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Protein level alteration and micronuclei induction in *Channa punctatus* fish exposed to chlorpyrifos and their alleviation by *Melissa officinalis* and *Mentha piperita* extracts

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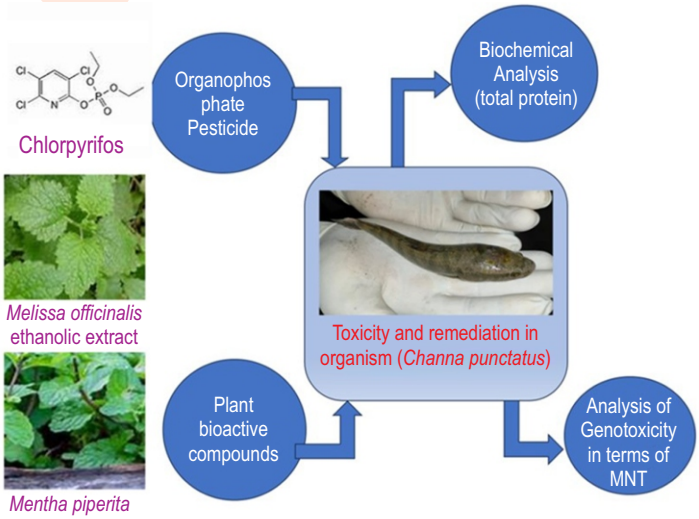
Abstract

Aim: To assess alteration in the protein levels of major organs and induction of micronuclei in the freshwater fish, *Channa punctatus* following exposure to chlorpyrifos, while also investigating the potential mitigating effects of extracts from *Melissa officinalis* and *Mentha piperita* on the adverse impacts induced by this pesticide.

Methodology: Two weeks of acclimatized healthy fish were divided into four different groups. Group G1 served as the control group, while G2 contained chlorpyrifos (LC_{50}/10, 96 hr). Group G3 was exposed to chlorpyrifos (LC_{50}/10, 96 hr) along with 8 mg l^{-1} of *Melissa officinalis* ethanolic extract, and similarly, G4 contained chlorpyrifos (LC_{50}/10, 96 hr) along with 8 mg l^{-1} ethanolic extract of *Mentha piperita*. Sampling was conducted over various durations of 7, 14, 21, and 28 days. The ethanolic extracts of *Melissa officinalis* and *Mentha piperita* were used as remedial compounds as they are rich in several bioactive compounds, primarily polyphenols.



Results: The investigation revealed a notable decrease in the total protein content of kidney, liver, gills and muscles following exposure to chlorpyrifos after each sampling duration. While, upon exposure to remedies containing the ethanolic extracts of *Melissa officinalis* and *Mentha piperita*, the protein levels exhibited a significant recovery ($p < 0.05$) in a duration-dependent manner, compared to the control group. This suggests a promising ameliorative capability of *Melissa officinalis* and *Mentha piperita* against chlorpyrifos toxicity. Further, the induction of micronuclei by chlorpyrifos and their subsequent recovery through the application of phytoextracts *Melissa officinalis* and *Mentha piperita* displayed a similar pattern, indicating the potential protective effects of these extracts against chlorpyrifos-induced genotoxicity.

Interpretation: It is plausible to conclude that standardized doses of *Melissa officinalis* and *Mentha piperita* hold the potential to enhance aquaculture practices and safeguard fish production in agriculturally contaminated water bodies. These findings emphasize their valuable contribution in sustaining fish health amidst toxic environments.

Key words: *Channa punctatus*, Chlorpyrifos, *Melissa officinalis*, *Mentha piperita*

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Introduction

A broad-spectrum pesticide chlorpyrifos belongs to the organophosphate family and is used throughout the world (Kwong, 2002) for pest control in crops (Ali *et al.,* 2009). Commercial application of this organophosphate chemical is used to manage foliar insects that damage crops (Rusyniak and Nanagas, 2004). It is one of the largest-selling organophosphate pesticides and is proven to be more hazardous to fish than organochlorine substances (Macirella *et al.,* 2020). Organophosphate compounds represent themselves as a serious health hazard worldwide carrying nerve-poisoning properties based on the primary mechanism of action that comes from acetylcholinesterase inhibition (AChE) (Lenina *et al.,* 2020). When it comes to producing AChE inhibition, it has been found that chlorpyrifos is more hazardous than diazinon and parathion (Yen *et al.,* 2011), and is one of the most widely used organophosphate insecticides (Sun and Chen, 2008).

The application of chlorpyrifos decreases the population of beetles (Hill *et al.,* 2017). It is known to inhibit acetylcholinesterase, which hydrolyzes the neurotransmitter acetylcholine quickly to produce choline and acetate. Acetylcholinesterase, involved in neurotransmission at cholinergic synapses (Colović *et al.,* 2013), transmits nerve impulses from one nerve cell to the next. The potential toxicity of chlorpyrifos includes various mechanisms but the major toxicity chlorpyrifos is neurotoxicity, causing inhibition of acetylcholinesterase (Yu *et al.,* 2008). Various other mechanisms of chlorpyrifos toxicity to animals has been reported by researchers, one such method is uncoupling of oxidative phosphorylation, which results in the formation of ROS and inhibit mitochondrial ATP synthesis (Ishii *et al.,* 2004). According to Tripathi and Shasmal (2010), it may cause oxidative stress, affect physiological processes, and result in an undesirable metabolic state for the organism. In general, the term "aquatic toxicity" describes how a chemical affects aquatic life. In an aquatic ecosystem, fish are ideal experimental models for the assessment of various stressors and toxic chemicals in an aquatic habitat, where animals are completely exposed to chemicals (Schuijt *et al.,* 2021).

The fauna of aquatic habitats is affected by the physio-chemical parameters of the medium which results in the altered physiological parameters of aquatic organisms. Chlorpyrifos has been found to be a the causative agent of aquatic toxicity even at low concentrations, causes fish mortality (Abdel-Halim *et al.,* 2006). Fish is directly linked to humans through food chain, as it is an important vehicle for carrying lyophilic toxicants from the aquatic environment to humans (Begum and Vijayaraghavan, 1996). A large number of terrestrial as well as aquatic plants are known for their potential use to counter the toxic impression of chemical hazards by strengthening the structural and biochemical properties of the organisms. Numerous plant extracts have demonstrated their ability to mitigate toxicity in the fish species, *Channa punctatus*. After being suitably included in the fish diet, the potential immunostimulatory qualities of medicinal herbs-*Asparagus racemosus* and *Withania somnifera* were noted by Trivedi *et al.,* (2023) in *C. punctatus*. Evidence of the protective effect of *Acacia catechu* against metal toxicity in *C. punctatus* has been elucidated by Dwivedi *et al.* (2015). The herbaceous plant, *Melissa officinalis* is rich in bioactive ingredients and has potent antioxidant properties (Doukani *et al.,* 2021). *Melissa officinalis* improves fish health and reduces oxidative stress and fish mortality (Wang *et al.,* 2022). Phytochemicals like phenolic acids, flavonoids, terpenoids and several others are present in *Melissa officinalis*. These compounds are active in stabilizing pharmacological effects and altering metabolism for health advantages (Zam *et al.,* 2022).

Similarly, *Mentha piperita* also known as peppermint is rich in a variety of bio-active compounds like flavonoids, volatile oils, luteolin, hesperidin, organic components *viz.,* menthol (45.34%), 1,8-cineole (4.46%), neo-menthol (4.24%), limonene (2.22%), menthone (16.04%), menthofuran (8.91%) and cis-carane (8.70%) (Taherpour *et al.,* 2017). Peppermint exhibits certain efficacy against tumors, germs, viruses, radiation, antioxidants, and inflammatory responses. (Chakraborty *et al.,* 2022). Recent studies have shown that plants with strong antioxidant phytochemicals give protection against a wide range of diseases (Urquiaga and Leighton, 2000). Fish serves as an excellent model for toxicity evaluation in aquatic environments. *C. punctatus* is employed as a test model in several research studies due to easy availability and adaptability, particularly to study the remedial impact of plant extracts against pesticide toxicity (Tiwari and Trivedi, 2019). This study correlates the remedial role of *Melissa officinalis* and *Mentha piperita* against chlorpyrifos toxicity, focusing on total protein values in major organs, and genotoxicity analysis using the micronuclei assay.

Materials and Methods

Model animal for toxicity test: Live *Channa punctatus* fish (Family Channidae, Order Perciformes) measuring an average length of 12 ± 2.0 cm and weight 25 ± 2.0 g were purchased from the local fish market. They were carefully transported to the laboratory with enough water in the bucket, and transferred into glass aquaria containing 40 l of chlorine-free water for acclimatization. Previously, the fish were given a prophylactic treatment, which involved bathing them twice in a 0.05% $KMnO_4$ solution for 60 sec, to prevent dermal bacterial infections. These fish were acclimatized for 14 days under following conditions: (Temperature of 24 ± 2ºC, pH-7.2 ±0.2, alkalinity-62 to 68 mg l^{-1}, dissolved oxygen-6.62 to 6.76 mg l^{-1} and CO_2 Nil) performing the experiment. Fish food (Optimum) produced by Perfect Companion Group Co. Ltd., in Thailand was given to the fish.

Test Chemicals: Chlorpyrifos (20% EC) used as a test chemical was manufactured by FINO Agro Industries Hapur, India under the brand name Chloro Gold. Other chemicals were purchased from Sigma Aldrich and Merck for experimental work.

Collection and preparation of ethanolic extracts of *Melissa officinalis* and *Mentha piperita*: The dried leaves of *Melissa officinalis* and *Mentha piperita* were procured from the local market in Lucknow, India. The samples were identified by a taxonomist Botany Department, University of Lucknow. Leaves of *Melissa officinalis* and *Mentha piperita* were shade dried for 10 days and then powdered with the help of a mechanical device. Both the leaf powders were subjected separately in a cellulose thimble and extracted in Soxhlet apparatus using ethanol (95%) in appropriate conditions (Onyebuchi and Kavaz, 2020). After 12 hr, the filtrate of both samples were concentrated to a semi-solid state in a rotary vacuum evaporator. The concentrated semi-solid ethanolic root extracts were kept in a refrigerator for experimental use.

Determination of sub-lethal (LC_{50}) concentration of chlorpyrifos: The LC_{50} value (96 hr) for chlorpyrifos was determined following the acute toxicity bioassay (APHA, 2017). For determining the LC_{50} value for *C. punctatus* different rising concentrations of chlorpyrifos (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 mg l^{-1}) were prepared from the stock solution. Chlorpyrifos was dissolved in acetone and poured into different aquaria containing 80 l of water each. Acclimatized healthy fish having a nearly equal size and weight were randomly selected and put into separate groups that were exposed to different concentrations of chlorpyrifos in respective aquaria.

To verify reproducibility, the experiments were carried out in three replicates. The optimum temperature and pH were maintained, and oxygen was provided with aerators. Fish mortality and survival of fish were noted at various intervals 6, 12, 24, 48, 72 and 96 hrs following exposure to chlorpyrifos. Careful observation was made to monitor fish mortality, and dead fish were removed immediately to maintain the physico-chemical properties of the aquatic environment in aquaria from any internal factors.The 96 hr-LC_{50} of chlorpyrifos was calculated (0.82 mg l^{-1}) by Trimmed Spearman–Karber Method (Hamilton *et al.,* 1977).

Experimental design: Four groups were formed to investigate the efficacy of *M. officinalis* and *M. piperita* extracts along with sub-lethal concentrations of chlorpyrifos, each comprising ten healthy and acclimated fish. These groups are labeled as G1 to G4: G1 (Control), G2 ($1/10^{th}$ of LC_{50} of CPF), G3 ($1/10^{th}$ of LC_{50} of CPF + 8 mg l^{-1} MO), and G4 ($1/10^{th}$ of LC_{50} of CPF + 8 mg l^{-1} MP). Three replicates were used for each concentration. At the end of stipulated exposure periods (7, 14, 21 and 28 days), fish were randomly selected, and samples were taken for further investigation from both the treatment groups and the control group. Sub-lethal concentrations of 1/10 of LC_{50} based on 96 hr-LC_{50} value, single and combined exposure were estimated and used for *in vivo* experiment.

Estimation of protein: Total protein content in different (kidney, liver, gills and muscles) of control and treated fish at the end of stipulated exposure periods (7, 14, 21 and 28 days) was estimated by Lowry's method (1951). For this, the tissues were washed in NaCl solution homogenized with 5% trichloroacetic acid. The homogenates were centrifuged for 10 min at 3000 rpm and the residues were dissolved in 0.1N NaOH. Using 0.1N NaOH, precisely 0.2 ml of this solution was increased to 1 ml and proceeded for protein estimation through a spectrophotometer.

Micronuclei assay: Micronuclei are small, extra-nuclear, circular, or ovoid structures made up of acentric chromosomal fragments. Fish blood from each experimental as well as control group at the stipulated time was drawn by cardiac puncture with the help of a 22-gauge needle and a plastic disposable syringe (Molnar,1960), and immediately used for the preparation of a thin and uniform smear on a pre-cleaned microscopic slide. The slides were left overnight for air drying in a dust and moisture-free environment at room temperature, followed by fixing with absolute methanol for 5 min and staining for 3 to 5 min with May-Grunwald's solutions 1 and 2. Further slides were stained with 5% Giemsa for 30 min. Stained slides were subjected to further drying overnight before mounting with DPX and finally observed under an oil immersion microscope using 40/100X objective lenses. Cellular anomalies like micronuclei, were observed for analysis (Fenech *et al.,* 2003). Each slide had 2000 erythrocyte cells that were evaluated for the presence or absence of micronuclei. MN was calculated using the following formula:

MN (%) = (Number of cells containing micronucleus × 100) / Total number of cells counted

Statistical analyses: The data obtained were presented as mean ± S.E. Statistical significance ($p < 0.05$) was assessed by One-way ANOVA. Post-hoc comparisons with the control were conducted by Tukey's test. Statistical analyses was performed using SPSS (Statistical Package for Social Sciences) software, version 20.0.

Results and Discussion

A significant ($p < 0.05$) decrease in protein percentage was observed in the kidney, liver, gills and muscle tissues of *C. punctatus* following sub-lethal exposure to CPF in Group G2 after each sampling duration. The decrease in protein levels in the tissues of fish exposed to sub-lethal concentrations of chlorpyrifos for 7 to 28 days suggests that protein degradation may be a compensatory mechanism in response to stress induced by toxicant metabolism and energy production. These findings highlight the impact of chlorpyrifos exposure on the depletion of total protein content in fish tissues, as evidenced by comparison with the control group. These findings corroborates with the study of Sadiqul *et al.* (2017) who reported a similar reduction in protein content and alteration in genotoxic parameters in response to quinalphos exposure in a freshwater fish, *Barbonymus gonionotus*. Moreover, in the present study, a significant ($p < 0.05$) recovery of protein levels in the kidney, liver, gills and muscle was recorded in G3 and G4 group fish that were exposed to combined treatment of chlorpyrifos and *M. officinalis* and chlorpyrifos and *M.*

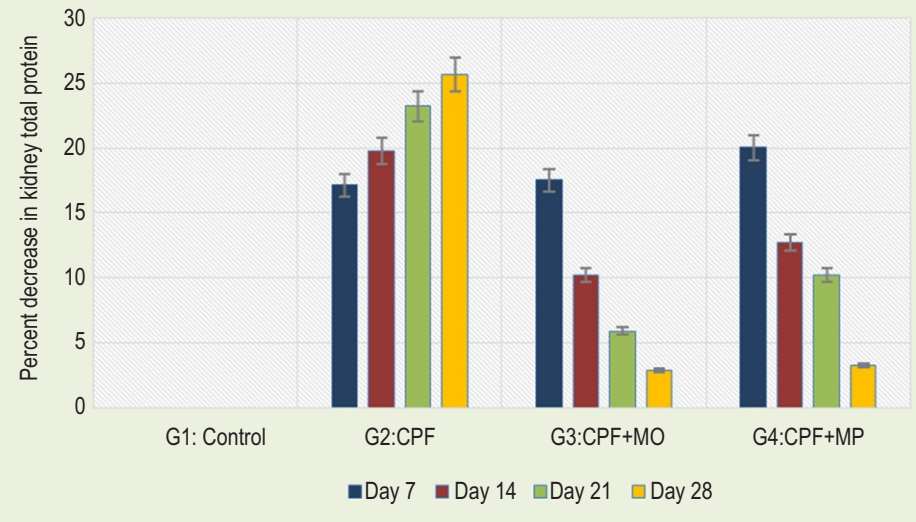


Fig. 1: Percent decrease in kidney total protein for different experimental groups over four exposure durations: 7, 14, 21 and 28 days. Groups include G1 (control), G2 (Chlorpyrifos-treatment), G3 (Chlorpyrifos + *Melissa officinalis* extract) and G4 (Chlorpyrifos + *Mentha piperita* extract).

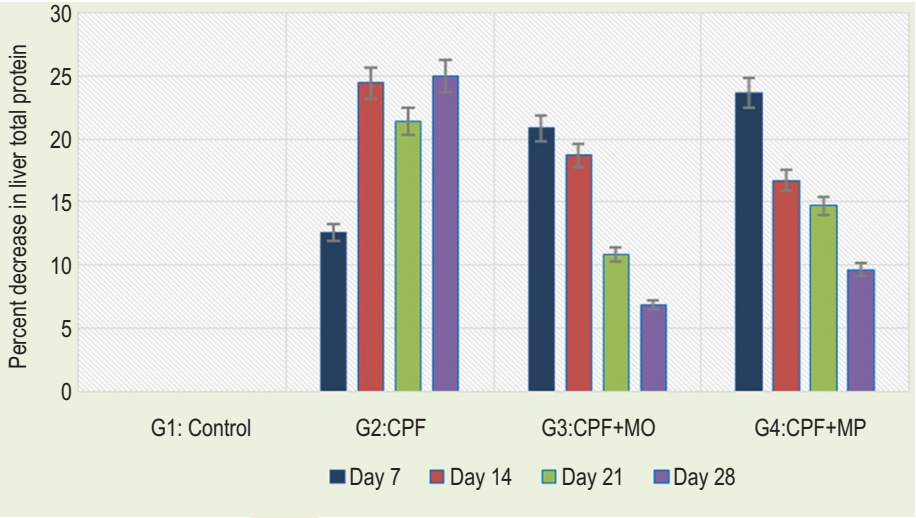


Fig. 2: Percent decrease in liver total protein for different experimental groups over four exposure durations: 7, 14, 21, and 28 days. Groups include G1 (control), G2 (Chlorpyrifos-treatment), G3 (Chlorpyrifos + *Melissa officinalis* extract) and G4 (Chlorpyrifos + *Mentha piperita* extract).

piperita extracts respectively, at different exposure duration (Fig. 1-4). The inclusion of *M. officinalis* and *M. piperita* seems to alleviate protein content in G3 and G4, as indicated by the decrease in the percentage reduction of protein levels in the liver, gills, muscles, and kidney tissues of test fish after each sampling period. Chlorpyrifos induces a spectrum of biochemical alterations in fish, manifesting as impaired metabolism of carbohydrates and proteins, alongwith lipid disturbances and changes in detoxification enzymes. These alterations disrupt the normal rate of biochemical reactions, rendering the organism less capable of tolerating chemically stressed environments. Protein degradation could be the cause of drop in protein levels to counter the stress developed by toxicant metabolism and energy production (De Smet and Blust, 2001) . Concurrently, the levels of kidney total protein in G3 group was 17.54%, 10.24%, 5.92% and 2.90% and G4 group 20.03%, 12.74%, 10.25% and 3.29%. The decline in protein content were significantly lower ($p < 0.05$) than those observed in the G2 (17.13%, 19.79%, 23.22% and

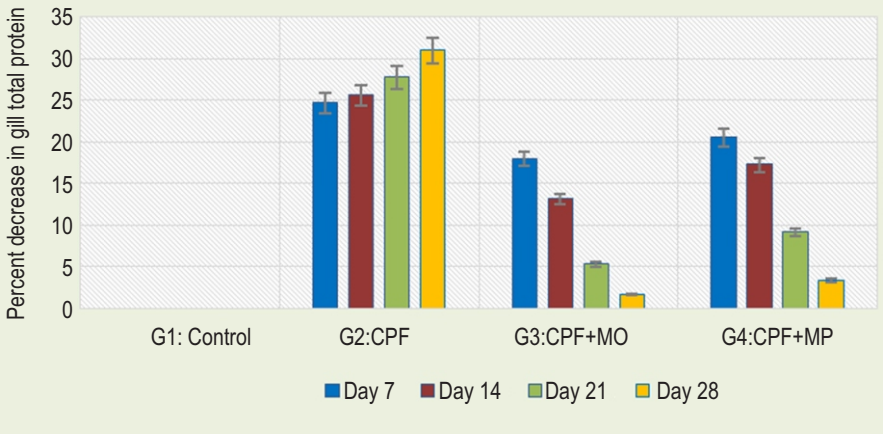


Fig. 3: Percent decrease in gill total protein for different experimental groups over four exposure durations: 7, 14, 21 and 28 days. Groups include G1 (control), G2 (Chlorpyrifos-treatment), G3 (Chlorpyrifos + *Melissa officinalis* extract) and G4 (Chlorpyrifos + *Mentha piperita* extract).

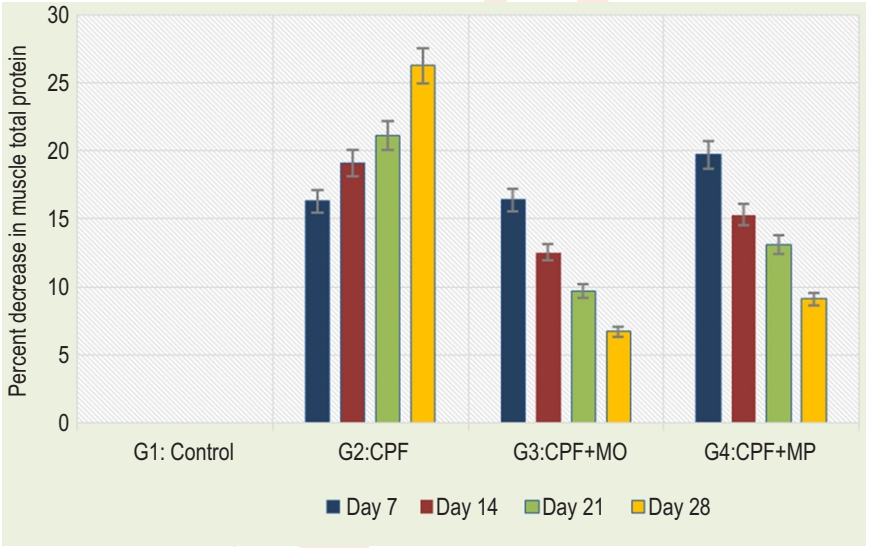


Fig. 4: Percent decrease in muscle total protein for different experimental groups over four exposure durations: 7, 14, 21 and 28 days. Groups include G1 (control), G2 (Chlorpyrifos-treatment), G3 (Chlorpyrifos + *Melissa officinalis* extract), and G4 (Chlorpyrifos + *Mentha piperita* extract).

25.66%) following treatment for 7, 14, 21 and 28 days, respectively.

Similarly, the values of liver total protein in G3 group was 20.85%, 18.71%, 10.83% and 6.83% and 23.64%, 16.73%, 14.73% and 9.67% in the G4 group, with the percentage decline in protein content significantly lower ($p < 0.05$) than G2 group (12.59%, 24.42%, 21.45% and 25.01%) after 7, 14, 21 and 28 days of treatment, respectively. Hence, it can be concluded that the decrease in protein degradation observed in the G3 and G4 groups, compared to the G2 group, is attributed to the remedial effects of the herbs utilized in the study. This suggests that the inclusion of *M. officinalis* and *M. piperita* extracts mitigated the decline in liver total protein levels in fish exposed to chlorpyrifos. The gill total protein in the G3 group was 18.01%, 13.15%, 5.32%, and 1.74% and 20.56%, 17.29%, 9.21% and 3.42% in the G4 group. The percent decrease in protein content was significantly lower ($p < 0.05$) in comparison to chlorpyrifos-exposed group (24.64%, 25.61%, 27.80% and 30.94%) after 7, 14, 21, and 28 days of treatment, respectively.

The muscle total protein in the G3 group was 16.39%, 12.58%, 9.73% and 6.72%, while in the G4 group 19.72%, 15.33%, 13.12% and 9.10%. The percent decrease in protein

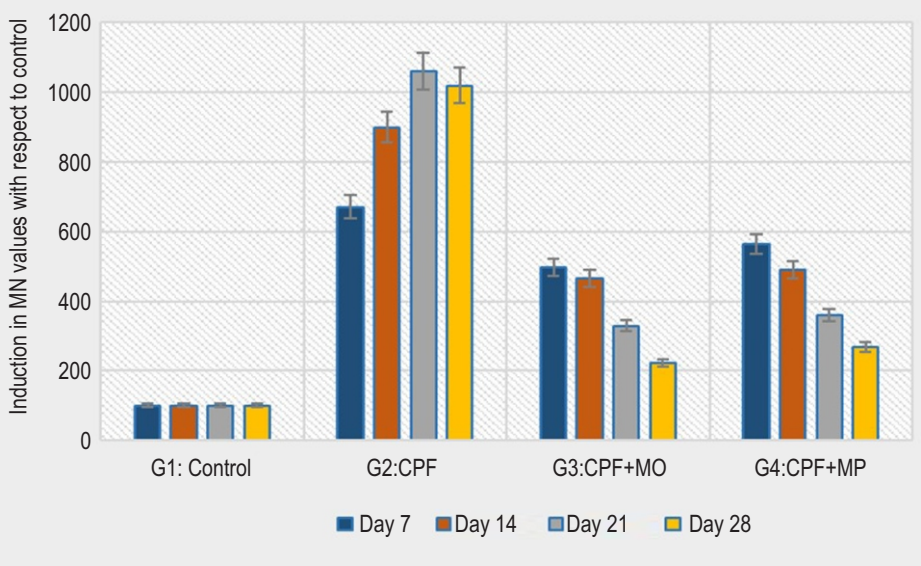
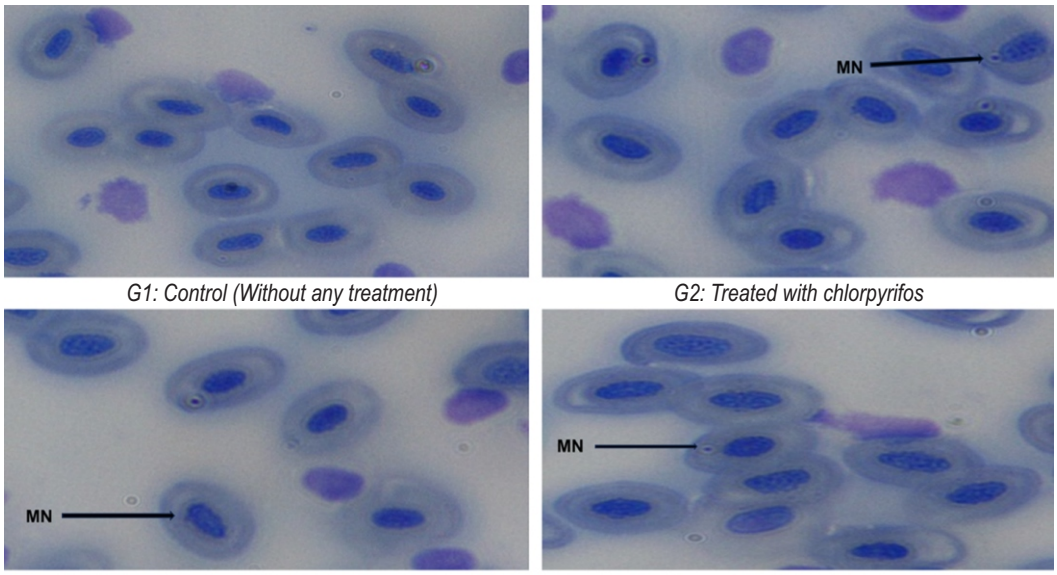


Fig. 5: Micronuclei induction across different treatment groups and exposure durations. (7, 14, 21 and 28 days) Groups include G1 (control, no treatment), G2 (Chlorpyrifos-treatment), G3 (Chlorpyrifos + *Melissa officinalis* extract), and G4 (Chlorpyrifos + *Mentha piperita* extract).



G1: Control (Without any treatment)

G2: Treated with chlorpyrifos

G3:Treated with chlorpyrifos and Melissa officinalis extract

G4:Treated with chlorpyrifos and Mentha piperita extract

Fig. 6: Micronuclei induction for different treatment groups G1: Control i.e. without any treatment, G2: Treated with chlorpyrifos, G3: Treated with chlorpyrifos and *Melissa officinalis* extract and G4: Treated with chlorpyrifos and *Mentha piperita* extract in erythrocytes of *Channa punctatus.*

content was significantly lower ($p < 0.05$) than chlorpyrifos-exposed group (16.33%, 19.10%, 21.15% and 26.28%) after 7, 14, 21 and 28 days of treatment, respectively. The reduction in protein levels across vital metabolic organs of fish species, including *Labeo rohita* and *Cirrhinus mrigala*, post-exposure to the insecticide fenvalerate, was documented by Susan *et al.* (2010). Similarly, Revathi *et al.* (2020) also documented the decrease in protein levels in *Channa punctatus* after exposure to chlorpyrifos pesticide. As proteins are the building blocks for cells and tissues, the bioactive and antioxidant-containing compounds are useful to stabilize and strengthen the tissue which is undergoing degradation against stress generated by toxicants. In

treatment groups G3 and G4, where *M. officinalis* and M. *piperita* were administered, respectively, significant reductions ($p < 0.05$) in the percentage decrease of kidney total protein, gill total protein, muscle total protein and liver total protein levels were observed compared to the G2 group. However, the kidney total protein, gill total protein, muscle total protein, and liver total protein levels could not recover in the treatment groups as compared to the control group. The decline in the total protein value in these vital organs can be attributed to exhaustive proteolysis and loss of total protein in the tissue when exposed to the stressed environment. Similar findings were also found by Revathi *et al.* (2020). The protein content in the remedy-exposed groups may have recovered because of the strengthening physiological reaction of an exposed organism by bioactive compounds present in the *Melissa officinalis* and *Mentha piperita*.

In addition to protein degradation, the increase in micronuclei frequency served as a significant indicator of genotoxicity in CPF-exposed fish. A significant ($p < 0.05$) increase in micronuclei (Fig. 5) was observed in the group-G2 for the varied duration of exposure. Induction of MN frequency was observed in the G2 groups exposed to CPF, while a decrease in MN frequency was noted in G3 and G4 following remediation with *M. officinalis* and *M. piperita* (Fig. 6). When compared with the control group, G2 group showed higher induction of micronuclei, however, a significant ($p<0.05$) decrease in micronuclei were recorded in G3 group (495.51%, 465.38%,328.85%, and 221.79%) as well as in G4 groups (564.10%, 491.02%, 358.97% and 369.23%) was found after 7, 14, 21, and 28 d of treatment. The recovery in G3 and G4 groups may be due to the strengthening nuclear integrity of cells of the exposed organism by bioactive compounds present in the *Melissa officinalis* and *Mentha piperita*.

The present findings indicate that chlorpyrifos-induced oxidative stress might be alleviated through bioactive compounds found in *M. officinalis* and *M. piperita*. Additionally, the presence of flavonoids in these extracts suggests potential benefits for antioxidant enzyme regulation, including the induction of phase II detoxification. Similar to the present results, Dwivedi *et al.* (2017) documented that *Melissa officinalis* may alleviate arsenite-induced oxidative stress and enhance antioxidant enzyme regulation in fish, *Channa punctatus*. *M. officinalis* was also found effective against malathion toxicity in rats (Seif *et al.*, 2014). Likewise, Tiwari *et al.* (2017) revealed the remedial role of *M. officinalis* against carbofuran toxicity in common food fish. Samarth *et al.* (2006) documented the protective role of *M. piperita* against benzo[a]pyrene-induced carcinogenicity in Swiss albino mice. Moreover, the notable difference in micronuclei induction between the chlorpyrifos-alone group and those treated with *M. officinalis* and *M. piperita* extracts may be attributed to the antioxidative properties and detoxification mechanisms conferred by the bioactive compounds present in the extracts. Consistent with the findings of the current study, Tiwari *et al.* (2016) documented the ameliorative potential of *Rauvolfia serpentina* (Sarpagandha) root extract against genotoxicity, specifically in terms of Random Amplified Polymorphic DNA (RAPD), observed in *Channa punctatus* exposed to a carbamate pesticide. Zeraatpishe *et al.* (2011) documented that the consumption of *M. officinalis* infusion by radiology unit workers led to a marked reduction in plasma DNA damage and impacts of *M. officinalis* on lowering the stress in living sea bass (Wang *et al.*, 2022). Hassan *et al*. (2012) found that *M. piperita* extract pretreatment in gamma-irradiated mice led to improvements in brain apoptosis, DNA cycle stability, cell cycle analysis, and antioxidant defense systems. Highlighting the role of therapeutic plants in mitigating the effects of environmental stressors, Trivedi *et al.* (2021) documented that *Rauwolfia serpentina*, a medicinal plant, alleviates copper-induced genotoxicity in *C. punctatus* Similarly *R serpentina* has shown therapeutic effect to manage the hypertension and hypercholesterolemia in albino rats (Shah *et al.*, 2020). Additionally, Trivedi *et al.* (2024) reported the health-promoting properties of *W. somnifera* and *A. racemosus* in *C. punctatus*, showing significant ($p<0.05$) increases in growth indicators. In conjunction with these findings, the present study suggests that the antioxidative and detoxifying properties of *M. officinalis* and *M. piperita* extracts contribute significantly to their ability to mitigate DNA damage and protein content alterations in test fish, *C. punctatus*. These mechanisms, such as scavenging reactive oxygen species, enhancing antioxidant enzyme activity, and promoting cellular repair processes, underscore the potential therapeutic value of these herbal extracts in addressing oxidative stress-related conditions.

The present study investigates the effectiveness of *Melissa officinalis* and *Mentha piperita* in mitigating chlorpyrifos toxicity in fish. Through their antioxidant properties, plant extracts exhibit a capacity to reduce micronucleus frequency and percent decrease in protein levels in vital organs like liver, kidney, gill and muscles. Significant ($p < 0.05$) reduction in micronucleus frequency and increase in protein levels were observed in fish treated with *Melissa officinalis* and *Mentha piperita* extract as compared to chlorpyrifos. This research highlights the potential of plant extracts to enhance fish health in contaminated environments, thus offering a promising strategy to enhance fish productivity amidst toxicant exposure. Ultimately, the utilization of MO and MP extracts holds considerable promise in ameliorating the adverse effects of toxicants, potentially leading to improved fish productivity in contaminated aquatic environments.

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MS correction and drafting; **V. Kumar:** Preparation of graphical abstract and MN slide analysis; **K.K. Yadav:** Sampling and MN slide analysis; **S.P. Trivedi:** Overall planning and experiment lay out, Editing of MS.

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Ethical approval: Institutional Animal Ethics Committee (IAEC) of the University of Lucknow (vide registration no 1861/GO/Re/S/16/CPCSEA, Lucknow).

Conflict of interest: The authors declare that there is no conflict of interest.

Data availability: This article contains all the data created or examined during this investigation.

Consent to publish: Publication of the manuscript in the *Journal of Environmental Biology* is approved by all authors.

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