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Ameliorative activity of medicinal plant fraction for neuroprotection against acrylamide-induced neurotoxicity in *Drosophila melanogaster*—a comparative study

G. K. Pratap, Danagoudar Ananda, Chandrashekhar G. Joshi and Manjula Shantaram*

Abstract

Background: Medicinal plant extracts used in folk medicine seem to be crucial since those are generally accepted by people without worrying about the toxicity. In our present study, we have compared the neuroprotective role of the rhizome of *Curculigo orchioides* Gaertn. and leaf extracts of *Olea dioica* Roxb., against acrylamide-induced neurotoxicity in *Drosophila melanogaster*.

Results: In-vivo neurotoxic study was carried out using 7-day-old flies (wild-type *D. melanogaster*). Prior to co-after exposing the flies with acrylamide (8 mM) along with or without OLE-2 and CU-3 fractions (0.2, 0.4 and 0.6% w/v), the heads of flies of both the control and treated groups were homogenized for biochemical assay. ACR-treated groups have shown higher elevation in AChE, SOD, LPO, and CAT activity when compared to control and treated (plant fraction) groups. Biochemical and histopathology studies show that both the plant fractions (OLE-2 and CU-3) have neuroprotective action against acrylamide.

Conclusion: The present study has demonstrated that dietary supplementation with plant fraction of OLE-2 and CU-3 has increased antioxidant enzymes and decreased AChE activity in *D. melanogaster*. This activity could be part of the probable mechanisms of action by which OLE-2 and CU-3 fractions have extended the lifespan and locomotory activity in fruit flies. These fruit flies continue to provide an exciting system for metabolic studies and should be more extensively exploited. Based on these results, further studies on the identified bioactive compounds from these two plants are being planned.

Keywords: *Drosophila melanogaster*, *Curculigo orchioides* Gaertn., *Olea dioica* Roxb., Histopathology, Anti-cholinesterase activities, Medicinal plant

*Correspondence: manjula59@gmail.com
Department of Studies and Research in Biochemistry, Jnana Kaveri Post Graduate Centre, Mangalore University, Chikka Aluvara, Kodagu 571 232, Karnataka, India

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Background

Acrylamide (ACR), a water-soluble vinyl monomer group, is formed in the production of polymers used in cosmetics, pulp, paper and food industries and wastewater management (Dearfield et al., 1988). ACR also has a significant application in the research laboratory in gel electrophoretic separation of molecules.

Neurotoxicity is one of the main consequences of exposure to acrylamide, whose neurotoxicity manifestations are characteristic weight loss, muscle weakness, degeneration of the axon and nervous system (LoPachin & Gavin, 2012). Some studies have shown autonomic, sensory, motor deficits resulting from the thalamus in experimental animals (Mehri et al., 2014). Acrylamide causes some alterations in the neurotransmitters released in the brain region, neurofilament distribution, demyelination of neurons, loss of body weight, reduction in RBC cells and haemoglobin level (Adewale et al., 2015; Imai & Kitahashi, 2012).

Medicinal plant extracts are being used from ancient times to treat a range of diseases including diabetes, cancer, skin diseases and cardiovascular diseases (Adewale et al., 2015). The rising popularity of plant bioactive compounds as an alternative to synthetic drugs is primarily because they are generally regarded as safe, affordable, easily accessible (Priyadarshini et al., 2010), ethnically acceptable form of health solutions and trusted by a number of people (Adewale et al., 2015; Prasad & Muralidhara, 2012). There are some plant bioactive compounds like Geraniol, Quercetin, Curcumin and Gallic acid which have natural flavonoid and phenolic compounds with antioxidant, and neuroprotective activity in neurodegenerative disease models (Niveditha et al., 2017).

Olea dioica Roxb., a member of the Oleaceae family, is an important ethnomedicinal plant and grows in open, evergreen forests at 1100–1200 m and is distributed throughout the Western Ghats region. Its plant parts such as roots, bark and leaves are used for anticancer, antioxidant, febrifuge, anti-AChE activity (Ashwathanarayana & Naika, 2017; Pratap & Shantaram, 2020; Pratap et al., 2020b).

Curculigo orchioides Gaertn is an ethnomedicinal plant that belongs to Hypoxidaceae family found in India and China. It has been used to alleviate human ailments like neurodegenerative diseases, bronchitis, diarrhoea, anti-AChE activity (Pratap et al., 2020a) improve memory and learning, etc., (Sharma et al., 2007).

D. melanogaster has been used as one of the best models for human diseases. In addition, these flies have other advantages, namely, easy to handle, shortened lifespan, increased oxidative stress sensitivity, learning and memory defects. Recently, its usage has been comprehensive to check the effectiveness of *ayurvedic* medicines and their action at the molecular level (Haddadi et al., 2014).

In the present study, *D. melanogaster* was used to study the neuroprotective activities in plant fractions (OLE-2 and CU-3) against acrylamide. Our results recommend that these two medicinal plants may be used as sources of neuroprotective agents**.**

Methods

Chemicals

ATCI (Acetylthiocholine iodide), DTNB, acrylamide, EDTA, TBA, SDS, TCA, DCFD, ethanol were sourced from Merck company.

Drosophila strain

D. melanogaster (Wild-type, Oregon K.) strain was obtained from *Drosophila* stock centre, Dept. of Zoology, Mysore University, Mysuru. The *Drosophila* were maintained on standard wheat media supplemented with dry yeast granules at 24 ± 2 °C and 60–80% humidity.

Collection of plant material

Rhizomes of *C. orchioides* Gaertn. and *Olea Dioica* Roxb (Leaves) were collected from the Western Ghat region of Madikeri (Lat:12.55817, Lng: 75.95315), Kodagu, Karnataka, India. The sample was authenticated by Dr. S. Leelavathi, Dept. of Botany, University of Mysore, Mysore, Karnataka, India. The specimen voucher number for the *C. orchioides* Gaertn is UOMBOT20CO01 and *Olea dioica* Roxb is UOMBOT20CO02. After authentication, medicinal plant was collected in bulk and taken for further studies. Ethanolic and methanolic extracts were subjected to column chromatography eluting with hexane and ethyl acetate in the ratio of 3:2 (Ethanolic extracts of rhizome) and chloroform and methanol in the ratio of 9:1 (methanolic leaf extracts). We have collected 15 fractions, and particularly a second fraction of the leaf extract and the third fraction of the rhizome extract showed good antioxidant (Pratap et al., 2020b), antimicrobial and anti-AChE activity (Pratap et al., 2020a).

Preliminary study for selection of acrylamide concentration for treatment of Drosophila flies

An initial study for the selection of an appropriate concentration of acrylamide to treat flies was done as per Prasad and Muralidhara (2012) with a slight modification (Prasad & Muralidhara, 2012). *D. melanogaster* flies were exposed to different concentrations of acrylamide (0, 2, 4, 6, 8, 10 mM) in the diet for 24 and 48 h to verify the lethality. They were monitored frequently for the incidence of locomotor and mortality deficits. Based on the preliminary result, 8 mM concentration was chosen as

suitable for treatment. The exposure of flies to acrylamide for seven days induced locomotor deficits and lethality. The modulatory effect was assessed by treating with plant fractions containing phytoconstituents of OLE-2 (*Olea Dioica* Roxb. leaf fraction) and CU-3 (*Curculigo orchioides* Gaertn rhizome fraction). Seven-day-old flies of *D. melanogaster* were co-exposed to acrylamide (8 mM) with or without OLE-2 and CU-3 fractions (0.2, 0.4and 0.6% w/v) in the medium. Lethality was recorded every 24 and 48 h, while locomotory dysfunction was assessed on day1 and 2. Flies from control and treated groups were subjected to negative geotaxis assay (50 flies per group/3 replicates) to assess the extent of locomotory deficits.

OLE-2 and CU-3 plant fraction concentrations for dietary supplement for flies

A preliminary study for the selection of plant extract concentrations for dietary supplement for flies was done following the method of with a slight modification (Hosamani & Muralidhara, 2009; Niveditha et al., 2017; Prasad & Muralidhara, 2012; Rzezniczak, et al., 2011). To determine suitable concentrations of OLE-2 and CU-3 plant fractions for feeding experiment and the duration of exposure required, each plant fraction diet supplement population ($n=50$ male flies) was split into six groups, including control and treated groups. The fly diet was supplemented with 0.01, 0.04, 0.2, 0.4, 0.6, 0.8 and 1%(w/v) plant extract and three replicates were used for each experiment. Based on the data, three concentrations of plant fractions were selected (0.2%, 0.4% and 0.6%; w/v), and the survival rates were compared between control and treated flies. Three concentrations of plant fractions represent lower and higher concentrations to measure the neuroprotective effect on ACR-induced changes in negative genotoxicity assay. The higher concentration of plant fractions (0.8 and 1% (w/v)) show the same neuroprotection activity. Hence, 0.6% (w/v) was chosen as an ideal concentration.

Treatment

Seven-day-old male flies of *D. melanogaster* were co-exposed to acrylamide (8 mM) as per prasada et al., with a slight modification (Hosamani & Muralidhara, 2009; Niveditha et al., 2017; Prasad & Muralidhara, 2012). For every tested group, three (3) replicates of 50 flies were tested with or without OLE-2 and CU-3 fraction (0.2, 0.4 and 0.6% w/v) in the medium. Briefly, transferred 7 (seven) day-old flies into vials (Bottle) containing 10 ml of media with 8 mM ACR + plant fraction (OLE-2 and CU-3) and kept for 7 (seven) days. Flies were transferred into a new medium for neuroprotection studies once in every two days, and after each ACR exposure, the experimental groups were maintained at 25 ± 1 °C and 60–80% humidity. Later, the *Drosophila* flies from both control and treatment groups were subjected to biochemical assays.

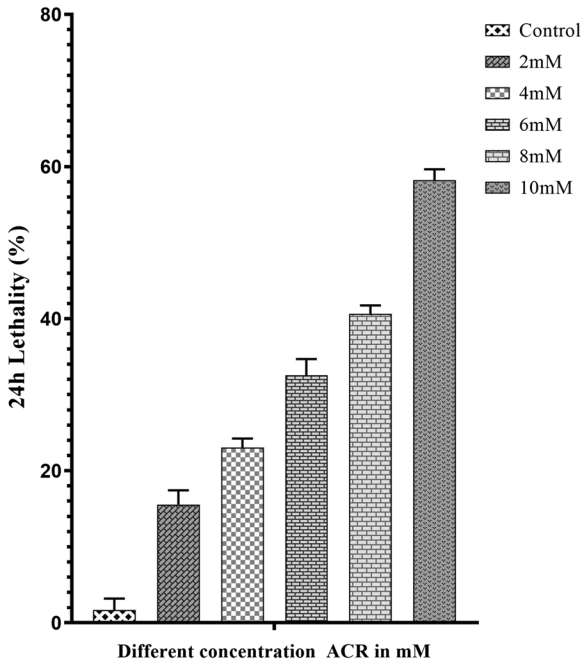

Fig. 1 24 h lethality

Biochemical assay

Tissue preparation

Fly heads from control and treated groups were homogenized in ice-cold PBS (sodium phosphate buffer, 0.1 M, pH7.0), cold centrifuged at 2500*g* for 5 min. and the supernatant was used for biochemical assay.

ROS assay

ROS levels measured by fluorometric method of Black with a slight modification (Black & Brandt, 1974; Haddadi et al., 2014). The microplate containing 50 µL of the supernatant of sample and 15 µL (5 µM) DCFH-DA was made up to 135 µL by adding homogenizing buffer, and the mixture was incubated for 1 h at RT. The microplate was read at 489 nm excitation and 525 nm emission by Spectro-fluorometer.

Lipid peroxidation (LPO) assay

LPO assay was performed following Buege and Aust with slight modifications (Buege & Aust, 1976; Haddadi et al., 2014). The homogenized sample (50 µL) was added to the test tube containing a reaction mixture of 200 µL of SDS solution (8.1%), 1.3 ml of acetic acid (20%) 1.3 mL of TBA (0.8%) and 150 µL of milli Q water. The reaction mixture

Table 1 Modulatory effect of OLE-2 and CU-3 plant fractions on antioxidant enzyme activities in the head of adult male *D. melanogaster* co-exposed with acrylamide (ACR) at the end of 7 days

Assays	Control	ACR	ACR + OLE-2 (0.2% w/v)	ACR + OLE-2 (0.4%w/v)	ACR + OLE-2 (0.6% w/v)	ACR + CU-3 (0.2%w/v)	ACR + CU-3 (0.4%w/v)	ACR + CU-3 (0.6% w/v)
LPO[a]	4.94 ± 0.691	6.16 ± 0.152	6.10 ± 0.100	5.57 ± 0.315	5.67 ± 0.050	5.73 ± 0.115	5.20 ± 0.005	5.14 ± 0.112
Catalase[b]	13.7 ± 0.173	15.6 ± 0.208	14.21 ± 0.037	12.67 ± 0.301	7.59 ± 0.353	15.25 ± 0.015	13.52 ± 0.205	11.63 ± 1.479
AChE[c]	124.6 ± 6.429	162.6 ± 2.081	150.6 ± 2.309	149 ± 1	145.6 ± 0.577	153.6 ± 2.081	151.6 ± 0.577	149 ± 1

Data analysed by one-way ANOVA followed by Tukey's test for comparison of control, ACR (8 mM) and co-exposed with ACR + plant fractions at different concentrations Mean ± SD

[a] Lipid peroxidation (LPO), MDA nM/mg protein

[b] Catalase, nmol of H_2O_2/min/mg protein

[c] AChE, nM/min/mg protein

was properly mixed and incubated for one hour at 60 °C and then cooled, which then was added to 1.5 mL of butanol and further incubated for 1 h and centrifuged for 5 min at 8000*g*. The yellowish supernatant was read at OD at 532 nm.

Catalase activity

Catalase activity was checked following the protocol of Haddadi with a slight modification (Haddadi et al., 2014). The homogenized sample (50 µL) was added to the reaction mixture contains 150 µL (8 mM, H_2O_2) and 50 µL of PBS (50 mM, 7.0 pH), and the OD was read at 240 nm.

AChE activity

Acetylcholinesterase (AChE) activities were determined by the Ellman method (Akinyemi et al., 2017; Ellman, 1959; Haddadi et al., 2014). The 50 µL of the homogenized sample contains 300 µl of 1.04 mM DTNB (Ellman's Reagent). The reaction mixture was initiated by adding of 200 µL of 0.83 mM AChI and 450 µL sodium phosphate buffer (0.5 M, pH 8) and incubated for 5 min. The reaction mixture measured at 412 nm in a spectrophotometer (UV–visible, Beckman Coulter, USA). AChE activity was expressed as nmol/min/mg protein.

Climbing assay

Locomotor activity and the climbing assay were performed as per Lee (Hwang et al., 2013; Lee et al., 2010; Valéria et al., 2014) with a slight modification. Spontaneous locomotion was measured on the sixth day. Ten male flies were introduced to labelled vertical climbing assay chamber (50 cm in length and 2 cm in diameter), and the *Drosophila* were tapped down to the bottom of the tube and counted the number of flies that have climbed to the top of the column in 20 s (five trials were carried out for each group at 5-min intervals) and the number of flies reaching the top of the column against the total number of flies (Climbing Scores) as well as those that remained below the mark were counted separately.

Moving behavior

Initially, a single *Drosophila* was placed inside the Petri plate to assess its moving behavior (Finelli et al., 2004; Singh et al., 2017; Mathew & Krishnamurthy, 2018; O'Keefe & Denton, 2018). Moving behavioral studies were carried out for both treated and untreated groups.

Histopathology studies

The flies were anaesthetized, fixed in Carnoy's fixative at 4 °C overnight. After the fixation of the flies' heads, the fly sample were dehydrated in alcohol (40–100%) and the heads were embedded in paraffin wax. Sections of 4 µm thick heads were stained with H–E stain (Kucherenko et al., 2010). The stained fly sections were observed under a light microscope (Olympus) for neuroanatomical studies to confirm the internal morphology of the flies' head sections, which showed a distinct pattern of brain degeneration in the hippocampus and optical lobe region.

Protein estimation

The protein concentration of homogenized sample was determined by Lowry's method using BSA standard (Lowry, 1951). The sample was incubated with FC phenols in alkaline condition for 30 (thirty) min and the absorbance was read at 750 nm using a spectrophotometer (Beckman Coulter, USA).

Results

ACR-induced lethality response

ACR co-exposure to flies (24 and 48 h) resulted in a time- and concentration-dependent lethality (Figs. 1, 2). Minor mortality was observed at lower to higher concentrations of ACR at 48 h, higher concentration of ACR (2–10 mM) showed cumulative mortality (Fig. 3).

A considerable mortality was observed when treated with ACR for 7 days. The plant fractions (OLE-2 and CU-3) improved the survival of flies against ACR toxicity than the control group. The flies fed with plant fractions exhibited dose-dependent survival against ACR toxicity.

The percentage (%) survival of plant fraction-treated flies was significantly higher compared to ACR-treated group, so it can be inferred that the OLE-2 and CU-3 plant fractions were effective against ACR toxicity. Based on efficient doses against acute ACR-toxicity, 0.2, 0.4 and 0.6% were used as the best concentrations to compare the neuroprotective action of the plant fractions against ACR. The ACR-induced neural dysfunctions and oxidative stress after seven-day ACR exposure flies fed with 0.2, 0.4 and 0.6% of OLE-2 and CU-3 plant fractions showed better survival than the different fly group fed with the plant fraction. The OLE-2 and CU-3 fractions showed neuroprotective action against ACR-induced mortality in flies (Fig. 3), and the flies in CU-3 fraction showed higher neuroprotective action and survivability activity compared to OLE-2.

Geotaxis assay and moving behavior

Locomotor-deficit flies exposed to ACR resulted in cruel locomotor destruction as evident from the negative geotaxis assay. Flies were transferred into the glass column; in each climbing activity, 10 flies were introduced into a vertical glass bottle or column. The flies lean to stay at the base of the vertical glass column which showed injurious locomotor effect of ACR on the climbing capability. Dietary supplementation of the plant fraction helped improve the locomotor deficit caused by exposure to ACR (Fig. 4). The protective action of CU-3 plant fraction on climbing ability of flies was higher compared to that of OLE-2 plant fraction. However, CU-3 fraction is preferable for defence against ACR-causing locomotor injury compared to OLE-2 plant fraction. The CU-3 plant fraction-treated group showed better moving behavior and higher distance cover compared to OLE-2 plant fraction-treated groups. Better moving behavior of flies was observed compared to ACR-treated group (Fig. 5).

Oxidative stress markers

ACR-treated flies indicate a remarkable elevation in Reactive Oxygen Species ROS (Fig. 6), LPO (Fig. 7) and catalase (Fig. 8) levels. All the four fly groups fed with the plant fraction of OLE-2 and CU-3 showed declining levels of ROS, catalase, AChE and lipid peroxidation when compared to the untreated groups. The CU-3 fraction caused higher reduction in ROS, catalase and LPO levels followed by OLE-2 fraction. Also, the administration of OLE-2 and CU-3 fractions caused a remarkable reduce in LPO and ROS levels compared to untreated groups. Treatment of OLE-2 and CU-3 fraction decreased the catalase content. The neuroprotective action of CU-3 fraction was higher than that of OLE-2 fraction. CU-3 fraction was more effective in the prevention of ROS, catalase, and LPO (Table 1).

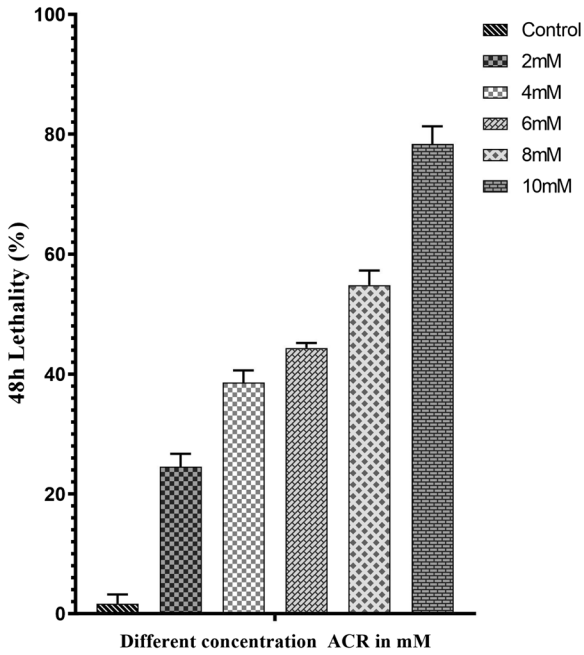

Fig. 2 48 h lethality

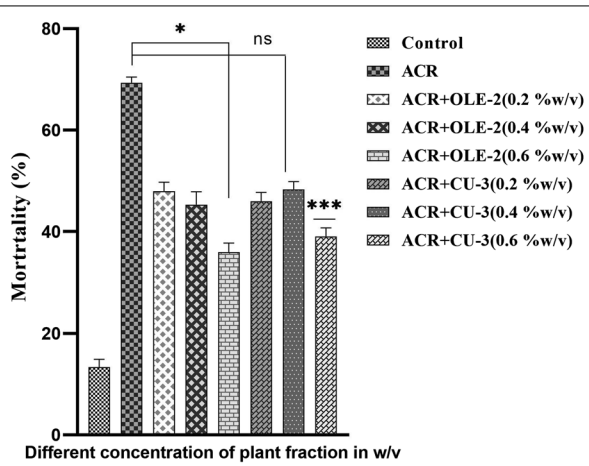

Fig. 3 Neuroprotective effective OLE-2 and CU-3 plant fractions on ACR-induced mortality of flies; Treatment group shows a significant increase of survivorship after 48 h of ACR (8 mM) exposure; Significance analysed by a one-way ANOVA; Error bar represents the Mean±SD. The *p* values *p < 0.05 and ***p < 0.0001 were considered as significant

Acetylcholinesterase (AChE)

The AChE activity was significantly increased when exposed to ACR but dietary supplementation of plant fractions of OLE-2 and CU-3 altered or prevented it

(Fig. 9). Among the two fractions, the CU-3 fraction had higher effect in the avoidance of changes in acetylcholinesterase activity than with OLE-2 fraction.

Histopathology analysis

Haematoxylin–eosin-stained histopathological sections of the *D. melanogaster* fly head were made ready. Flies treated with ACR (8 mM) along with various concentrations of plant fractions (OLE-2 and CU-3) showed lesser gap in their brain indicating that these plant fractions provided neuroprotective support against ACR. The plant fraction treated fly's brain showed less neuronal loss compared to the control groups (Fig. 10).

Discussion

Acrylamide is water-soluble, and, as a result, is quickly absorbed and distributed throughout the body (Besaratinia & Pfeifer, 2007). ACR significantly increases the production of oxidative stress and lipid peroxidation in the body. Humans are continuously exposed to low levels of acrylamide through the eating of processed carbohydrates (Kommuguri et al., 2014). It is very important to investigate regular food components which can alleviate the likely neurotoxicity. Intake of acrylamide is reported in western countries to an average daily in the range of 0.2–1.4 mg/kg body weight for adults and about 3.4 mg/kg BW for children (Dybing et al., 2005). Further, daily intake of acrylamide-caused the neurotoxicants 40 mg/kg BW/day (Tardiff et al., 2010). Fruit fly models are highly useful in the primary molecular mechanisms in different human neurodegenerative diseases (Feany, 2010). In recent times, use of fruit fly is considered as the best model.

The present study showed that the OLE-2 and CU-3 fraction-infused diet supplement improved the antioxidant property in *D. melanogaster* model.

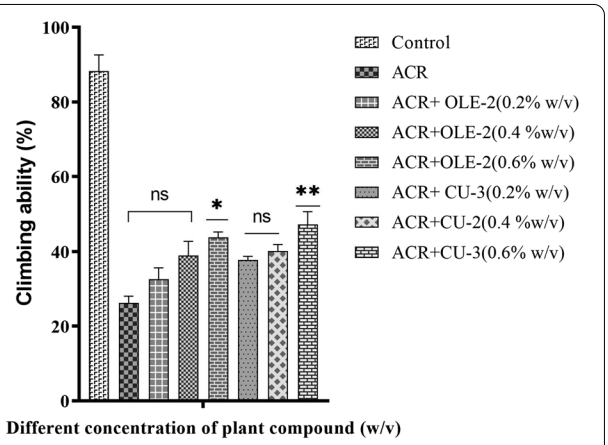

Fig. 4 Climbing assays can be used to examine the locomotor activity of co-exposed of ACR + plant fraction flies compared with control and treated groups. Plant fraction showed (OLE-2 and CU-3) improvement in the climbing ability. Significance analysed by one-way ANOVA. Error bar represents the Mean ± SD. The *p* values *$p < 0.05$ and **$p < 0.01$ were considered as significant while comparing ACR treated group and co-exposed to ACR + plant fraction group

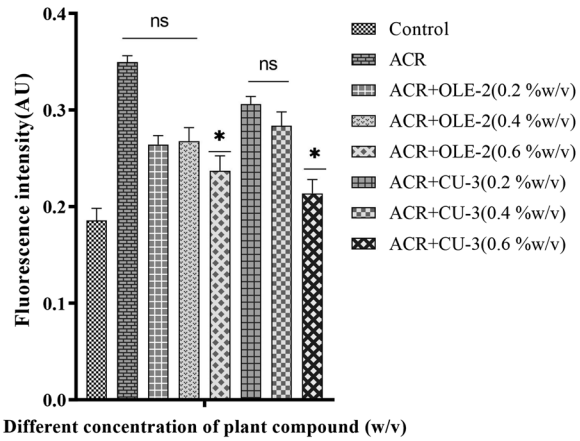

Fig. 6 ROS levels in control and treated groups of male D. melanogaster. Plant fraction supplementation decreases the level of ROS in plant fraction treated flies' group in comparison to control. Statistical significance was analysed by one-way ANOVA. Error bar represents the Mean ± SD. The *p* value *$p < 0.05$ were considered as significant while comparing ACR treated group and co-exposed to ACR + plant fraction group

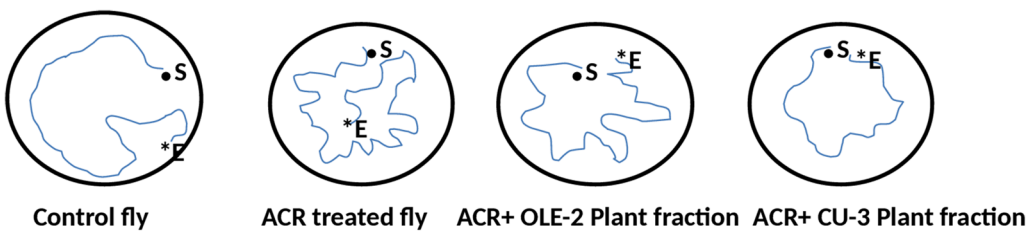

Fig. 5 Moving behaviour control and ACR and ACR + plant fraction treated fly, ACR treated fly move irregularlycompared to ACR + plant treated fraction groups showed less irregular movement. 'S' is the starting point of fly, 'E' is theendpoint of fly

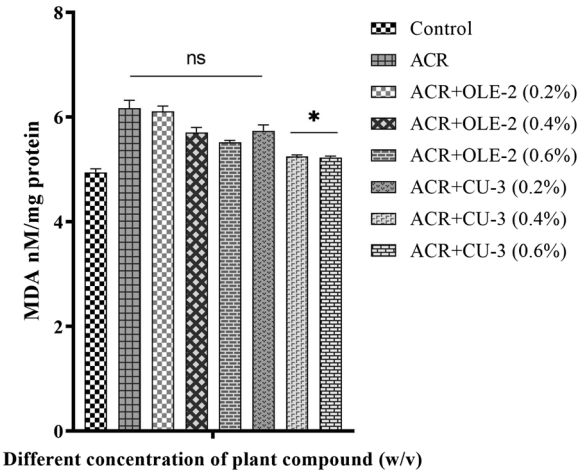

Fig. 7 Effect of plant fraction on LPO which is a oxidative stress marker in the ACR treated flies. The level of MDA was more in the ACR treated group. Plant fraction supplementation decreased the level of MDA in the treated flies' group. Statistical significance was analysed by one-way ANOVA. Error bar represents the Mean ± SD. The *p* value **p* < 0.05 were considered as significant while comparing ACR treated group and co-exposed to ACR + plant fraction group

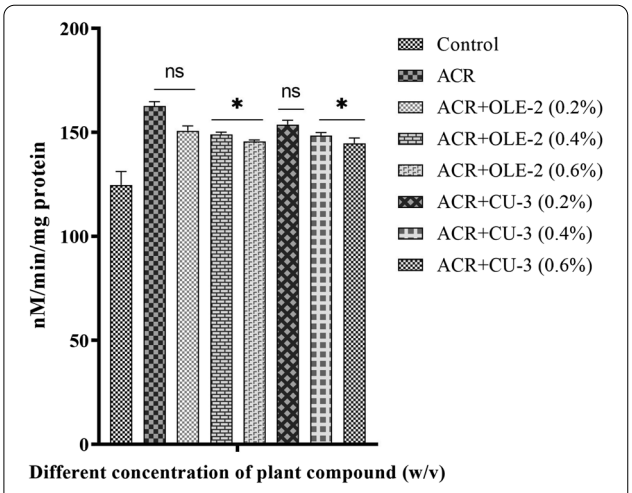

Fig. 9 AChE activity was more in ACR treated group. AChE activity of co-exposed ACR + plant fraction flies compared with control and treated groups. Plant fraction showed (OLE-2 and CU-3) significant decrease in the AChE activity expressed in nM/ min /mg protein. Statistical significance was an analysed by one-way ANOVA. Error bar represents the Mean ± SD. The *p* value **p* < 0.05 were considered as significant while comparing ACR treated group and co-exposed to ACR+ plant fraction group

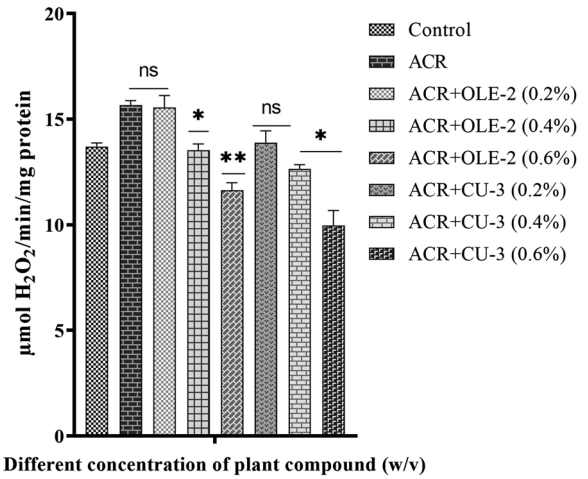

Fig. 8 Effect of plant fraction on catalase, an oxidative stress marker in the ACR treated flies. The level of catalase wasmore in ACR treated group. Plant fraction supplementation decreased the level of catalase in the treated flies group.Statistical significance was by one-way ANOVA. Error bar represents the Mean ± SD. The *p* values **p* < 0.05 and ***p* < 0.01were considered as significant while comparing ACR treated group and co-exposed to ACR + plant fraction group

The reduction in the action of antioxidant and superoxide dismutase in the ACR-treated flies and the administration of OLE-2 and CU-3 could reverse these effects to the level found in controls. The ability of plant fraction of OLE-2 and CU-3 to inhibit lipid peroxidation in the flies of acrylamide-treated group has been increased. The plant fractions could inhibit membrane lipid peroxidation thereby preventing free radical scavenging or by the enhancement of the activity of glutathione (Deiana et al., 2011).

We studied the consequence of acrylamide in fruit fly head regions. Acrylamide effect CNS and PNS system in both higher animals and humans (Pennisi et al., 2013). Studies that describe the neuroprotective effects of OLE-2 and CU-3 fractions in the *D. melanogaster* system are limited.

Thus, the plant fractions are indicative of their ability to suppress oxidative stress, hence the capability to reduce ACR neurotoxicity. The plant fractions (OLE-2 and CU-3) suppressed ACR-induced oxidative stress as conspicuous from the decline in the LPO and ROS level, and enhanced lifespan and climbing activity. ACR-treated fruit flies with plant fraction-supplemented diet showed a reduction in the activity of LPO, catalase and ROS.

Acetylcholine is a neurotransmitter of the cholinergic system and plays an significant role in modulating functions such as learning, memory and locomotory activities (Deiana et al., 2011). It has some significant role in cholinergic system in neurodegenerative diseases like Alzheimer's and Parkinson's disease (Zhang et al., 2013). Our study showed that plant fraction-supplemented diet caused decrease in AChE activity significantly when compared to control groups.

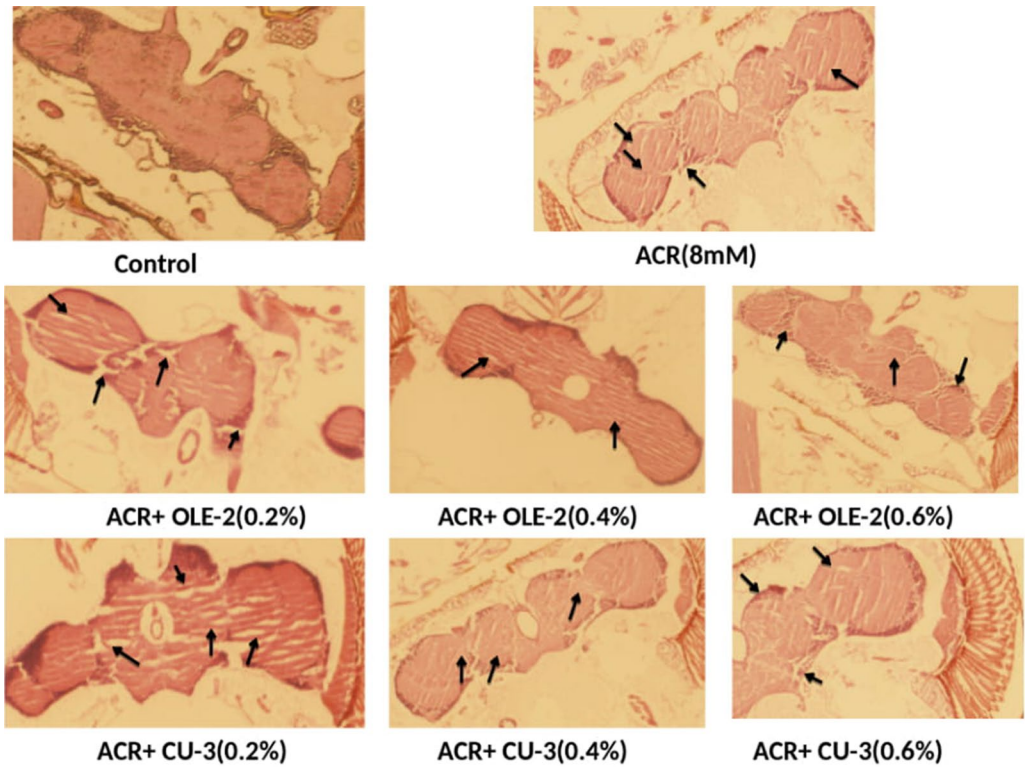

Fig. 10 The control fly's brain showed no vacuolated areas and no tissue gaps in central body complex but ACR treated fly brain showed more tissue gap in central and optical lobe, more over ACR + plant fraction (OLE-2 and CU-3) treated group showed less vacuolated areas and tissue gap compared to ACR treated group

The plant fractions working in our study showed altering degrees of neuroprotection against ACR and showed no variation in their action. The neuroprotective effect of plant fractions was comparable to that of CU-3 which was better than that of OLE-2 fraction against ACR-induced oxidative stress. Our study shows that the neuroprotection of OLE-2 and CU-3 fractions may act via reduction in oxidative stress, LOP and AChE activity, which are implicated in neuronal dysfunction. Hence, we surmize that OLE-2 and CU-3 fractions are possible to provide major neuroprotection against acrylamide-induced neurotoxicity and neuropathy in fly models (LoPachin & Gavin, 2008, 2012).

Conclusion

Plant fractions (OLE-2 and CU-3) contained some natural bioactive compounds that exhibited an increase in lifespan, antioxidant enzyme and decreased the activity of AChE. On the other hand, this may be appropriate to the synergistic effect of many plant compounds in the plant fractions. Further, the isolation and detection of active compounds should be able to consider the outlet mechanism of neuroprotective action in *Oleo dioica* Roxb. and *Curculigo orchioides* Gaertn. The semi-purified plant fraction contains certain bioactive compounds such as tannins, phenolics, polyphenols, terpenes and flavonoids. The types of natural compounds present in the plant fractions and their concentration that protect the neurons against the acrylamide in the plant fraction are yet to be explored. In general, the bioactive compound proportion of any semi-purified plant fraction is < 1–2% and different types of plant bioactive compounds would successfully protect the neurons and thus may exhibit AChE inhibition activity.

The present study has demonstrated that dietary supplementation with plant fraction of OLE-2 and CU-3 has increased antioxidant enzymes and decreased AChE activity in *D. melanogaster*. This activity could be a part of the probable mechanisms of action by which OLE-2 and CU-3 fractions have extended the lifespan and locomotory activity in fruit flies. These fruit flies continue to provide an exciting system for metabolic studies and should be more extensively exploited. Based on these results, further studies on the identified bioactive compounds from these two plants are being planned.

Abbreviations
ATCI: Acetylthiocholine iodide; ACR: Acrylamide; AChE: Acetylcholinesterase; CAT: Catalase; CU-3: *Curculigo orchioides* Gaertn rhizome fraction; DCFDA: 2′,7′-Dichlorofluorescin diacetate; DTNB: Dithionitrobis benzoic acid; EDTA: Ethylenediaminetetra acetic acid; LPO: Lipid peroxidation; OLE-2: *Olea Dioica Roxb*. Leaf fraction.

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Authors' contributions
This work was carried out in consultation and collaboration with all the authors. First author, PGK had designed and conducted the study. Second author AD did the data analysis. Authors CGJ and MS conceived the study and were in charge of overall directions, planning and manuscript correction. All authors read and approved the final manuscript.

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