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Oxidative stress response, epigenetic and behavioral alterations in *Caenorhabditis elegans* exposed to organophosphorus pesticide quinalphos

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ABSTRACT

Quinalphos (QP), an organophosphate pesticide is used worldwide in agricultural practices. Extensive use of pesticides creates toxicological and environmental nuisances on non-target organisms, where it severely affects cholinergic neurons in higher animals and also induces Parkinson, Alzheimer's disease and male sterility in humans. This study evaluated the sublethal effects of QP using *Caenorhabditis elegans* on the endpoints of body bend frequency, head thrash frequency, feeding activity for the assay as toxicity parameters. To assess the molecular-level effect, expression pattern of genes associated with locomotion (*unc-47, unc-13*), pharyngeal pumping (*egl-30*), epigenetic modulation (*utx-1*), and oxidative stress (*daf-2, daf-16, age-1* and *glod-4*) was investigated by semi-quantitative PCR. The LC_{50} value of QP was 0.0323 mM for 1 h of exposure, and L4-stage worms were exposed to sublethal concentrations of 0.00344 mM. After QP exposure, body bend frequency, head thrash frequency and feeding behavior of worms was significantly decreased. Expression analysis results indicate that QP exposure leads to the up-regulation in expression of some stress genes (*daf-16* and *glod-4*) and pharyngeal pumping (*egl-30*), while other genes responsible for stress like *daf-2* and *age-1*, locomotion (*unc-13* and *unc-47*) and epigenetic modulation (*utx-1*) are down-regulated. Subsequently, transgenic worms expressing *daf-16*::GFP were exposed to QP, where induced expression of DAF-16 was evident. Overall, it appears to recruit *daf-2*/insulin receptor and *daf-16*/FOXO-dependent pathways to rescue the animals from QP-mediated oxidative stress. Alterations in the expression of many genes by QP are unveiled for the first time in this study, which could serve as biomarkers for monitoring QP exposure.

1. Introduction

Quinalphos (QP; *O*, *O*-diethyl *O*-quinoxalin-2-yl phosphorothioate) is a synthetic organophosphorus (OP) pesticide being used extensively as an insecticide and acaricide for controlling most of pests that infest variety of crops due to low bioaccumulation and higher rate of biodegradation (Rahman et al., 2004; Mishra and Devi, 2014). QP produces various metabolites during its degradation that include QP oxon, *O*-ethyl-*O*-quinoxalin-2-yl phosphoric acid, 2-hydroxy quinoxaline, and quinoxaline-2-thiol, among them, 2-hydroxy quinoxaline and oxon were more toxic than the parent compound and persist in the ecosystem for quite longer period (Gupta et al., 2011). Like other organophosphates, QP is neurotoxic to the target pests by irreversible inactivation of acetylcholine esterase (AChE) in the central and peripheral nervous system (Vasilic et al. 1992). QP has been classified as moderately hazardous pesticide by WHO but has become a matter of concern because of its potentiality and hazardous effect to non-target organisms.

Extensive usage of pesticides in the intensive agricultural and public health practices has increased its persistence in the environment leading to the ecological imbalance and thus many non-target organisms have become vulnerable to those toxicants. Such pesticide load in agricultural ecosystem exerts harmful effects on the microbial flora and fauna (You and Liu, 2004), which leads to destruction of agricultural ecosystem structure and function. Humans are inevitably exposed to pesticides and its degradation byproducts through environmental contamination or occupational exposure, which resulted in hypercholinergic toxicity characterized by miosis, lacrimation, salivation, diarrhea, bradycardia, bronchospasm, confusion, nausea, vomiting and dizziness at acute exposures (Morris et al., 2014). Chronic low level exposures of OP pesticides are also shown to be associated with

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neurobehavioral impairments and audiovisual disturbances (Srivastava et al., 2000; Ross et al., 2010). It is also reported that OP pesticides induced chromosome breaks/fragments in fish and human alveolar cells (Nataraj et al., 2017; Zerin et al., 2015).

Organophosphorous toxicity is known to induce oxidative stress in an organism exposed that lead to generation of free radicals and inactivation of antioxidants and scavenging enzymes, and lipid peroxidation (Banerjee et al., 1999; Etemadi-Aleagha et al., 2002), whereas the stress response pathways are recruited in the same organism to metabolize these hazardous materials for the detoxification of their obnoxious effects and also exercise protective mechanisms to reduce the extent of damages that resulted by toxicants. The induced stress in the host constitutes primary signals that are transduced in to cytoplasm and ultimately modulate the expression levels of transcriptional regulators which in turn control the expression of specific genes related to stress response cascade contains a number of gene pathways.

Studies aimed at deciphering of pesticide induced stress-responsive genes have implications for human health and well being, environmental safety, and for understanding of basic biological knowledge. Several model organisms have been examined for this purpose however, *Caenorhabditis elegans* is the most commonly employed animal model for the toxicity studies because of its unique features that include short life span, defined life cycle, simple anatomy (it has a total of 959 highly differentiated cells), transparency, defined genome and tractable genetics, ease of maintenance and growth (Dhawan et al., 1999). In addition, it is a simple multicellular eukaryote that possesses analogs for many human physiological functions as many of the human genes have orthologs in the genome of this nematode (83% of worm proteins have domains with significant similarity to human proteins (O'Brien et al., 2005; Kaletta and Hengartner, 2006) and whose developmental process and behavior can easily be traced. Moreover, the use of *C. elegans* has been demonstrated as a successful mammalian equivalent neurological model based on its behavioral responses to OP insecticides (Cole et al., 2004). Similarities in genetic and physiologic components and functions of cholinergic systems between *C. elegans* and higher animals are also established. As in the higher eukaryotes, the primary excitatory neurotransmitter acetylcholine governs motor function in *C. elegans* (Rand and Nonet, 1997), where similar inhibition in AChE activity by OP toxicity was observed with defects in locomotion (Rajini et al., 2008).

As a group, OP pesticides are structurally diverse in nature, while elicit a range of neurotoxic effects that are quite distinct for all compounds (Mileson et al., 1998). In spite of the promising toxicity in various animal and *in vitro* cell culture models, QP is extensively used worldwide, and systematic studies on the QP toxicity at whole animal model in terms of neurotoxicity associated body behavior and gene expression are lacking. Hence, this study was designed to use *C. elegans* as a model organism to explore the responses against sublethal concentrations of QP to underscore the expression pattern of genes associated with neurotoxic effects in locomotion (*unc-47*, *unc-13*), pharyngeal pumping (*egl-30*), epigenetic modulation (*utx-1*), and oxidative stress responses (*daf-2*, *daf-16*, *age*-1 and *glod-4*).

2. Materials and methods

2.1. Culture and maintenance of C. elegans

The wild-type *C. elegans* N2 Bristol and *C. elegans* zls356 (strain TJ356) [DAF-16p::DAF-16a/b::GFP + rol-6(su1006)] were generously provided by Dr. Balamurugan Krishnaswamy, Alagappa University, India and grown on Nematode Growth Medium (NGM) with bacterial culture *E. coli* OP50 as feed at 20 °C till they were gravid adults (Gandhimathi et al., 2015). Gravid adult animals were treated with hypochlorite-NaOH solution and eggs were collected from these animals to obtain a synchronized population by transferring to standard NGM plates with feeding bacteria to hatch at 20 °C. Synchronized worms at L4 stage were scored, washed with M9 buffer (containing 0.01% Triton X-100), pelleted gently by centrifugation, and washed several times to remove residual OP50 bacteria, which were further used for the pesticide exposure studies.

2.2. Determination of lethality

Lethality assessment with wild-type *C. elegans* against various concentrations of quinalphos (Sigma-Aldrich, USA) was performed using K-medium in 24-well cell culture plates as described earlier (Rajini et al., 2008). Using stereo microscope (Micro Zoom 1280, Micros, Austria), 100 ± 10 age synchronized fourth-stage larvae (L4) were transferred into each well containing appropriate aliquots pesticide stock solutions at a final concentrations ranging from 0.0034 to 0.034 mM and plates were incubated at 20 °C for 1 h. Worms added with K-medium alone was treated as control. After the 1-h exposure, the wells were observed under a stereo microscope and the live and dead worms were counted in each well. The worms that did not move or respond to gentle touch with a needle were counted as dead. The experiment was performed in triplicates on different days with newly age synchronized worms and freshly made QP solutions. The LC_{50} value (concentration at which there was 50% mortality in worms) at a given concentration was determined using the PROBIT program version 1.5.

2.3. Body behavior assay of C. elegans under pesticide treatment

Head thrash and body bend frequency were used to assess behavior of *C. elegans* upon the exposure of pesticide as previously described (Tsalik and Hobert, 2003). To assay the head thrash and body bend frequency, nematodes were washed with M9 buffer. Animals were transferred into cavity slides containing K medium alone for control and LC_{50} concentration of QP prepared in K medium for the treated ones and incubated for 1 h. After a 1 min recovery period, the head thrash and body bend frequencies were scored for 1 min in an interval of 20 s. A thrash was defined as a change in the direction of bending at the mid-body. Body bend was counted as a change in the direction of the part of the animals corresponding to the posterior bulb of the pharynx along the y-axis, assuming that the animal was travelling along the x-axis. Ten age synchronized fourth-stage larvae (L4) were examined per treatment. The experiment was performed in triplicates.

2.4. Feeding inhibition assay of C. elegans under pesticide treatment

Feeding inhibition assays with *C. elegans* worms exposed to quinalphos was performed as previously described (Jones and Candido, 1999). For this, *E. coli* OP50 cultures were grown to an OD_{600} of approximately 0.6. A plate of adult *C. elegans* was washed with 3 ml of K medium and the suspension with worms was incubated on ice for 10 min. The supernatant was removed and the suspension containing worms were stored on ice until needed. Feeding inhibition experiments were conducted in 6 well culture plates. A feeding control consisted of aliquots of 170 µl of *E. coli* OP50 suspension, 20 µl of nematode suspension and 10 µl of K medium. The test sample consisted of 170 µl of *E. coli* OP50 suspension, 20 µl of nematode suspension and 10 µl of LC_{50} concentration of QP. The plates were incubated at 25 °C on a rocking platform. Later, culture suspensions were transferred from each well into sterile disposable cuvettes and the OD_{600} of the bacterial suspension were measured until 6 h incubation with 2 h intervals.

2.5. Semi-quantitative RT-PCR

To study the expression pattern of genes associated with neurotoxic effects (*unc-47*, *unc-13* and *egl-30*), embryonic development (Utx-1), and stress responses (*daf-2*, *daf-16*, *age*-1 and *glod-4*) at mRNA levels in the *C. elegans* exposed to low concentrations of QP, semi-quantitative RT-PCR assay using gene-specific primers was performed. For this, total

Table 1
Primers used in this study for semi-quantitative RT-PCR.

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
unc-47	TGGTCAAGGCTCTTCTAT	TTTCCAACTAATCCCATC
unc-13	AGTGAGCCGCTTTCTTAT	AATCCTCCACCACTTTCA
daf-16	CATCGGCTCTTCCAATTGAT	ATGAATATGCTGCCCTCCAG
utx-1	GCAGAACACCAGCTCATCAG	ATCAACGCCATTCTTCTCGC
egl-30	TCAGAAAGGCGGAAGTGGAT	GGTTCTCGTTGTCACACTCG
age-1	ATTAGAGCTCCACGGCACTTT	TGTCAAGCACGTTTTCTTCG
daf-2	TCGAGCTCTTCCTACGGTGT	CATCTTGTCCACCACGTGTC
glod-4	TCTTCAAAGTGGCGAATCGTG	TCTTGCTCCATCGTCCGTTAT
β-actin	ATCGTCCTCGACTCTGGAGATG	TCACGTCCAGCCAAGTCAAG

RNA was isolated from the whole synchronized young adult *C. elegans* worms exposed to QP as described previously (Gandhimathi et al., 2015). RNA samples isolated were treated with DNAse and further checked for its integrity and purity. Subsequently, total RNA (2 µg) was reverse transcribed in to cDNA using oligo(dT) primers and M-MuLV RNaseH$^+$ reverse transcriptase (DyNamo cDNA synthesis kit, Finnzymes, USA) according to the manufacturer's instructions. Semi-quantitative PCR was then performed using cDNA as the template with gene specific primers for the candidate genes and the house keeping gene β-actin at the exponential phase of PCR cycles. The gene-specific primers used in this study are listed in Table 1. For each 50 µl PCR reaction, 2 µl of cDNA was used as template and the amplification set up consisted of 1 cycle at 94 °C with a 4 min hold followed by 32–40 cycles at 94 °C for 45 s, optimized gene-specific annealing temperature (52–58 °C) with 45 s, 72 °C with 45 s for extension. The resultant PCR products were resolved in a 2.0% agarose gel for further visualization of amplicons and quantification of band intensity. Densitometry values were measured for each gene at each cycle sampling using the ImageJ software. RT-PCR values were presented as the ratio of the specified gene's band intensity in the selected linear amplification cycle divided by the band intensity of β-actin as described previously (Udhayabanu et al., 2018).

2.6. Responses of transgenic GFP expressing C. elegans exposed to pesticide

C. elegans TJ356 daf16::GFP were seeded into a 6 well plate and exposed to QP at a concentration of LC_{50}. After 1 h, worms were collected and subjected to total RNA isolation to quantify the *daf*-16 gene expression by semi-quantitative PCR using gene-specific primers as described above. Tubulin was used as internal control in this study. To further validate the *daf*-16 expression, live cell fluorescence microscopy imaging of *C. elegans* was performed with live TJ356 DAF16::GFP nematodes that were paralyzed using sodium azide after 1 h exposure to pesticide followed by imaging using Nikon Ti-S inverted fluorescence microscope at excitation wavelength of 485 nm and the emission wavelength of 535 nm (Gandhimathi et al., 2015).

2.7. Statistical analysis

Statistical analysis was performed using one-way ANOVA (http://vassarstats.net/anova1u.html) followed by Tukey's HSD test, with statistical significance set at 0.05. All semi-quantitative RT-PCR analyses were performed on at least 3 separate occasions with comparable results, and data presented are from representative sets of experiments.

3. Results and discussion

3.1. Lethality assessment in C. elegans in response to QP

Organophosphorous compounds are characteristic to exert neurotoxicity by irreversible inhibition of AChE, resulting in excessive accumulation of acetylcholine (ACh) in the neurons, and leading to cholinergic toxicity. Besides, OP pesticides induce oxidative stress, which is implicated as pathophysiological factor in several metabolic disorders. Several studies investigate the relationship between exposure to OP pesticides and toxicity in terms of stress response, neurotoxicity and reproduction using various animal models, nevertheless studies pertaining to elucidate the molecular insights at the levels of expression of specific genes related to stress response and physiological behavior simultaneously is very much limited. Moreover, OP pesticides are greatly varied in the range and mechanism of toxicity exerted in a host upon its exposure due to their absorption and biotransformation in different tissues, bioactivation and their metabolic byproducts that are more toxic than the parent compound. Hence, this study was targeted to ascertain the toxicity of QP, one of the least understood, at sublethal concentrations in terms of stress response, behavior, feeding and reproduction using *C. elegans* as a model system. For this, lethality of worms was assessed by exposing them to QP at various concentrations ranging from 0.0034 to 0.034 mM. The worms showed 0% lethality for the control for all tests (i.e., 100% survival). QP evidenced a concentration dependent reduction in survival of the wild-type N2 worms tested and the calculated LC_{50} amounted to 0.0323 mM. Based on the LC_{50} values obtained in this study, the sublethal dose of QP was fixed to be 0.0034 mM and worms were exposed at this concentration for further endpoint toxicity assays. Previously, Rajini et al. (2008) have made a comparison on toxicological endpoints of OP pesticides and shown that dichlorvos was most toxic for *C. elegans* with an LC_{50} of 0.039 mM than acephate and methamidophos. Likewise, LC_{50} was determined as 300 mM/ml for malathion in *C. elegans* (Kamaladevi et al., 2013). It is noteworthy to mention that LC_{50s} in *C. elegans* were normally higher than LD_{50s} reported in mammals (Williams and Dusenbery, 1988), due to the robust genetic mechanisms that help the worms to adapt any harsh environments.

3.2. Body behavioral defects in C. elegans by QP

OP pesticides are well-known for their neurotoxicity in various animals causing damage to both central and peripheral nervous system. In addition to ataxia, skeletal muscle weakness and body weight loss, OPs have been reported to markedly induce gait abnormalities and affect locomotion behavior in the exposed animals. The responsiveness of animals exposed to neurotoxicants in terms of movement behavior has attracted several researchers to explore locomotion in the determination of chemicals known to be neurotoxic. Thus, the body behavior of *C. elegans* after the exposure to sublethal concentrations of QP was assessed in this study. Fig. 1A shows severe head thrash defects in age synchronized L4-larva stage nematodes when exposed to QP at a sublethal concentration of 0.00344 mM. Likewise, body bend frequency of worms was significantly decreased in the QP exposed L4-larva stage worms (8 ± 1/20 s) than control (15 ± 2/20 s). Therefore, the assessment of QP toxicity with the endpoint of head thrash was very well consistent with that of body bends and can be noted that the defects in body behavior of the worms ascertaining the acute neurotoxicity of QP at sublethal concentrations for the first time in this study. Previously, Cole et al. (2004) demonstrated the behavioural abnormalities in *C. elegans* against five OP pesticides such as dichlorvos, parathion, methyl parathion, methidathion, and fensulfothion. Such a decrease in the locomotion as an effect of AChE activity inhibition has also been noticed in *C. elegans* exposed to chlorpyrifos (Ruan et al., 2009), and monocrotophos (Ali and Rajini, 2012). Similarly, exposure of worms to acephate and dimethoate were also shown to affect their movement significantly (Rajini et al., 2008), and the same study highlighted the reliability of behavioral endpoint assays instead of measurement of AChE activity for the assessment of OP pesticides toxicity.

It is obvious that movement of nematodes was more sensitive to QP, which is a well known for neurotoxic insecticide. To further decipher the molecular mechanisms corresponding to this susceptibility, we were intended to assess the expression pattern of few of the candidate genes like *unc*-13 and *unc*-47 in the worms that are exposed to QP. For this, we

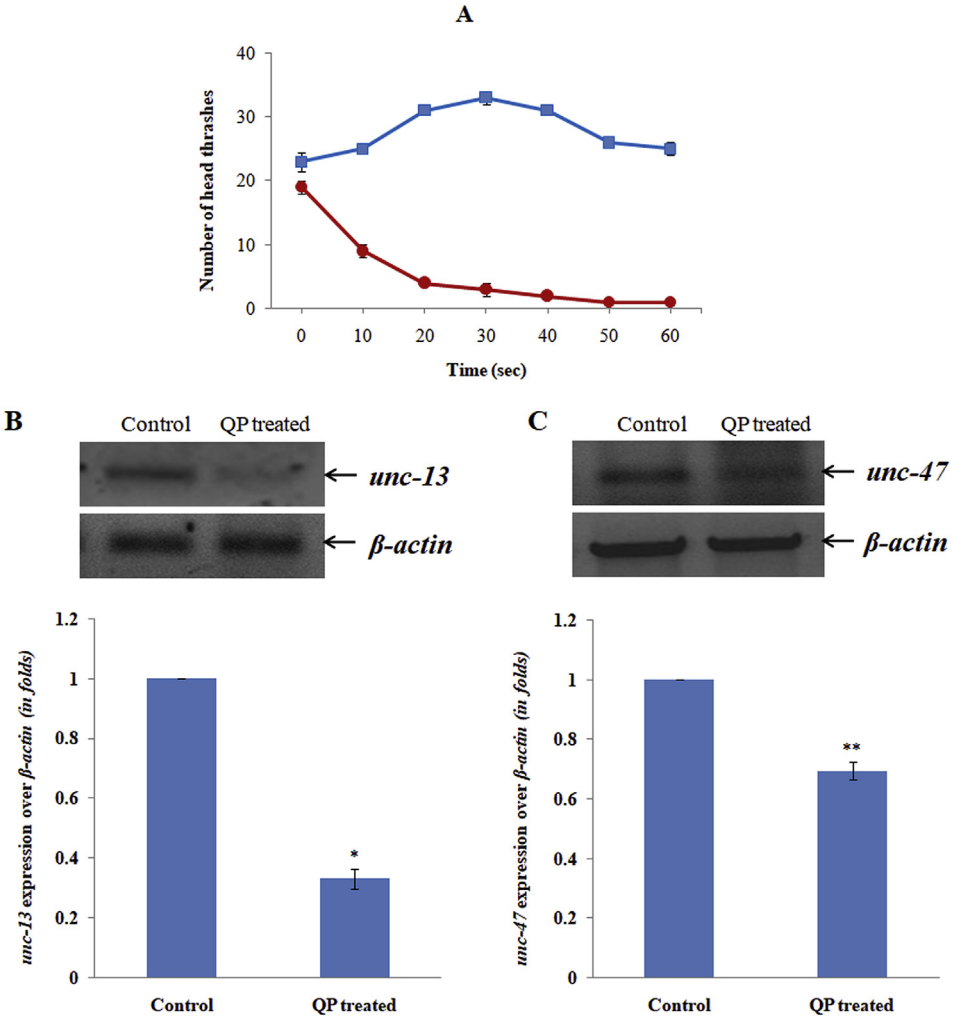

Fig. 1. Body behavior of *C. elegans* exposed to sublethal concentrations of quinalphos. (**A**) Head thrash defects in animals subjected to quinalphos toxicity. Semi-quantitative RT-PCR assay to quantify expression of (**B**) *unc-13* and (**C**) *unc-47* at transcript levels in worms treated with quinalphos. Bar diagram represents relative expression of genes corresponding to *β-actin* in folds. Bars represent SD. $*P < 0.01$; $**P < 0.05$.

investigated the expression pattern of the genes *unc*-13 and *unc*-47 by semi-quantitative RT-PCR assay using total RNA as the template, which was isolated from the worms exposed with sublethal concentrations of QP and unexposed worms as control. Exposure of worms with QP significantly decreased the expression of both the *unc*-13 ($P < 0.01$), and *unc*-47 ($P < 0.05$) than the control (Fig. 1B and C), which is indicative of the involvement of both *unc*-13 and *unc*-47 genes in QP exerted defects in the behavior of animals by at least partly via the regulation of their expression at transcriptional levels. Thus, it can be certain that the reduced expression of *unc*-13 and *unc*-47 transcripts that reflected phenotypically in terms of defects in the locomotion of worms upon the exposure to QP and it is reported for the first time in this study. The gene *unc*-13 had been characterized first in *C. elegans* that encodes a protein with a function of synaptic neurotransmitter release (Maruama and Brenner, 1991) and thus essential for normal locomotion. Meantime, strains of UNC-13 mutant proteins were characterized to be defective in behavior, normal pharyngeal pumping and head thrashing with reduced brood sizes than control (Kohn et al., 2000). *Unc-47* encodes a transmembrane vesicular GABA transporter in *C. elegans* with highest expression in pharyngeal muscle cell and required in GABAergic neurons for all GABA neurotransmission, specifically for loading of GABA into synaptic vesicles (McIntire et al., 1997). Moreover, *unc-47* mutant worms were developmentally defective, lacking of muscle contraction and morphologically shrink (Schuske et al., 2004). Previously, it has been reported that aldicarb, a carbamate insecticide potentially inhibits AChE, thereby accumulates more amount of acetylcholine at the synapse and causing paralysis and lethality (Nguyen et al., 1995). Overall, our findings corroborate with the earlier observations and clearly evidenced that the inhibition of neurotransmitter release is the confounding factor in the neurotoxicity exerted by the QP.

3.3. Feeding inhibition of C. elegans by QP

Living organisms respond to any stress in different ways by means of physiological or cellular effects. *C. elegans* exposed to chemical and physical stress respond rapidly by cessation of feeding on their bacterial food as there is interference in the pharyngeal muscular pumping. This feeding inhibition prevents the worms from the exposure of toxicants from the environment. In this study, the response of wild-type N2 worms with respect to feeding behavior upon the exposure to QP at sublethal concentrations was tested. For this, we used OP50 as a food source for liquid feeding assay at initial OD units 0.6 for both control and pesticide treated worms, where the OD_{600} declined significantly at a steady state in the control having a bacterial culture incubated with wild-type N2 worms (Fig. 2A) and found that 46% of feeding activity was inhibited for 6 h exposure. In contrast, there was only a slight decrease in OD_{600} of the pesticide containing culture suspension incubated with same number of N2 worms (only15% of feeding activity

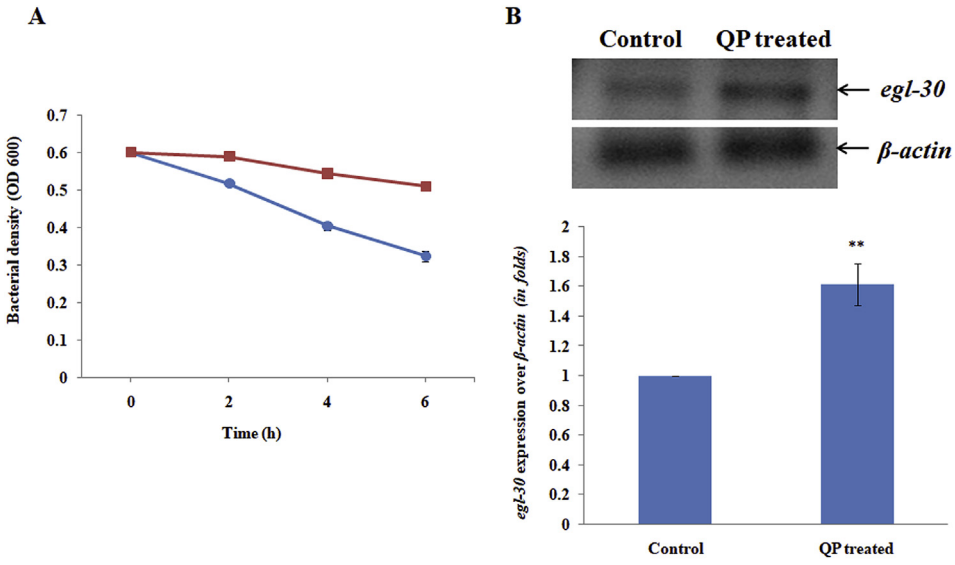

Fig. 2. Feeding inhibition assay in *C. elegans* exposed to quinalphos. (**A**) Reduction in OD_{600} of a suspension of *E. coli* OP50 over time incubated with N2 worms in the presence (■) or absence (●) of quinalphos. Values shown are the means ± S.E of three replicates. (**B**) Semi-quantitative RT-PCR assay for the quantification of *egl-30* expression at transcript levels in worms treated with quinalphos. Bar diagram represents relative expression of genes corresponding to *β-actin* in folds. Bars represent SD. $^{**}P < 0.05$.

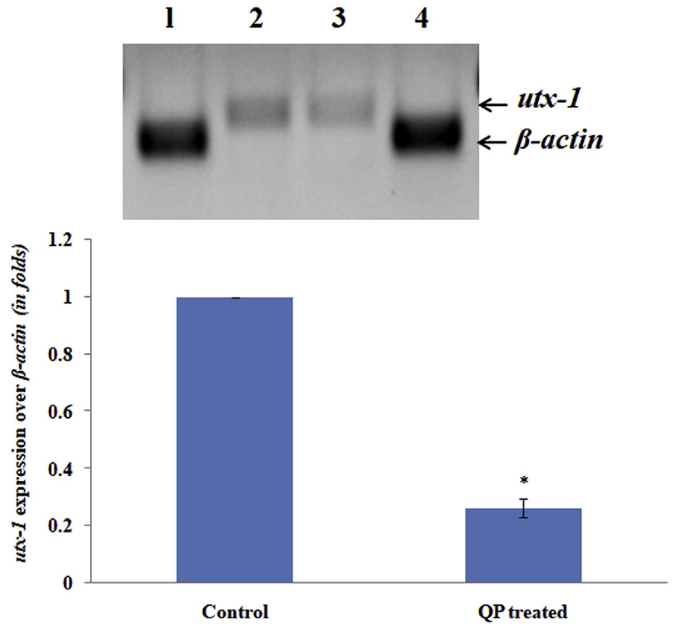

Fig. 3. Expression of epigenetic modulator histone H3K27 demthylase by quinalphos treatment. Semi-quantitative RT-PCR assay to quantify expression of *utx-1* at transcript levels in control (1 and 2) and worms treated with quinalphos (3 and 4). Bar diagram represents relative expression of genes corresponding to *β-actin* in folds. Bars represent SD. $^{*}P < 0.01$.

was inhibited for 6 h exposure), thus QP appeared to inhibit the feeding rate of worms at a concentration of 0.00344 mM. Similar inhibition in feeding is observed in L4 nematodes exposed to dichlorvos for 4 h (Jadhav and Rajini, 2009). Boyd et al. (2007) found that 50% of feeding activity was inhibited at concentrations of 1.0–2.2 µM of chlorpyrifos with a longer exposure of 24 h.

Feeding inhibition has been postulated as a survival mechanism against a stress (Jones and Candido, 1999) that could activate various stress responsive genes as a protective mechanism to limit its exposure to the toxicants or to minimize the damage. The gene *egl*-30 encodes a coupling G-protein binding receptor in *C. elegans*, which is largely expressed in neurons and pharyngeal muscle and functionally responsible for acetylcholine secretion, transmission and regulation of pharyngeal pumping (Bastiani et al., 2003). Our relative transcript quantification analysis of *egl*-30 gene in the worms exposed to QP at sublethal dose revealed significant induction ($P < 0.05$) in its expression than in worms untreated (Fig. 2B). As the pesticide treated worms found it difficult to feed and had reduced pharyngeal pumping activity, the genes essential for pharyngeal pumping should be upregulated in order to compensate for the decreasing functional activity as an adaptive mechanism. It is previously reported that the gene *numr-1*, which is expressed in the muscles and neurons in the pharynx of *C. elegans* and essential for feeding, was upregulated upon cadmium exposure especially in the nuclei of the intestine and the pharynx along with the stress-responsive heat-shock transcription factor HSF-1 as protective mechanism to combat heavy metals induced oxidative stress (Tvermoes et al., 2010). Hence, the inducible expression of *egl*-30 gene could be a protective mechanism for the survival of worms when exposed to QP. Such induction in *egl*-30 expression is reported for the first time in this study, which could serve as a potential biomarker to monitor pesticide exposure.

3.4. Epigenetic modulation by QP

In *C. elegans*, histone H3K27 demthylase is encoded by *utx-1* gene that specifically catalyses the demethylation of di- and tri-methylated lysine 27 of histone H3 (H3K27me2/3) and required for embryonic viability, vulval development, and for high brood sizes, locomotion, and growth sizes. Vandamme et al. (2012) had shown that UTX-1 regulates methylation levels of H3K27me2/3 globally in *C. elegans*, which is concomitant with silencing of expression of several genes. In this study, it is of our interest to examine any modulation in the expression of *utx-1* following QP exposure in the worms for the indicated time period. From our data, it is strongly evident that sublethal concentrations of QP down-regulated the expression of *utx-1* gene ($P < 0.01$) at mRNA levels (Fig. 3). To the best of our knowledge the influence of OP pesticide on expression of histone demethylase UTX-1 is investigated here, which revealed a promising decrease in its expression, suggesting that epigenetic mechanisms may contribute to pesticide-induced toxicity. Further studies are warranted to gain definite molecular mechanisms of this response. It is also noteworthy to highlight that pesticide induced variations in DNA methylation patterns resulted in increased carcinogenic risk in humans by modulating oxidative stress, alkylation of DNA, AChE inhibition, disruption of endocrine and S-adenosyl-methionine (Krieger, 2001). Earlier, an environmental pollutant methylmercury exposure has shown to cause epigenetic landscape modifications of histone H3K4 trimethylation marks in *C. elegans*. Recently, Camacho et al. (2018) have shown that ancestral bisphenol A exposure caused a transgenerational decrease in

the germline levels of H3K9me3 and H3K27me3, which is dependent on the activity of the JMJD-2 and JMJD-3/UTX-1 demethylases and responsible for germline dysfunction and elevated progeny lethality.

3.5. QP responsive expression of genes associated with oxidative stress

Numerous studies have evidenced that enhanced oxidative stress as one of the major confounding mechanism of OP toxicity besides the most prominent effect on AChE inhibition. Pesticides results in oxidative stress either by increasing reactive oxygen species (ROS) generation or decreasing antioxidants levels (Kwong, 2002). It is also known that oxidative stress activate or repress many genes that result in metabolic dysfunction and also recruit stress response pathways to detoxify the pesticides or exercise protective mechanisms to reduce the possible damages by pesticides. QP has greater potential of inducing oxidative stress through ROS generation in humans (Abdollahi et al., 2004) and also evidenced ROS induced histopathological changes, lipid peroxidation, and decreased liver FRAP in rats (Subramaneyaan et al., 2012). QP generated ROS is also shown to play a role in the modulation of expression of apoptosis- and antioxidants-related genes in human lung A549 cells (Zerin et al., 2015). Here, we have evaluated the expression pattern of oxidative stress responsive genes (*daf-2*, *daf-16*, *age-1* and *glod-4*) that are differently expressed in *C. elegans* following QP treatment at sublethal concentrations (Fig. 4). From the results, it is evident that *daf-2* and *age*-1 expression at transcript levels were prominently down-regulated ($P < 0.01$), while the expression of *daf-16* and *glod-4* were significantly up-regulated ($P < 0.01$) (Fig. 4A).

In *C. elegans*, a profound increase in lifespan is correlated with modulation of certain gene activities (Guarente and Kenyon, 2000), most notably, reduced activity of the *C. elegans daf-2*/insulin-like signaling pathway has increased lifespan more than tow folds (Kenyon et al., 1993; Paradis et al., 1999). Such a decrease is observed in the expression of *daf-2* following QP exposure, which can be considered as an adaptive mechanism at molecular levels for the survival of worms in the harsh environment. In general, signaling from the *daf-2*/insulin-like receptor antagonizes the fork head (*FOXO*) transcription factor *daf-16*, which is the major effector of *daf-2*/insulin-like regulation of *C. elegans* lifespan (Lin et al., 1997; Ogg et al., 1997) and responsible for activating genes involved in longevity, lipogenesis, heat shock survival and oxidative stress responses (Lin et al., 1997). It is overt from this study that the expression of *daf-16* gene is prominently up-regulated in the QP treated worms (~19 folds than control), where *daf-2* expression was down-regulated (~0.55 folds to control) (Fig. 4B).

Glyoxalase-1, an enzyme encoded by *glod-4* gene in *C. elegans*, which is essential for detoxification of methylglyoxal and the other reactive aldehydes produced during normal metabolism. This enzyme reduces oxidative stress and thereby increasing the lifespan (Morcos et al., 2008). From the observations of QP exposure, the expression of *glod-4* increased prominently as a protecting mechanism to reduce the oxidative stress induced by pesticide (Fig. 4C). Thus, it appears to involve *daf-2*/insulin receptor- and *daf-16*/FOXO-dependent pathways, for reducing oxidative stress exerted by OP pesticide exposure and also to activate detoxification systems like glyoxalase-1 (*glod-4*). Similarly, the transcript levels of *age-1* (~0.5 folds to control) were also reduced

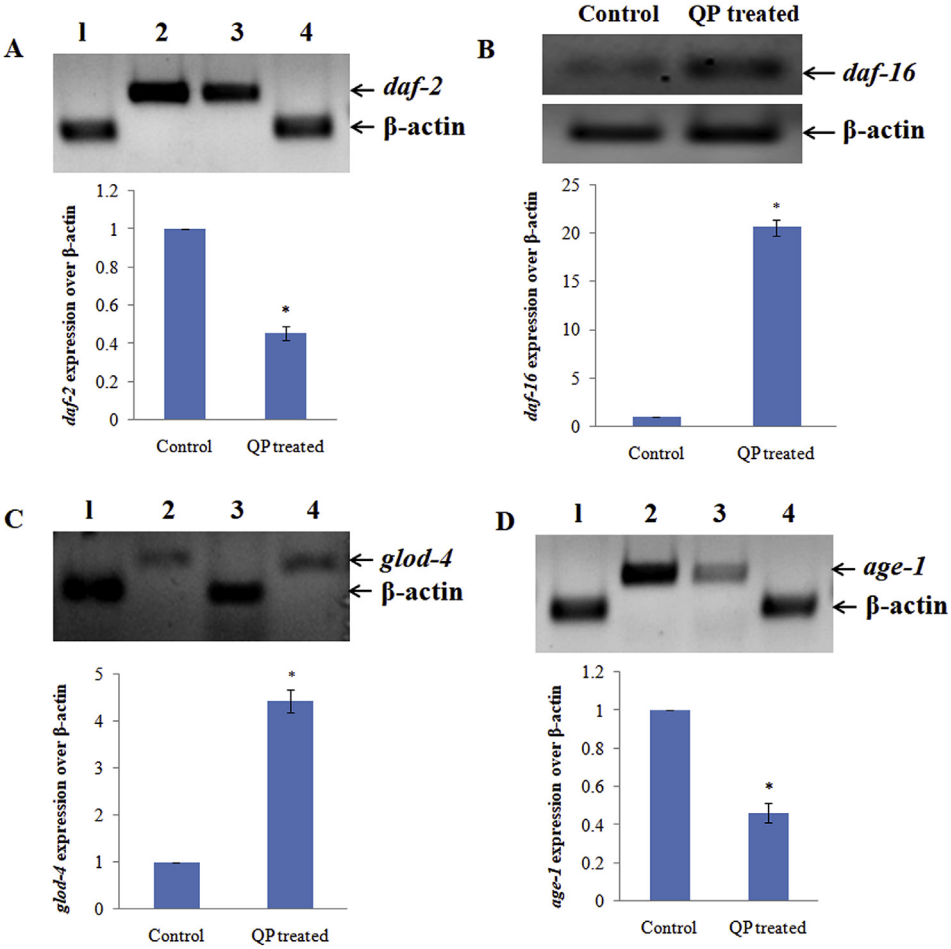

Fig. 4. Expression of oxidative stress related genes by quinalphos treatment. Semi-quantitative RT-PCR assay to quantify expression of (**A**) *daf-2*, (**B**) *daf-16,* (**C**) *glod-4* and (**D**) *age-1* at transcript levels in control (1 and 2) and quinalphos treated worms (3 and 4). Bar diagram represents relative expression of genes corresponding to *β-actin* in folds. Bars represent SD. *$P < 0.01$.

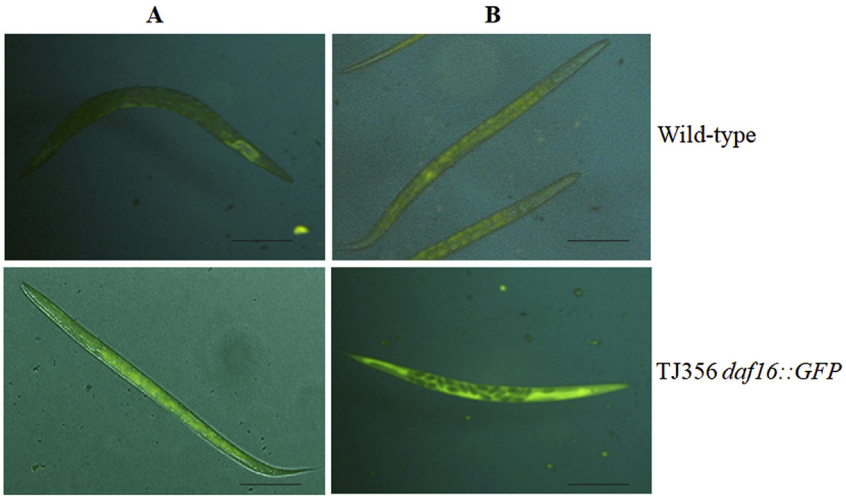

Fig. 5. Fluorescent Microscopy imaging for DAF-16 expression in *C. elegans* transgenic strain TJ356 expressing *daf-16*::GFP. (**A**) Untreated and (**B**) QP treated wild-type and TJ356 *daf-16*::GFP *C. elegans* strains. Scale bar, 100 µm.

in the pesticide exposed worms (Fig. 4D). In *C. elegans*, *age-1* encodes a phosphatidylinositol 3-kinase that functionally assist for the increased longevity by a reduction in IIS signaling, which is entirely dependent on DAF-16 (Kenyon et al., 1993).

To further validate these findings, *daf-16*::GFP fusion reporter strain TJ356 was exposed to low concentrations of QP and followed by live cell fluorescence microscopy imaging to observe intensity of fluorescence. Fig. 5 showed very intense fluorescence in the TJ356 *daf-16*::GFP worms exposed to QP than the wild-type N2 worms that were untreated or treated with QP as well as untreated TJ356 *daf-16*::GFP worms, respectively. The enhanced expression of *daf*-16 in the cell nuclei is shown by increased localization of GFP lead to increase in the fluorescence intensity. The induced nuclear localization of DAF-16 was noticed earlier during the exposure of worms to heat stress, anoxia, oxidative stress, starvation and exposure to pathogenic bacteria (Henderson and Johnson, 2001; Dues et al., 2016). Since, *daf-16* mutants are highly sensitive to various stress (Li et al., 2008; Rodriguez et al., 2013) and overexpression of DAF-16 results in enhanced resistance to stress (Henderson and Johnson, 2001). Thus, DAF-16 could play an important role in stress resistance. In addition, *daf*-16 gene expression at transcript levels was quantitated in the QP treated TJ356 strain by semi-quantitative RT-PCR and the untreated TJ356 strain was used as control. An increase in the expression of *daf-16* transcript was observed in the pesticide treated transgenic worms (~1.0 fold; $P < 0.05$) than control (Fig. 6), which clearly substantiated the above observations.

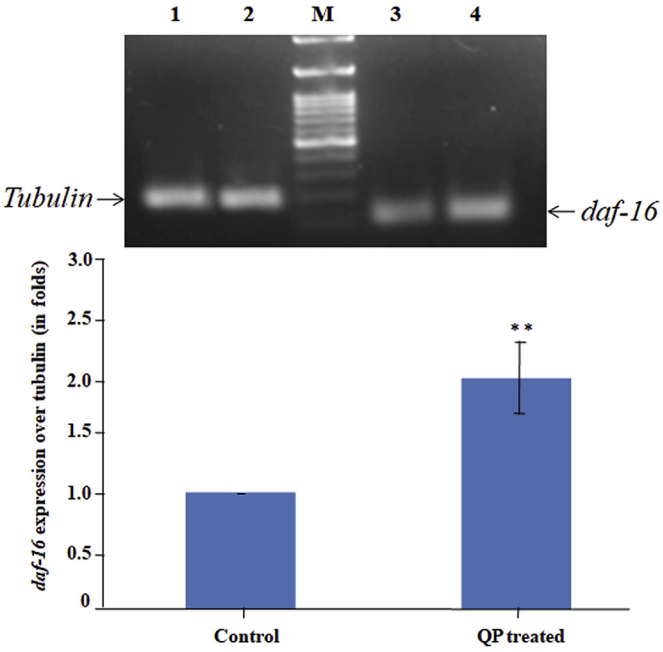

Fig. 6. Expression *daf-16* gene in a transgenic TJ356 strain expressing *daf-16*::GFP. Semi-quantitative RT-PCR assay to quantify expression of *daf-16* at transcript levels in control (1 and 3) and quinalphos treated worms (2 and 4). Bar diagram represents relative expression of genes corresponding to *β-actin* in folds. Bars represent SD. $^{**}P < 0.05$.

4. Conclusion

The systemic exposure of QP was investigated on *C. elegans* by assessing the body behavior, locomotion, feeding activity, and stress-responsive gene expression, as endpoints. The overall results suggest that QP exposure at sublethal concentrations exert prominent decrease in head thrash and body bends; inhibition of feeding activity and, altered expression of *unc-47*, *unc-13*, *egl-30*, *utx-1*, *daf-2*, *daf-16*, *age*-1 and *glod-4* genes, which imply that QP toxicity on *C. elegans* happened in several biological parameters that warrant further studies to delineate the association between the molecular and biochemical effects of QP exposure for its complete understanding. Further, it is unveiling that *unc-47*, *unc-13*, *egl-30*, *utx-1*, *daf-2*, *daf-16*, *age*-1 and *glod-4* genes could serve as potential biomarkers for monitoring QP exposure.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.01.031.

Conflicts of interest

Authors confirm that this article content has no conflict of interest.

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