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EFFECT OF QUINALPHOS ON EISENIA FETIDA EARTHWORM BIOMARKERS FOR ASSESSING QUINALPHOS TOXICITY: EVIDENCE FROM *EISENIA FETIDA*

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ABSTRACT
Earthworms are an integral part of an agricultural ecosystem, contributing to soil fertility, increasing soil structure, and nutrient cycling. Crop quality and, consequently, soil fertility have drastically declined as a result of the growing use of pesticides in agricultural areas. Earthworms, which greatly improve the fertility and quality of agricultural soil, are reportedly among the animals most negatively impacted by these conditions. The purpose of this study is to investigate the effects of the quinalphos organophosphate insecticide on the beneficial non-target organism *Eisenia fetida* as well as the biomarkers that show these effects. The most suitable and dependable model organism for a wide variety of environmental studies is the species *E. fetida* . Following 48 h of exposure to varying concentrations of quinalphos (2, 4, 6, 8, and 10 µg/ml), the median lethal concentration (LC50) for *E. fetida* was found to be 1.2 µg/ml, suggesting a significant and lethal effect on the earthworms. For 24, 48, and 72 h, the *E. fetida* were exposed to a sublethal dosage of LC25 (0.57 µg/ml). A protein oxidation assay using protein carbonyl activity was also used to assess the antioxidant responses, which included the activity of the neurotransmitter enzyme acetylcholinesterase (AChE), antioxidant enzymes like catalase (CAT), lipid peroxide (LPO), and detoxification enzymes like glutathione sulfotransferase (GST), glutathione peroxidase (GPX), and glutathione (GSH). After 24, 48, and 72 h, the group exposed to quinalphos demonstrated a significant elevation in these biochemical activities when compared to controls, suggesting that exposure for 72 h had a significant effect. These results shed important light on the increased awareness of *E. fetida* 's physiological defences following quinalphos exposure in agricultural environments.

KEYWORDS: Quinalphos, *Eisenia fetida*, Toxicity, Enzymes,

1. INTRODUCTION

Earthworms are prevalent in most types of soil, and they help make soil ecosystems more fertile and more structured (Bartlett et al., 2010). They change the chemical and physical properties of soil organic matter, mix leaf litter with the soil, help soil aggregates develop and stay stable, and make soil more porous (Zhang et al., 2022). Earthworms make up as much as 60–80% of the entire animal biomass in soil (Zhang et al., 2022; Alengebawy et al., 2021). Earthworms are more sensitive to soil chemicals than other soil creatures because they don't have a thick cuticle on the outside of their bodies (Dzul-Caamal et al., 2024; Campani et al., 2024).

Agricultural growth and the careless use of pesticides may harm the soil ecology, kill a lot of people, make the soil hazardous, and pollute it (Rani et al., 2021). It is estimated that $38 billion are spent on pesticides every year throughout the world (Mali et al., 2023). The pesticides used on farms should only be harmful to the pests they are meant to kill, and they should also be biodegradable and safe for the environment to some degree. But sadly, most pesticides are not targeted and kill creatures that are harmless and quite helpful to the ecosystems they are in.

Quinalphos (QP; O, O-diethyl O-quinoxalin-2-yl phosphorothioate) is a synthetic organophosphorus (OP) pesticide that is widely used as an insecticide and acaricide to control most pests that infest a wide range of crops (Govindarajan et al., 2019). It is effective because it does not build up in the body and breaks down quickly (Raffa and Chiampo, 2021; Talwar et al., 2014). During its breakdown, QP makes a number of metabolites, such as QP oxon, Oethyl-O-quinoxalin-2-yl phosphoric acid, 2-hydroxy quinoxaline, and quinoxaline-2-thiol. Of these, 2-hydroxy quinoxaline and oxon were more toxic than the parent compound and stayed in the environment for a long time (Raffa and Chiampo, 2021). QP is neurotoxic to the pests it targets because it permanently stops the activity of acetylcholine esterase (AChE) in the central and peripheral nervous systems (Dzul-Caamal et al., 2024). World Health Organization has classed QP as a moderately hazardous pesticide, however it has become a worry since it might harm non-target creatures (WHO 2020). The widespread use of pesticides in intensive farming and public health has made them last longer in the environment, which has caused ecological imbalance and made many non-target creatures more susceptible to these hazardous substances. This quantity of pesticides in the agricultural environment impact the microbes and animals of the exposed area (Yatoo et al., 2022), which breaks down the structure and function of the agricultural ecosystem.

When an organism is exposed to OP toxicity, it can cause oxidative stress, which makes free radicals and stops antioxidants and scavenging enzymes from working. This can also cause lipid peroxidation (Qiao et al., 2022; Campani et al., 2024). At the same time, the organism's stress response pathways are used to break down these harmful substances and protect itself from the damage they cause. The stress that is put on the host sends out main signals that are sent to the cytoplasm and change the amounts of transcriptional regulators, which in turn affect the expression of certain genes (Govindarajan et al., 2019). The stress response cascade has a variety of gene pathways.

The QP is used a lot all over the globe, however there haven't been any comprehensive research on how hazardous it is to entire animals in terms of antioxidant reactions. This research used *E. fetida* as a model organism respond to low levels of QP. Because these organisms respond in unique ways to changes in the environment, they are commonly utilised as sensitive bioindicators for ecotoxicology testing and monitoring soil contamination (Gu et al., 2021). To learn more about how pesticides affect organisms and how they interact with them, we need to do more monitoring. We can learn a lot about how hazardous substances affect behaviour, development, reproduction, and oxidative stress in animals by looking at how they react to pollutants (Yasmin and D'Souza, 2010; Hou et al., 2022; Qiao et al., 2022).

Some studies have said that using too many pesticides may have varied effects on the physiology and populations of earthworms (Bardiya and Dixit, 2024; Astaykina et al., 2022). Toxicological tests showed that the skin and stomach of earthworms are permeable to water and harmful chemicals, which is thought to be the major way that they take in contaminants (Dzul-Caamal et al., 2024; Yatoo et al., 2022).

It is commonly known that a lot of pollutants, such as pesticides, cause oxidative stress (OS) in a lot of living things (Hou et al., 2022; Mali et al., 2023). Pesticides are hazardous because they cause OS by making too many reactive oxygen species (ROS) in an organism (Sharifi-Rad et al., 2020; D'Souza et al., 2024). The ROS family includes unstable forms of molecular oxygen, such as hydroxyl radical (OH) and non-radical hydrogen peroxide (H_2O_2) (Ansari et al., 2025). The cells have several antioxidant mechanisms that protect them, such as enzymatic and non-enzymatic defence systems that get rid of free radicals (Qiao et al., 2022). It is known that too much ROS may directly harm the parts of cells, which causes oxidative stress, which in turn causes inflammation, lipid oxidation, and cell death.

Based on all of the aforementioned information, the present study was set up to look at the sublethal toxic effects of QP on earthworms *E. fetida* using filter paper contact method. The research looked at antioxidant enzymes including CAT, GST, GPX, GSH, AChE, LPO, and protein oxidation after being exposed to low levels of the chemicals for 24, 48, and 72 hours. There should be a clear difference in the amounts of antioxidants in quinalphos.

2. MATERIAL AND METHODS

2.1 Chemicals:

Quinalphos (35.70 % w/w) organophosphorus pesticide was purchased from local market Hyderabad, Telangana. Reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5'-Dithiobis (2-nitrobenzoic acid (DTNB), Acetyl thiocholine (ATC), Tricarboxylic acid (TCA), NADPH, Bradford reagent, 2,4 2,4-Dinitrophenylhydrazine (DNPH), Guainidine HCl, K_2HPO_4, and KH_2PO_4 were purchased from Sisco research laboratories (SRL) Pvt. Ltd., India. H_2O_2 was purchased from Merck Life Sciences Private Limited, India.

2.2 Experimental Animal

Eisenia fetida was collected from a local agricultural market in Hayathnagar, Hyderabad, India, as healthy and active specimens. The selected earthworms had an average length of 6.5 ± 1.5 cm. Before the experiment, the earthworms were acclimatized under laboratory conditions for at least 10 days using an organic fertilizer substrate. The substrate was maintained in a cool area of the laboratory and covered with a jute sack to retain moisture. The containers were covered with a fine mesh net to prevent the earthworms from escaping and to protect the substrate from external contaminants. Dead earthworms were removed immediately upon observation. The organic fertilizer substrate was replaced weekly to maintain consistent nutritional conditions throughout the acclimatization period.

2.3 Toxicity test method:

2.3.1 Contact filter paper test and lethal concentration:

A modified contact filter paper test was conducted (OECD, 1984). A piece of filter paper was put in a 9-cm Petri dish and 2 cc of acetone was added to the test ingredient QP. Once the solvent had dried, 2 cc of distilled water was added to the filter paper, and an earthworm was put on it. The dish was put in the dark at 20±1°C for 24, 48, and 72 hours and the number of dead earthworms was recorded. If an earthworm didn't move when touched gently on the front end, it was considered to be dead. Earthworms were kept on moist filter paper in the dark for 24 hours at 20±1°C to empty their guts before the dosage response test. A test was done first to find the chemical concentration range that mortality is from 0% to 100% of the earthworms.

A semi-statistical method devised by Finney (1971) was employed to determine the median lethal concentration (LC50) of quinalphos for *E. fetida*. Three sets of each concentration were used for the experimental purpose. For 24, 48, and 72 h, a set of six *E. fetida* was exposed to five different doses of 2, 4, 6, 8, and 10 µg/ml, as well as a control. Acetone was used as the control. The treated *E. fetida* were kept in the dark at 20±1°C and 80–85% relative humidity.

The dead *E. fetida* exposed to the QP were utilised to determine the LC50 values using a regression analysis based on the probit approach developed by Finney (1971). The LC50 value (1.159 µg/ml) was used to calculate the LC25 concentration (0.57 µg/ml). Then, *E. fetida* was treated with the LC25 concentration for 24, 48, and 72 h. After the treatment, the *E. fetida* were collected and put in a refrigerator at -20°C until further analysis.

2.4 Tissue preparation

Samples were taken from both the treatment and control groups. We put *E. fetida* in ice-cold 50 mM potassium phosphate buffer (1:8, w/v), pH 7.0, and sliced them up. Under cold conditions, the tissues were mixed together in phosphate buffer using a mortar and pestle. We next spun the homogenates in a centrifuge for 15 min at 13,000 rpm. The supernatants were used for studying the enzyme activity in both control and treated groups.

2.5 Enzyme assays:

2.5.1 Acetylcholinesterase (AChE) assay

The AChE test was conducted using the methods outlined by Ellman et al (1961). The reaction mixture for the test was prepared to a final volume of 2.5 mL by including 1.4 mL of 20 mM potassium phosphate buffer at pH 7.0, 0.5 mL of 1 mM DTNB, 0.5 mL of 1 mM ATC, and 100 µL of cell extract. A UV-Visible spectrophotometer measured absorbance at 412 nm.

2.5.2 Catalase (CAT) assay

The CAT test was conducted using the protocol outlined by Aebi (1984). For the experiment, a 1 mM H2O2 solution was produced, and the reaction mixture included 2.4 mL of 20 mM potassium phosphate buffer at pH 7.0, 0.5 mL of 1 mM H2O2, and 100 µL of cell extract. A UV-Visible spectrophotometer was used to measure the absorbance at 240 nm.

2.5.3 Glutathione sulfotransferase (GST) assay

The GST was evaluated using a spectrophotometric technique using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate, as outlined by Habig et al. (1974). The test mixture comprises 1.9 mL of potassium phosphate buffer (20 mM, pH 7.0), 0.5 mL of 1 mM GSH, 0.5 mL of 1 mM CDNB, and 100 µL of cellular extract. Subsequent to the production of the S-conjugate, its absorbance at 340 nm was assessed.

2.5.4 Glutathione peroxidase (GPX):

The activity of the GPx enzyme was assessed according to the methodology established by Beutler (1984). Employing 1 mM NADPH and 1 mM H2O2 as substrates. The test mixture comprises 1.4 mL of potassium phosphate buffer (20 mM, pH 7.0), 0.5 mL of NADPH (1 mM), 0.5 mL of hydrogen peroxide (1 mM), and 100 µL of cellular extract. The oxidation of NADPH to NADP+ was assessed by measuring absorbance at 340 nm.

2.5.5 Glutathione (GSH):

The GSH concentration in *E. fetida* was quantified with the technique described by Ellman (1959). A volume of 0.9 mL of 5% TCA was combined with 0.1 mL of tissue homogenate. Following centrifugation for 15 minutes at 2,300×g, 2.0 mL of 0.01% DTNB was added to 0.5 mL of the supernatant. Absorbance was then quantified via a spectrophotometer at 412 nm.

2.5.6 Lipid peroxide (LPO) assay:

The LPO test was assessed using the methodology of Ohkawa et al. (1979). The assay comprises the following combination. 0.1 mL of tissue extract was combined with 1.9 mL of 20 mM phosphate buffer solution and incubated for 1 hour at 37°C. One millilitre of 5% trichloroacetic acid was added to the incubation solution to generate a precipitate. The resultant solution was subjected to rotation at 2200 rpm for 15 minutes and thereafter allowed to reach room temperature. The absorbance of the resulting supernatant was measured using a UV-Visible spectrophotometer at 532 nm.

2.5.7 Protein carbonyl:

The activity of PC was assessed using the methodology outlined by Levine et al. (1994). A 100 µL aliquot of the supernatant was extracted from the mixture. Subsequently, 500 µL of a 1 mM solution of 2,4-Dinitrophenylhydrazine was included and blended with the supernatant, followed by a one-hour incubation. Following incubation, 500 µL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 500×g for 5 minutes, resulting in protein precipitation. The precipitated protein was then washed extensively with ethanol or ethyl acetate. The protein was dissolved in 6M Guanidine HCl, formulated in 20mM PBS at a pH of 2.3. Absorbance was measured at 370 nm.

2.5.8 Protein Estimation:

The quick and sensitive Bradford technique (1976) was used to quantify total protein concentration using bovine serum albumin (BSA) as the standard curve. The protein standard was established using bovine serum albumin, and the protein content in *E. fetida* tissues was evaluated against this standard and measured at 595 nm.

2.6 Statistical analysis

Each experiment was performed in triplicate, resulting in a total of six repetitions (n = 6). A one-way analysis of variance (ANOVA) was conducted, followed by a comprehensive pairwise multiple comparison method (Tukey's test). The Mann-Whitney U test and Levene's test were used to assess normal distribution and homogeneity of variance, respectively. The significance threshold was established at $P < 0.05$.

3. RESULTS AND DISCUSSION

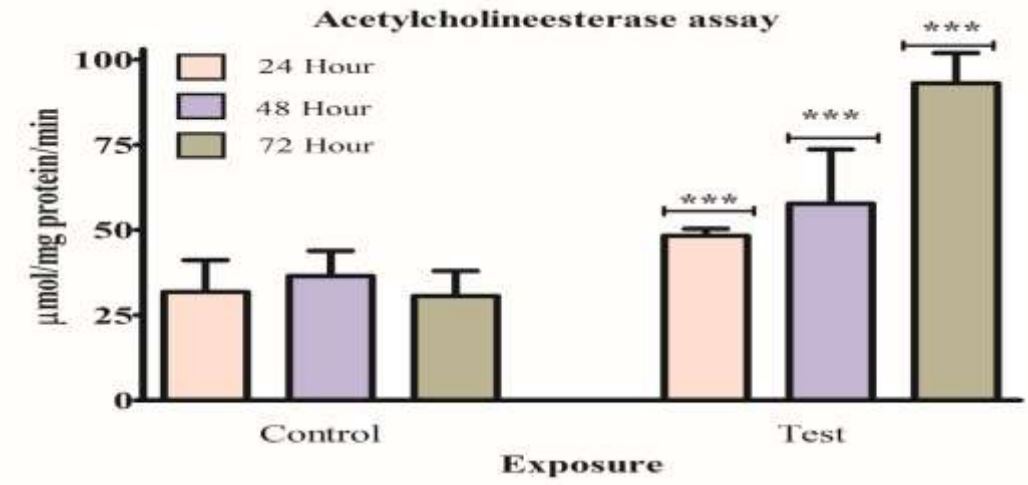

Figure 1: Acetylcholine esterase activity

AChE activity

This investigation determined the effect of exposure to a concentration of QP equal to the LC25 on AChE activity in *E. fetida.* The activity of AChE was quantified in µmol/mg protein/min. The results showed that the average AChE activity was significantly increased by 54.84%, 68.57%, and 206.67% after 24, 48, and 72 hours of exposure, respectively. These data suggest that AChE activity was affected by QP exposure as a function of time. The results showed that AChE activity was significantly increased only in the organisms exposed to the LC25 dose of QP for 72 hours.

The increase in AChE activity with exposure time (54.84% at 24 h, 68.57% at 48 h, and 206.67% at 72 h) indicates an adaptive compensatory response to the cholinergic disruption caused by QP. The mechanism of action of organophosphates is well established, involving the incorporation of a phosphoryl group into the active serine residue of AChE, thereby interfering with the hydrolysis of acetylcholine (Aroniadou-Anderjaska et al., 2023). The increase in AChE activity over time could be due to reactivation of the enzyme or transcriptional upregulation in response to stress as a detoxification mechanism, suggesting an attempt to restore neurotransmitter homeostasis in the presence of persistent stress. In *E. fetida* exposed to isoprocarb and chlorpyrifos, compensatory AChE responses have been reported, with AChE being part of a larger stress response cascade (Gu et al., 2021; Hou et al., 2022).

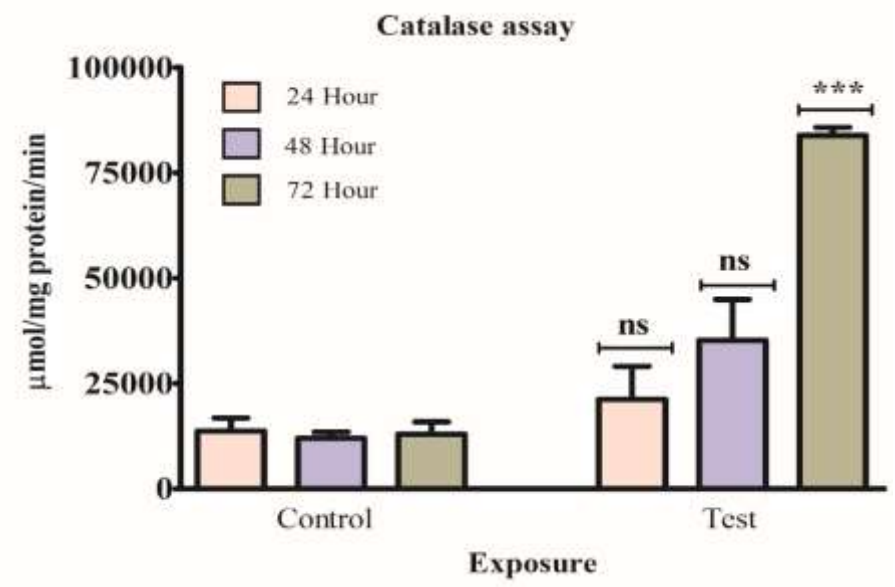

Figure 2: Catalase activity

CAT activity

The impact of exposing *E. fetida* to a concentration of QP corresponding to the LC25 was investigated in this study (Fig. 2). CAT activity was quantified in µmol/mg protein/min. The results showed a significant rise in catalase activity after 24, 48, and 72 hours of exposure, by 66.67%, 209.09%, and 608.33%, respectively. The data showed a time-dependent effect of QP exposure on CAT activity in the studied group. The results showed that after 72 hours, the organisms exposed to the LC25 concentration of QP had significantly increased CAT activity.

Catalase (CAT) is one of the main enzymes that scavenges hydrogen peroxide and reduces it to water and molecular oxygen, thereby minimizing the damage caused by ROS to cells. The increased expression of CAT at 72 hours (608.33%) is similar to an increase in intracellular ROS accumulation due to quinalphos-induced mitochondrial dysfunction. The continuous and progressive rise in CAT activity at all three time points indicates a definite time-dependent response, which implies that exposure to QP may induce a chronic state of oxidative stress in earthworm tissues, leading to constant upregulation of antioxidant defence mechanisms. A similar increase in CAT activity has been observed in organophosphate-exposed *E. fetida*, and Hou et al. (2022) and Gu et al. (2021) found that significant upregulation of CAT activity was a key oxidative stress response to sublethal concentrations of pesticides.

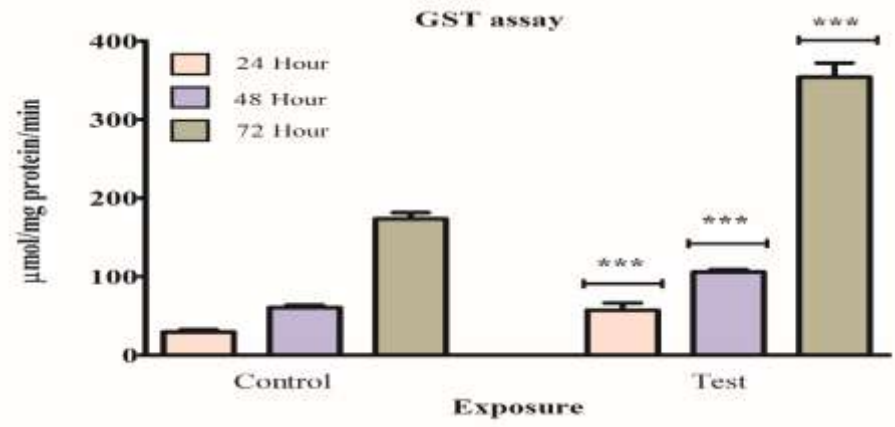

Figure 3: GST activity

GST activity

The effect of exposing *E. fetida* to a concentration of QP equivalent to the LC25 on GST activity was investigated in this work (Fig. 3). GST activity was measured in units of µmol/mg protein/min. Results showed that mean GST activity increased significantly by 57.29%, 105.89%, and 354.16% after 24, 48, and 72 hours of exposure, respectively. These data suggest a time-dependent effect of QP exposure on GST activity in the group studied. The results showed that only after 72 hours did the organisms exposed to the LC25 concentration of quinalphos show a significant increase in GST activity. Phase II xenobiotic detoxification was assessed by measuring glutathione S-transferase (GST) activity, which was found to increase in a time-dependent manner. GST facilitates the conjugation of electrophilic compounds with reduced glutathione, an important mechanism for the biotransformation and excretion of organophosphate metabolites. The significant induction of detoxification capacity after 72 hours (354.16%), as observed, aligns with findings in earthworms exposed to organophosphate insecticides, where GST induction was found to be proportional to the level of xenobiotic stress (Hou et al., 2022; Campani et al., 2024).

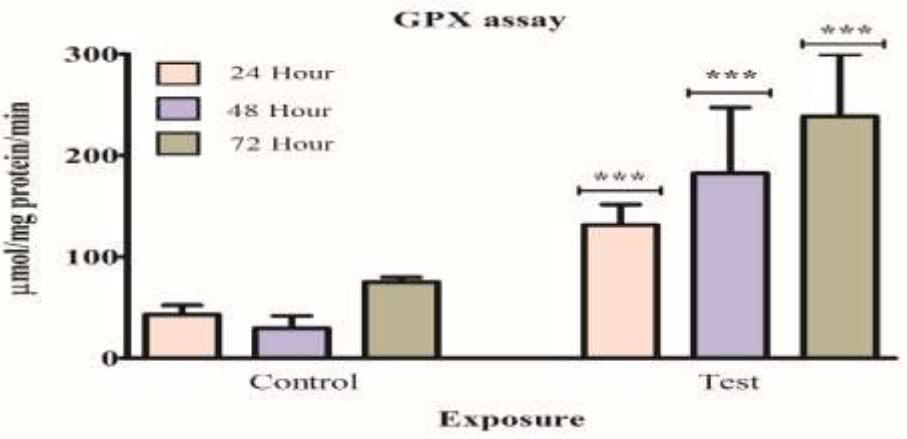

Figure 4: GPX activity

GPX activity

The impact of exposing *E. fetida* to a QP concentration equal to the LC25 on GPX activity was investigated in this work (Fig. 4). GPX activity was measured in µmol/mg protein/min. The results showed that mean GPX activity increased by 131.29%, 182.20%, and 238.47% after 24, 48, and 72 hours of exposure, respectively. These data suggest that GPX activity in the studied group was affected by QP exposure in a time-dependent manner. The results showed that GPX activity was significantly increased in organisms exposed to the LC25 concentration of quinalphos after 72 hours.

Glutathione peroxidase (GPX) is a major enzyme that scavenges lipid hydroperoxides and hydrogen peroxide in concert with the glutathione system to neutralise ROS. The progressive rise in GPX activity at 72 hours is in agreement with the rise in oxidative burden in earthworm tissues due to mitochondrial dysfunction caused by QP. Long-term exposure to QP causes chronic oxidative stress, as indicated by the sustained upregulation of GPX together with CAT. Campani et al. (2024) and Hou et al. (2022) also reported similar GPX induction in *E. fetida* exposed to sublethal levels of organophosphates, further supporting the use of GPX as a sensitive biomarker of oxidative stress caused by pesticide exposure in soil invertebrates.

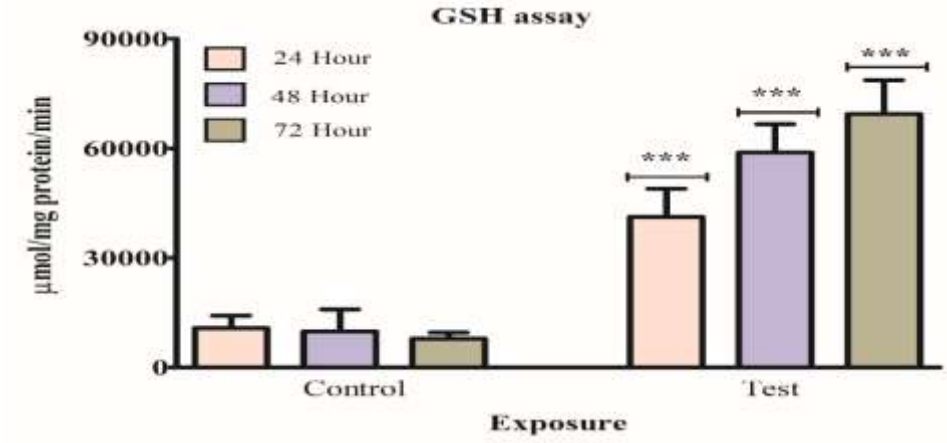

Figure 5: GSH activity

GSH activity

The effect of exposure of *E. fetida* to a QP concentration equivalent to the LC25 on GSH activity (Fig. 5) was examined. GSH activity was quantified in µmol/mg protein/min. After 24, 48, and 72 hours of exposure, the mean GSH concentration showed a significant increase of 411.76%, 588.23%, and 694.11%, respectively. These data suggest that GSH activity was affected by QP exposure over time in the studied group. After 72 hours, only the organisms exposed to the LC25 concentration of quinalphos showed a significant increase in GSH activity.

The remarkable elevation in GSH concentration, reaching a 694.11% increase at 72 hours, highlights how the cellular redox defence system was mobilised in response to the toxicity induced by QP. GSH serves as a cofactor for GST and GPX and is also a direct, non-enzymatic scavenger of ROS. The parallel increase of GSH with GST and GPX supports the coordinated activation of the glutathione-dependent antioxidant pathway. These results are similar to those of Gu et al. (2021) and Dzul-Caamal et al. (2024), who found that earthworms and other invertebrates showed a significant increase in GSH levels in response to sublethal exposure to organophosphates, as part of an effort to replenish the antioxidant pool depleted by oxidative challenge.

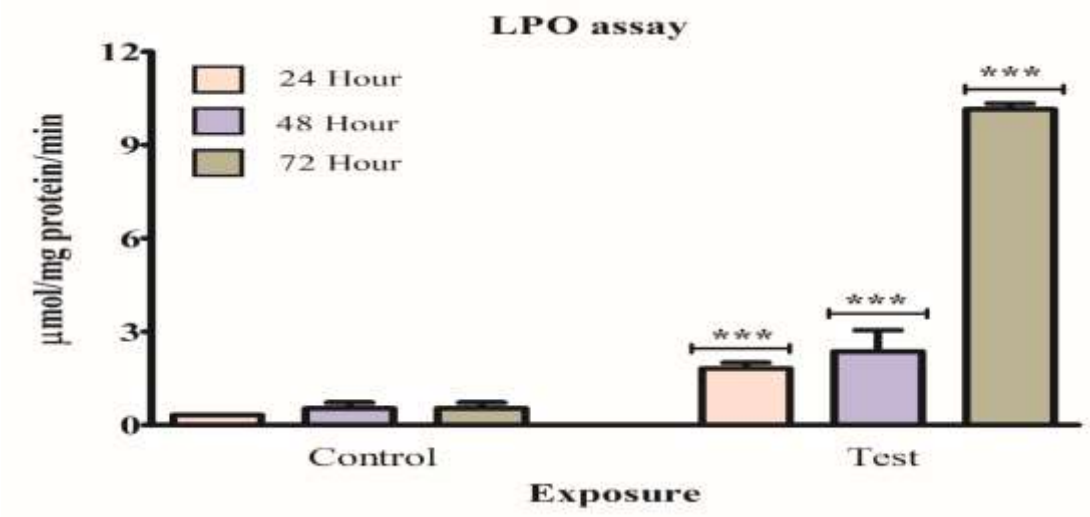

Figure 6: LPO activity

LPO activity

The effect of exposure of *E. fetida* to an LC25 concentration of QP on LPO activity was investigated (Fig. 6). LPO activity was expressed in µmol/mg protein/min. The results showed that after 24, 48, and 72 hours of exposure, the mean LPO increased by 1.81%, 2.36%, and 10.14%, respectively. These data suggest that LPO activity in the group examined was affected by exposure to QP over time. The results showed that LPO activity alone was significantly increased in the organisms exposed to the LC25 concentration of quinalphos at 72 hours.

Quinalphos treatment significantly increased lipid peroxidation (LPO), expressed as malondialdehyde (MDA) equivalents, with the highest level observed at 72 hours. This finding is in line with ROS attack on polyunsaturated fatty acids in cell membranes, resulting in loss of membrane integrity and disruption of cellular homeostasis. The percentage changes in LPO are of the same order of magnitude as the enzymatic biomarkers, but represent downstream and irreversible oxidative membrane damage rather than a protective enzymatic response, and are therefore of toxicological significance even when small. A similar progressive elevation in LPO was observed in *E. fetida* exposed to organophosphate pesticides by Hou et al. (2022) and Campani et al. (2024), which further confirmed LPO as a good indicator of membrane-level oxidative damage.

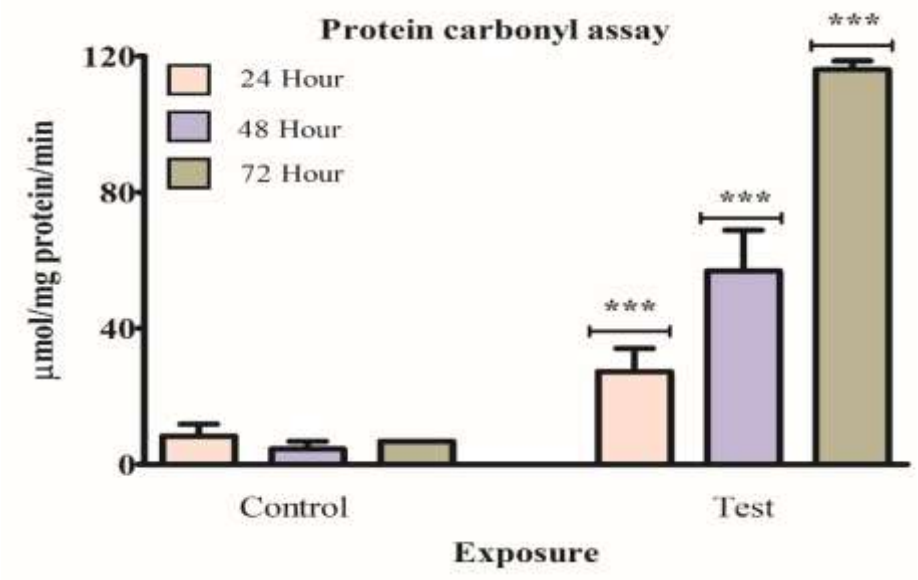

Figure 7: Protein carbonyl activity

Protein carbonyl (PC) activity

The influence of LC25 exposure of *E. fetida* on the activity of PC was investigated in this study (Fig. 7). PC activity was expressed in µmol/mg protein/min. It was found that the mean PC content after 24, 48, and 72 hours of exposure increased by 27.27%, 56.81%, and 116.08%, respectively. These data suggest that QP exposure had a time-dependent effect on PC

activity within the group studied. The results showed that at the 72-hour exposure period, there was a significant increase in PC activity for organisms exposed to the LC25 concentration of quinalphos.

Protein carbonyl (PC) content, which is widely accepted as a biomarker of irreversible protein oxidation, increased gradually as exposure time increased, indicating structural and functional changes in proteins caused by oxidative modification via carbonyl-reactive species produced during QP metabolism. The 116.08% rise after 72 hours suggests a decline in the ability of antioxidants to counteract protein damage, indicating a shift from adaptive stress response to actual oxidative injury. In line with the present results, Hou et al. (2022) and Campani et al. (2024) reported increased PC levels as a terminal marker of oxidative protein damage in earthworms exposed to sublethal concentrations of organophosphates.

The biochemical findings presented in this study suggest that quinalphos causes progressive oxidative stress in *E. fetida*, with compensatory enzyme induction occurring at short exposure periods and an increase in damage markers at longer exposure periods. Increased levels of LPO and PC at 72 hours indicate that the ability of the antioxidant defence system to compensate for oxidative damage caused by QP is progressively depleted over time, suggesting a dose-duration relationship in toxicological response. The results show the sensitivity of earthworm biochemical systems to stress caused by organophosphate use and support the use of oxidative stress biomarkers as useful tools for ecotoxicological assessment. The findings highlight the ecological threat of quinalphos to beneficial soil invertebrates and the importance of careful and targeted pesticide use practices to protect soil biodiversity and ecosystem function.

CONCLUSION

The gradual rise in important biochemical indicators, such as AChE, catalase, GST, GPX, GSH, LPO, and protein carbonyl content, indicates that quinalphos has time-dependent harmful effects on earthworms (*Eisenia fetida*). The heightened concentrations of antioxidant and detoxifying enzymes, together with indicators of oxidative damage, suggest that earthworms endure considerable physiological stress with extended exposure. These reactions indicate both an adaptive defence mechanism and evidence of progressive cellular damage over time.

The results underscore the vulnerability of earthworm biochemical systems to quinalphos and emphasise the need of using these biomarkers in ecological risk evaluation. Considering the crucial function of earthworms in sustaining soil health and ecosystem integrity, the findings need prudent use and enhanced control of organophosphate pesticides such as quinalphos to avert possible detriment to non-target soil species.

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