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EVALUATION OF PESTICIDE CHLORPYRIFOS TOXICITY ON *DROSOPHILA MELANOGASTER*

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ABSTRACT: Chlorpyrifos is one of the commonly detected pesticides found in food and water. Extensive use of it in agriculture and its persistence in the environment have raised public concern to overcome the pollution and toxicity problems. Exposure to chlorpyrifos poses several harmful effects to animal and human health. And the pathology in neurodegenerative diseases is mainly because of mitochondrial dysfunction. GSH depletion proceeding with mitochondrial dysfunction is therefore considered as the earliest triggering factor of neurodegeneration. In the present study using *Drosophila melanogaster* a fly model for human neurodegenerative disease, we investigated the effects of chlorpyrifos on glutathione, reactive oxygen species, mitochondrial Complex I, and Complex II activities. We found that chlorpyrifos generates reactive oxygen species, diminished the levels of reduced glutathione (GSH) concurrently increasing oxidized form of glutathione (GSSG), this preceded with inhibition of mitochondrial enzymes such as complex I and complex II activities, ultimately leads to motor dysfunction and death of Drosophila flies.

INTRODUCTION: Chlorpyrifosis one of the most widely used organophosphates *i.e.*, synthetic insect pesticides. The widespread use of these in modern agriculture is of increasing concern mainly due to environmental contamination and subsequent biodiversity loss. Repeated applications of chlorpyrifos modify the soil microbial community structure and pose potential health risks to the other non-targets. The inhibition of cholinesterase activity caused by chlorpyrifos, leads to accumulation of acetylocholine at the synapse, causing over stimulation and disruption of neuro-transmission in both central and peripheral nervous systems [1, 2].

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Earlier studies on neurodegenerative diseases modeled in the fly include accumulation of disease proteins in abnormal aggregates, the toxicity of the proteins to induce neuronal dysfunction and loss, among other features reflective of the human diseases. Extensive use of chlorpyrifos has stimulated research into the possible existence of effects related with their toxic reproductive activity in various organisms.

In one research cladoceran *Daphnia magna* was exposed to malathion, chlorpyrifos, and carbofuran to estimate acetylcholinesterase (AChE) and carboxylesterase (CbE) inhibition and recovery patterns, which revealed survival of *Daphnia* juveniles was impaired at AChE inhibition levels higher than 50% affected mortality [3]. Also, short-term pesticide exposure has shown deleterious effects on children's speed of attention, sequencing, mental flexibility, visual search, concept formation, and conceptual flexibility.

Since the nervous system undergoes rapid growth and development in the first years of life, children are more likely to have neurological problems [4]. When chemicals destroy cells in the developing brain, there is a risk that results in dysfunction of the brain, which would be irreversible [5]. Chlorpyrifos also brought about a marked reduction in epididymal and testicular sperm counts in exposed males, induces severe testicular damage, which results in a reduction in sperm count and thus affects fertility [6]. There is evidence that glochidia and juvenile life stages of a freshwater mussel (*Lampsilis siliquoidea*) are highly sensitive to this toxin. Some pesticide formulations are more toxic than their active ingredient because of the presence of surfactants, adjuvants, or other ingredients in the formulation [7]. The joint toxicity of esfenvalerate and chlorpyrifos to the fathead minnow (*Pimephales promelas*) and the aquatic midge larvae (*Chironomus tentans*) was determined using comparisons to independent and combined action [8]. The toxicity of contaminants their potential hazards in the natural aquatic environment was assessed in *Americamysis bahia* [9].

Drosophila melanogaster is very much commonly used insect for various experimental purposes. It occasionally becomes a pest in home, restaurant, and fruit markets. Some species are attracted to human and animal excrement; they serve as disease carriers by feeding on uncooked foods. The approach to understand human neurodegenerative diseases in the simple fruit fly Drosophila offers many advantages for studying molecular and cellular pathology of human disease. The studies will be beneficial as it includes faster time frame due to the shorter life span of the fly, also because of availability of large number of progeny for many tools and techniques to manipulate gene expression, and relatively well-known for anatomy and phenotypes [10, 11]. Comparisons between the fly and human genomes indicate a high degree of conservation in fundamental biological pathways[12]. Drosophila cells in S2 stage mimic early events in *Chlamydia trachomatis* [13], and act as a vector of life-threatening causative pathogen, *Staphylococcus aureus* [14].

Earlier studies *in-vivo* and *in-vitro* suggested that mitochondrial dysfunction occurs with exposure of Chlorpyrifos, results in depletion of ATP production [15]. The respiratory chain consists of 3 proton pumps linked by 2 mobile electron carriers that are present within the inner membrane of mitochondria. Electrons from NADH or $FADH_2$ flow-through 3 specific transmembrane complexes called NADH: ubiquinone oxidoreductase (EC 1.6.5.3, Complex I), ubiquinol: ferricytochrome C oxidoreductase (EC 1.10.2.2, Complex III), and errocytochrome *C*: oxygen oxidoreductase (EC 1.9.3.1, Complex IV). Complex II (succinate dehydrogenase: ubiquinone oxidoreductase, EC 1.3.99.1) also contributes to electron flow as FADH2 is converted to FAD. The synthesis of ATP is directly dependent on the concentration of ADP and flow of electrons from NADH or FADH2 to O2, creating a proton gradient across the inner mitochondrial membrane [16]. GSH depletion in dopaminergic cells in culture increases oxidative stress and decreases mitochondrial function. As mitochondrial complex formation and GSH functioning are important for cellular integrity, the effect of Chlorpyrifos on these at different concentrations and time response studies were done in Drosophila model.

MATERIALS AND METHODS:

Culturing of Drosophila Flies and Treatment with Chlorpyrifos: Drosophila flies were maintained in bottles containing sufficient volume of media. The media was prepared by boiling 900ml water with 100g jaggery; added 100gm Rava and 10gm agar by avoiding the formation of clumps. Contents were boiled and stirred constantly till the medium is cooked and begins to thicken; 7.5ml Propionic acid, and remaining 100ml water was added and mixed well. The molten medium is poured into glass culture bottles (about 50ml/ bottle) using the funnels and allowed undisturbed for 30 min and plugged the mouth with cotton.

Flies were exposed to various concentrations of chlorpyrifos (250nM, 500nM, 750nM, and 1000nM) and were killed at 72 hours after exposure to chlorpyrifos and separated the head from rest of the body, then used for biochemical assays.

Estimation of Total Glutathione (GSH + GSSG): Total glutathione estimations in the brain extracts were carried out by the DTNB recycling method as described earlier [17]. All estimations were conducted in triplicate and total glutathione concentrations

were normalized per milligram protein. Glutathione-S-transferase was assayed by the 1-chloro 2-4-dinitro benzene (CDNB) method [18]. To 1 ml of reaction mixture containing phosphate buffer (0.1 M, pH 6.5; 0.5 mM EDTA), CDNB (1.5 mM) and 50 ml of GSH (1 mM), 30 mg of protein (sample) were added, and the increase in absorbance at 340 nm was monitored for 5 min. The enzyme activity was expressed as nanomoles of S-2,4, dinitrophenyl glutathione formed/min/mg protein. Solubilize brain protein extracts (100 mg) was assayed at 25 °C in 0.1 M Tris-HCl (pH 8.1), and 0.2 mM NADPH and the reaction was initiated by the addition of 1 mM GSSG and followed spectro-photometrically at 340 nm, and the activity was expressed as nanomoles of NADPH oxidized/ min/mg protein (MEC = 6.22/mM/cm) [19].

Measurement of Reactive Oxygen Species (ROS): Total ROS in Drosophila was measured by adding 1 mlof Locke's solution (154 mM NaCl, 5.6 mM KCl, 3.6 mM NaHCO3, 5 mM HEPES, 2 mM $CaCl_2$ and10 mM Glucose, pH 7.4). Ten micromoles of dihydrodichlorofluorescein diacetate (DCFDA) was then added, and the cells were incubated at 37 ºC (10 min) in a CO_2 incubator. The Locke's solution was then removed, and the cells were harvested. The cell pellet was washed with 1X phosphate-buffered saline (PBS), pH 7.4, twice, and reconstituted in lysis buffer (10 mM Tris–HCl containing 0.5% Tween-20). The lysate was centrifuged at 1000g (10 min), and the fluorescence of the supernatant was measured (Excitation-480 nm; Emission-530 nm [20].

Mitochondrial Complex I Assay: Mitochondrial complex I (CI) is believed to be the central player to the mitochondrial dysfunction during neuro-logical disorders. CI enzyme assay was initiated by addition of aliquots of tissue homogenates (10ug/10ul suspended in hypotonic buffer to 200ul of the reaction mix containing 25 Mm phosphate buffer pH 7.2 + 5 mM$MgCl_2$) to 0.1M Tris-HCl pH 7.2, 500 µM EDTA, 1% bovine serum albumin (BSA), 200 µM NADH, and 200 µM decylubiquinone with or without 50 µM rotenone, in the presence of 2mM NaCN with 0.002% DCIP as a secondary electron acceptor. The decrease in the absorbance at 600 nm was recorded as a measure of the rate of enzyme reaction at 37 °C for 10 min, and specific activity was calculated. The reaction was measured by a spectrophotometer in a reaction volume of 150ul in an ELISA plate reader. The specific activities with and without rotenone were calculated independently. The activity obtained in the absence of rotenone corresponds to total NADH dehydrogenase activity since rotenone is a specific inhibitor of CI. The activity in the presence of rotenone corresponds to non-mitochondrial CI NADH dehydrogenase activity. Hence the difference between the two gives the activity specific to mitochondrial CI. Thus results were plotted as a relative rotenone-sensitive specific activity. The reaction was measured spectrophotometrically in a reaction volume of 200ul in an ELISA plate reader.

Mitochondrial Complex II Assay: The mitochondrial machinery for ATP synthesis includes the electron transport chain, which comprises respiratory complexes (CI, CIII, and CIV) coupled with ATP synthase. Electron transfer through the complexes is coupled to the generation of free radicals. The complex II assay was initiated by the addition of aliquots of tissue homogenates (15ug/10ul suspended in the hypotonic buffer to 190ul of the reaction mix containing 10 mMTris-HCl pH 7.8, 2 µM EDTA, 10mM of succinate, 0.1% bovine serum albumin (BSA), 3µM rotenone,1uM of antimycin,0.3mM of KCN, 80uM of DCIP and just before the reaction 50 µM decyl-ubiquinone. The decrease in the absorbance at 600 nm was recorded as a measure of the rate of enzyme reaction at 37 °C for 10 min, and specific activity was calculated. The reaction was measured by a spectrophotometer in a reaction volume of 200ul in an ELISA plate reader.

Negative Geotaxis Assay: Geotaxis is generally measured for ten to twenty groups of ten individuals of the same genotype or treatment (100-200 flies total for each genotype/treatment). We Sorted groups of male flies on a CO_2 anesthesia apparatus and placed each group to allow resting for one hour so that flies recover completely from anesthesia. Then a group of ten flies were transferred into a climbing apparatus and immediately covered the lower vial with the top vial and allowed flies to settle down for 1 min before conducting the assay. Then gently tapped the flies down to the bottom of the vial and measured the number of flies that can climb above

the 10 cm mark in 10 sec. This assay was repeated 3 times, allowing 1-minute rest between each trial, and recorded the number of flies as a percentage of total flies.

Survival Assay: The flies were exposed to different concentrations of toxin and mortality of the flies was noted down every day. The number of dead flies was noted every day both in the control vials and in the treatment vials. The readings were taken at the interval of 24 h until the complete mortality is seen.

Statistical Analysis: Quantitative data were accumulated from at least three independent experiments and expressed as mean ± SD followed by an analysis of variance (ANOVA) and Pearson's correlation of linear regression (*r*-value). In all the experiments, data with $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION: Neuro-degenerative diseases are seen as more common in aged human populations. Aged individuals may therefore be differentially sensitive to toxicants affecting cholinergic neurotransmission. Old male Drosophila flies of different ages such as 3, 10, and 30 days were exposed to various concentrations of Chlorpyrifos such as 250nM, 500nM, 750nM and 1000nM for 72 h time interval. Flies were killed at 72 h after exposure to Chlorpyrifos followed by separation of the head from body, and levels of GSH were estimated in both head and body. GSH levels are expressed as µg of GSH/mg protein and are shown in **Fig. 1**.

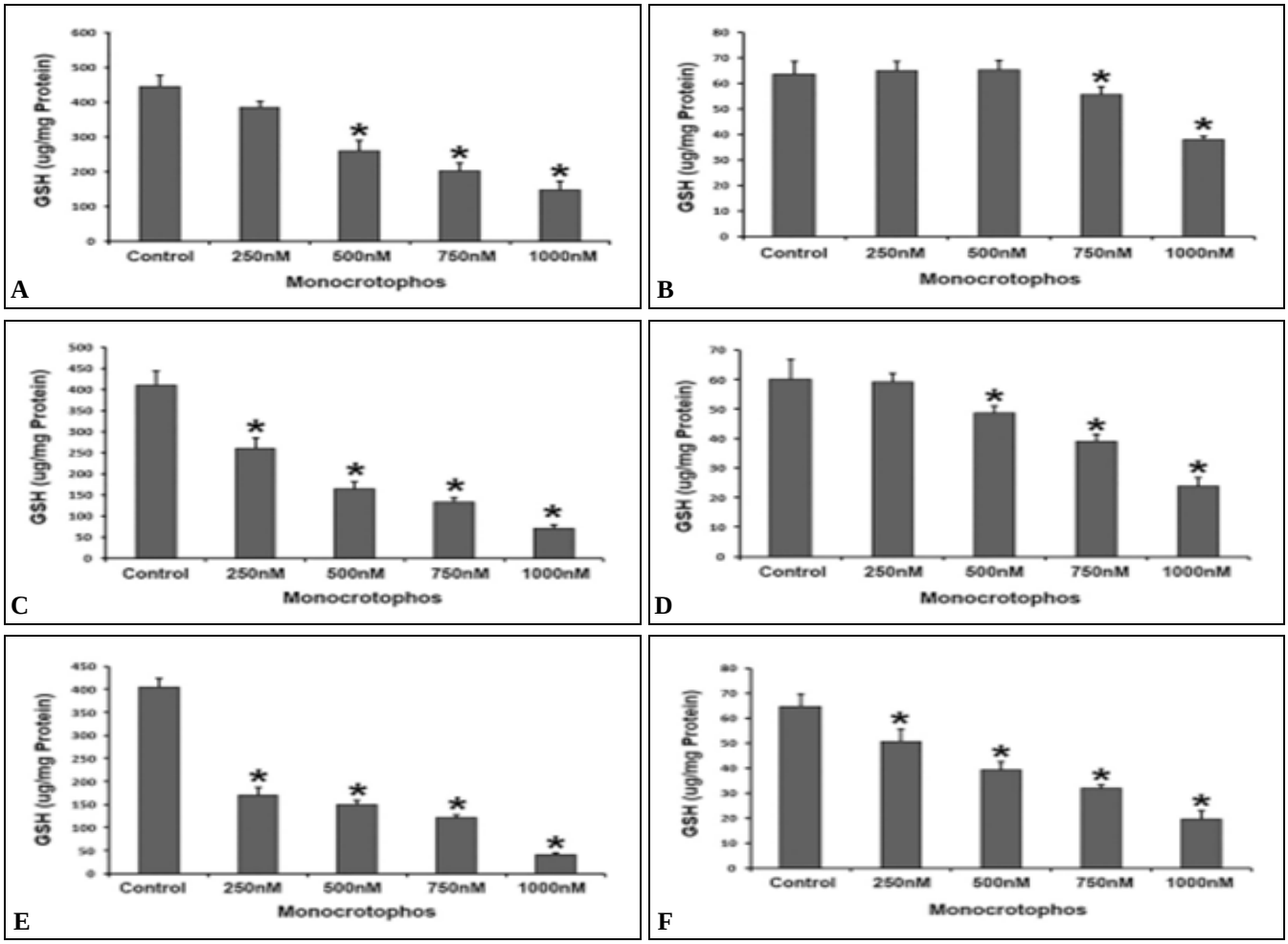

FIG. 1: PESTICIDE CHLORPYRIFOS EXPOSURE DECREASES REDUCED GLUTATHIONE (GSH) LEVELS IN HEAD AND BODY OF DROSOPHILA MELANOGASTER: 3 DAYS (A AND B), 10 DAYS (C AND D) AND 30 DAYS OLD (E AND F) MALE DROSOPHILA FLIES WERE EXPOSED TO VARIOUS CONCENTRATIONS OF CHLORPYRIFOS (250 nm, 500 nm, 750 nm AND 1000NM) OR VEHICLE ALONE (CONTROL). FLIES WERE KILLED AT 72 h AFTER EXPOSURE TO CHLORPYRIFOS AND SEPARATED HEAD FROM REST OF THE BODY. GSH LEVELS WERE ESTIMATED IN HEAD (A, C AND E) AND BODY (B, D AND F). GSH LEVELS ARE EXPRESSED AS µG OF GSH/mg PROTEIN. VALUES ARE MEAN ± SD, n = 3 INDEPENDENT EXPERIMENTS. * INDICATE VALUES SIGNIFICANTLY DIFFERENT FROM CONTROL FLIES WITH P<0.05

In a similar way, male flies with different age groups such as3 days, 10 days, and 30 days old were exposed to various concentrations of chlorpyrifos as mentioned above for 72 h. Then files were killed and separated the head from the rest of the body and estimated GSSG levels. GSSG levels are expressed as µg of GSH/mg protein as shown in **Fig. 2**. All these toxic effects are moderate at low doses and become severe at higher dose levels, and older flies are more sensitive to pesticides even at lower concentrations. GSH is synthesized in the cytosol, and approximately 10% of this pool is imported into the mitochondria. The mitochondrial pool is buffered during physiological and pathological changes such that any alteration in the cytosolic pool might not significantly affect the mitochondrial GSH [21]. We observed higher levels of GSSG in 1000nM treated flies. GSH and GSSG data in **Fig. 1** and **2** clearly show that the head is more responsive than the rest of the body, and older flies are highly liable to chlorpyrifos toxicity. GSH decreased directly corrected with increased GSSG levels at different concentrations of pesticide and also on an age-dependent manner.

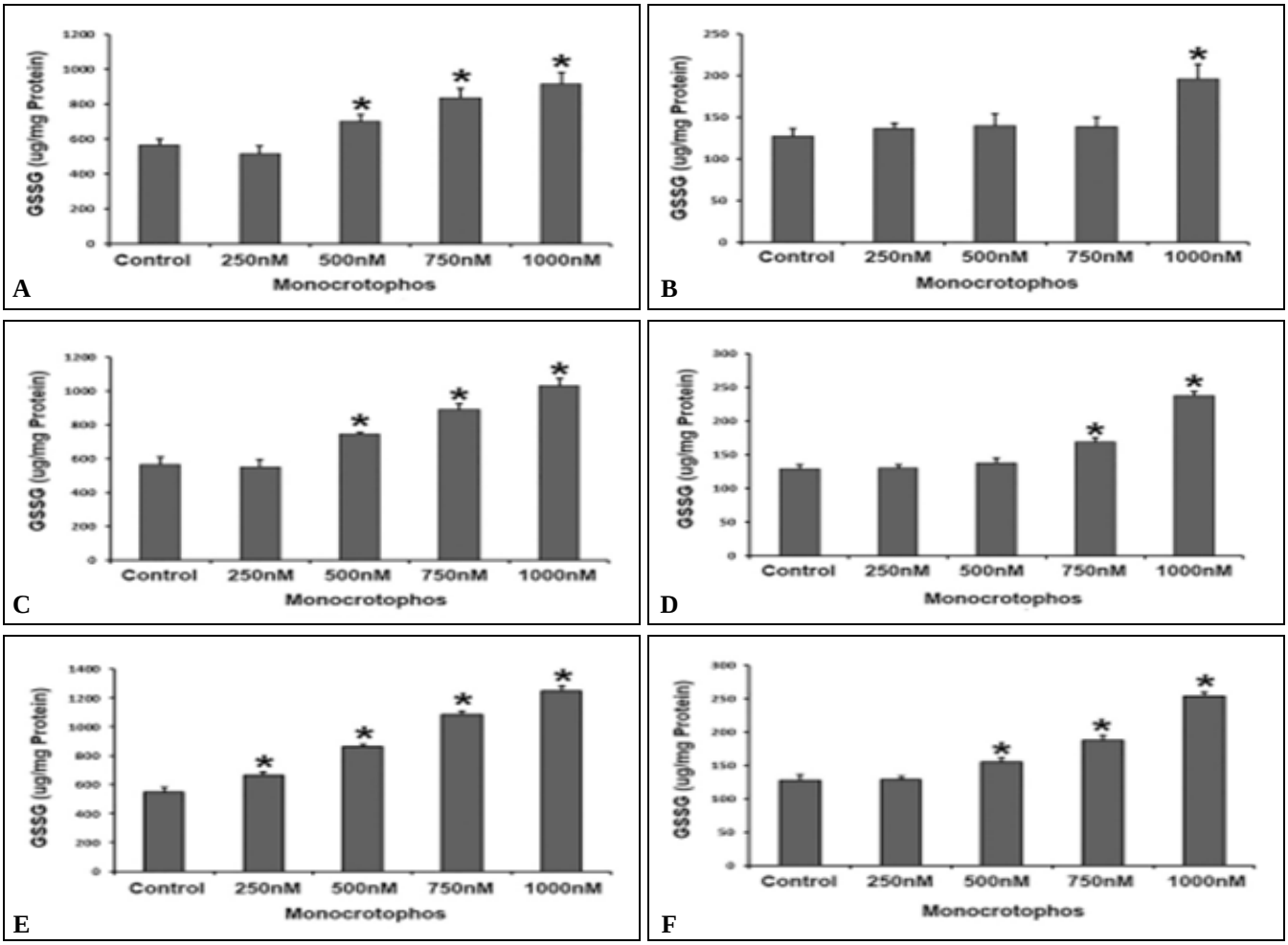

FIG. 2: PESTICIDE CHLORPYRIFOSEXPOSURE INCREASES OXIDIZED FORM OF GLUTATHIONE (GSSG) LEVELS IN HEAD AND BODY OF DROSOPHILA MELANOGASTER: MALEFLIES WITH DIFFERENT AGE GROUPS SUCH AS3 DAYS (A AND B), 10 DAYS (C AND D) AND 30 DAYS OLD (E AND F) WERE EXPOSED TO VARIOUS CONCENTRATIONS OF CHLORPYRIFOS (250 nm, 500 nm, 750 nm, AND 1000 nm) OR VEHICLE ALONE (CONTROL) FOR 72 h. THEN FILES WERE KILLED AND SEPARATED HEAD FROM REST OF THE BODY, AND ESTIMATED GSSG LEVELS IN HEAD (A, C AND E) AND BODY (B, D, AND F). GSSG LEVELS ARE EXPRESSED AS µG OF GSH/MG PROTEIN. VALUES ARE MEAN ± SD, N = 3 INDEPENDENT EXPERIMENTS AND* INDICATE VALUES SIGNIFICANTLY DIFFERENT FROM CONTROL FLIES WITH P<0.05

Exposure of pesticide chlorpyrifos to *Drosophila Melanogaster* flies generates reactive oxygen species (ROS) in a concentration-dependent manner. 3, 10 and 30 days old male flies were exposed to 250nM, 500nM, 750nM, and 1000nM of the pesticide chlorpyrifos, and untreated serves as vehicle control. After 72 h flies were killed, the head and body were taken separately and estimated

for ROS levels. The assay was done by using cell-permeable 2',7' –dichlorofluorescin diacetate fluorogenic dye, which gets oxidized by ROS and produces fluorescent DCF. DCF levels are monitored and expressed as fluorescent units/mg protein, which is depicted in **Fig. 3**. Exposure of pesticide chlorpyrifos to male flies generated reactive oxygen species in age-dependent and dose manner those 3, 10, and 30 days old in response to different concentrations 250 nM, 500 nM, 750 nM, and 1000 nM. Older files (30 days) are highly responsive to chlorpyrifos toxicity compared to younger ones, and the head is more susceptible than the rest of the body **Fig. 3**.

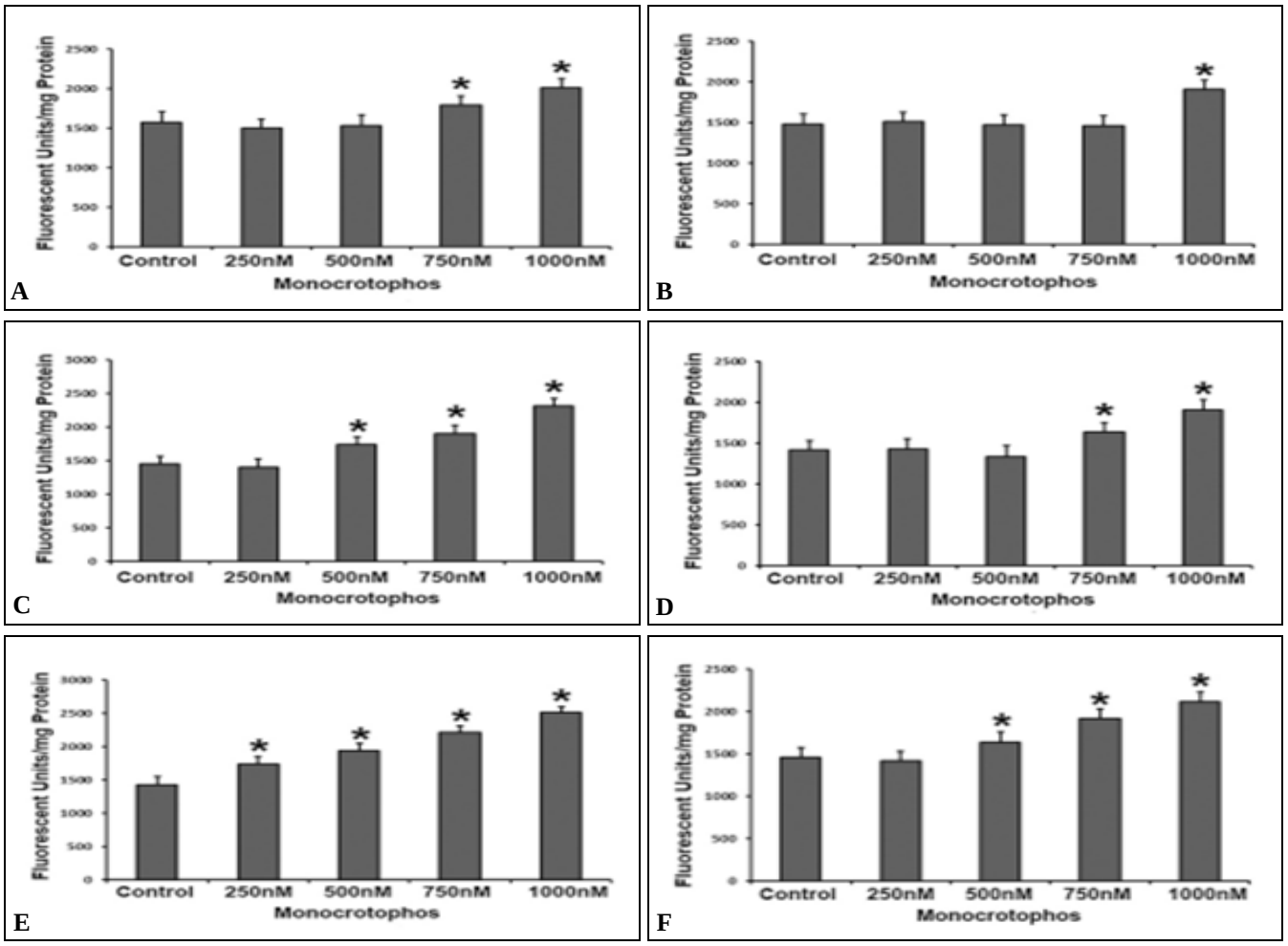

FIG. 3: EXPOSURE OF PESTICIDE CHLORPYRIFOS TO DROSOPHILA MELANOGASTER FLIES GENERATES REACTIVE OXYGEN SPECIES (ROS): 3 DAYS (A AND B), 10 DAYS (C AND D) AND 30 DAYS OLD (E AND F) MALE FLIES WERE EXPOSED TO 250 nm, 500 nm, 750 nm AND 1000NM OF PESTICIDE CHLORPYRIFOS OR VEHICLE ALONE (CONTROL) FOR 72 h. AFTER 72 f FLIES WERE KILLED SEPARATED HEAD FROM REST OF THE BODY. ROS LEVELS WERE ESTIMATED IN HEAD (A, C AND E) AND BODY (B, D AND F) BY USING CELL PERMEABLE 2',7' – DICHLOROFLUORESCIN DIACETATE (DCFDA), A FLUOROGENIC DYE WHICH GETS OXIDIZED BY ROS AND PRODUCES FLORESCENT DCF, DCF LEVELS ARE MONITORED AND EXPRESSED AS FLUORESCENT UNITS/mg PROTEIN. VALUES ARE MEAN ± SD, N = 3 INDEPENDENT EXPERIMENTS. * INDICATE VALUES SIGNIFICANTLY DIFFERENT FROM CONTROL FLIES WITH P<0.05

Mitochondrial complex I activity decreases by increasing age. Mitochondrial complex I and its activity are directly linked to oxidative stress with implications for aging and neurodegeneration [22]. Effect of chlorpyrifos exposure on mitochondrial complex I activity in head and body of *Drosophila Melanogaster* was estimated in 3, 10, and 30 days old male Drosophila flies for 72 h. Flies were killed, heads and bodies were collected, and mitochondria were isolated from both head and body separately. Complex I activity was measured and expressed as nmoles of NADH oxidized/ min/mg protein, as shown in **Fig. 4**. Decreased complex I activity was seen in both head and body of the flies with more effect in the head at lower concentrations of chlorpyrifos than the body, and older flies are more sensitive than younger ones **Fig. 4**.

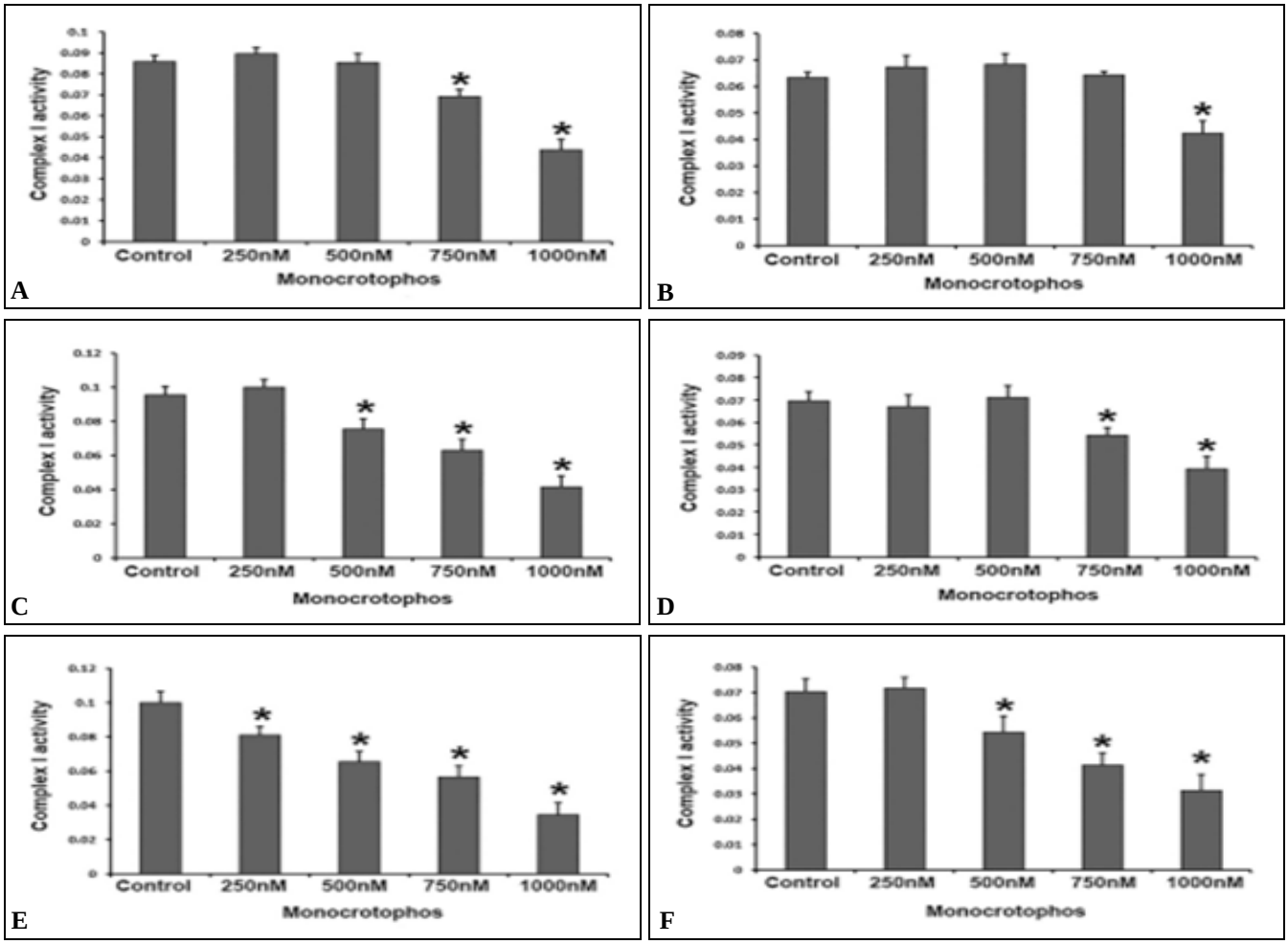

FIG. 4: EFFECT OF CHLORPYRIFOS EXPOSURE ON MITOCHONDRIAL COMPLEX I ACTIVITY IN HEAD AND BODY OF *DROSOPHILA MELANOGASTER*: VEHICLE (CONTROL) OR 250 nm, 500 nm, 750 nm, AND 1000 nm CONCENTRATIONS OF CHLORPYRIFOS WERE EXPOSED TO 3 DAYS (A AND B), 10 DAYS (C AND D) AND 30 DAYS (E AND F) OLD MALE DROSOPHILA FLIES FOR 72 h. FLIES WERE KILLED, HEADS AND BODIES WERE COLLECTED, AND MITOCHONDRIA WERE ISOLATED FROM BOTH HEAD AND BODIES. COMPLEX I ACTIVITY WAS MEASURED IN HEAD (A, C AND E) AND BODY (B, D, AND F). COMPLEX I ACTIVITY IS EXPRESSED AS NMOLES OF NaDH OXIDIZED/MIN/MG PROTEIN. DATA ARE MEAN ± SD, N = 3 INDEPENDENT EXPERIMENTS. * DENOTES SIGNIFICANT DIFFERENCE COMPARED TO CONTROL WITH P<0.05

Similarly, the effect of chlorpyrifos exposure on mitochondrial complex II activity was performed. Complex II activity was measured in mitochondria isolated from head and body separately. Complex II activity is expressed as nmoles of 2, 6 dichloro phenol indophenol (DCIP) reduced/min/mg protein and data is recorded in **Fig. 5**. Our results demonstrated significant alterations in Complex II activity following exposure to chlorpyrifos, and it is downstream of oxidative stress response **Fig. 1, 2** and **3**.

Exposure of chlorpyrifos to *Drosophila melanogaster* male flies also impacted climbing abilities shown in **Fig. 6**. 3, 10, and 30 days old flies were exposed to various concentrations of chlorpyrifos (250nM, 500nM, 750nM, and 1000nM) for 72 hrs. An assay was conducted to check its climbing ability with negative geotaxis and counted the number of flies climbs target line of 10 cm in 10 sec. Data is expressed as percent flies crossing target line against gravity in 10 sec as shown in **Fig. 6**. 3 and 10 days old flies didn't show any signs of climbing defects; however, and 30 days old flies showed climbing defects at 500nM of the chlorpyrifos, indicating older motor cells more susceptible to chlorpyrifos toxicity. In the same way, the mortality rate of flies were observed in chlorpyrifos exposed 3, 10, and 30 days old male drosophila flies. 24 h post-exposure flies were monitored for mortality and repeated this at 24 h interval until complete morality is observed.

The mortality rates are as shown in **Fig. 7**. Results showed that chlorpyrifos exposure significantly decreased the percent live flies in time, concentration, and age-dependent manner, whereas statistically same results were obtained with DPDS treatment following 28 days of treatment [23]. In another co-exposure study, toxin exposure significantly increased flies' mortality while the survivors exhibited significant locomotor deficits with decreased acetylcholinesterase (AChE) activity.

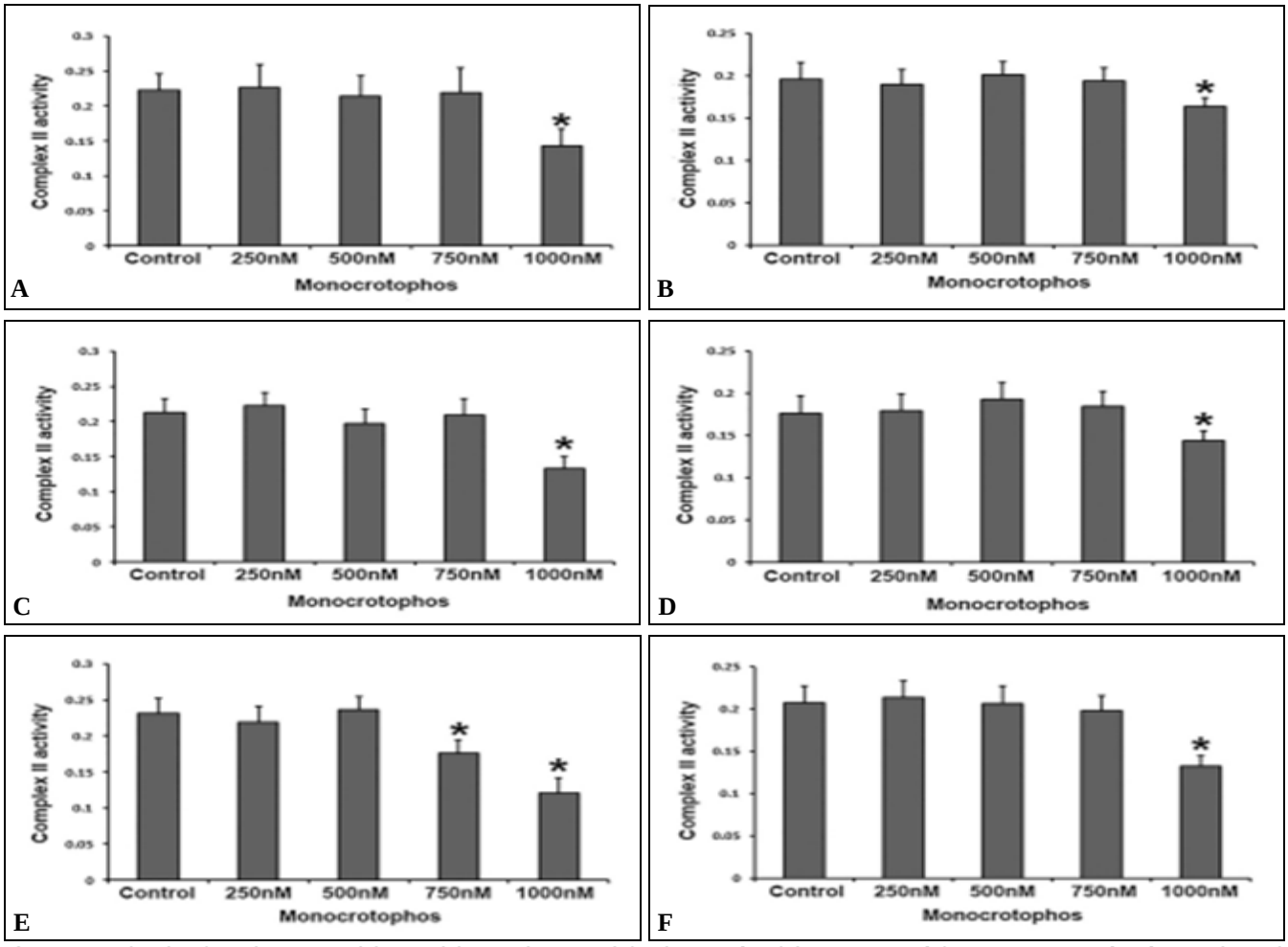

FIG. 5: EFFECT OF CHLORPYRIFOS EXPOSURE ON MITOCHONDRIAL COMPLEX II ACTIVITY IN HEAD AND BODY OF DROSOPHILA MELANOGASTER: VEHICLE (CONTROL) OR 250 nm, 500 nm, 750 nm, AND 1000 nm CONCENTRATIONS OF CHLORPYRIFOS WERE EXPOSED TO 3 DAYS (A AND B), 10 DAYS (C AND D) AND 30 DAYS (E AND F) OLD MALE DROSOPHILA FLIES. 72 h AFTER EXPOSURE FLIES WERE KILLED, HEADS AND BODIES WERE COLLECTED AND ISOLATED MITOCHONDRIA. COMPLEX II ACTIVITY WAS MEASURED IN MITOCHONDRIA ISOLATED FROM HEAD (A, C AND E) AND BODY (B, D AND F). COMPLEX I ACTIVITY IS EXPRESSED AS NMOLES OF 2, 6DICHLORO PHENOL INDOPHENOL (DCIP) REDUCED/MIN/MG PROTEIN. DATA ARE MEAN ± SD, N = 3 INDEPENDENT EXPERIMENTS. * DENOTES SIGNIFICANT DIFFERENCE COMPARED TO CONTROL WITH P<0.05

Overall, chlorpyrifos exposure significantly decreased catalase, and glutathione-S-transferase activities, total thiol level with significant concomitant elevation in the levels of reactive oxygen species, and thiobarbituric acid reactive substances in the head and body regions of the treated flies [23]. The recovery effect of chlorpyrifos on antioxidant enzymes, locomotor behaviour and the target enzyme acetylcholinesterase interaction were also studied in mosquitofish, *Gambusia affinis*. Earlier results showed from few researchers depict that chlorpyrifos, besides its inhibitory effect on target enzyme acetylcholinesterase, also inhibits antioxidant enzymes, which can be used as biomarkers in the pesticide-contaminated aquatic streams [24]. To combat electron transport chain degradation by ROS generation, the mitochondrion can reduce the proton gradient of the proton motive force by leaking protons back to the matrix from the inter-membrane space through the uncoupling proteins [25].

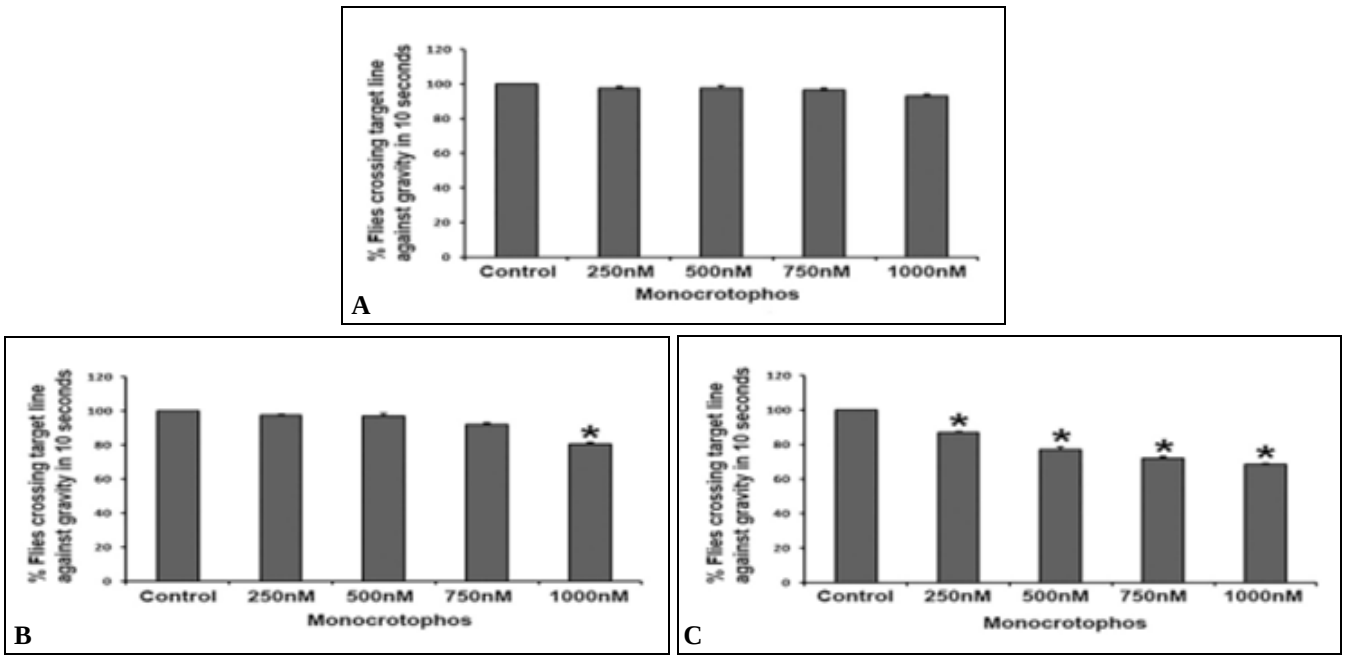

FIG. 6: EXPOSURE OF CHLORPYRIFOS TO DROSOPHILA MELANOGASTER MALE FLIES IMPACTS CLIMBING ABILITIES: 3 DAYS (A), 10 DAYS (B) AND 30 DAYS (C) OLD FLIES WERE EXPOSED TO VARIOUS CONCENTRATIONS OF CHLORPYRIFOS (250 nm, 500 nm, 750 nm, AND 100 nm) OR VEHICLE (CONTROL) FOR 72 h. THEN ASSAYED FOR CLIMBING ABILITY WITH NEGATIVE GEOTAXIS ASSAY AND COUNTED THE NUMBER OF FLIES CLIMBS TARGET LINE (ABOVE 10 cm LENGTH) IN 10 SECONDS. DATA IS EXPRESSED AS PERCENT FLIES CROSSING TARGET LINE AGAINST GRAVITY IN 10 SECONDS. DATA ARE MEAN ± SD, N = 3 INDEPENDENT EXPERIMENTS. * DENOTES SIGNIFICANT DIFFERENCE COMPARED TO CONTROL WITH P<0.05

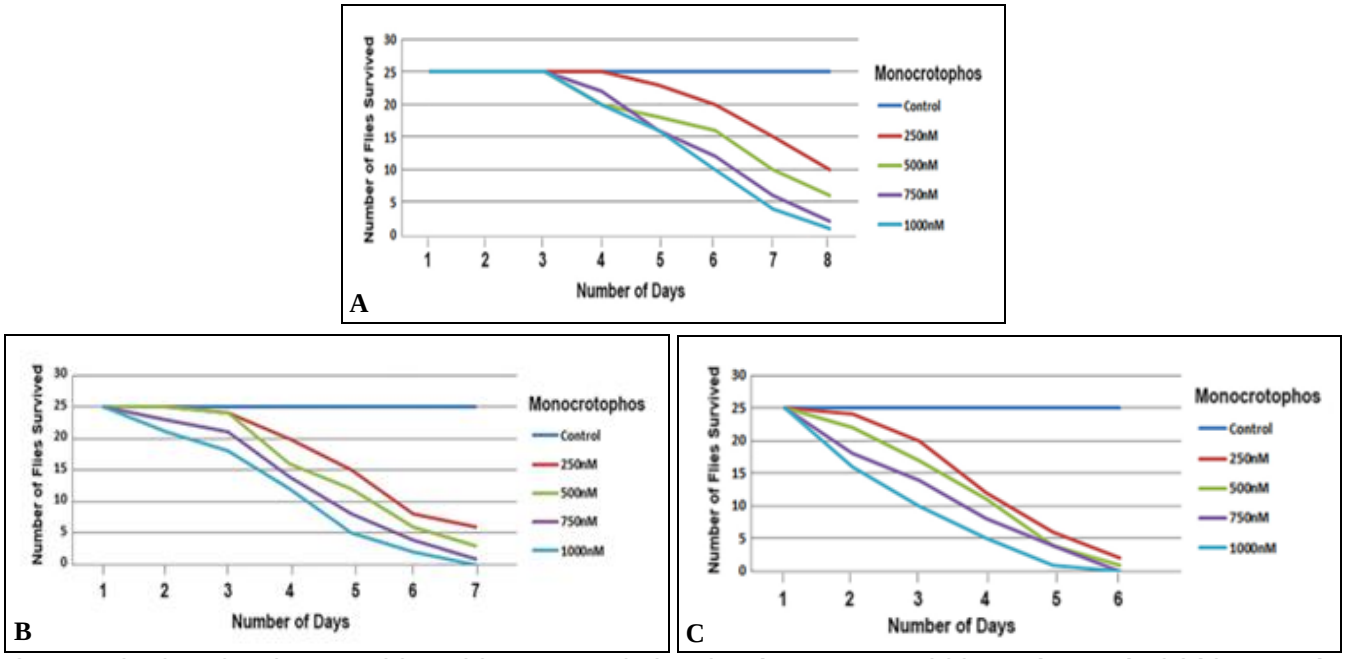

FIG. 7: PESTICIDECHLORPYRIFOSEXPOSURE INDUCES MORTALITY IN DROSOPHILA MELANOGASTER MALE FLIES: 3 DAYS (A), 10 DAYS (B) AND 30 DAYS (C) OLD DROSOPHILA MELANOGASTER FLIES WERE EXPOSED TO 250 nm, 500 nm, 750 nm AND 1000 nm OR VEHICLE (CONTROL). 24 h FOLLOWING EXPOSURE TO CHLORPYRIFOS STARTED MONITORING THE FLIES FOR MORTALITY AND REPEATED THIS AT 24 h INTERVAL UNTIL COMPLETE MORALITY IS OBSERVED. DATA ARE FROM 3 INDEPENDENT EXPERIMENTS AND EXPRESSED AS NUMBER OF FLIES SURVIVED

The power of these genetic systems has revealed many genetic factors involved in the various pathways affected, as well as provide potential drug targets for therapeutics. This review focuses on fruit fly models of human neurodegenerative diseases, with emphasis on how fly models have provided new insights into various aspects of human diseases [26]. Chlorpyrifos was also evaluated for potential teratogenicity and developmental toxicity in mice, which suggested that chlorpyrifos

is teratogenic and embryotoxic in mice at doses below those that cause significant maternal toxicity [27]. The present findings are generally consistent with previous studies conducted on different experimental models. Further research is needed to identify successful strategies to reduce exposure and concentrations of Chlorpyrifos. Drugs that enhance the total cellular GSH levels could be modeled to manipulate the kinetics of GSH metabolism, thereby rendering protection against oxidative/nitrosative stress [28].

CONCLUSION: Treatment with Chlorpyrifos showed a significant decrease in GSH levels and showed maximum oxidative stress. GSH depletion might be the first biochemical event followed by oxidative stress and mitochondrial complex inhibition. Additional research is needed to understand different modes of action, on the role of different routes of exposure, and compare with other species to evaluate similarities and differences of a toxic response. Chlorpyrifos, previously shown to be resistant to enhanced degradation, have now been proved to undergo enhanced microbe-mediated decay. Special emphasis should be given to degradation methods such as ozonation, Fenton treatment, photodegradation, and advanced oxidation processes along with microbial degradation. Finally, we should focus on the degradation process of chemicals at enzyme and molecular levels and standardize minimum dose levels, which will not harm non-targets.

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CONFLICTS OF INTEREST: Nil

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