates the free radical-mediated oxidative damage to neurons (Wang and Michaelis, 2010). Since oxidative stress have been implicated to play a central role in the pathology of PD; antioxidants, especially those from natural sources are useful in mediating oxidative stress and therefore, could serve as preventive therapeutic agents for neurodegenerative diseases (Havsteen, 2002; Halliwell *et al.*, 2005; Feng *et al.*, 2018). Experimental studies have shown that plants offer protective potentials to neurodegenerative diseases (Ravikumar, 2009; Chandran, 2012; Jahromi *et al.*, 2013; Blanco-Ayala *et al.*, 2014; Akinyemi *et al.*, 2018)

Drosophila melanogaster is a widely used convenient model to study the mechanisms involved in neurodegenerative disorders (Bilen and Bonini, 2005) and environmental toxin-induced parkinsonism (Dinis-Oliveira *et al.*, 2006). Toxic compounds like rotenone and paraquat have been successfully used in *Drosophila* to reproduce PD phenotypes (Coulom and Birman, 2004; Dinis-Oliveira *et al.*, 2006; Dawson & Dawson 2010). *Drosophila* disease models provide convenience studies of neurodegeneration at the molecular level and also for the search for candidate therapeutics agent (Pienaar *et al.*, 2010). Notably, the fly has homology among five of the six genes related to PD in humans (Whitworth *et al.*, 2006).

Lannea microcarpa (Engl. and K. Krause) is a wild fruit tree belonging to the family *Anacardiaceae*. The tree is native to Senegal and Cameroon where it is locally abundant (Arbonnier, 2004). The fruits are commonly consumed (used in the production of local drinks) and are also utilized in traditional medicine to treat scurvy, rickets and cough (Bationo *et al.*, 2012). Studies on extracts of the fruit of *Lannea microcarpa* showed good levels of flavonoids and polyphenols, as well as antioxidant, antibacterial and anti-inflammatory activities with active compound including myricetin, vitexin, isovitexin, gallic acid, epicatechin among others (Picerno *et al*, 2006; Bationo *et al.*, 2012).

In this study, the protective effect of *Lannea microcarpa* fruit pulp extract (LMFE) on PQ-induced locomotor deficits and oxidative stress in *D. Melanogaster* was investigated. More specifically, the protective potential of LMFE was investigated in terms of its ability to attenuate paraquat induced mortality, locomotor dysfunction and oxidative impairment in whole body homogenates of *Drosophila melanogaster*.

Materials and Methods

Sample Collection and Extraction

Fresh and ripe sample of *L. microcarpa* fruits were obtained from Adewole market in Ilorin, Kwara State, Nigeria. The sample was identified and authenticated at the Herbarium unit of Forestry Research Institute of Nigeria, Ibadan, Oyo state with voucher no: FHI. 109507. The edible pulp of *Lannea microcarpa* fruit (200 g) was extracted with 2 L of methanol with continuous shaking at room temperature for 24 h using an electric mixer. The residue was re-extracted twice, and all supernatants were combined and concentrated using a rotary evaporator. The total yield obtained (21.75 g) was kept in an airtight container prior to use.

Reagents

PQ, methanol, thiobarbituric acid (TBA), 1-Chloro, 2, 4-dinitrobenzene (CDNB) and reduced glutathione were procured from Santa Cruz Biotechnology, Germany. Diphenylamine, guanidine hydrochloride, 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide and methyl p-hydroxybenzoate were sourced from Research Organics (Cleveland, OH, USA). All other reagents used were of analytical grade.

Fruit Fly (*Drosophila melanogaster*) Culturing

D. melanogaster (wild-type, Oregon R^+) were reared on a standard diet. The diet (100 mL) contains corn flour (6.5 g), sucrose (4 g), yeast (2 g) and agar (1 g). Methyl p-hydroxybenzoate (0.2 g) was added to inhibit mould growth. Flies were maintained at room temperature with 12 h alternate light-dark cycle and 70–80% relative humidity for all the experiments.

Experimental procedure

Mortality

The effect of LMFE treatment on survival of flies following PQ treatment was investigated with acute exposure to PQ (20 mM, 24 h) and two concentrations of LMFE (0.25, 0.5 %). For this assay, 20 adult flies per replicate (three replicates) were exposed to a diet combination of LMFE and PQ or PQ alone. The number of dead flies was documented and expressed in percentage.

Negative geotaxis assay for analysis of locomotor function

The effect of PQ on locomotor ability of the flies was determined by the method described by Feany and Bender, (2000). Briefly, test flies were placed into a graduated measuring cylinder (25 cm length, 2 cm diameter). After 10 min recovery period, flies were gently tapped to the bottom of the jar and then allowed to climb. The number of flies that climbed beyond the 20 cm mark in 1 min was recorded. For each tested group, twenty flies were used and assay were carried out in triplicates. Data were expressed as percentage of flies that escaped beyond minimum distance of 20 cm in 60 s.

Paraquat exposure and LMFE treatment

To assess the protective effect of LMFE against PQ toxicity, a preliminary study was carried out to determine the concentration of LMFE to be used in the study. Flies (50) were fed standard diet containing 0.05, 0.1, 0.25, 0.5 and 1% (w/v) of LMFE for 10 days. The experiment was repeated three times. Only two concentrations of LMFE (0.25 and 0.5 %) were used based on survival and negative geotaxis assays.

Flies (5–7 days old) were randomized into six groups: Group A served as the control and were fed the normal diet. Flies in group B fed on the standard diet for six days followed by paraquat (20 mM) exposure for 24 h on the last day of experimental period. Group C and D were fed 0.25 and 0.5% of LMFE respectively. Group E and F were pretreated with 0.25 and 0.5% of LMFE respectively followed by exposure to paraquat (20 mM) for 24 h. In each of the treated group, the paraquat or LMFE was prepared in the cornmeal agar media as described (Rzezniczak *et al.,* 2011). The experimental period lasted for 7 days. The choice of dose (PQ 20 mM) was based on previous studies where PQ causes oxidative stress and reproduces PQ-incuced locomotor deficits (Chaudhury *et al.,* 2007; Srivastav *et al.,* 2018).

Preparation of Homogenate

The flies were immobilized on ice and homogenized in 0.1M phosphate buffer, pH 7.4. The homogenates were centrifuged at 2500 g for 10 min at 4°C. The supernatant was separated from the pellet into labeled tubes and used for biochemical assays.

Biochemical assays

Acetylcholineasterase

Acetylcholinesterase (AChE) activity was assayed by the method described by Ellmann *et al.* (1961). The reaction mixture contains 1 mL phosphate buffer (0.1M, pH 8.0), 50 µl of DTNB (10mM), 50 µl of the sample and 20 µl acetylthiocholine iodide (78 mM). The change in absorbance was monitored for 3mins using spectrophotometer at 412nm and expressed as µmoles of ATCI hydrolyzed/min/mg protein.

Glutathione-S-transferase (GST)

Glutathione-S-transferase (GST) activity was assayed according to the procedure described by Habig and Jakoby, (1974) using 1-chloro- 2, 4-dinitrobenzene (CDNB) as substrate. The assay mixture (270 mL) contain (20 mL of 0.25 M potassium phosphate buffer, pH7.0, with 2.5 mM EDTA, 10.5 mL of distilled water, and 500 mL of 0.1 M GSH), 20 mL of sample and10 mL of 25 mM CDNB. The reaction was monitored for 5 min (10 s intervals) at 340 nm and the data were expressed as mm/min/mg protein using the molar extinction coefficient (ε) of 9.6 mM-1 cm-1 for CDNB conjugate.

Catalase (CAT)

Catalase activity was assayed according to the procedure described by Sinha, (1972). The assay mixtures contain 2ml of (0.036% w/w) H_2O_2 and 5ml of 0.01M phosphate buffer (pH 7.8). Sample homogenate (0.5ml) was added to initiate the reaction. 2ml of dichromate/glacial acetic acid solution (1:3) was added to 1 mL of the reaction mixture at one-minute interval for three minutes. The resulting solution was incubated in boiling water bath for 10mins and the color developed was read at 570nm. The activity of catalase was expressed as µmole of H_2O_2 decomposed /min/mg protein.

Superoxide Dismutase (SOD)

The activity of SOD was determined according to Misra and Fridovich, (1972). Briefly, tissue homogenate (0.2 mL) was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate and the reaction was started by addition of 0.3 mL of substrate (0.3 mM epinephrine). The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds. One unit of enzyme activity is 50% inhibition of the rate of autooxidation of pyrogallol as determined by change in absorbance/min at 480 nm.

Reduced Glutathione (GSH)

The concentration of GSH was measured by the method described by Ellman, (1959). The sample (1.0 mL) was added to 0.1 mL of 25% TCA and precipitate was removed by centrifuging at 500 g for 10 minutes. Supernatant (0.1 mL) was added to 2 mL of 0.6 mM DTNB prepared in 0.2 M sodium phosphate buffer pH (8.0). Absorbance was read at 412nm. The GSH concentration was calculated with molar absorption coefficient of 13,600$M^{-1}cm^{-1}$.

Malondialdehyde

Malondialdehyde levels were assayed by the method of Reilly and Aust, (2001). Briefly, fly homogenate was mixed with TBA/TCA/HCl (15%, 0.2 N, 0.37%) at reagent/sample ratio of 2:1 (v/v), placed in a boiling water bath for 15 min, cooled to room temperature and centrifuged at 1000 *g* for 10 min at room temperature. The absorbance of the solution was read at 535 nm against the blank (containing all reagents except the homogenate). MDA content was determined using the extinction coefficient of 1.56×10^6 $nm^{-1}cm^{-1}$.

Protein carbonyl

Protein carbonyl levels in the samples were quantified (Levine *et al.*, 1990) with minor modifications. Two equal aliquots of supernatant fraction were taken, one treated with an equal volume of 2,4-Dinitrophenylhydrazine (10mM dissolved in 2M HCl) (test sample) and the other with 2M HCl (blank). Each mixture was incubated for 1h, followed by precipitation with 20% TCA and subsequently extracted

with ethanol: ethyl acetate mixture (1:1). The pellets were then dissolved in 1.0 mL 6M guanidinehydrochloride. The spectrum of the DNPH treated sample versus the HCl blank was determined at 370nm, and results were expressed as nmol carbonyl/mg protein (e = 22,000/M/cm).

Fragmented DNA

The quantity of fragmented DNA in the homogenates was determined using the procedure described by Burton (1956). Briefly, homogenate was centrifuged at 15,000 × *g*, for 15 min at 4°C. The supernatant was separated from the pellet and treated with trichloroacetic acid (1.50 ml, 10%). The pellet was treated with trichloroacetic acid (0.65 ml, 5%) as well. The reaction mixtures were allowed to precipitate overnight (≥ 4 h) in a refrigerator (4°C), centrifuged at 2500 × *g* for 10 min. The reaction mixtures were boiled at 100°C for 15 min, cooled to room temperature and further centrifuged at 2500 × *g* for 5 min. The supernatants (0.5 ml) were treated with diphenylamine reagent (1 ml) and incubated at 37°C for 4 h. Absorbance was read at 600 nm using a spectrophotometer. The fragmented DNA was calculated using the following expression:

$$\text{Fragmented DNA (\%)} = \frac{\text{Absorbance of supernatant}}{\text{Absorbance of supernatant + Absorbance of pellet}}$$

Statistical Analysis

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. The p value of 0.05 was considered as the minimum level of significance.

Results

Mortality

PQ exposure (fig. 1) results in 35% mortality in flies after 24 h. Pretreatment of flies with 0.25 and 0.5% LMFE reduced the incidence of mortality to 20 and 18% respectively.

Climbing ability (Negative geotaxis assay)

PQ treated flies exhibited significant locomotor impairments as evident by (52%) number of flies that crossed the 20 cm mark in 60 s compare to the control (88%), interestingly pretreatment of flies with LMFE (0.25 and 0.5%) significantly improved the locomotor deficits in a concentration-dependent manner producing 70 and 78% (Fig. 2).

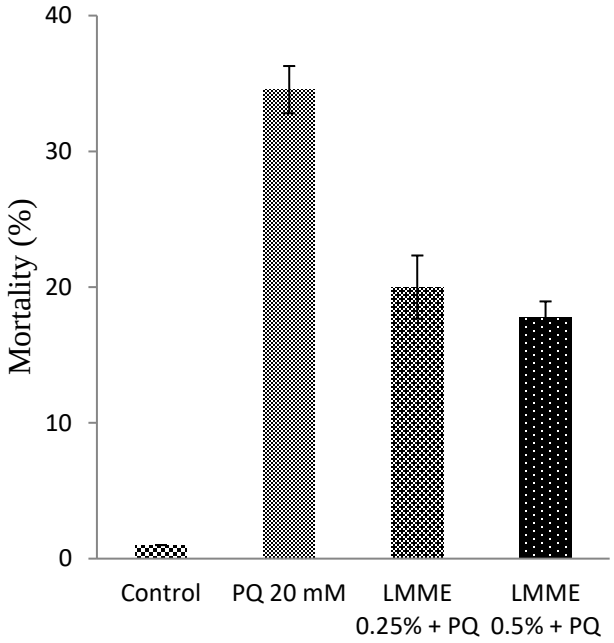

Figure 1: Protective effect of LMFE on paraquat-induced mortality in flies

Pretreatment of flies with LMFE show significant decrease in mortality after 24 h of PQ (20 mM) exposure. Data analyzed by one-way ANOVA followed by Duncan's test ($n = 3$, $p < 0.05$). Values are mean ± SE.

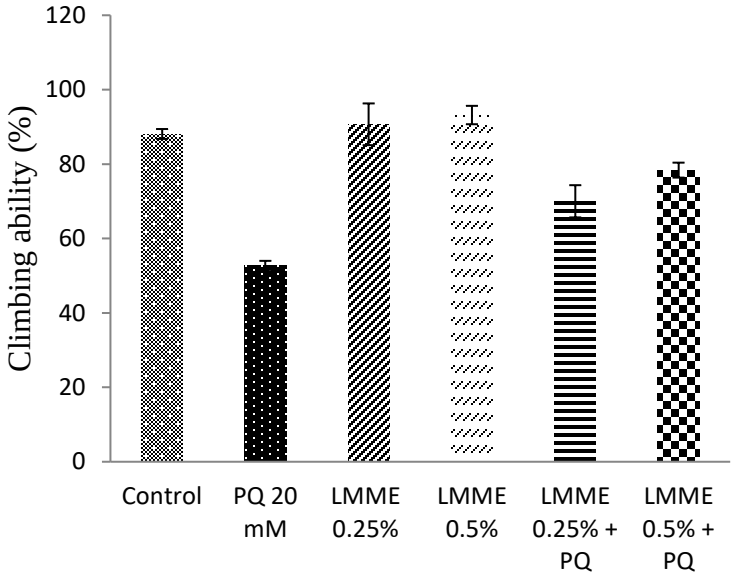

Figure 2: Modulation of paraquat induced locomotor deficits in flies pretreated with LMFE

Data analyzed by one-way ANOVA followed by Duncan's test ($n = 3$, $p < 0.05$). Values are mean ± SE.

Acetylcholinesterase

PQ exposure result in significant elevation (28 %) in the activity of AchE compare to the untreated flies (Fig. 3). Pretreatment of flies with LMFE reversed the PQ mediated increase in the activity of AchE, although it did not compare with the control.

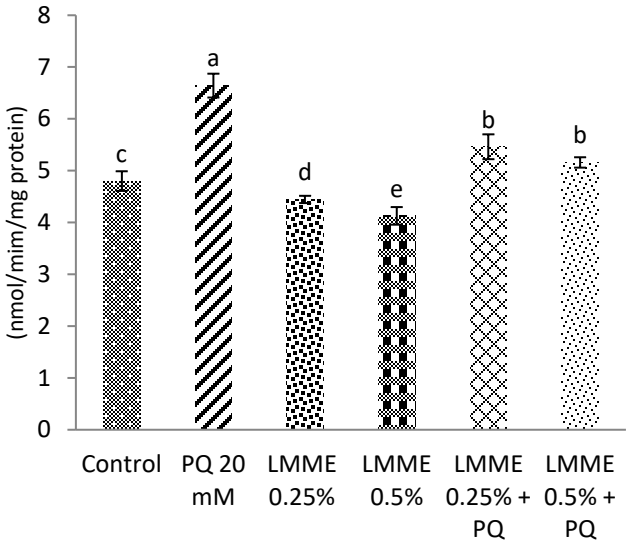

Figure 3: Protective effect of LMFE on acetylcholine esterase activity in flies exposed to paraquat

Data analyzed by one-way ANOVA followed by Duncan's test ($n = 3$, $p < 0.05$). Values are mean ± SE.

Modulatory effect of LMFE on PQ induced alterations in antioxidant enzymes and Non-enzymatic antioxidants

PQ induced alterations in the activities of antioxidant enzymes was measured by assaying for the activity of GST, SOD, and CAT, which are enzymes involved in cell response to oxidative stress. PQ exposure induced a significant elevation in the activities of CAT (Fig. 4), SOD (Fig. 5) and GST (Fig. 6) compare to the control. However, LMFE treatment alone boosted the antioxidant defense mechanism by increasing the activity of both SOD and CAT while that of GST remain unchanged. Interestingly, pretreatment with LMFE reversed the PQ-mediated increase in the activity of CAT, SOD and GST to normalcy.

PQ treatment led to mark depletion of GSH in flies. Treatment of flies with LMFE alone at both concentrations elevated GSH levels by11% and 31%. Pretreatment of flies with LMFE attenuated the PQ mediated depletion in GSH and compare favorably with the control (Fig. 7).

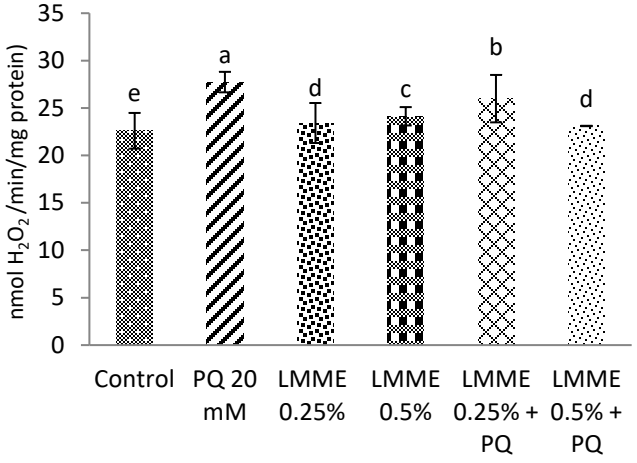

Figure 4: Effect of LMFE on catalase activity in flies exposed to paraquat

Data analyzed by one-way ANOVA followed by Duncan's test (n = 3, $p < 0.05$). Values are mean ± SE.

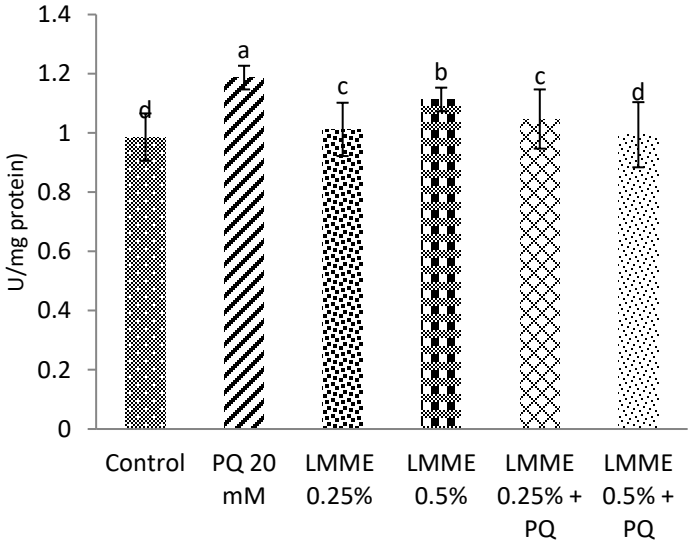

Figure 5: Effect of LMFE on superoxide dismutase activity in flies exposed to paraquat

Data analyzed by one-way ANOVA followed by Duncan's test (n = 3, $p < 0.05$). Values are mean ± SE.

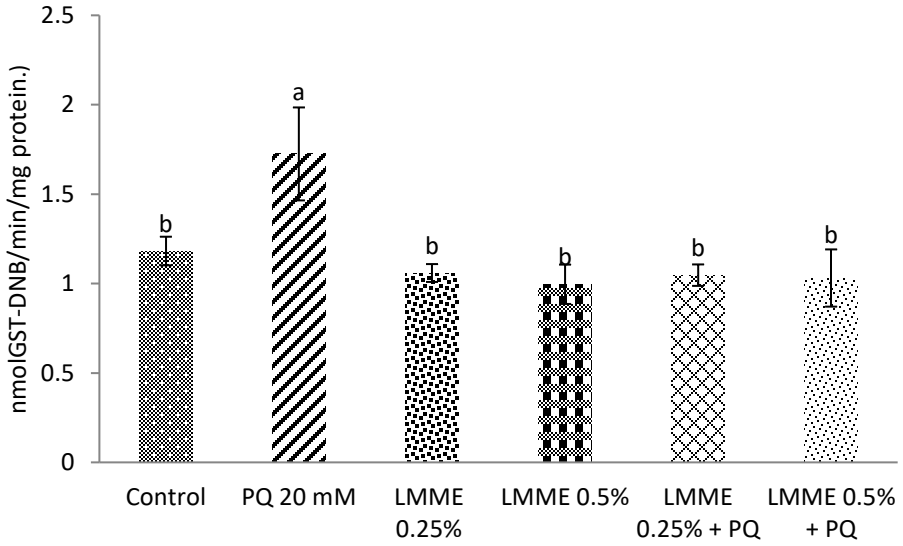

Figure 6: Effect of LMFE on Glutathione transferase activity in flies exposed to paraquat

Data analyzed by one-way ANOVA followed by Duncan's test ($n = 3$, $p < 0.05$). Values are mean ± SE.

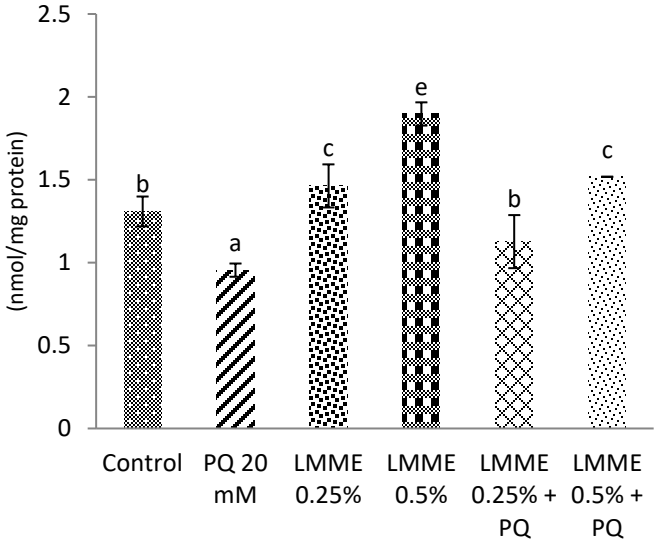

Figure 7: Protective effect of LMFE on PQ-induced alteration in GSH concentration

Data analyzed by one-way ANOVA followed by Duncan's test ($n = 3$, $p < 0.05$). Values are mean ± SE.

Effects of LMFE on PQ induced oxidative stress markers.

PQ exposure induced a marked increase in PC (44%), MDA levels (26%) and fragmented DNA (8%) when compare to the control flies (Table. 1). Interestingly, pretreatment of flies with LMFE reversed the PQ mediated increase in the level of the oxidative markers. The decrease observed following pretreatment of flies with 0.25 and 0.5% LMFE was PC (41 – 42%), MDA (21 – 27%) and fragmented DNA (14%).

Table 1: Modulatory effect of LMFE on paraquat induced alteration in the endogenous levels of oxidative markers in *Drosophila melanogaster*

Group	Protein carbonyl	MDA	% DNA
Control	1.719 ± 0.004^{c}	5.576 ± 0.243^{c}	4.105 ± 0.184^{b}
Paraquat 20 mM	3.052 ± 0.027^{a}	7.526 ± 0.077^{a}	4.454 ± 0.438^{a}
LM 0.25%	1.450 ± 0.002^{d}	5.440 ± 0.168^{d}	3.404 ± 0.185^{d}
LM 0.5%	1.376 ± 0.010^{e}	5.189 ± 0.074^{e}	3.344 ± 0.501^{d}
LM 0.25% + Paraquat	1.805 ± 0.019^{b}	5.947 ± 0.028^{b}	3.846 ± 0.178^{c}
LM 0.5% + Paraquat	1.767 ± 0.007^{b}	5.488 ± 0.134^{c}	3.842 ± 0.014^{c}

Data analyzed by one-way ANOVA followed by Duncan's test ($n = 3$, $p < 0.05$). Values are mean ± SE

Discussion

Paraquat toxicity occurs as a result of formation of a cation (PQ^{+}) that is re-oxidized by oxygen to initiate an oxidative cascade that leads to the generation of superoxide radicals and other ROS, mainly hydrogen peroxide and hydroxyl radical (Suntres, 2002; Thiruchelvam *et al.,* 2005; Cocheme and Murphy, 2008). Accumulation of the ROS results in the degeneration of dopaminergic neurons along with deterioration in locomotion behavior leading to Parkinson-like symptoms in exposed organism (Peng *et al.,* 2004; Shukla *et al.,* 2014). Also, ROS production via the PQ redox cycle can promote lipid peroxidation, impair the recycling of oxidized glutathione, promote oxidation of protein and DNA fragmentation, all contributing to oxidative damage (Suntres, 2002; Amin and Hamza, 2005). In recent times, the potential of natural compounds to attenuate the endogenous redox status in vivo has been considered as an effective approach mitigate oxidative stress-induced diseases (Rio *et al.,* 2009; Dumont and Beal, 2011). Since PQ is a widely employed model for inducing oxidative stress and PD both in mammalian and the fly model, the fly model of *Drosophila melanogaster* was chosen to test the hypothesis that dietary feeding of LMFE could mitigate PQ induced toxicity.

Acute exposure to PQ is associated with high mortality and reversibility of locomotor impairment (Jahromi *et al.,* 2013; Navarro *et al.,* 2014; Niveditha *et al.,* 2017). In this study, PQ exposure (20 mM, 24 hrs) results in high mortality in *Drosophila*. Low incidence of mortality among flies pretreated with LMFE indicates protective ability of the extract against PQ induced lethality. Studies have reported that exposure of flies to PQ reproduced the characteristic pathological features of PD (i.e., alteration of locomotor behavior and progressive deficiency of dopaminergic neurons) (McCormack *et al.,* 2002; Zhou, 2011; Jahromi *et al.,* 2013). In this study, PQ-induced locomotion deficit was evaluated by measuring climbing ability. PQ exposed flies tends to stay at the middle or bottom areas of the vials as they could n0ot coordinate their legs while climbing, indicating that PQ may pose toxic effects to fly locomotion (Fig.7). In addition, the decreased mobility observed in the flies was due to the loss of dopaminergic neurons that induce the mobility deficits characterized by decreased walking activities (Park *et al.,* 2012). Dopamine (DA) is an essential neuromodulator in the nervous system of mammals and insects, where it plays a central regulatory role in the neural networks controlling locomotor activity and stereotypical behaviors (Riemensperger *et al.,* 2011). Interestingly, the flies pretreated with LMFE showed improved locomotor activity indicating the ameliorative effect of the extract on PQ-induced locomotor deficit. The improved performance of the flies may be attributed to the restoration of DA levels. Interestingly, studies have shown that phytonutrients

enhance the DA levels in fly model of neurodegenerative diseases (Faust *et al.,* 2009; Muralidhara *et al.,* 2012; Prasad and Muralidhara, 2014).

Oxidative damage to cellular macromolecules (lipid, protein, DNA), arising from redox imbalances, is normally counteracted by ROS detoxifying enzymes (SOD, CAT, GSH-Px, GSH-Red and Glc 6-PD) (Ajiboye *et al.,* 2010). In the present study, there were significant increase in the activities of antioxidant enzymes SOD, CAT and GST following acute PQ exposure. The increase observed in the activities of these enzymes suggests an increased generation of ROS and also may be an attempt by the enzymes to abate the adverse effect of free radicals generated by PQ metabolism. Several studies have reported that increase in the activities of antioxidant enzymes suggests a compensatory mechanism in response to oxidant effects triggered by PQ (Cocheme and Murphy, 2008; Jahromi *et al.,* 2013; Nunes *et al.,* 2016).

Thus, the significant attenuation of PQ-mediated increase in the activities of antioxidant enzymes by LMFE indicates the capability of the extract to modulate anti-oxidative pathways in the cell by restoring the activities of antioxidant enzymes. The restoration of alteration in the activities of these enzymes following LMFE treatment buttress the protective potentials of the extract in protecting against paraquat mediated oxidative stress. These findings are consistent with earlier investigations on flies fed with *Bacopa monnieri* and also *Decalepis hamiltonii* that modulate both SOD and CAT activities in *Drosophila* following PQ treatment (Hosamani and Muralidhara, 2010; Jahromi *et al.,* 2013).

GSH-mediated metabolism is the most important non-protein thiol that confers cellular protection against oxidative stress mediated toxic injury either by reacting with radicals non-enzymatically or as a substrate in GST catalyzed reactions**.** As an anti-oxidant, GSH plays an important role in protecting dopaminergic nigrostriatal neurons from the damage inflicted by neurotoxin (Shukla *et al.,* 2014), and depletion in GSH content has been reported in the postmortem brain of PD patients (Sofic *et al.,* 1992). Enhancement of GSH levels have been used as a therapeutic agent for the treatment of several neurodegenerative conditions (Pocernich and Butterfield 2011). In the present study, severe depletion in cellular GSH levels upon paraquat exposure in *Drosophila* validate that a state of oxidative stress exists. GSH levels were significantly restored in flies pretreated with LMFE. The enhanced GSH level may in part be responsible for the observed protection against PQ induced toxicity. The result obtained fall in line with (Jahromi *et al.,* 2013). Thus, the preservation of GSH in the PQ-treated flies following treatment with LMFE indicates possible antioxidant activity.

Acetylcholinesterase (AchE) is an enzyme in the cholinergic pathway where its activity is required in synaptic transmission and conduction of signals across the synapses (Schmatz *et al.,* 2009). AchE hydrolyzes the neurotransmitter acetylcholine to acetate and choline. Acetylcholine as a neurotransmitter regulates cognitive function, motor function, learning/memory and locomotion (Peres *et al.,* 2016). Increase in AchE activity causes more acetylcholine degradation and consequent reduction in acetylcholine receptor stimulation leading to decrease in cholinergic neurotransmission and related functions such as cell proliferation and enhanced apoptosis (Jin *et al.,* 2004; Bernhardi *et al.,* 2005; Schmatz *et al.,* 2009). Previous studies have demonstrated that rotenone, PQ and acrylamide increased the activity of AchE in *Drosophila* (Hosamani and Muralidhara 2010; Prasad and Muralidhara, 2012; Haddadi *et al.,* 2013). Deficiency in acetylcholine and consequent cholinergic neurotransmissions is one of the cognitive symptoms of AD and PD (Elufioye *et al.,* 2010). Lately, phytochemicals with AchE inhibitory capacity are being extensively used as therapeutic adjuvants (Lee *et al.,* 2011; Oboh *et al.,* 2018). In the present study, PQ exposure caused elevation of AchE activity. Co-exposure of flies to both LMFE and paraquat completely prevented the enhancement in the activity of AchE thereby protecting the flies against neurotoxic effect of PQ, thus, suggesting the effect of the extract on cholinergic function. This finding corroborates earlier reports from various plants *viz*., *Decalepis hamiltonii*, *Bacopa monnieri, Z. rhizome* on AchE activity in *Drosophila* (Hosamani and Muralidhara 2010; Park, 2012; Jahromi *et al.,* 2013). Hence, the capability of LMFE to offset PQ-induced toxicity in *Drosophila* suggests that it may be exploited as an agent in the management of oxidative stress mediated neurodegenerative pathologies.

PQ has been reported to induce lipid peroxidation in experimental fly model of PQ-induced toxicity (Hosamani and Muralidhara, 2010; Jahromi *et al.,* 2013; Niveditha *et al.,* 2017; Srivastav *et al.,* 2018). Thus, the significant increase in the levels malondialdehyde (a lipid peroxidation product) shows oxidative

assaults on the cellular lipids. This can lead to loss of membrane structure and function (Niki, 2009). The reduction of PQ-mediated increase in MDA levels by LMFE reveals protection of membrane lipids. The capability of LMFE to reverse the PQ-mediated increases in MDA might have resulted from the ROS detoxification potentials of the extract.

Protein carbonyl is an indicator of irreversible oxidative damage to cellular proteins and may have lasting detrimental effects on cells and tissues (Dalle-donne *et al.*, 2003). Thus, the significant increase in protein carbonyl following PQ exposure could have resulted from the oxidation of protein by ROS generated during PQ metabolism. The attenuation of PQ-mediated increase in the level of protein carbonyl by LMFE shows the capability of the extract to promote the detoxification of ROS via the induction of antioxidant enzymes. Similar attenuation of PQ-mediated increase in protein carbonyl level following the administration of *Bacopa monnieri* leaf powder has been reported (Hosamani and Muralidhara, 2010).

Agricultural chemicals such as herbicides (PQ), pesticides (rotenone) and heavy metals are capable of reacting with DNA, thereby causing DNA damage which is implicated as a causative factor in PD (Wilson *et al.,* 2003). Oxidative stress may induce cellular damage, impairment of the DNA repair system and mitochondrial dysfunction, all of which have been known as key factors in acceleration of aging process and the development of neurodegenerative disorders (Gandhi and Abramov, 2012; Song and Zou, 2015). Oxidative stress have been reported to mediate DNA fragmentation (Amin & Hamza, 2005). In this study, PQ exposure result in significant increase in level of fragmented DNA. The increase in the level of fragmented DNA in the PQ-treated flies show genotoxic ability of PQ. The reduction in the level of fragmented DNA in the PQ-treated flies by LMFE shows antioxidants and antigenotoxic activity of the extract. The extracts could have acted to enhance the DNA repair system or induce DNA synthesis (Brahmi *et al.*, 2011).

In conclusion, the significant protection rendered by LMFE against PQ-induced mortality, locomotor deficits as well as the oxidative markers and cholinergic function strongly suggests its role in modulating oxidative stress.

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